ABSTRACT BOOK

25TH INTERNATIONAL PAPILLOMAVIRUS CONFERENCE CLINICAL & EDUCATIONAL WORKSHOP

May 8 - 14 2009 Malmö, Sweden

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I. SmPC, August 2008

[#] For individual HPV 18 the significance was not reached. Safety with AS04 is unknown beyond 6.4 years. The vaccination should be done in line with official recommendations. Cervarix is a trademark of the GlaxoSmithKline group of companies

Abstracts of the 25th International Papillomavirus Conference

Dear Colleagues!

It is with pleasure and pride we hereby provide the abstracts of the 25th International Papillomavirus Conference to you.

With the amazing amount of high quality abstracts submitted, the International Papillomavirus Conference is consolidating its position as the leading conference encompassing all areas of Papillomavirus research, from clinical vaccinology to molecular biology.

Overall, about 3300 authors submitted 966 abstracts. We would like to take this opportunity to extend our gratitude to the 104 members of the 25th IPV Scientific Committee who completed >4500 abstract reviews. This abstract book contains 887 accepted abstracts, making the 25th IPV the largest International Papillomavirus Conference ever.

To promote ample time for interactions, all 887 posters will be displayed throughout the conference (Sunday-Thursday). The poster display is complemented with 2 parallel plenary sessions with 237 oral abstract presentations in a succinct presentation format with time for discussion.

The need for a comprehensive HPV conference has never been greater. We know today that HPV causes about 5% of all cancers in man, ranking among the top 3 causes of cancer. There are several ways to prevent HPV-associated diseases as we now have access to effective primary, secondary and tertiary prevention as well as treatment. Limited knowledge about how to prevent and treat HPV-associated disease is recognized as a major reason why these diseases are still a major disease burden globally. Advancing and disseminating this knowledge is of utmost importance in the fight against cancer.

As there are almost 2000 Medline-indexed scientific publications on papillomaviruses every year, the IPV has an important role in facilitating that the scientific advances in the field rapidly reach the entire HPV scientific community.

Finally, an important role of the International Papillomavirus Conferences has always been to bring people together for intellectual discussions. We believe that the scientific progress in the field is significantly furthered by an annual forum that has so comprehensive attendance that most of the scientists active in the field can meet there in person. Indeed, many major advances on linking HPV to human diseases and the ways how to prevent them have been conceived during discussions at the IPV conferences.

The mighty Öresund bridge that is part of the official logo of the 25th IPV symbolizes not only the dynamic Danish-Swedish Öresund region where the conference is held, but is also a symbol of our ambition to provide a bridge for communication between scientists working in many different disciplines in many parts of the world.

We hope that this Abstract Book will provide you with the foundation for an interesting and inspiring conference on the timely subject of Papillomavirus research.

Joakim Dillner Chairman of the Organizing Committee



NOTE ADDED IN PROOF: NEW ROOMS

Please observe that because of the extraordinary high number of participants, the Rooms for the Oral Presentations are changed. All presentations previously scheduled for Room Scania will now be held in Hall C. All presentations previously scheduled for Room K1-K3 will now be held in Scania.

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HERA CONSEIL

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HPV PROPHYLACTIC VACCINES

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THE 25TH INTERNATIONAL PAPILLOMAVIRUS CONFERENCE MAY 8-14 2009, MALMÖ,	
SWEDEN	

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O-01.00 HPV VACCINATION AND PUBLIC HEALTH: OPPORTUNITY AND CHALLENGE

J Paavonen, Department of Obstetrics and Gynecology, University Hospital, Helsinki, Finland

Human papillomavirus (HPV) infection is the most common viral sexually transmitted infection. HPV infections are extremely common, and most young adults are exposed to HPV soon after sexual debut. Although most HPV infections resolve, persistent infection by one or more of the oncogenic HPV types can cause cervical neoplasia. HPV can also cause other anogenital neoplasias and a smaller proportion of oropharyngeal neoplasias. Worldwide, cervical cancer is the second most common cancer in women and the 3rd leading cause of cancer death in women worldwide. Secondary prevention by cytologic screening has been effective in some countries, but the screening policies vary widely. Therefore, the incidence rates of cervical cancer differ strikingly between countries, also within Europe. Due to mass screening, the disease burden has shifted to management of cervical intraepithelial neoplasias (CIN). This causes problems and drains health care resources. The development of prophylactic HPV vaccines that target HPV16/18 which account for at least 70% of cervical cancers has been a remarkable success. These vaccines can substantially reduce the public health and economic burden of cervical precancer and cancer and other HPV-associated diseases. The vaccines are safe and highly immunogenic. International phase III trials among 15-26 year old women have demonstrated that among HPV naive women the vaccines are nearly 100% efficacious against persistent infection and high grade cervical precancer (CIN2/3) caused by the vaccine HPV types. This is extremely reassuring since CIN2/3 is considered a valid surrogate marker of cervical cancer. Thus, primary prevention by vaccination of young adolescents before sexual debut will be the most effective strategy to prevent cervical (and other HPV related) cancer. Not surprisingly the efficacy among those already exposed to HPV is lower. However, it appears that only a small subpopulation of young adults have been infected by more than one vaccine HPV type suggesting that catch-up vaccination may still be beneficial resulting in less HPV infections and decreasing need for invasive cervical procedures to evaluate atypical Pap smear findings. Regarding catch-up vaccination, public health benefit and overall resources need to be taken into account when making decisions how to implement vaccination programs. An important bonus effect of the vaccines is cross-protection against infection and disease caused by 16/18-related HPV types, specifically against HPV31 and 45 which are the next most important HPV types causally attributed to cervical cancer, with an attributable proportion of approximately 10% of cases. Vaccines providing significant degree of crossprotection mey therefore increase the overall impact. Clinical trials have also raised several questions and challenges to be addressed to assess the public health impact, and to design the most effective vaccination strategy. These include duration of immune response, vaccination of males, efficacy against other HPV-related cancers, postmarketing surveillance, impact of vaccination on screening programs, potential type-replacement after widespread vaccination, and finally feasibility of HPV vaccination in developing countries where the disease burden is enormous.

O-01.01

IMPACT OF HPV VACCINATION DEPENDS ON EFFECTIVE STRATEGY

M Lehtinen, National Institute of Health& Welfare, Tampere, Finland

Human papillomavirus (HPV) vaccination for the prophylactic prevention of associated diseases is recent and the impact of vaccination strategies has yet to be assessed. In 1975 a live rubella vaccine was introduced for young girls in Finland primarily to prevent congenital rubella syndrome, however it was not until 1982 when MMR vaccination of boys and girls was introduced that the number of rubella cases significantly declined. By 1997 indigenous rubella was eliminated in Finland.

Herd-immunity against high-risk (hr) HPVs is most effective because of assortativeness of sexual behaviour/transmission. A dynamic transmission model suggests that vaccination of boys and girls has an impact on hrHPV occurrence: Moderate vaccine coverages reduce HPV16 prevalence by 20 % in the unvaccinated population; vaccination of also boys decreases the incidence of cervical high-grade lesions (HSIL) by an extra 20%. Most analyses find that vaccination of boys is not cost-effective if high levels of coverage are achieved in girls. On the other hand, significant proportions of marginalized females are currently neither taking HPV vaccine nor attending screening, and will eventually present with HSIL+. This threatens to jeopardize effectiveness of intervention programmes against hrHPVs and their sequelae.

Finland is running a phase IV trial in 33 randomized communities (A: boys&girls get HPV16/18 vaccine, B: girls get HPV16/18 vaccine and boys get hepatitis B vaccine, C: boys&girls get hepatitis B vaccine) to study effectiveness of different vaccination strategies. When the participants, living in the original community, reach 18-years of age they are screened for hrHPV to find out relative reduction of hrHPV. In the first 18 months 32 000 1992-95 born boys (35%) and girls (65%) have volunteered. The study is expected to complete by 2014.

O-01.02

IMMUNE RESPONSE AFTER PRIMARY VACCINATION COURSE: A COMPARATIVE TRIAL OF TWO HPV PROPHYLACTIC VACCINES

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Background: Vaccine-induced protection against HPV-16/18 has been demonstrated for two prophylactic human papillomavirus (HPV) vaccines: HPV-16/18 AS04-adjuvanted vaccine (Cervarix[™]; GlaxoSmithKline Biologicals) and HPV-6/11/16/18 vaccine (Gardasil[®]; Merck). It is unclear whether differences exist in immune responses elicited by these vaccines and whether they represent determinants of long-term protection, or protection against HPV types other than 16 and 18.

Objectives: To compare immunogenicity and safety one month after third vaccine dose (Month 7) of Cervarix[™] or Gardasil[®] in healthy women aged 18–45.

Methods: In this observer-blind study (NCT00423046), women (n=1,106) were stratified by age (18–26, 27–35, 36–45 years) and randomized (1:1) to receive Cervarix^m (Months 0, 1, 6) or Gardasil^{*} (Months 0, 2, 6). Antibody responses were evaluated (sera and cervicovaginal secretions [CVS]) by pseudovirion-based neutralization assay (PBNA, developed by NCI) and ELISA, and memory B-cell responses (sera) by ELISPOT assay.

Results: In ATP cohort (seronegative/DNA-negative before vaccination for HPV type analyzed), GMTs of serum neutralizing antibodies measured by PBNA at Month 7 were 2.3–4.8-fold higher for HPV-16 and 6.8–9.1-fold higher for HPV-18 with Cervarix^m compared with Gardasil[®], across all age strata. In TVC cohort (women who received ≥ 1 dose), Cervarix^m induced significantly higher HPV-16 and -18 serum neutralizing antibody titers; p<0.0001 for each antigen in each age stratum. Positivity rates for anti-HPV-16/18 neutralizing antibodies in CVS and circulating HPV-16/18-specific memory B-cell frequencies were higher for Cervarix^m. Both vaccines were generally well-tolerated. Rates of solicited symptoms were higher for Cervarix^m, injection site reactions being most common. Compliance was high (84%) for both vaccines.

Conclusions: Higher immune response was observed with Cervarix[™] than with Gardasil[®], which may represent a determinant of duration of protection against HPV-16/18. Long-term studies evaluating duration of vaccine efficacy are needed to assess the clinical relevance of observed differences in immune response.

O-01.03

LONG-TERM EFFICACY OF A PROPHYLACTIC HUMAN PAPILLOMAVIRUS TYPE 16 VACCINE

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JP Hughes, University of Washington, Seattle, WA, USA

NS Weiss, University of Washington, Seattle, WA, USA

LA Koutsky, University of Washington, Seattle, WA, USA

BACKGROUND: Prophylactic human papillomavirus (HPV) L1 virus-like particle (VLP) vaccines have demonstrated high levels of protection against HPV infection and cervical intraepithelial neoplasia (CIN) in randomized controlled trials (RCTs) through limited follow-up. OBJECTIVE: To provide efficacy data after administration of a prophylactic HPV-16 L1 VLP vaccine at a mean of 8.5 years (range: 7.2-9.5 years), the longest duration of follow-up reported to date. METHODS: Between March 2006 and May 2008, 290 women who had participated in a phase IIb RCT of this vaccine in Seattle (November 1998-January 2004) were enrolled in an extended follow-up study. RESULTS: During the RCT period, one woman exhibited HPV-16 infection by HPV DNA detection at a single visit (month 12) in the vaccine group; 15 women exhibited HPV-16 infection and 5 women developed HPV-16-associated CIN in the placebo group. During the extended follow-up period, no woman exhibited HPV-16 infection or developed HPV-16-associated CIN in the vaccine group; 6 women exhibited HPV-16 infection (vaccine efficacy [VE] = 100%; 95% confidence interval [CI]: 25%-100%) and 3 women developed HPV-16-associated CIN (VE = 100%; 95% CI: <0%-100%) in the placebo group. Approximately 86.3% of vaccine recipients remained HPV-16 competitive Luminex® immunoassay seropositive at 8.5 years. Overall, throughout the combined RCT and extended follow-up periods, 1 woman exhibited HPV-16 infection and no woman developed HPV-16-associated CIN in the vaccine group; 21 women exhibited HPV-16 infection (VE = 96%; 95% CI: 73%-100%) and 8 women developed HPV-16-associated CIN (VE = 100%; 95% CI: 47%-100%) in the placebo group. CONCLUSIONS: The prophylactic HPV-16 L1 VLP vaccine remains highly efficacious against HPV-16 infection 8.5 years after its administration. While there was limited power to definitely address the efficacy against cervical lesions during the extended follow-up period, it was reassuring that no vaccine-recipient developed HPV-16-associated CIN.

O-01.04

QUADRIVALENT HPV (TYPES 6/11/16/18) VACCINE EFFICACY AGAINST LOW-GRADE GENITAL DISEASE

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Objectives: To evaluate the prophylactic efficacy of the quadrivalent HPV types 6/11/16/18 vaccine in preventing lowgrade cervical, vulvar, and vaginal intraepithelial neoplasias (CIN 1, VIN 1, and VaIN 1, respectively) and condylomata acuminata.

Methods: Women were enrolled from primary care centers and university/hospital associated health centers in 24 countries and territories around the globe. 17,622 women aged 16-26 years were enrolled between December 2001 and May, 2003. Major exclusion criteria were pregnancy and history of abnormal Pap test results. 17,599 women aged 16-26 were randomized to three doses of quadrivalent HPV 6/11/16/18 vaccine or placebo at day 1, month 2, and month 6. Efficacy against low-grade cervical and anogenital disease in a per-protocol susceptible population that included subjects who received 3 doses, were sero- and DNA-negative to the relevant vaccine HPV type(s) at day 1 and remained DNA-negative to the relevant vaccine HPV type(s) through month 7, and had no major protocol violations as well is in a generally HPV-naïve population.

Results: In the per-protocol susceptible population, vaccine efficacy against HPV 6/11/16/18-related lesions was 95.9% for CIN 1 (95% confidence interval [CI]: 91.3, 98.4), 100% for both VIN 1 (95% CI: 74.1, 100.0) and VaIN 1 (95% CI: 64.0, 100.0), and 99.0% (95% CI: 96.2, 99.9%) for condylomata. The protection against any disease (regardless of HPV type) in the generally naïve population was 20.3% for CIN 1 (95% confidence interval [CI]:12.4-27.5), 32.3% for VIN 1 (95% CI: <0-60.0), 30.9% for VaIN 1 (95% CI: 64.0, 100.0), and 82.8% (95% CI: 74.3-88.8%) for condylomata.

Conclusions: Quadrivalent HPV 6/11/18/18 vaccination provided sustained protection against vaccine-HPV-type-related low grade diseases (CIN 1, VIN 1, VaIN 1, and condylomata) and provided a substantial reduction in the burden of low-grade diseases through 44 months of follow-up.

Trial Registrations: NCT00092521 and NCT00092534

O-01.05

IMPUTED GLOBAL VACCINATION BENEFIT BY KEY RISK PREDICTORS

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Background: Predictors and benefit from HPV vaccination among adults have not been clearly established. Objective: To impute absolute and relative impact of HPV-16/18 vaccination on cumulative rate of CIN2+ and/or persistent HPV infection by selected HPV risk predictors.

Methods: 7,466 women enrolled in the NCI-sponsored community-based Costa Rica HPV Vaccine Trial (CVT) were evaluated (median follow-up: 35 mos). To estimate vaccine impact without unblinding the trial we 1) calculated the trial's overall attack rate of CIN2+ and/or oncogenic type-specific HPV persistent (>10 mos) infection, 2) applied published vaccine efficacy estimates to the attack rates to estimate study-arm-specific event numbers, and 3) imputed vaccine benefit using absolute (rate reduction) and relative (% reduction) measures, stratified by age, sexual behavior, and other variables of interest.

Results: The overall reduction in burden was 35.7 (per 1,000 women), which represented a 17.3% decline. Rate reduction was 35.7 for 18-19 years old and 27.1 among 24-25 year old women; the respective relative measures were 20.2% and 12.8%. Rate reduction estimates by time since sexual debut were 56.6 (23.3%) for women reporting sexual debut within a year and 31.7 (12.6%) for those reporting initiation 6+ years before enrollment; virginal women had a rate reduction of 17.1 despite a 36.5% relative measure of effect. Rate reduction estimates increased from 29.2 for monogamous women to 51.3 for women with 4+ partners, despite the small difference on the relative scale (16.4% versus 14.4%, respectively).

Conclusions: Time since sexual debut was a better risk stratifier of vaccine benefit than age, consistent with its being a better predictor of HPV exposure risk among young adult women. Direct estimates will be obtained once unblinded data become available. Nonetheless, these results highlight the importance of evaluating vaccination benefit using both absolute and relative measures of impact.

O-01.06 WHO RECOMMENDATIONS: USE OF HPV VACCINES IN NATIONAL IMMUNIZATION PROGRAMMES

KL Irwin, World Health Organization, Geneva, Switzerland *J Hombach,* World Health Organization, Geneva, Switzerland *MT Aguado,* World Health Organization, Geneva, Switzerland

Background: The World Health Organization (WHO) issues recommendations about vaccine use in national immunization programmes. Recommendations strongly influence vaccine introduction decisions in public sector programmes of lowand middle-income countries and organizations that subsidize vaccines for low-income countries such as the Global Alliance for Vaccines and Immunization (GAVI). Most cases of cervical cancer cases occur in low- and middle income countries: more than half occur in the 72 countries eligible for GAVI subsidies.

Development of WHO policy on HPV vaccines: In November 2008, Strategic Advisory Group of Experts (SAGE) on Immunization, the leading body that advises WHO on immunization, advised WHO to recommend routine use of the two currently marketed HPV vaccines for national immunization programmes under certain conditions. This advice was based on the recognition that cervical cancer and other HPV-related diseases are important global health problems and a review of evidence on the safety and efficacy of the two vaccines in preventing cervical cancer and other HPV-related diseases. SAGE provided advice on circumstances where routine vaccine use would be warranted, characteristics of primary and secondary target populations, special populations, vaccine administration, interchangeable use of the two vaccines, considerations for vaccine product selection, delivery strategies, monitoring vaccination programme impact, integration of vaccination and cervical cancer screening, and education of vaccination candidates and parents. WHO is considering SAGE's advice and is expected to issue a Position Paper on HPV vaccines by April 2009.

Conclusions: This presentation will describe WHO's latest position on HPV vaccines for national immunization programmes. A WHO recommendation for HPV vaccine use in national immunization programmes would promote introduction in public sector programmes of low- and middle income countries and GAVI investment.

O-01.07

QUADRIVALENT HPV VACCINE EFFFICACY AGAINST MALE GENITAL DISEASE AND INFECTION

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BACKGROUND: In males, anogenital infection with human papillomavirus (HPV) causes genital warts, penile, perineal, perianal, and anal neoplasia and cancer. In addition, male HPV infection contributes significantly to infection and subsequent cervical disease in women.

OBJECTIVE: This study examined the efficacy of the quadrivalent HPV (type 6/11/16/18) L1 virus-like particle vaccine against incidence of HPV 6/11/16/18-related external genital lesions (EGL) (external genital warts, penile/perineal/perianal intraepithelial neoplasia, and penile/perineal/perianal cancer) as well as genital HPV 6/11/16/18 infection in young men (heterosexual men and men having sex with men).

METHODS: In this randomized, double-blind, placebo-controlled trial, 4,065 young men aged 16-26 years were administered quadrivalent HPV vaccine or placebo at enrollment, month 2, and month 6. Subjects underwent detailed genital exams as well as sampling from the penis, scrotum, and perineal/perianal region at enrollment, month 7 and at 6-month intervals afterwards. After enrollment, all new lesions were biopsied for pathological diagnosis and PCR testing. Efficacy analyses were performed in a per-protocol population seronegative at day 1 and HPV DNA-negative from day 1 through month 7 to the relevant vaccine HPV type. Median follow-up was 2.3 years (starting from month 7).

RESULTS: Among 1,397 vaccine subjects and 1,408 placebo subjects, efficacy against any HPV 6/11/16/18-related external genital lesion was 90.4% (95% CI: 69.2, 98.1). Vaccine efficacy against condyloma and PIN was 89.4% (95% CI: 65.5, 97.9) and 100% (95% CI: <0, 100), respectively. Vaccine efficacy against HPV 6/11/16/18 persistent infection and DNA detection at one or more visits was 85.6% (97.5% CI: 73.4, 92.9), and 44.7% (95% CI: 31.5, 55.6), respectively. Slightly more injection-site adverse experiences were seen among vaccine recipients.

CONCLUSION: The quadrivalent HPV vaccine is efficacious in reducing the burden of HPV 6/11/16/18-related EGL and infection in young men aged 16-26 years naïve to the relevant HPV type at baseline.

O-01.08

IMPACT OF HPV6/11/16/18 VACCINE ON ABNORMAL PAP TESTS AND PROCEDURES

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J Paavonen, University Central Hospital, Helsinki, Finland

Background: Organized screening has reduced cervical cancer rates but is costly when considering the Pap/HPV testing and need to follow-up on abnormalities and treat precancers. We report end-of-study data from 2 pivotal Phase III clinical trials of the quadrivalent vaccine.

Methods: 17,622 women were enrolled in 1 of 2 randomized, placebo-controlled, efficacy trials. Vaccine or placebo was given at Day 1, Month 2 and 6. Subjects underwent cervicovaginal sampling at Day 1. Pap testing occurred at Day 1 and every 6-12 months thereafter. Colposcopy referral was Pap algorithm/HPV test-based. Definitive therapy referral was algorithm based, using generally accepted standards of care. All analyses were regardless of causal HPV type and conducted in a population of women who, at Day 1, had a negative Pap test and were negative to 14 common HPV types. This population approximates a primary target population for HPV vaccination: sexually-naïve girls and women shortly post-sexual debut and/or with few lifetime sex partners.

Results: After an average follow-up of 3.6 years post-Day 1, significant reductions in Pap tests, colposcopy, cervical biopsy, and definitive therapy were observed. There was a 17% reduction in ASC-US HR-Positive or Worse (95% CI: 10 to 24). The reduction was highest for HSIL (45%: 95% CI: 4 to 69). The incidence of colposcopy, cervical biopsy, and cervical definitive therapy in the group vaccinated with quadrivalent HPV vaccine was reduced by 20% (95% CI: 12 to 27%), 22% (95% CI: 14 to 29%

14 to 29%), and 42% (95% CI: 28 to 54%), respectively, compared to placebo.		#Cases Vaccine (n=4616)	#Cases Placebo (n=4679)	% Reduction	95% CI
Conclusions:	Pap test result				
Administration of	ASC-US HR-Positive or Worse	1002	1205	17	10, 24
quadrivalent HPV	ASC-US HR-Positive	276	357	22	9,34
1	LSIL	813	980	17	9,24
vaccination to HPV-	ASC-H	55	87	36	10, 55
naïve women reduced	HSIL	22	40	45	4,69
the incidence of cervical	AGC	3	4	NA	NA
procedures and abnormal	Procedure				
	Colposcopy	850	1061	20	12,27
Pap tests within 4 years,	Cervical Biopsy	728	935	22	14, 29
irrespective of the HPV	Definitive therapy	131	229	42	28, 54
type involved.					, 0 1

O-01.09

MISCARRIAGE RISK IN TWO RANDOMIZED CONTROLLED TRIALS OF HPV VACCINE

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G Macones, Washington University, St. Louis, USA

P Gonzalez, Proyecto Epidemiologico Guanacaste, San Jose, Costa Rica, B Befano, IMS, Silver Spring, USA A Hildesheim, NCI, Bethesda, USA, A Rodriguez, Proyecto Epidemiologico Guanacaste, San Jose, Costa Rica D Solomon, NCI, Bethesda, USA, R Herrero, Proyecto Epidemiologico Guanacaste, San Jose, Costa Rica M Schiffman, NCI, Bethesda, USA, for CVT Group

Context. Randomized trials show that vaccines based on an antigen consisting of HPV L1 protein viruslike particles can interrupt natural history of cervical cancer. Given that young women are the main target for vaccination, possible effects of vaccination on reproduction are important to explore.

Objective. To assess whether vaccination against HPV types 16 and 18 increases risk of miscarriage.

Design and Setting. Pooled data from two multicenter, phase-3, masked, randomized trials.

Participants. 26,131 randomized women 15 to 25 years.

Intervention. Participants were randomly assigned to 3 doses of a bivalent HPV-16/18 L1 protein VLP AS04 vaccine (n=13,074) or a control hepatitis A vaccine (n=13,055) over 6 months.

Main Outcome Measures. One-sided p-value from permutation tests of the null hypothesis that vaccine has no effect on miscarriage against numerous possible alternative hypotheses.

Results. Of 3,599 pregnancies, 2,850 (79.2%) ended in live births, of which 2,620 (91.9%) were of gestational age 37 weeks or more; 373 (10.3%) ended in miscarriage, most (58%) at 7 to 12 weeks of gestational age. The 1-sided P-value for the primary analysis using nearest vaccination as the reference date was 0.16, well above the standard critical value of 0.025 for a 1-sided test; thus, overall, there was no significant increase in the miscarriage rate in the women assigned to the HPV vaccine arm. In secondary descriptive analyses, miscarriage rates were 15.4% in the HPV arm and 9.6% in the comparison arm among pregnancies that began within 3 months after vaccination.

Conclusion. These results do not establish a relationship between HPV vaccination and miscarriage risk but are insufficient to rule out a small effect in pregnancies conceived in the three months immediately after vaccination.

O-01.10 HPV VACCINATION UPTAKE AND REGISTRATION OF HPV VACCINATION IN SWEDEN

P Sparén, Karolinska Institutet, Stockholm, Sweden

Background: Registration of HPV vaccination is crucial for monitoring and evaluation of HPV vaccination.

Objectives: Monitor HPV vaccination uptake in Sweden, and evaluate registration thereof.

Methods: HPV vaccination in Sweden started late 2006, and simultaneously individual registration of HPV vaccination (SVEVAC) was introduced. Currently HPV vaccination is re-imbursed by around 50% for girls aged 13-17 years, and an organised HPV vaccination program for girls aged 10-12 will start in January 2010.

Results: Until December, 2008 there were 134 835 doses of HPV vaccine distributed in Sweden, and 102 810 doses registered in SVEVAC. This yields a completeness of registration of 76% at this time point, which is an underestimation since there is a time lag from distribution to injection of vaccine, and finally registration in SVEVAC. Allowing a time lag of 1 month yields 80% completeness, while a time lag of 2 months yields 85%. The number of first doses of HPV vaccine registered in SVEVAC was 46 165. The age specific proportion per 1000 women peaked at 139 and 161 for women 15 and 16 years, respectively. Before age 13 the proportion of vaccinated women was very low and after age 17 the proportion of vaccination dropped heavily. At ages 13-17 years the overall proportion of HPV vaccinated women in Sweden was 120 per 1000. The counties with the highest proportion of HPV vaccinated women were Halland and Skåne with 176 per 1000 women, followed by Stockholm at 152, and Uppsala, Dalarna, Jämtland and Västerbotten with around 130 vaccinated per 1000 women.

Conclusions: Registration of HPV vaccination in Sweden shows a completeness of 80-85%. Despite subsidies of HPV vaccination for girls age 13-17 in Sweden a fairly small proportion are yet vaccinated. This highlights the importance of an organised HPV vaccination program for young girls in Sweden.

POSTER ABSTRACTS SESSION 01

POSTER SESSION I MONDAY 10.00: ODD NUMBERS

POSTER SESSION II

TUESDAY 10.00: EVEN NUMBERS

P-01.11 EVALUTING HPV VACCINE IMPACT IN GENERAL FEMALE POPULATION

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Background: In the implementation of mass HPV vaccination of the general young female populations, several theoretical questions need to be answered, including, 1) how much the vaccination will decrease the vaccine-type associated and overall incidence of HPV-related diseases; 2) if mass vaccination against HPV vaccine types will change the equilibrium of other non-vaccine high-risk HPV types; and 3) if vaccination will affect sexual practice.

Objectives: To evaluate impact of a quadrivalent HPV vaccine (qHPV) on a population level.

Methods: Linking existing nationwide registries in the 4 participating Nordic countries, qHPV impact on overall incidence of cervical, vaginal and vulvar precursors and cancers will be assessed annually before and after the implementation of qHPV. Cross-sectional HPV surveys take place in pre- (2004-2006) and post-vaccination (2010-2011) eras: Each country collects representative CIN and cervical cancer tissues samples, as well as 2,000-4,000 cervical swab samples from women in the general population for HPV testing. These HPV surveys can help monitor any shift in HPV type distribution in cervical diseases and in the general population after the introduction of qHPV. Large-scale population-based questionnaire surveys are conducted before and 5 years after qHPV launch to collect information on life style risk factors and sexual practices. By linking the questionnaire survey data and data from different health-related registries to the Vaccine registry, recipients and non-recipients of HPV vaccine can be characterized to help identify potential differences in health outcomes between these two groups.

Results: To date, approximately 167000 women had received at least one dose of qHPV in participating countries. Conclusion: The results from the VIP study will not only establish the "pre-GARDASIL[®] baseline" and the first look of GARDASIL[®] impact in 5 years, but also serves as stepping stone to potentially subsequent longer term surveillance.

P-01.12

GARDASIL AND TWINRIX CO-ADMINISTRATION: PRELIMINARY SAFETY DATA

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Background. In the province of Quebec (Canada) the Gardasil is co-administered with Twinrix to 9-10 year-old girls. Objectives. 1) To assess the immunological response to Gardasil and Twinrix when co-administered and when administered one month apart; 2) To assess vaccines comparative safety profiles.

Methods. This open-labelled randomized long term clinical trial is conducted in Quebec City, Canada. 415 9-10 yearold girls eligible for HPV and HA/HB vaccination were randomized in two groups to receive Gardasil and Twinrix in co-administration or at one month interval. Vaccine immunogenicity and tolerability are assessed post-first dose, preand post-second dose given 6 month later, and pre- and post-third dose given at the age of 14-15 years. Here we present preliminary safety data after the first dose of vaccines when co-administered.

Results. 46% of vaccinees reported at least one general and 75% at least one local adverse event (AE) after vaccination. The most frequent general AE was fatigue (27%), followed by headache (23%), gastro-intestinal symptoms (14%), muscle aches (9%), joint pain (4%), fever (3%), rash (3%), and hives (1%). 91% of general AE were mild and 100% resolved within 4 days after vaccination. Pain at injection site was reported by 74% of vaccinees, redness by 13%, and swelling by 9%. 96% of local AEs resolved within 4 days and 100% within 7 days after vaccination. No statistically significant difference was observed when comparing local AE at Gardasil and Twinrix site administration. Only one vaccinee consulted a local clinic in relation with potential vaccine related muscle aches 9 days after vaccination. The family physician assessed the symptom as non-vaccine related.

Conclusion. Gardasil and Twinrix co-administration is well tolerated by 9-10 year-old girls. The local AEs after Gardasil are similar to those observed after Twinrix which is known to have an excellent longterm safety profile.

P-01.13

SAFETY AND IMMUNOGENICITY OF BPV-1 VLP VACCINATION IN HORSES

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Background: Equine Sarcoids are benign yet locally invasive skin tumours which affect horses and all other equids. Sarcoids persist, are highly therapy resistant and tend to recur following treatment. Today it is widely accepted that bovine papillomavirus type 1 (BPV-1) and, to a much lesser extent, type -2 (BPV-2) are the main causative agents for equine sarcoids. BPV-1 and BPV-2 are related serotypes.

Objective: As prophylaxis against this disease, a vaccine candidate based on BPV-1 L1 virus-like particles (VLPs) was evaluated for bio-safety and immunogenicity.

Methods: 15 experimental horses which had tested negative for BPV-1/-2 neutralising antibodies were immunised with BPV-1 VLPs. Three groups each comprising four animals were injected on days 0, after 2 weeks and after 6 months with 50, 100 or 150 µg of BPV-1 VLP plus adjuvant (AlOH 250µg/dose), respectively. A control group consisting of three horses received adjuvant only. The horses were monitored daily to record possible side effects. Blood was collected before and two weeks after each immunisation. Serum antibody titres were determined by BPV-1 pseudovirion (PsV) neutralisation assay.

Results and Conclusions: No unacceptable side effects were observed. Two weeks after the second immunisatio 12/12 VLP animals developed neutralising antisera. Two weeks after the third immunisation 12/12 of the horses had raised neutralising antibody titres \geq 1600 and 10/12 (83,3%) had titres \geq 1:6400, whereas no titres were measured in the control group. Measured titres compare to those affording protection from infection in other animal species and man. Accordingly, it is likely that we have achieved to generate a prophylactic vaccine that protects horses from BPV-1 and -2 infection. Effectivity of protection will be tested in a phase II challenge study in the near future.

P-01.15

A QUADRIVALENT HPV VACCINE EFFICIENTLY INDUCES IMMUNE RESPONSES IN WHIM-IMMUNODEFICIENCY

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WHIM syndrome (warts, hypogammaglobulinemia, recurrent bacterial infections and myelokathexis) is a rare primary immunodeficiency disorder associated with CXCR4 mutation. Laboratory abnormalities include peripheral neutropenia, B-cell lymphopenia and T-cell abnormalities. WHIM patients suffer from a specific susceptibility to HPV infections that may cause extensive cutaneous and anogenital warts or high-grade dysplasia. We have determined the immune response to a quadrivalent HPV vaccine in a 12 year old girl, suffering from WHIM syndrome. The patient is maintained on regular immunoglobulin substitution and free of opportunistic infections and is not suffering from warts. The patient and 3 immunocompetent volunteers were immunized intramuscularly at months 0, 2 and 6 according to the licensed vaccination protocol. The vaccine was well tolerated without adverse effects. Serum samples were collected prior to each vaccination and 2 months after the last administration. Antibodies against vaccine types were tested by type 6, 11, 16 and 18 virus-like particle (VLP)-ELISAs. The WHIM patient developed titers of 400 for types 6, 11, 16, and a titer of 100 for HPV 18. In comparison, immunocompetent controls mounted titers of 6400 to 25600 after vaccination. Sera were further examined by HPV6, 11, 16, 18 pseudovirion neutralization assays. In the WHIM patient titers ranged from 1:50 to 1:200, whereas immunocompetent controls developed neutralization titers of 1600 to 6400 to the four types, respectively. Determination of cellular immune responses to vaccine-type VLP is in progress. This is the first report on immune responses to HPV vaccination in WHIM immunodeficiency, indicating induction of sterilizing immunity as correlate for protection. Lower antibody titers compared to immunocompetent controls might indicate that patients with WHIM syndrome, and probably other immunodeficiencies, might further benefit from augmented HPV vaccination regimens.

P-01.16 STRATEGIES TO MONITOR HPV VACCINE IMPACT IN THE U.S.

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ER Unger,

Background: Monitoring the impact and effectiveness of HPV vaccine in the U.S. will allow evaluation of the duration of protection, health disparities in implementation, and potential changes in HPV epidemiology. The sustainability of a vaccine program may depend on measuring outcomes of the program. We describe initial plans to comprehensively monitor HPV vaccine impact in the U.S.

Objectives: To describe HPV vaccine monitoring activities on biologic and clinical outcomes in the U.S.

Methods: We describe the background on decision-making for outcomes to target for surveillance, describe the plans to monitor these principal outcomes, describe the status of the evaluations, and the challenges encountered to date.

Results: To measure impact of the HPV vaccine, we targeted the outcomes of type-specific HPV infection, genital warts, cervical cell abnormalities, and HPV-associated cancers. Type-specific HPV prevalence is currently monitored by self collected vaginal swabs in a national population based survey. Genital warts will be monitored through a network of STD clinics and billing data. Projects to monitor precancers have been initiated, some of which will include typing of lesions and central histology review through a web-based virtual slide archive. Use of billing data to track precancers and other HPV related outcomes are being investigated. Finally, existing cancer registries will provide a framework for monitoring cervical cancer, precancers, and other HPV related cancers, as well as HPV types contributing to cancers.

Conclusions: A wide range of surveillance activities have been initiated in the U.S. to measure the impact of the HPV vaccine on select clinical outcomes. We anticipate that the variety of these activities targeting different outcomes will allow for a comprehensive evaluation of the impact of the HPV vaccine in a dynamic environment. Baseline feasibility evaluations to determine long term viability for specific monitoring programs will be useful.

P-01.17 SURVEY OF EUROPEAN WOMEN'S INTENTION TO UNDERGO CERVICAL SMEAR TESTING

M Perrin, IPSOS, Paris, France *C Melinand*, SP-MSD, Lyon, France *A Darras*, SP-MSD, Lyon, France

Objectives: To compare women's attitude to undergoing cervical cancer screening prior to and after the introduction of Gardasil(R), a quadrivalent HPV6/11/16/18 vaccine. In most European countries cervical cancer screening is recommended, generally at a frequency of every three years.

Methods: An Internet-based survey was carried out in women in 2006, prior to the introduction of Gardasil and then repeated in 2008. Among other questions, women were asked how frequently they usually underwent cervical cancer screening. To be able to compare the frequency we report data from six countries for both surveys: Denmark, France, Germany, Portugal, Spain, and Sweden.

Results and conclusions: 2816 and 4106 women replied to this question in 2006 and 2008, respectively. In all countries screening rates either did not change significantly or increased between the pre-vaccination and post-vaccination era (Sweden 55% to 63%, p<0.01; Spain 72% to 77% p=0.05). In general, the screening rates reported are higher than those reported using national reporting methods. This may be explained by the self-declarative nature of our data, but since the methods used were the same for both surveys, the results can be compared. In the 2008 survey vaccinated women were also asked about their intention regarding future attendance to screening and the vast majority said being vaccinated would not change their attitude to attending screening.

Also, analysis of declared screening attendance in the 2008 survey showed that mothers of vaccinated girls tended to attend screening for themselves more often than other mothers. These results show that the fears that vaccinated women would change their screening habits are unfounded, and that in some countries there is a trend for increased compliance with the recommendation for screening at least every three years.

P-01.18

MONITORING THE IMPACT OF HPV-16/18 VACCINATION IN THE NETHERLANDS

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National Institute for Public Health and the Environment, Bilthoven, the Netherlands

In 2009, HPV-16/18 immunization will be implemented in the National Immunization Programme for 12-year old girls and a catch-up programme will be performed for 13-16 year old girls. Alongside introduction of HPV-vaccination a monitoring programme will be carried out.

Vaccination coverage will be monitored using national vaccination registration data and specific studies on vaccine acceptance. The possibility to study potential associations between mothers' attendance for screening and vaccination of their daughters is explored.

In addition to monitoring adverse events through an enhanced passive surveillance system, further studies on reactogenicity will be performed as well as registration of acute incidents after vaccination. Also baseline incidences of autoimmune disorders will be established since a temporal association – relatively frequent appearance of these illnesses in adolescents – is expected.

Two population-based serum collections of the general population in the Netherlands (1995/6; 2006/7) will be used to obtain insight into the prevalence of type-specific HPV-antibodies before vaccine introduction. Repeating similar studies at a regular interval could be used to explore the impact of vaccination on type-specific prevalence of antibodies both in vaccinated and unvaccinated females (and males).

To monitor possible shifts in (non) vaccine HPV types, the prevalence of type-specific HPV DNA and antibodies will be studied in STI clinic attendees aged 16-24 years (females and males). We currently explore the feasibility to utilize an existing collection of swabs collected in girls aged 16-29 years to assess pre-vaccination occurrence of type-specific HPV-infections. Both studies may also give insight in the interaction of HPV with other STIs. Furthermore, possibilities to perform a cohort study of vaccinated and unvaccinated 16-year-olds from whom both serum and HPV-DNA will be collected are explored.

Linkage of vaccination and disease registrations of precancer lesions, cervical cancer and other HPV-related cancers will be necessary to monitor the long-term impact of vaccination.

P-01.19 INTERIM ANALYSIS OF CLINICAL TRIAL OF HPV-16/18-AS04 VACCINE IN JAPAN

R Konno, Saitama Medical Center, Jichi Medical University, Saitama, Japan **H Yoshikawa**, Tsukuba University, Tsukuba, Japan

A phase II double-blind controlled randomized multicenter study with HPV-16/18 AS04-adjuvanted vaccine is ongoing in Japanese women aged 20-25 years. An interim analysis was performed at Month 7 (one month after the third dose of vaccine) to determine reactogenicity, safety and immunogenicity of the vaccine and to evaluate the baseline HPV16/18 seropositivity and DNA status of women. In the HPV-16/18 group (according-to-protocol [ATP] cohort for immunogenicity analysis), 100% seroconversion was observed against HPV-16 and HPV-18 at Month 6 (five months after the second dose) and at Month 7. At Month 7, anti-HPV-16 geometric mean titer (GMT) was 7441.0 EL.U/mL and anti-HPV-18 GMT was 3805.4 EL.U/mL, which is respectively 250-fold and 168-fold higher than GMTs observed after natural infection with HPV-16 or HPV-18. In the total vaccinated cohort (TVC), the seropositivity rates against HPV-16 and HPV-18 at study entry were 17.3% and 15.8%. At the same time point, HPV-16 and HPV-18 DNA was detected in 6.5% and 4.0% of the women respectively. The immunogenicity of the HPV-16/18 vaccine and the HPV prevalence pre-vaccination in Japanese women are in line with what was observed in other populations. Injection site symptoms and some general symptoms were reported more frequently in the HPV-16/18 group than in the HAV group but had no impact on compliance with completion of the vaccination course. Overall, the HPV-16/18 vaccine had a good safety profile, was well tolerated, and highly immunogenic in the study population of Japanese women.

P-01.20

MARKERS OF HPV INFECTION IN CZECH WOMEN BEFORE HPV VACCINATION

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Background: Currently two prophylactic HPV vaccines are commercially available to prevent HPV16/18 infection and associated lesions. During clinical studies it has been shown that vaccination of females incidentally or persistently infected with vaccinal HPV types is less effective or ineffective.

Objectives: The aim of the study was to assess the proportion of sexually active women who were at risk of reduced vaccine efficacy.

Methods: Altogether 173 women (16-49 years, mean 23.4 years) were enrolled. Before the first vaccine dose a sample for HPV DNA detection and typing and blood for anti-HPV antibodies assessment were taken. HPV DNA detection and typing in cervical smears was done by means of PCR and RLB/sequencing, sera were tested using ELISA. VLPs derived from HPV6, 11, 16, 18, 31 and 33 were used as an antigen.

Results: HPV DNA prevalence in the whole cohort (35.8%) was age dependent, culminated in the age group 27-30 years (48.7%), in older women decreased to 25%. Vaccinal types were found in 11.7% of females with the maximum between 27-30 years of age (17.4%). In older women these types were not detected. Overall 32.4% of women were anti-HR VLP seropositive, in women above 30 years the positivity was as high as 69%. One quarter of females possessed antibodies to VLP16/18, about 30% to VLP6/11. Incident infection (HPV DNA+/Ab-) with vaccinal HPV types was observed in 6.4% and persistent infection (HPV DNA+/Ab+) in 4.0%. There were 16% women VLP16/18 seropositive and 35% VLP6/11/16/18 seropositive with coincident negative HPV DNA finding (cleared infection).

Conclusions: Our study has shown that about 60% of women enrolled have already encountered the HPV infection. HPV vaccination might have reduced efficacy in about 10% of vaccinated women who were positive for HPV DNA of the vaccinal types at the moment of vaccination.

P-01.21

HPV RISK FACTOR ASSESSMENT OF EARLY-ADOPTERS OF THE HPV VACCINE

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Background: Differences between trial inclusion criteria and late 'catch-up' demographics may effect real-time populationbased efficacy of the vaccine and consequentially recommendations and cost-effectiveness. In addition, this knowledge enables providers to adequately assess individuals' benefits of prophylactic HPV vaccination.

Objectives: To investigate the sociodemographic and HPV risk factors of early-adopting users of the HPV vaccine in the late 'catch-up' population (ages 19-26) in a US ethnically diverse, urban population.

Methods: Patients from March 2007 to October 2008 who sought vaccination at a large, centralized HPV vaccine clinic were asked to complete an extensive questionnaire for HPV risk assessment which included questions regarding sexual history, contraceptive practices, and other major factors abstracted. Data was compared to the surrounding community data using the 2007 Youth Risk Behavior Surveillance System (YRBSS), and comparisons were made to the published data from the Phase II and III HPV vaccination trials.

Results: 375 women ages 19-26 visited the vaccine clinic during this period. Nearly half (43%) of the women who visited the vaccine clinic reported having had an abnormal Pap. These women, who were similar to the surrounding community with regards to race and ethnicity, reported having had a significantly higher number of sexual partners than those on both the FUTURE and PATRICIA trials (p<0.001). In fact our group was 76% more likely to have ever had sex than participants of the vaccine trials, with 30% of the women reporting 6 or more sexual partners.

Conclusions: These data suggest, early users of the HPV vaccine in the late catch-up population do not resemble those on the Phase II and III clinical trials, which questions the ability to generalize trial data to actual vaccine users. Real time prospective data collection for efficacy is imperative to further maximize HPV vaccination policies, recommendations, and cost-utilization.

P-01.22 LESSONS LEARNED FROM PHASE 1 OF HPV VACCINATION IN PERU

I Ramos, PATH, Peru DS LaMontagne, A Jumaan,

Background: In 2007, the Peruvian Ministry of Health (MINSA), working with PATH, initiated a demonstration project to deliver the human papillomavirus (HPV) vaccine to young girls. Vaccination occurred in two phases: a small, initial study comparing two approaches to vaccination, followed by a scaled-up study that applied lessons learned from the first phase.

Method: The first phase assessed the benefits and costs of "active follow-up" of school-based immunization, compared with simple provision of vaccine without active follow-up. Active follow-up included home visits for girls who missed first, second, or third doses of vaccine. The first phase also evaluated the impact of utilizing lengthy consent forms for HPV vaccination. Results: The study found that a similar percent of girls accepted vaccination, regardless of type of follow up. Because follow-up did not increase first-dose coverage, and had the potential to increase costs, follow-up after a missed first dose was judged not to be a valuable approach. Drop-out rates were very low between doses of the vaccine, however, follow-up was considered important for ensuring that those who initiated vaccination were fully protected. Finally, it was found that the length and complexity of the consent forms seem to have increased parental concern and to have been a significant barrier to vaccine acceptance. Based on these findings, in the scaled-up phase of the project, a simpler vaccination authorization form was used. In terms of follow-up: only girls who received the first dose, but missed the second or third dose, became candidates for active follow-up. On Dec. 10, 2008, the second phase of Peru's vaccination was completed. More than 9000 girls were vaccinated in over 700 schools. Preliminary results show high levels of acceptance of the vaccine and high continuation rates once a girl and her family consented to the first dose.

Conclusion: Evaluating vaccine delivery strategies using a small-scale phased approach played a key role in guiding design of scale-up and improving HPV vaccine acceptance and coverage.

SESSION 02

THERAPEUTIC VACCINES AND IMMUNE MODULATION

PROGRAMME

THE 25TH INTERNATIONAL PAPILLOMAVIRUS CONFERENCE MAY 8-14 2009, MALMÖ, SWEDEN

TIME	NUMBER	TITLE	ROOM
009-05-10			
SESSI	ON 02: THE	ERAPEUTIC VACCINES AND IMMUNOMODULATION	K1-3
13.30-14.05	O-02.00	THE SPONTANEOUS HPV-SPECIFIC IMMUNE RESPONSE AS A GUIDE FOR IMMUNOTHERAPY S H van der Burg	
14.05-14.16	O-02.01	IMMUNOTHERAPY OF VIN WITH IMIQUIMOD FOLLOWED BY PDT OR VACCINATION P L Stern, S Daayana, E Elkord, U Winters, H Kitchener	
14.16-14.27	O-02.02	POISED HPV16-SPECIFIC T-CELLS IN CERVICAL CARCINOMA AND DRAINING LYMPH NODES P J de Vos van Steenwijk, M Heusinkveld, TH Ramwadhdoebe, AM van der Hulst, SJ Piersma, R Goedemans, GG Kenter, SH van der Burg	
14.27-14.38	O-02.03	REGRESSION IS ASSOCIATED WITH CD8 RESPONSES TO HPV 16 E6 M Nakagawa, S Gupta, H Coleman, M Sellers, J Banken, W Greenfield	
14.38-14.49	O-02.04	VIRAL VECTOR-BASED PRIME-BOOST IMMUNIZATIONS: A POSSIBLE INVOLVEMENT OF T-CELL COMPETITION T Daemen, A. de Mare, A.J.A. Lambeck, J. Regts, G.M. van Dam, H.W. Nijman, H. Snippe, J Wilschut	
14.49-15.00	O-02.05	IFN-KAPPA IS ABOLISHED BY CERVICAL CANCER-ASSOCIATED HUMAN PAPILLOMAVIRUS TYPE16 B Rincon - Orozco, G Halec, F Bosch, F Roesl	
15.00-15.11	O-02.06	GENITAL IMMUNIZATION WITH HPV PSEUDOVIRIONS INDUCES GENITAL CD8+ T CELLS N J Cuburu, RC Kines, J Roberts, J Nicewonger, BS Graham, CB Buck, D Lowy, J Schiller	
15.11-15.22	O-02.07	DMXAA ENHANCES THERAPEUTIC HPV VACCINE-INDUCED CTL RESPONSES AND ANTITUMOR EFFECTS S Peng, A Monie, Cf Hung, TC. Wu	
15.22-15.33	O-02.08	NOVEL IMMUNOTHERAPY TO INDUCE E7-SPECIFIC CTL IN MURINE GENITAL MUCOSA L Decrausaz, M Duc, Ar Goncalves, M Bobst, N Zosso, D Nardelli - Haefliger	
15.33-15.44	O-02.09	HPV-16 E5 DOWN-REGULATES MHC I AND CONTRIBUTES TO VIRAL IMMUNOEVASION M S Campo, S Man, MS Cortese, K Smith, E Dornan	
15.44-15.55	O-02.10	REGULATION OF LANGERHANS CELL NUMBER ACROSS HPV GENERA M Hibma, C-M Leong, J Doorbar, H-S Yoon	

THE SPONTANEOUS HPV-SPECIFIC IMMUNE RESPONSE AS A GUIDE FOR IMMUNOTHERAPY

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The HPV E6 and E7 proteins play a pivotal role in carcinogenesis and are expressed in both pre-malignant and advanced cervical lesions. Because HPV proteins are foreign to the body, one would expect the immune system to mount a response against these antigens when expressed in the cervical epithelium. Indeed, circulating HPV16 E6, E7 and E2-specific Th1and Th2-type CD4+ T-cells as well as E6-specific CD8+ T-cells, able to migrate into the skin upon antigenic challenge, were frequently detected in healthy individuals, showing that successful defense against HPV16 infection is commonly associated with the installment of a systemic effector T-cell response against these viral early antigens. In contrast, the development of high-risk HPV-positive CIN to cervical cancer is associated with a HPV-specific T-cell response that fails at least at three different levels. In about half of the patients, PBMC lack the capacity to mount a detectable proliferative response against HPV16 E6, E7 and E2, whereas the other half displayed antigen-specific proliferative responses exhibiting a non-inflammatory cytokine profile. Analysis of the local immune response revealed that in many cases HPV-specific effector T-cells failed to home to CIN lesions or tumors and fail to infiltrate the cancer cell nests. Furthermore, HPV E6and E7-specific CD4+ regulatory T-cells can be isolated from CIN lesions, lymph node biopsies and the tumors of cervical cancer patients indicating that the immune response in these patients is suppressed at both the induction and effector level. We have used these data to guide us in developing an immunotherapeutic vaccine strategy and showed that the restoration of Th1/CTL response against the early antigens results in the regression of HPV-induced lesions in the majority of patients. However, the counterattack of regulatory T-cells and improved homing is still required for this strategy to become successful in patients with cancer.

O-02.01 IMMUNOTHERAPY OF VIN WITH IMIQUIMOD FOLLOWED BY PDT OR VACCINATION

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Objectives: HPV associated vulval intraepithelial neoplasia (VIN) is a difficult condition to treat. We report on studies using a topical immunomodulator, imiquimod, followed by either photodynamic therapy (PDT) or therapeutic HPV vaccination with TA-CIN (fusion protein HPV16L2E6E7).

Methods: Twenty women with VIN were recruited in each trial; 74% previously treated, 44% > 3; mean duration 6.5 years. Biopsy, blood samples and HPV testing occurred at 0, 10, 20 and 52 weeks. Lesion immune infiltration was assessed using immunofluorescence detecting T-cell subsets and HPV response by lympho-proliferation/cytokine release. A complete response (CR) was an absence of vulvoscopic evidence of VIN. A partial response (PR) was clearance of disease from 50% or more of the total disease. Responders showed no VIN histologically. Results: At wk 52, Imiquimod / PDT treated patients showed 35% CR and 25% PR and Imiquimod / TA-CIN patients showed 55% CR and 20% PR. Imiquimod treatment is characterised by increased local infiltration of CD8 T cells but in non-responders this is accompanied by increased T- regulatory cells whereas in the responders these are at significantly lower density. In the imiquimod / PDT trial, responders had significantly increased pre-existing lympho-proliferative responses to HPV16 compared to non-responders but there was no stimulation of HPV immunity with treatment. Following vaccination in the imiquimod / vaccine trial, patients showing clinical responses had significantly increased lympho-proliferation to TA-CIN, HPV16 E6, E7 and L2 antigens compared to the non-responders. Data from cytokine responses and further followup will also be presented.

Conclusion: Imiquimod followed by PDT or TA-CIN both show promise as non surgical therapies for VIN. Nonresponders showed higher level of T-regulatory cells in situ consistent with their negative prognostic value in some cancers. The therapeutic impact of treatment may depend on the differential immune response of responders and non-responders

POISED HPV16-SPECIFIC T-CELLS IN CERVICAL CARCINOMA AND DRAINING LYMPH NODES

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Introduction: Cervical cancer is mainly caused by human papillomavirus (HPV) type 16 and 18 and constitutively expresses the two tumor-specific antigens E6 and E7. Previously, we showed that HPV16/18 E6/E7-specific T-cells are present in tumor draining lymph nodes and can home to the tumor site in order to exert their function but little is known about the magnitude and character of these local HPV-specific anti-tumor responses.

Objective: To characterise the magnitude and polarisation of local HPV-specific anti-tumor responses.

Methods: Multiparameter flowcytometric analysis of CD4 and CD8 positive cells with the specific activation markers CD154 and CD137 in combination with IFNy and IL-2 enabled the enumeration of HPV-specific T-helper cells and CTL in draining lymph nodes and cervical tumors directly ex-vivo.

Results: Our preliminary data showed that up to 1% of lymph node cells were HPV-specific. In depth analysis of HPVspecific lymphocytes by co-staining for different TCR v β chains revealed the full breath of the response as the number of epitopes recognized ranged from 0-8 per patient and the number of different T cells responding to a given epitope ranged from 1-6. Notably, in many cases HPV-specific T cells did not display an overt effector cytokine profile suggesting that although in principle broad T-cell reactivity can be mounted against HPV, the activation in vivo is not optimal. Indeed, stimulation of lymph node cells in the presence of DC-activating compounds resulted in a stronger effector type profile. Conclusion: Patients with cervical cancer display a large reservoir of tumor-specific T cells awaiting proper activation either by vaccination strategies or in vitro for adoptive T-cell therapies.

O-02.03

REGRESSION IS ASSOCIATED WITH CD8 RESPONSES TO HPV 16 E6

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OBJECTIVE: The goal was to examine the role of CD8 T-cell responses to HPV 16 in regression of cervical lesions.

METHODS: Women being followed but untreated for abnormal Pap smear results were enrolled. HPV-DNA testing using the Linear Array HPV Genotyping Test (Roche Diagnostics, Indianapolis, IN), and enzyme-linked immunospot assay using the HPV 16 E6 and E7 antigens were performed. The subjects were catagorized into three groups: regressor (n=32), persistor/progressor (n=33), or indeterminate (n=20) based on comparisons of pathological diagnoses between the last clinic visit and the current clinic visit.

RESULTS: There was a higher rate of CD8 T-cell responses to the HPV 16 E6 antigen in the regressor group (17 of 32 or 53.1%) compared to the persistor/progressor group (8 of 33 or 24.2%) (p=0.023) but not for E7 (4 of 32 or 12.5% for the regressor group and 4 of 33 or 12.1% for the persistor/progressor group, p=1.000). The results were the same when the analyses included only subjects who were HPV 16-positive (n=27, p=0.046 for E6 and 1.000 for E7), HPV 16-related positive (n=48, p=0.041 for E6 and 1.000 for E7) or high-risk HPV positive (n=64, p=0.003 for E6 and 1.000 for E7), but not low-risk HPV positive (n=19, p=1.000 for E6 and not applicable for E7). The E6 91-115 region was immunodominant, and the comparison between the two groups was significant (p=0.044).

CONCLUSIONS: CD8 T-cell immune responses to the HPV 16 E6 antigens but not to E7 antigens are associated with SIL regression, and such responses appear to be cross-reactive to other high risk HPV types. These data support the use of HPV 16 E6 antigens in the development of therapeutic vaccines for the prevention and treatment of cervical cancer caused by high risk HPV types.

VIRAL VECTOR-BASED PRIME-BOOST IMMUNIZATIONS: A POSSIBLE INVOLVEMENT OF T-CELL COMPETITION

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Recombinant viral vectors are being developed for immunotherapeutic applications, including immunotherapy of cervical neoplasia. Although these approaches are promising, it is generally recognized that viral vectors in vivo may induce immunity against the (non-)structural proteins of the vector, which may impede the efficacy of booster immunizations with the same vector. In general, neutralizing antibodies are thought to be the main effector mechanism of vector-specific immunity, while cellular immunity would appear to play a secondary role.

We previously demonstrated that immunizations with recombinant Semliki Forest virus (rSFV) is very effective in inducing transgene-specific CTLs (Riezebos-Brilman A, Gene Ther.2007,14:1695). We also demonstrated that to achieve optimal responses and specifically to induce strong memory CTL responses, at least two immunizations with rSFV are required. Now we investigated the effect of vector-specific immunity, induced by a priming immunization with rSFV, on transgene expression and CTL activation by a subsequent injection of SFV expressing E6E7 from human papillomavirus (HPV) (SF-VeE6,7). We furthermore determined which immune mechanisms may be involved in SFV vector-specific immunity.

Secondary immune responses against E6E7 were neither affected by vector-specific antibodies nor by CTL-mediated killing of infected cells. Instead, the presence of the antigen during the prime immunization is the main determinant for the boosting efficacy. After priming with rSFVeE6,7, a homologous booster stimulated the primed E6E7-specific CTL response and induced long-lasting memory. Conversely, in mice primed with irrelevant rSFV, induction of E6E7-specific CTLs was inhibited presumably due to vector-specific responses induced by the priming immunization. When during the priming with irrelevant rSFV, E7-protein was co-administered, the inhibitory effect was abolished.

These observations indicate that T cell competition may determine the outcome of secondary immunizations in viral vector immunization strategies. For rSFV this immune mechanism however does not hamper homologous prime-boost immunization.

Study supported by Dutch Cancer Society grant RUG-2001-2361 (de Mare).

O-02.05

IFN-KAPPA IS ABOLISHED BY CERVICAL CANCER-ASSOCIATED HUMAN PAPILLOMAVIRUS TYPE16

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Expression of IFN-k is mostly restricted to keratinocytes, activated monocytes and dendritic cells. In the present study we show that primary human foreskin keratinocytes (HFS) and keratinocytes immortalized with HPV16-E7 expressing recombinant retroviruses exhibit constitutive IFN-k expression. In contrast, keratinocytes transduced with HPV16-E6 or HPV16-E6/E7 as well as cervical carcinoma cells have suppressed IFN-k expression. However, expression could be restored when cells were treated with 5-aza-deoxy-cytidine, indicating that the inhibition of IFN-k is a consequence of de novo methylation. In fact, methylation analysis of the putative IFN-k promoter 2.5-kb upstream of the transcriptional start site revealed that in HPV16-E6 keratinocytes, as well as in the HPV16 positive cervical carcinoma lines, a CpG island localized at positions -237 to -96 near the TATA box is completely de novo methylated, contrasting the situation found in HPV16-E7 keratinocytes and HFS. Moreover, when IFN-k was ectopically expressed in cervical carcinoma cells, a strong antiviral activity could be induced which was accompanied by an up-regulation of IFN target genes such as p53, MxA and Interferon Response Factors (IRFs). Conversely IFN-k siRNA experiments significantly reduces p53, IRFs and MxA. Furthermore, our in vitro findings were confirmed in biopsy samples from cervical cancer patients, where IFN-k was also down-regulated in contrast to normal donors. These results demonstrate that HPV16-E6 can suppress the host immune response by targeting IFN-k

GENITAL IMMUNIZATION WITH HPV PSEUDOVIRIONS INDUCES GENITAL CD8+ T CELLS

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HPV pseudoviruses (PsV) composed of the viral L1 and L2 proteins can encapsidate virtually any 6-8 kb circular target plasmid. They are attractive gene transfer vehicles, as infectious titers of up to 10e11 can easily be generated in culture. Previous studies from our laboratories have demonstrated that cervico-vaginal immunization of mice with HPV PsV induced broad-based systemic and respiratory immune responses against the antigen encoded in the packaged plasmid. In addition, these responses were similar those observed after intramuscular infection with an adenoviral vector (rAd5). In the present study, we sought to determine whether genital immunization with HPV PsV could induce CD8+ T cell responses in the female genital tract. Using respiratory syncytial virus (RSV) M2 as a model antigen and an MHC class 1 tetramer conjugated with an immunodominant peptide from M2, we have been able to enumerate M2-specific CD8+ T cells in various tissues, including the female genital tract, after HPV PsV immunization. At day 8 and 15 post-secondary immunization, we observed a massive (5-fold) expansion of total CD8+ T cells in mice immunized with HPV-M2 PsV compared to control mice. Importantly, the increase in the number of CD8+ T cells was mainly attributable to the expansion of M2-specific CD8+ T cells, which represented up to 80% of CD8+ T cells present in the genital tract. Surprisingly, the magnitude of the genital CD8+ T cell response was much higher than the response observed in draining lymph nodes, spleen, and blood. These data indicate that genital HPV immunization induces CD8+ T cell responses that seed the genital tract and may have implication in the design of vaccines against sexually transmitted diseases in which CD8+ T cells may be required for protection.

O-02.07

DMXAA ENHANCES THERAPEUTIC HPV VACCINE-INDUCED CTL RESPONSES AND ANTITUMOR EFFECTS

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Objectives/Hypothesis: Since the combination of multiple modalities for cancer treatment is more likely to generate more potent therapeutic effects for the control of cancer, we have explored the combination of chemotherapy using 5,6-dimethylxanthenone-4-acetic acid (DMXAA), an investigational anti-cancer drug with immunotherapy using therapeutic human papillomavirus (HPV) vaccines in a preclinical model.

Methods: We characterized the combination of DMXAA with therapeutic HPV vaccination for its potential ability to generate antigen-specific CD8+ T cell immune responses as well as antitumor effects against the E7-expressing tumor, TC-1.

Results: We observed that treatment with a DNA vaccine encoding calreticulin (CRT) linked to HPV-16 E7 (CRT/E7) in combination with DMXAA could significantly enhance the E7-specific CD8+ T cell immune responses and antitumor effects compared to DNA vaccination alone in tumor-bearing mice as well as naïve mice. In addition, treatment with CRT/E7 DNA vaccine in combination with DMXAA enhanced the memory T cell immune responses in naive mice. Furthermore, DMXAA was capable of enhancing the antigen-specific immune responses and antitumor effects induced by CRT/E6 DNA vaccine as well as a recombinant vaccinia vaccine encoding E7 antigen linked to the lysosome-associated membrane protein type 1 (Sig/E7/LAMP-1).

Conclusions: Thus, our data suggest that treatment with DMXAA can further enhance the antigen-specific immune responses as well as antitumor effects generated by various therapeutic HPV vaccines and may potentially be translated into the clinical arena.

NOVEL IMMUNOTHERAPY TO INDUCE E7-SPECIFIC CTL IN MURINE GENITAL MUCOSA

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Background: Therapeutic vaccines designed to eliminate lesions in already HPV-infected women, only showed limited clinical results, which poorly correlate to immune responses measured in blood of vaccinee and contrast with the 100% regression of ectopic TC-1 tumors observed in pre-clinical trials. This may be linked to an inefficient targeting of protective immune responses to the genital mucosa, that is in addition associated to a basic immunosuppressive environment.

Objectives: Develop novel immunotherapies that effectively enhance the vaccine-specific immune responses in the genital mucosa.

Methods: Mice were immunized via aerosol-like or subcutaneous routes with adjuvanted synthetic HPV16 E71-98 polypeptide vaccines. Tumor regression was evaluated using TC-1 cells. Antibody-mediated in vivo depletion of CD4 T, CD8 T or NK -cells were performed during tumor protection assays. E7-specific CD8 T cell effector responses were determined by ex-vivo IFN-y ELISPOT and in vivo cytotoxic assays. Intravaginal administration of immunostimulants (Toll-like receptors agonists) were used in combination with vaccination.

Results: Both vaccination routes induced 100% regression of subcutaneous tumors in both prophylactic and therapeutic settings. In vivo cell-depletions demonstrated that tumor regression was essentially mediated by CD8 T cells. Indeed, E7-specific CD8 effector cells were detected in the blood, different lymphoid organs and more importantly in the genital mucosa it-self. There was no correlation between the responses measured in the periphery with those measured in the genital mucosa of individual mice. The additive topical application of different TLR agonists greatly enhanced the E7-specific responses locally in the genital mucosa. Finally, data using a vaginal tumor protection assay will be presented.

Conclusion: Our data highlight the necessity to determine the immune responses directly in the genital mucosa and suggest that combination of an adjuvanted E71-98 peptide with topical immunostimlants could be an efficient immunotherapy against HPV-16 and cervical cancer.

O-02.09

HPV-16 E5 DOWN-REGULATES MHC I AND CONTRIBUTES TO VIRAL IMMUNOEVASION

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Background. Reducing antigen display by major histocompatibility complex class I (MHC I) at the cell surface is one approach used by viruses, including papillomavirus, to escape detection by the immune system. HPV-16 E5 reduces levels of MHC I on the cell surface and retains the complex in the Golgi apparatus. The N-terminal 30 residues of HPV16-E5 are necessary and sufficient for this relocalisation.

Objectives. 1) The identification of the amino acid residues in the N-terminus of E5 necessary for MHC I down-regulation. 2) Confirmation that E5-induced MHC I down-regulation contributes to escape from CTL.

Methods. For objective 1), mutation analysis, cell transformation, qRT-PCR, flow cytometry. For objective 2), cell transformation, peptide loading, CTL assays.

Results. 1) Four distinct di-leucine motifs (LL) are present in the N-terminus 30 residues of E5. Conservative mutation of each LL motif to VV motifs has shown that all four LL motifs are necessary for MHC I down-regulation.

2) To see if MHC I down-regulation by E5 has functional consequences, cells expressing E5 and HLA-A2 were pulsed with varying doses of HPV16 E6 29-38 peptide, before co-culture with a T cell clone specific for HPV16 E6 29-38. T cell activation as measured by secretion of gamma-IFN by the CTL was considerably lower for E5-expressing cells than for control cells.

Conclusions. 1) The four N-terminus LL motifs of E5 appear to be equally important for down-regulation of MHC I. 2) E5 down-regulation of MHC I has a functional consequence with reduced CTL recognition, and therefore by extrapolation, E5 contributes to immunoevasion by HPV.

REGULATION OF LANGERHANS CELL NUMBER ACROSS HPV GENERA

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Human papillomaviruses (HPV) cause a range of pathologies, including latent infection, benign proliferative lesions and cancer. Many HPV infections are persistent, which may be contributed to by viral regulation of immunity at the infected site. We have previously reported that Langerhans cells (LC), the primary antigen presenting cells in the skin, are depleted in HPV16 infected cervical tissues, which we propose contributes to viral evasion of the immune response. The objective of this study was to determine if LC regulation by HPV was widely conserved immune evasion mechanism in other HPV genera.

HPV-infected epidermis was obtained from the cervix, genital region and other cutaneous sites. LC were enumerated following immunohistochemical staining with an antibody to CD1a. Further staining of HPV16 and HPV6/11 tissues was carried out to identify regulatory T cells, using an antibody to FoxP3.

LC numbers were reduced by around 10 to 100 fold in HPV epidermis infected with α , μ and γ types when compared with normal cutaneous epidermis. Although LC were depleted overall in condylomata, patches of LC were identified in these tissues which was inconsistent with the staining pattern of other α types. FoxP3 staining revealed the presence of regulatory T cells in condylomata, which were not frequently observed in HPV16-infected lesions. Epidermis infected with β types had comparable numbers of LC compared with normal skin.

The wide conservation of LC loss in HPV-infected tissue from all the genera tested except the β genus supports a contribution of LC loss to viral immune evasion. Although patches of LC can be found in condylomata, the regulatory T cells also found in those lesions are likely to have an immunosuppressive effect. Infection with β types is generally associated with latent infection that may be driven, at least in part, by their inability to regulate LC.

EPIDEMIOLOGY OF

HPV-ASSOCIATED DISEASES

SESSION 03

PROGRAMME

THE 25TH INTERNATIONAL PAPILLOMAVIRUS CONFERENCE MAY 8-14 2009, MALMÖ, SWEDEN

TIME NUMBER TITLE

ROOM

2009-05-11

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O-03.01

GLOBAL PERSPECTIVE ON HPV AND HPV-ASSOCIATED DISEASES

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Background: Over the last decades, the International Agency for Research on Cancer (IARC) has carried out case-series and case-control studies of cervical cancer that have substantially contributed to establishing the role of some HPV types in disease causation.

Objectives: To elucidate the infection burden and the distribution of individual HPV types worldwide.

Methods: A series of HPV prevalence surveys with similar protocols for population sampling and HPV testing. IARC HPV surveys currently include complete information on almost 20,000 women 15-64 years of age, including approximately 1,000 women from each of 20 world areas (Georgia, Italy, Poland, Spain, Argentina, Colombia, Chile, Guinea, Nigeria, Korea, Mongolia, Nepal, Pakistan, two areas in both Thailand and Vietnam, and three areas in China). Additional surveys are on-going in Algeria, Iran and Tonga.

Results: IARC HPV surveys show that not only does HPV prevalence vary many-fold between, and even within, countries but also that the distribution of HPV prevalence by age group reported in high-resource countries (i.e., peak below age 25 years) cannot be generalized. HPV prevalence remains high in the 15-59-year age range in some populations in Asia and sub-Saharan Africa, whereas U-shaped age curves are found in some Latin American populations.

Conclusions: HPV prevalence in different parts of the world correlates well, though not perfectly, with cervical cancer incidence rates as reported in IARC's Cancer Incidence in Five Continents. Furthermore, mapping of cancer incidence (i.e., IARC's Globocan), and of HPV prevalence worldwide, share the problem of substantial gaps in the availability and quality of relevant information. Examples will show that caution is required in making inferences from neighbouring countries or regions. In the case of HPV prevalence, further difficulties derive from selection bias (especially among the youngest women), the use of different assays for HPV testing and a steady tendency towards increasingly sensitive assays.

O-03.02

TIME TRENDS IN THE HPV TYPES DISTRIBUTION IN CERVICAL CANCER

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Background: HPVs are reported to be stable DNA viruses with a low rate of HPV genomic change. However, HPV type-specific distribution in cervical cancer may be modified by factors other than the virus genetic characteristics.

Objective: To evaluate time trends in the relative contribution of HPV16, 18 and other high risk types in invasive cervical cancer cases (ICC) over a period of 87 years (1920-2007).

Material and Methods: Paraffin embedded ICC cases were collected from historical archives (1920-2007). The study includes cases from 35 countries worldwide. HPV detection was done through amplification of HPV DNA by SPF-10 broadspectrum primers PCR subsequently followed by DEIA and genotyping by LiPA25 (version 1). Samples were tested at HPV laboratories at ICO (Barcelona, Spain) and at DDL (Voorburg, The Netherlands). Quality controls between the two labs were occurring regularly. Time trend univariate and multivariate analyses for HPV16, 18 and other HPV genotype distributions taking into account single infections has been performed according to decades.

Results: Out of 11,248 cases of ICC HPV analysed, 9,172 cases were found to be HPV-DNA positive. In the univariate analyses the relative contribution of HPV16, 18 and other HPV types showed variations over time: HPV16-from 65.7% (1920-29) to 53.0% (2000-07) trend test p<0.05; HPV18-from 5.7% (1920-29) to 11.6% (2000-07) trend test p<0.05; Other HPV types-from 25.7% (1920-29) to 27.1% (2000-07) trend test p>0.05. These patterns were also observed in the restricted analysis for squamous cell carcinoma cases. Region and country specific variations in these trends were observed.

Conclusions: The study shows a downward trend in the relative contribution of HPV16 to cervical cancer and a relative increase in the contribution of HPV18. Multivariate analyses on the possible impact of age, geography, histology and screening programs in these long term cross sectional observations will be presented.

O-03.03

THE ROLE OF CO-FACTORS IN CERVICAL CANCER IN EPIC COHORT

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Background: A number of environmental exposures have been reported as putative co-factors involved in cervical carcinogenesis. However, evidence is still conflicting and mostly coming from retrospective case-control studies.

Objectives: To explore potential associations between putative co-factors and the risk of developing cervical cancer in a large European prospective cohort study.

Methods: Between 1992 and 2000, 343,009 women mostly aged 35 to 70 years, participating in the European Prospective Investigation Into Cancer and Nutrition [EPIC] study, were recruited from 10 European countries. Participants completed lifestyle and diet questionnaires at recruitment and were followed-up for incidence of cancer until 2005. Relative risks for invasive cervical cancer [ICC] and cervical intraepithelial neoplasia [CIN] 2/3 or carcinoma in situ were estimated by Cox proportional hazard regression models stratified by age at recruitment and study centre. Attained age was used as the primary time variable. All estimates were mutually adjusted for the explored co-factors.

Results: During the 2,775,235 person-years follow-up (median, 8.96 years), a total of 261 ICC cases and 843 CIN2/3 cases were reported. Statistically significant increased risks of ICC were found in women with low educational attainment, in short-term former and heavy current smokers, in divorced or separated women and in long-term (5+ years) oral contraceptive (OC) users. No associations were found with number of full-term pregnancies, age at first pregnancy and IUD use. A similar co-factor profile was observed for CIN2/3 risk, with the exception that a positive association was additionally found with number of full-term pregnancies. The effect of dietary variables will also be presented.

Conclusions: These results, derived from a large prospective cohort study, confirm the role of environmental co-factors in cervical cancer. Low education, divorced/separated marital status, smoking, and long-term OC use were the co-factors most strongly associated with cervical cancer risk.

O-03.04

IMMUNITY-RELATED CANDIDATE GENES AFFECTING CERVICAL CANCER SUSCEPTIBILITY

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Background: Persistent infection with high-risk HPV is a necessary cause of cervical cancer. The fact that only a subset of the infected develops cervical tumors indicates that there are also other risk factors influencing cervical cancer susceptibility. There is a genetic predisposition to the disease and genes of the immune response are obvious candidate genes for cervical cancer susceptibility.

Objectives: To analyze the impact of polymorphisms in CCR-2, IL-4R, IL-10, FasL, TNF, LTA, TAP1 and TAP2 as well as variation at HLA-DQB1 on cervical cancer susceptibility in the Swedish population.

Methods: Association was analyzed in 1306 cases diagnosed with cervical cancer in situ and 288 blood donor controls. All cases were selected from a family material which increases the statistical power to detect associations. In total, 15 SNPs were genotyped using either Taqman assays or the Inflastrip assay; a reverse hybridization linear array with sequence specific probes. Polymorphism at DQB1 was genotyped using a reverse hybridization linear array with sequence specific probes.

Results: Association with cervical cancer in situ was detected for CCR-2 V64I and IL-4R I75V. Strong association was demonstrated for the DQB1 locus where alleles *0301, *0402 and *0602 increased and *0501 and *0603 decreased risk. There were no associations of other SNPs in the MHC that were independent of DQB1.

Conclusions: The results support the role of immunologic host factors in determining susceptibility to cervical cancer. Our findings underscore the importance of sufficient statistical power and incorporation of previously known risk factors when looking for genetic association.

O-03.05

REDUCED RISK OF CERVICAL AND VULVAR CANCERS ASSOCIATED WITH ALLERGIES

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Background: Many studies have shown an inverse association between allergies and risk of various cancers, most recently including cervical carcinoma. It is unknown whether this association is consistent across histologic types of cervical cancer or may exist with other anogenital cancers.

Objectives: To determine how the relationship between allergies and cervical and vulvar cancers may be modified by histology, tumor behavior, and HPV type.

Methods: Cases were diagnosed with cervical squamous cell carcinoma (SCC), invasive or in situ cervical adenocarcinoma, or invasive or in situ vulvar SCC in western Washington state between 1986 and 1998. Controls were recruited from the source population using random-digit telephone dialing. Participants completed a comprehensive in-person interview that included questions about lifetime history of allergies. Odds ratios (OR) and 95% confidence intervals (CI) were calculated using logistic and polytomous regression.

Results: History of allergies conferred a reduced risk of cervical cancer (OR 0.8, 95% CI 0.6-0.9). Pollen allergies, the most commonly reported allergy (32% controls, 24% cervical cases, 17% vulvar cases), were associated with a reduced risk of invasive cervical cancer (OR 0.8, 95% CI 0.6-0.9) and vulvar SCC (OR 0.5, 95% CI 0.4-0.7). This association was not present for in situ cervical adenocarcinoma (OR 1.5, 95% CI 0.9-2.3, p for heterogeneity = 0.01). Food allergies were associated with a significantly reduced risk of vulvar SCC (OR 0.6, 95% CI 0.4-0.8), but not cervical cancer (OR 0.8, 95% CI 0.6-1.1). However, wheat allergies were associated with reduced risk of invasive cervical cancer (OR = 0.1, 95% CI 0.0-0.9).

Conclusion: Allergies to pollen and certain foods were associated with a reduced risk of cervical and vulvar cancers in this study. It is worth exploring what is driving this association, possibly a common immunogenetic path such as toll-like receptors that link innate and adaptive immune response.

O-03.06

GENE POLYMORPHISMS ASSOCIATED WITH CERVICAL CANCER IN SWEDEN

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Background: High-risk human papillomavirus (hrHPV) infection is the major risk factor for cervical cancer (CxCa). The role of genetic susceptibility in the disease has been suggested, but the existing data lack consistency.

Objectives: To examine the association of common genetic variants with CxCa risk in the Swedish population

Methods: We conducted a case-control study on 973 CxCa cases and 1763 matched controls from two population-based cohorts. Human leukocyte antigen (HLA) alleles and 24 other polymorphisms in 10 immune response and cell cycle-related candidate genes were selected on the basis of mechanistic plausibility with an HPV infection or reported association with cervical cancer development. Genotyping was conducted using multiplex PCR and Luminex technology.

Results: A significant association of CxCa with various polymorphisms was observed. Three variants, one from the interleukin-6 (IL-6) gene and one from the lymphotoxin-alpha (LTA) gene, indicated a lower susceptibility to CxCa, and one variant in the cyclin D1 (CCDN1) gene indicated higher susceptibility. Additionally, three alleles of the HLA class II DRB1 locus were associated with CxCa, two of them increased (DRB1*0401 and DRB1*1501) and one decreased (DRB1*1301) the risk for the disease. The effects of the cell-cycle gene and the HLA*DRB1 alleles were independent of the effect of smoking. We did not find any association of risk with polymorphisms in genes related to the innate immune system. Conclusions: Our study provides evidence for genetic susceptibility to CxCa due to variations in genes involved in the

immune system and in cell cycle.

POSTER ABSTRACTS SESSION 03

POSTER SESSION I MONDAY 10.00: ODD NUMBERS

POSTER SESSION II

TUESDAY 10.00: EVEN NUMBERS

PARITY AS A CO-FACTOR FOR SUBSEQUENT CIN3+ AMONG HPV-POSITIVE WOMEN

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BACKGROUND: In addition to human papillomavirus (HPV), parity has been suggested as a possible risk factor for developing cervical abnormalities.

OBJECTIVES: To assess the additional risk of cervical abnormalities associated with either deliveries or pregnancies in high-risk HPV positive women, and in women with a persistent HPV infection.

METHODS: A population-based cohort of younger women was established from 1991 to 1993. The participants were interviewed about background and lifestyle factors, and cervical cells were obtained for cytological examination and HPV testing. Two years later the women were re-examined. In the present study we included 1352 women who at the second examination were high-risk HPV positive and had normal cytology. These women were followed passively in the Danish Pathology Data Bank for all cervical cytological and histological examinations. We used a parametric regression model to estimate the risk (Hazard Ratio (HR)) of cervical abnormalities (1: > atypia; and 2: > high-grade cervical intraepithelial neoplasia (HSIL) or > cervical intraepithelial neoplasia grade 3 (CIN3)) associated with either deliveries or pregnancies among initially high-risk HPV positive women in a follow-up period of 12 years. Similar analyses were made in a sub-cohort of women (n=381) with a persistent HPV infection (positive to the same high-risk HPV type at both examinations). RESULTS: The risk of > HSIL/CIN3 was statistically significantly increased in women with one or more deliveries (HR=1.38; 95% CI: 1.01-1.89) compared to no deliveries (adjusted for potential confounders), whereas this did not apply to pregnancies. The same risk pattern was found for > atypia. Finally, number of deliveries significantly increased the risk of both > atypia and > HSIL/CIN3 (HR=1.96; 95% CI: 1.16-3.29) in women with persistent HPV infection.

CONCLUSIONS: The risk of developing cervical lesions when being high-risk HPV positive is significantly affected by number of births but not by number of pregnancies.

P-03.08

HPV, HSV-2, C. TRACHOMATIS AND CERVICAL CANCER IN EPIC COHORT

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Background: HPV infection is the main cause of cervical cancer (CC), but other sexually transmitted infections may play a role as co-factors in the development of cervical cancer.

Objective: To estimate, prospectively, the association between serological markers of HPV infection, HSV-2 and Chlamydia trachomatis (CT) and CC risk.

Methods: Between 1992 and 2000, 343,009 women mostly aged 35 to 70 years, participating in the European Prospective Investigation Into Cancer and Nutrition [EPIC] study, were recruited from 10 European countries. Participants provided sera and risk-factor data at recruitment and were followed-up for incidence of cancer until 2005. We performed a nested case-control study within the EPIC cohort that included a total of 402 CC cases (140 invasive CC and 262 CIN2/3 or carcinoma in situ) and 808 matched controls in whom sera was available for this study. Enzyme and membrane-based immunoassays were used to detect serum antibodies to HSV-2. CT IgG serum antibodies were detected using a micro-immunofluorescence assay. HPV sera antibodies against L1 proteins of types 11,16,18,31,33,35,45,52 and 58 were detected using GST-L1 capture based ELISA and bead-based multiplex methods. Adjusted odds ratios (ORs) were computed by conditional logistic regression.

Results: Fully adjusted OR and 95% CI for combined CC and CIN2/3 risk were: 2.60 (1.83-3.70) for sero-positivity to any HPV type; 2.02 (1.34-3.05) for HSV-2 sero-positivity; and 1.60 (1.09-2.35) for CT seropositivity. These associations were stronger for invasive CC than CIN2/3. Seropositivity to C. pneunoniae was not associated with CC risk (OR=1.12; 95% CI, 0.83-1.52). Interaction analyses between HSV2, CT and HPV on CC risk and the effect of other environmental co-factors will also be presented.

Conclusions: This study adds prospective evidence that past infection with HSV-2 and CT may act in conjunction with HPV as co-factors in cervical carcinogenesis.

IL6 AND IL6 RECEPTOR POLYMORPHISMS AND VULVAR CANCER RISK

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Interleukin-6 (IL6) and its receptor IL6R are hypothesized to have a role in host response to oncogenic HPV infection, and thus risk of HPV-related anogenital cancers, but studies in vitro and in vivo have been small and inconclusive. Since IL6 and IL6R polymorphisms contribute to the respective levels of these proteins, we determined whether common genetic variation in IL6 and IL6R are associated with vulvar carcinoma risk. Nine IL6 tagging single nucleotide polymorphisms (tagSNPs) and 7 IL6R tagSNPs spanning the region 4000 basepairs upstream and downstream of each of these genes were genotyped in 463 vulvar carcinoma cases and 799 controls from a population-based study in western Washington. Nine and 14 haplotypes were inferred from the IL6 and IL6R tagSNPs, respectively. Odds ratios (OR) and 95% confidence intervals (CI) were estimated under log-additive (i.e., per SNP allele or haplotype copy) models, adjusting for age and race. No associations were observed with individual IL6 or IL6R SNP alleles. Compared to the most common IL6 haplotype (17% allele frequency among controls), two haplotypes—one containing the minor alleles at 5' flanking region rs2069827 and 3' untranslated region rs2069861 (17% allele frequency) and the other containing those variants plus the minor allele at 5' flanking region rs1800795 (9% allele frequency)-were statistically significantly associated with a reduced risk of vulvar cancer (OR = 0.66 [95% CI = 0.49, 0.88] and OR = 0.67 [95% CI = 0.47, 0.94], respectively). Three other haplotypes with minor alleles at rs2069827, rs2069861, and one or more additional tagSNPs, were not associated with risk. The results were similar when analyses were restricted to HPV DNA positive vulvar cases. No statistically significant associations were observed with IL6R haplotypes. These results suggest that common variation in IL6, or an as yet unidentified linked locus, is associated with vulvar carcinoma risk.

P-03.10

IUD, HPV AND CERVICAL CANCER: POOLED ANALYSIS IN 20263 WOMEN

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BACKGROUND: IUD use has been shown to reduce the risk of endometrial cancer, but little is known about its relationship with cervical HPV and cervical cancer (CC).

METHODS: We performed pooled analyses of individual data from 10 case-control studies of CC conducted in 8 countries and from 17 HPV prevalence surveys conducted in general population from 14 countries worldwide. A total of 2,508 women with CC and 2,483 matched control women were included from the case-control studies and 15,272 women from the HPV surveys. Information on IUD use was obtained by personal interview. HPV DNA was tested for by PCR-based assays. ORs and 95% confidence intervals were estimated using multivariate logistic regression models.

RESULTS: After adjusting for relevant covariates including cervical HPV and number of previous Pap smears, a strong inverse association was found between ever IUD use and CC (OR=0.55, 95%CI:0.42-0.70). Subgroup analyses showed a consistent protective association for both squamous-cell carcinoma and adeno-/adenosquamous carcinoma as well as among HPV-positive women. The effect was similar after taking into account Pap screening history. In contrast, no association was found between IUD use and cervical HPV among the control women recruited in the IARC case-control study (OR=1.13) nor among the women recruited in the large HPV prevalence surveys (OR=0.96).

DISCUSSION: These data suggest that IUD use may reduce the risk of CC and act as a potential protective cofactor in cervical carcinogenesis. IUD use, however, does not seem to modify HPV DNA detection in the cervix, suggesting that the potential biological mechanism would act by reducing HPV persistence rather than HPV acquisition. Even though our results do not seem to be explained by differences in screening practices or other known differential characteristics between users and non-users, the potential effect of residual confounding or selection bias cannot be completely ruled out.

THE ROLE OF HUMAN PAPILLOMAVIRUS IN CERVICAL ADENOCARCINOMA

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Background: Cervical cancer is the second most common malignant disease in women worldwide. In Sweden the incidence of adenocarcinoma of cervix has increased during the last decades and accounts for about 15-20% of invasive cervical cancers.

Objective: To asses the risk of adenocarcinoma of the cervix regarding presence of HPV DNA.

Methods: In a prospective case-control study we identified 126 cases diagnosed with adenocarcinoma of the cervix who also had an initial cytologically normal Pap smear by linking the Swedish National Cancer Register with the National Cervical Cancer Screening Register. For each case one control with an initial cytologically normal Pap smear was selected. Using validated and sensitive PCR-assays, the presence of the most common oncogenic HPV types were analyzed in all available smears from cases and controls. Conditional logistic regression models were applied to analyse the risk of adenocarcinoma of the cervix for type specific HPV DNA exposure.

Results: The most frequently occurring HPV type among cases was 18 (31%), followed by 16 (25%). The odds ratio for adenocarcinoma of the cervix for was 10.85 (95% CI=4.11-28.64) for HPV18 and 9.95 (95% CI=3.40-29.17) for HPV16. HPV types 45, 31, and 33 were less common among cases (6%, 2%, and 2%, respectively), and were not associated with a significantly increased risk of this cancer.

Conclusion: This is the first prospective study investigating the association of HPV DNA in the development of adenocarcinoma of cervix uteri. Although the results are preliminary they give further evidence for HPV 18 and 16 as major risk factors for cervical adenocarcinoma.

P-03.12 HPV TYPE DISTRIBUTION IN 47 NEUROENDOCRINE TUMORS OF DE CERVIX

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Background: Neuroendocrine carcinoma (NEC) of the cervix is a rare entity with poor prognosis and there is little information regarding its pathogenesis. The WHO classification includes under the term "Neuroendocrine tumors": carcinoid, atypical carcinoid, large cell neuroendocrine carcinoma and small cell carcinoma.

Objective: To describe the HPV type distribution in NEC.

Methods: In the RIS HPV TT study, among 10289 cervical carcinomas, pathologists identified 47 carcinomas with neuroendocrine features based on morphological characteristics. WHO classification was applied taking into account cell size, nuclear features and architectural pattern. HPV detection was done through amplification of HPV DNA by SPF-10 broad-spectrum primers PCR subsequently followed by DEIA and genotyping by LiPA25 (version 1)

Results: Mean age of the patients was 53 y.o. Pathological classification showed 15 small cell NEC, and 30 large cell NEC (two cases were not classified due to small amount of tissue). HPV detection yielded 38 HPV positive cases (80.9%) and 9 HPV negative. The types detected were HPV 16 (n=20, 52.6%), HPV 18 (n=14, 39.5%), HPV 58 (n=1; 2.6%), HPV 35 (n=1, 2.6%) and one case with both HPV 18 and 52 (2.6%).

Conclusion: This study confirms that cervical NEC is related to HPV. HPV 16 and 18 are identified in more than 90% of these tumors. The four-fold increase detection of HPV 18 when compared to that observed among all cervical cancer cases in this study deserves further research.

RISK FACTORS FOR EARLY ONSET CERVICAL CARCINOMA & HIGH-GRADE DYSPLASIA

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Background: Delayed onset of cervical screening is now recommended, therefore knowledge of risk factors for early onset cervical carcinoma is important.

Aim: To examine the association between reproductive factors and risk of early onset cervical carcinoma and high-grade cervical dysplasia (CIN 3 and ACIS) \leq 25 years of age.

Methods: We present pilot-data from an on-going case-control study with an intended sample size of 438 women. Subjects who attended Royal Women's Hospital (RWH) and Mercy Hospital for Women (MHW), diagnosed with cervical cancer or high-grade dysplasia between 1983-2007 by the age of 25 years (cases) and those diagnosed after 25 years (controls) completed a self-administered survey. Pilot data was analysed using frequency tables, calculating odds ratio (OR), 95% confidence interval (CI), and interquartile range (IQR). Ethics approval was obtained from the study institutions.

Results: Analysis of 42 cases and 10 controls demonstrated a trend towards cases having later age of menarche [13.5 years, IQR 12-14; versus 12, IQR 11-14 (OR 0.29, 95% CI 0.05-1.38)] earlier first sexual intercourse [16 years, IQR 15-18; versus 17, IQR 16-19 (OR 1.39, 95% CI 0.30-6.70)], shorter interval between menarche and first sexual intercourse [3 years, IQR 2-4; versus 4, IQR 3-7 (OR 2.88, 95% CI 0.49-30.23)], earlier first child-birth [25.5 years, IQR 22-28; versus 30, IQR 18-32] and earlier oral contraceptive use [16 years, IQR 15.5-18.5; versus 18, IQR 16-20]. Final data of 438 subjects will be presented.

Conclusions: Pilot data suggests a trend for women diagnosed with early onset cervical cancer/high-grade dysplasia having later age of menarche, earlier age of first sexual intercourse, shorter gynaecological age, earlier age of childbirth, and earlier age of first oral contraceptive use, compared to women diagnosed later. Analysis of the complete sample will allow for adjustment of confounding variables, and thus a stronger inference can be made.

P-03.14

CLINICAL AND MOLECULAR CHARACTERIZATION OF SOLITARY/MULTICENTRIC LOWER GENITAL TRACT LESIONS

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Background: Many lower genital tract lesions (vulvar, vaginal, perianal) are associated with HPV infections. An increasing incidence of lower genital tract lesions has been reported over the last decades especially in young women with an increase of multicentric and metachronic lesions.

Objective: We analyzed solitary and multicentric lower genital tract lesions in women attending a large colposcopy clinic for clinical characteristics and molecular markers.

Material and Methods: 130 patients with lower genital tract disease attending a colposcopy clinic were analyzed. 200 histological specimens, including 136 vulvar, 15 vaginal, 25 perianal and 24 cervical lesions were analyzed by histology, HPV typing , and immunohistochemical p16 staining as a marker of HPV transformation.

Results: We identified two different patient groups in our clinic: Women with solitary lower genital tract lesions (39 women) and women with multicentric and metachronous lesions (91 women with 159 lesions). 88% of the lesions in the first group were grade 3 or higher as compared to 66% of the lesions in the second group. The mean age of women with solitary lesions was 50 years, while the mean age of women with multiple lesions was 42 years; their mean age was lower in all disease stages. 20 of 37 (54%) lesions from the first group were HPV positive and 138 of 148 (93%) lesions from the second group were HPV positive. 21 of 33 (64%) lesions from the first group, but 111 of 147 (76%) lesions from the second group were p16 positive.

Conclusion: Our results suggest that there is a subset of women with a higher risk of repeated HPV-induced lesions of the lower genital tract. More data on risk factors and sexual behavior is necessary to determine whether this phenomenon is mainly related to these factors or if there is a strong genetic predisposition.

TRENDS AND INTRA-FAMILIAL CLUSTERING OF HPV IN SWEDISH ARCHIVAL CIS

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Background: The introduction of vaccines against HPV 16 and 18 has raised questions regarding the temporal dynamics of HPV types. Also, there is a genetic predisposition to development of cervical tumors that is probably caused by host factors of the immune response. If these act in a type-specific manner then women from the same family may show a tendency towards infection by the same type. Archival tissue stored at pathology clinics could be used to evaluate these questions. Objectives: To investigate temporal trends in the distribution of oncogenic HPV types and to analyze if related women

were infected by the same HPV type more often than expected by chance. Methods: The HPV type prevalence in 1.079 Swedish women diagnosed with cervical cancer in situ 1965-1993 was studied

Methods: The HPV type prevalence in 1,079 Swedish women diagnosed with cervical cancer in situ 1965-1993 was studied using a convenient protocol for DNA extraction, real-time-PCR based typing of archival material and logistic regression analysis. Families with multiple affected were evaluated to analyze intra-familial clustering of HPV types.

Results: HPV type was obtained for 974 samples. HPV 16 (61%) was the dominant type followed by HPV 33/52/58 (24%), HPV 31 (13%) and HPV 18/45 (12%). The prevalence of HPV 16 decreased over the study period, while no other temporal trends were seen. Related women were not prone to infection by the same HPV type.

Conclusions: We demonstrate the feasibility of HPV typing in large archival materials. Our results support that genetic susceptibility to cervical cancer operates through general susceptibility to persistent HPV infection. Complex factors determine frequencies of individual HPV types in a population and the changes in HPV prevalence due to vaccines may be difficult to predict.

P-03.16

TGF-B/SMAD SIGNALING GENE IN HUMAN CERVICAL CANCER

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Transforming growth factor- β 1 (TGF- β 1) is a multipotent cytokine that elicits many tumor suppressive actions including growth inhibition and apoptosis induction, and acquisition of resistance to TGF- β 1 correlates with malignant progression of a variety of human cancers.

Paradoxically, however, elevated expression of TGF-\beta1 has been observed in many late-stage cancers and often correlates with the invasiveness and metastatic potential of tumors. To define the molecular basis of TGF-B1 function in cervical carcinogenesis, we explored the expression and mutational status of Smad7, a negative regulator of the TGF-B1 signaling pathway, in human cervical cancers. Expression of Smad7 transcript was determined by quantitative reverse transcriptionpolymerase chain reaction (RT-PCR), and sequence alteration was analyzed using RT-PCR-single-strand conformation polymorphism (SSCP) analysis. Genomic levels of Smad7 was also measured by quantitative genomic PCR. Expression of Smad7 transcript was observed at a similar level (Smad7/GAPDH, 0.78-1.46) in all 10 normal cervix epithelial tissues we tested. By contrast, primary cervical cancer tissues showed variable expression (0.72-4.88) of Smad7, and its level was significantly higher compared to that of normal tissues (P < 0.05). Twelve of 50 (24%) primary tumors exhibited expression levels more than 2 folds of normal mean. All of the 12 overexpressing tumors were identified to have Smad7 gene levels comparable to normal tissues, suggesting that elevated expression might be caused by transcriptional activation rather than abnormal amplification of gene itself. In addition, no evidences for sequence alterations of the gene were found by RT-PCR-SSCP analyses, while three specimens displayed abnormal SSCP pattern from the region comprising of codon 208, which has been reported to have a polymorphism. Together, our study demonstrates that Smad7 is abnormally overexpressed in a substantial fraction of cervical cancers, suggesting that elevated Smad7 activity might contribute to the malignant progression of human cervical tumors via suppressing TGF-β1's tumor suppression function.

A XENOTRANSPLANT MODEL FOR PRIMARY CERVICAL CANCER IN SCID-BEIGE MICE

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Background: The limited number of available cervical carcinoma lines and difficulties in establishing primary transplants in SCID mice made testing of new therapeutic agents for cervical cancer particularly challenging. Methods: We demonstrate a technique which allows for the first time reliable and efficient engraftment of human cervical cancer. Primary tumor material is transferred subcutaneously into SCID beige mice. Results: About 70% of transplanted tumors exhibited potent proliferation and retransplantation up to 12 passages was possible in 40% of tumors. The histological analysis of retransplanted tumors revealed preserved tumor characteristics throughout xenotransplantation and multiple passages in mice. In this model we analysed therapeutic effects of a novel immunotoxin (SA2E) targeting the EGF-receptor, which is highly expressed in the majority of cervical cancer tissues. Subcutaneous application of 15 μ g SA2E resulted in a regression of 90% of tumor volume. Conclusion: The high rate of engraftment, reproducibility, expansion of primary tumor material, and easy preparation make this newly established tumor model an attractive tool for testing of efficiency of new therapeutics for cervical cancer in vivo.

P-03.18

A LONG-TERM COHORT STUDY ON CERVICAL PRE-CANCEROUS LESIONS IN TAIWAN

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Background: Human papillomavirus (HPV) has been detected in almost 100% of cervical carcinomas. Since 1995, National Health Insurance introduced National cervical neoplasia screening program offered the annual Pap smear screening for women aged over 30 which was reimbursed by insurance in Taiwan. The results of Pap were collected by a computerized registry system. In this follow-up study, we estimate the incidence of squamous intraepithelial lesions (SIL) of cervix for women infected with HPV in Taiwan.

Method: There were 11,923 women from seven townships participated in this community-based cervical neoplasia screening project (CBCSP) in 1991-1993. They gave their informed consent for Pap smear examination, cervical cells collection, and cancer status follow-up through computerized data linkage with national cancer registry profile. Deep-frozen cervical cells were used for HPV DNA amplification by polymerase chain reaction and genotyping by HPV Blot to identify 39 types of HPV. Through data-linkage with the nationwide cervical neoplasia screening registry, we followed up the cohort members till December 2006.

Result: There were 10,123 cytologically normal women with available cervical cells recruited for analysis to follow-up. The incidence (per 10,000 person-year) of any SIL for HPV negative women was 136, 464 for women had any HPV infection, and 685 for women had high-risk types HPV infections, and 835 for women infected with HPV 16/18/52/58/31/33 at baseline. In compared with HPV negative women, the corresponding age-adjusted hazard ratios during follow-up were 3.5, 5.2, and 6.3, respectively. Regarding high grade SIL, the adjusted hazard ratios were 7.2, 11.3 and 15.9. Comparing with women infected with any single type, multiple types HPV infection had significantly higher risk (HR: 2.2, 1.6-3.4) to developing any SIL. Our results demonstrated the importance of HPV types 16, 52, 58, 18, 31 and 33 are important in Taiwan.

PREDICTING PROGRESSION OF CERVICAL PRECURSOR LESIONS: A PROSPECTIVE COHORT STUDY

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BACKGOUND: Only a subset of cervical intraepithelial neoplasia (CIN) progress to cervical cancer, while we cannot predict which lesion will progress or not.

METHODS: To identify determinants of CIN progression, we followed 570 Japanese women with cytological LSIL (lowgrade squamous intraepithelial lesion) and histological CIN1-2 lesions (479 grade1 and 91 grade2) at 3-4 month intervals for a mean follow-up period of 39.1 months.

RESULTS: Over the period of follow-up, 46 lesions progressed to CIN3 while 362 regressed to normal cytology. After adjusting for CIN grade and women's age, hazard ratios for progression to CIN3 varied greatly by human papillomavirus (HPV) genotype: type 16 (4.56, 95% confidential interval 1.25-16.5): 18 (4.39, 0.44-43.9): 31 (12.1, 2.69-55.1): 33 (6.89, 1.23-38.4): 35 (4.22, 0.41-43.9): 52 (3.84, 1.03-14.4): 58 (4.01, 1.00-16.0): other oncogenic types (2.02, 0.52-7.91): non-oncogenic types (1.00, reference). HPV45 was not detected in our study subjects. The cumulative risk of CIN3 within 5 years was 19.8% for HPV16/18/31/33/35/52/58: 6.7% for other oncogenic types: 3.1% for non-oncogenic types (P=0.0001). In addition, HLA class II DRB1*1302 allele exerted a protective effect against progression (P=0.03). A significant association between DQB1*03 alleles and progression was observed among HPV16-positive women only (P=0.01). Environmental factors –women's age, smoking, sexual behavior and Chlamydia trachomatis infection– significantly correlated with CIN persistence (P<0.05), but not with progression.

CONCLUSIONS: In women with LSIL cytology, type-specific HPV testing is most useful for identifying populations at increased or decreased risk for disease progression. Genetic variations in HLA class II regions may modify risk of progression.

P-03.20

SMOKING IS AN INDEPENDENT RISK FACTOR FOR INVASIVE CERVICAL CANCER

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Background: The strong correlation between smoking and exposure to oncogenic human papillomaviruses (HPVs) has made it difficult to verify the independent role of smoking in cervical carcinogenesis. Thus, the authors evaluated this role.

Materials and methods: Five large Nordic serum banks containing samples from more than 1,000,000 subjects were linked with nationwide cancer registries (1973–2003). Serum samples were retrieved from 588 women who developed invasive cervical cancer and 2,861 matched controls. The samples were analyzed for cotinine (a biomarker of tobacco exposure) and antibodies to HPV types 16 and 18, herpes simplex virus type 2, and Chlamydia trachomatis.

Results: Smoking was associated with the risk of squamous cell carcinoma (SCC) among HPV16- and/or HPV18seropositive heavy smokers (odds ratio: 2.7, 95% confidence interval: 1.7, 4.3). A similar risk of SCC (odds ratio: 3.2, 95% confidence interval: 2.6, 4.0) was found in heavy smokers after adjustment for HPV16/18 antibodies. The point estimates increased with increasing age at diagnosis and increasing cotinine level. This study confirms that smoking is an independent risk factor for cervical cancer/SCC in women infected with oncogenic HPVs.

Conclusion: These findings emphasize the importance of cervical cancer prevention among women exposed to tobacco smoke.

COMPREHENSIVE ANALYSIS OF HPV AND CHLAMYDIA TRACHOMATIS IN CERVICAL ADENOCARCINOMAS.

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Objective: Chlamydia trachomatis (CT) has been implicated as a co-factor in cervical carcinogenesis. Serological studies reported a correlation between serum detection of CT antibodies and development of squamous cell carcinomas of the cervix. The goal of the current study was to investigate if CT may play a role in pathogenesis of cervical adenocarcinoma and, specifically, if there is a co-infection between CT and Human Papillomavirus (HPV) in cervical adenocarcinomas. Methods: Biopsies of 71 cervical adenocarcinomas (31 in-situ and 40 invasive) were tested for the presence of CT using two novel PCR assays. In addition, all cases were tested for HPV using SPF10-PCR assay and genotyped using LIPA25 test. Results: None of the cases were found to be positive for CT using two independent PCR assays. All lesions, however, were positive for HPV (with the exception of a case of adenoma malignum) and HPV 16 and 18 were detected in 94.2% of cases. Of the cases positive for HPV 16, 59.5% accounted for European variant, 33.3% for Asian-American variant and 7.1% for African 2 variant, respectively. Conclusion: The study demonstrated lack of co-infection between Human Papillomavirus and Chlamydia trachomatis in adenocarcinoma in-situ and invasive adenocarcinoma of the uterine cervix. The role of CT as a carcinogenetic co-factor may be restricted to cervical squamous cell carcinomas.

P-03.22

EBV PRECEDES THE DEVELOPMENT OF CERVICAL DYSPLASIA IN HIV+ WOMEN

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Objectives:Previous data has demonstrated a 2-fold increase risk of cervical dysplasia in HIV+ women shedding Epstein-Barr virus (EBV) and high-risk HPV. To explore the temporal relationships of this interaction, longitudinal cervical specimens from women enrolled in the WIHS who developed SIL were tested for EBV shedding.

Methods:A study of 57 HIV+ women from the WIHS was undertaken, 29 developed SIL and 28 did not. Cervical-vaginal lavage specimens were tested for EBV at the time of the development of SIL, 5 visits prior and 5 after. EBV was detected utilizing a highly sensitive PCR assay. EBV viral loads were measured using a real-time PCR assay and normalized using an RNase-P real-time assay. HPV DNA status and Pap smear results were obtained from the WIHS.

Results:The detection of HR-HPV DNA was associated with the development of dysplasia. Women developing dysplasia had detectible EBV in cervical fluids on more visits (49.1%) than those not developing dysplasia (40.7%). Eighty-six percent (25 of 29) of the women developing SIL had EBV detected at the time of initial diagnosis. EBV was detected within one clinic visit in 78% (53 of 68) of all SIL found at any time during the follow-up period. EBV shedding started on average, 3.5 clinic visits prior to the first diagnosis of SIL. EBV viral loads were dropping in 10 subjects, and were at low levels in 13 others consistent with the development of latency.

Discussion:In a pilot study of women who developed dysplasia, there is a striking correlation between EBV shedding and cervical dysplasia. This adds to the growing data that there may be a direct or indirect interaction between EBV and HPV in the development of cervical dysplasia in HIV+ women.

EARLY SEXUAL EXPERIENCE AND AGE OF CERVICAL CANCER DIAGNOSIS

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Background: Given the established links between young age at first intercourse (AFI), number of sex partners, high-risk human papillomavirus infection, and squamous cell cervical cancer (SCC), we hypothesized that women diagnosed with SCC at younger ages would be more likely to report young AFI than women diagnosed later in life.

Methods: We performed a population-based investigation among invasive SCC cases who were diagnosed between 1986 and 2004, were 22 to 53 years old, and lived in the metropolitan Seattle-Puget Sound region (n=333). Using multivariate linear regression, we estimated coefficients and 95% confidence intervals (CI) to assess the association between age at SCC diagnosis and AFI (<15, 15-18, \geq 19) and number of sex partners before age 20 (0, 1, 2-4, 5-14, 15+), accounting for birth year and other factors. Interactions were assessed using the likelihood ratio test.

Results: The interval between AFI and SCC diagnosis ranged from 4 to 35 years. In a multivariate model, compared to SCC cases reporting AFI \geq 19, the mean age of diagnosis was 3.1 years younger for SCC cases reporting AFI<15 (CI:-5.8,-0.5) and 2.6 years younger for SCC cases reporting AFI 15-18 years (CI:-4.6,-0.6). Although number of sex partners before age 20 was associated with age at SCC diagnosis in a crude analysis, the association was not independent of AFI. However, in the AFI \geq 19 and AFI<15 groups, differences in effect were seen by number of sex partners before age 20 (p for interaction=0.08), with the association remaining strong and significant only in the AFI<15 group that had 2 or more partners before age 20 (coefficient:-4.2, CI:-6.3,-2.1).

Conclusion: Among younger and middle-aged women with SCC, early age of diagnosis was associated with early AFI, though the effect appeared to be modified by number of sex partners before age 20.

P-03.24 WORLDWIDE HPV CONTRIBUTION AND GENOTYPE DISTRIBUTION IN VULVA-VAGINA CANCERS

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Objective: To describe HPV contribution and genotype distribution in vulvar and vaginal invasive cancer in large series of cases using a common protocol.

Material and Methods: Paraffin embedded tissue blocks diagnosed as vulvar and vagina invasive cancers were consecutively collected from archival material. HPV detection was done through amplification of HPV DNA by SPF-10 broadspectrum primers PCR subsequently followed by DEIA and genotyping by LiPA25 (version 1). Samples were tested at the ICO HPV laboratory (Barcelona, Spain). Detailed histological evaluation was done using standardized criteria with special emphasis to identify basaloid and warty changes. Results presented here originate from samples collected in Australia, Austria, Bangladesh, Bosnia-Herzegovina, Brazil, Chile, Colombia, Czech Republic, France, Greece, Guatemala, Honduras, India, Korea S., Kuwait, Lebanon, Mexico, Mozambique, Paraguay, Philippines, Poland, Portugal, Spain, Taiwan Turkey and Venezuela. The study is still assembling cases covering a wide international geographical distribution. Results: The preliminary analysis included 662 vulva and 199 vagina cancer cases.

69.2% of the vulvar cases were classified as non warty-basaloid squamous cell carcinoma (SCC) and 23.1% as warty-basaloid (WB) with significant variability across continents. The HPV positivity was 12.4% for SCC and 62.1% for WB. The four most common types detected as a single infection were HPV16(63.6%), HPV18(4.6%), HPV45(4.6%) and HPV33(3.9%). Multiple infections accounted for 6.1%. Mean age of the cases was 70.1 years.

The overall HPV positivity in vaginal cases was 70.4%. The most common types detected as a single infection were HPV16 (58.7%), HPV31, 33, 51 and 58(4.5%); HPV52(3.7%) and HPV18 and 39(3.0%). Multiple infections accounted for 5.0%. Mean age of the cases was 61.7 years.

Conclusion: The preliminary data indicate a consistently high HPV prevalence in vulvar cases with a warty-basaloid component and in vaginal tumors. Evaluation of geographical and age differences will be described.

SCREENING HISTORY OF 200 HPV-GENOTYPED CERVICAL CANCERS IN ITALY

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In Italy, as in most western countries, cervical cancer still occurs for two main reasons: lack of screening or screening failure. Recent evidence showed that neoplasia positive to high-risk (HR) HPV16 appears to be more easily detected during cervical cancer screening than the other HR-HPVs. Our aim was to investigate the screening history of genotype specific cervical cancers. Two-hundred sixty-eight patients diagnosed with invasive cervical cancer who were referred for the first surgery to the European Institute of Oncology, were tested for the presence of HR-HPV types. All cervical specimens were sent to the IARC for HPV typing by the multiplex PCR/APEX assay. In 200 HPV-DNA positive cervical cancer cases we had information on the cervical cytology history; 28 (14%) patients never had a Pap-smear or had one more than 6 years before the cervical cancer diagnosis, 45 (22.5%) had a negative cytology, while 127 (63.5%) had an abnormal cytology result. Thirty (17.4%) HPV 16 and/or 18 positive cases had a negative Pap-smear result, while 105 (61.1%) had an abnormal cytology. Of the cases positive for other high-risk HPV genotypes only, 15 (8.7%) had a negative Pap-smear, while 22 (12.8%) had an abnormal cytology (chi-square test, p = 0.025). As expected, in patients who never had a pap smear performed during their life, the proportion of invasive cervical cancers due to HPV 16 and/or 18 or due to other HR-HPV genotypes only was 70.8% (17 cases) and 29.2% (7 cases) respectively. Our results show that screening failure is preferentially associated with the development of non-HPV 16 and/or 18 invasive cervical cancers. If this result will be confirmed by others, cervical screening using cytology in the vaccinated population might not be as effective as it has been in the pre-vaccination era, and should, therefore, be modified accordingly.

P-03.26

HPV 16/18/52/58 VIRAL LOAD PREDICTS VIRAL PERSISTENCE AND CERVICAL CANCER

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Background: HPV16, 18, 52, 58 were major risk types for cervical cancer in Asian regions. HPV persistence might be caused high viral load. We explored the viral characteristics of HPV persistence and incident cervical cancer in a longitudinal study with repeated measurement.

Method: In this community-based cervical neoplasia screening project (CBCSP) with two health examinations in 1991-1993 and 1993-1995, 11,923 women from seven townships participated and gave their informed consent for Pap smear examination and cervical cells collection. HPV DNA amplification and genotyping were performed by polymerase chain reaction and HPV Blot (39 types). Viral loads (copies/50ng DNA) of HPV16, 18, 52 and 58 were measured by typesspecific real-time PCR on E6 regions.

Result: There were 807 subjects included for viral load analysis. The percentage of high viral load (>10^4) was increased with severity of cervical abnormality (p<0.0001). Cytologically normal women at baseline (n=391) received both examinations were further analyzed. Compared with low viral load (<10^3) at baseline, the ORs were 4.3 (2.4-7.7) and 8.4 (4.8-14.8) for medium (10^3-10^4) and high (>10^4) respectively, after adjustment of menopause (OR: 1.9, 1.2-3.0) and multiple sexual partner (OR: 7.9, 2.0-31.5). The incidence (per 100,000 person-year) of cervical cancer were 181.0 (<10^3), 193.4 (10^3-10^4) and 669.4 (>10^4). In multiple Cox regression model, high viral load at baseline had a 3.4-fold risk to developing cervical cancer in a dose-response relationship. Considering viral load at F/U, while viral load lowered from medium/high to low, a significantly protective effect (HR: 0.1, 0.03-0.3) was observed. The risk of HPV persistence increased with viral load increasing, also HPV persistence strongly predicted the cervical cancer development with HR of 14.4 (2.3-45.3). In conclusion, HPV viral load of HPV16/18/52/58 predicts HPV persistence and cervical cancer. Lowering viral load could be helpful to prevent cervical cancer among HPV infected women.

HPV16 AND HPV18 VARIANTS IN 126 CERVICAL CANCERS

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Introduction: Because certain HPV variants appear to have differing biologic potential, we analyzed the variant status of 90 HPV16 and 36 HPV18 cancers in an attempt to correlate them with histologic cell type. Methods: DNA from HPV16- and HPV18 –positive cancers were amplified by PCR using primers for HPV16 E6 and HPV18 LCR. Purified PCR products were sequenced bidirectionally. Variant category was assigned using published sequence patterns. All cancers and cell type were verified histologically.

Results: For HPV16+ cancers, 71 (79%) harbored European patterns. 29 (37%) squamous cancers (SQ) and 35 (39%) of all cervical cancers harbored the European prototype (16-EP) while 36 (46%) of squamous cancers were associated with European variants (16-EV) that differed from EP by one or more single nucleotide changes. HPV16-European patterns in adenocarcinoma (3/7) and adenosquamous cancers (3/4) were 16-EP only. By contrast, 16-EV sequences were found in squamous cancers only.

Of 19 cases that harbored HPV16 non-European (NE) sequences, 13 contained the Asian-American (16-AA) variant. These were found in squamous and non-squamous cancers, particularly adenocarcinomas (4/7).

The most common HPV18 variant was the European pattern (18-E) - found in 17 (45.9%) cases including all histologic types except small cell neuroendocrine. The Asian-Amerindian variant (18-AsAi) was present in 15 (40.5%) cases of all histologic types. 18-AsAi was found in 5/8 HPV18 adenocarcinomas and all small cell cancers. The African variant (18-Af) was found in only 4 cases with no particular tropism for cell type.

Conclusions: HPV variant patterns appear to be associated with histologic cell type. HPV16-AA and 18-AsAi seem to be associated with adenocarcinomas while 18-AsAi was found in small cell cancers. These histologic types have been reported to have worse prognosis than squamous cancers, suggesting that variant sequence may influence cell type and biologic behavior in cervical cancers.

P-03.28

AGE AND STAGE ARE GENOTYPE RELATED IN 385 CERVICAL CANCERS

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There is evidence that HPV 16 infection can lead to CIN development quicker than infection with other high risk genotypes. We here report the relation of age, stage and survival to HR HPV genotypes in a large series of invasive cervical cancers from two Italian tumour Institutes. Paraffin embedded cervical specimens were obtained from 251 cervical cancer patients referred to the European Institute of Oncology (IEO) and 134 patients referred to Regina Elena Institute (IRE). Following preparation, cervical samples from IEO were sent to laboratories at the International Agency for Research on Cancer (IARC, Lyon, France) for DNA extraction and HPV typing by the multiplex PCR/APEX assay. Samples from IRE were sent to ICO (Barcelona, Spain) or DDL (The Netherlands) where HPV detection was performed by SPF-10 broad-spectrum primers PCR subsequently followed by DEIA and genotyping by LIPA25. A total of 385 cases had been genotyped; two hundred and seventy-three specimens (70.9%) tested positive for either HPV 16 or HPV 18 or both, whereas one hundred and twelve (29.1%) resulted positive for other HR HPV genotypes. A statistically significant association with younger age and earlier stage (FIGO stage I vs FIGO stage > I; 2 test, p = 0.07) was observed for HPV16/18 related invasive cervical cancers. The age related difference was statistically significant either considering patients younger than 35 years or considering three age groups, below 35, between 36 and 45 and older than 45 (2 test, p = 0.01). On 194 cases from IEO a further analysis on mortality showed a worse overall survival in cervical cancer cases non 16 or 18 genotype related, even if the difference was not statistically significant (log rank; p = 0.08). The results demonstrate that some important clinical features of invasive cervical cancers are genotype related

5'-CPG ISLAND HYPERMETHYLATION OF THE FHIT GENE IN CERVICAL CARCINOMA

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The abnormal expression of fragile histidine triad (FHIT) gene, a candidate tumor suppressor, has been frequently reported in a variety of epithelial malignancies including cervical carcinoma. Furthermore, in a recent study it was proposed that transcriptional inactivation of FHIT, as a consequence of aberrant 5'-CpG island methylation, plays an important role in the carcinogenesis of human cervical carcinoma. The authors sought to determine whether abnormal FHIT transcription occurs in human cervical carcinoma, and if so, whether this abnormal expression is associated with aberrant 5'-CpG island methylation. In addition, the clinical significance of FHIT inactivation was investigated in Korean women with cervical cancer.

Quantitative RT-PCR analysis demonstrated that FHIT was down-regulated in 15 of 58 (25.9%) cervical carcinomas but in none of the seven normal cervical tissues. Methylation specific PCR (MSP) was applied to detect the methylation status, and aberrant 5'-CpG island methylation of FHIT was detected in all 15 cervical carcinomas but in none of the seven normal cervical tissues. Bisulfite DNA sequencing confirmed these findings and showed that all methylated samples were densely methylated. Furthermore, a significant correlation was found between CpG site hypermethylation and low FHIT expression. However, no significant correlation was found between reduced FHIT expression and clinicopathological characteristics.

In this study, FHIT inactivation in cervical cancer was found to be strongly correlated with 5'-CpG island hypermethylation rather than a genetic alteration. Furthermore, no significant relation was found between a lack of FHIT expression and the presence of cervical cancer in our Korean cohort.

P-03.30

HPV VACCINE PREVENTABLE CANCERS IN MEN AND WOMEN IN AUSTRALIA

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Background: Given increasing evidence that in addition to cervical cancer, HPV is causally linked with other anogenital and oropharyngeal cancers in both sexes, there is a need to quantify the total vaccine preventable burden, particularly as to the benefit of extending Australia's National HPV Vaccination Program to males.

Objectives: Analyse HPV-associated cancer burden in Australia.

Methods: Incidence of invasive cancer was obtained from National Cancer Statistics Clearing House database. Cases (mapped to ICD10) diagnosed between January 1982 and December 2004 included cancer of the cervix (C53), anus (C21), vulva (C51), vagina (C52), penis (C60) and oropharynx (C01/C09/C10). Fractions attributable to HPV and HPV 16/18 were determined from the literature.

Results: From 2000 – 2004 there were 970 cases of HPV 16/18-associated invasive cancers pa. Non-cervical HPV 16/18-associated cancers accounted for 48% comprising oropharyngeal (15%), anal (21%), vulval (7%), vaginal (2%) and penile (2%) cancers. Oropharyngeal HPV 16/18-associated cancers were more common in males than in females (109 and 32 pa respectively) whereas HPV 16/18-associated anal cancers were similar in males and females (93 and 111 pa respectively). HPV 16/18-associated cancers in males accounted for 225 invasive cancers pa (23% of total). From 1982 – 2004 there was a significant fall in the incidence of cervical cancer of 3.4% pa (95%CI: 2.8 - 3.9) but no change in incidence of vulval, vaginal and penile cancers. Incidence of anal cancer increased by 2.3% pa in males (1.5 – 3.1) and 1.1% pa in females (0.5 – 1.9). HPV 16/18-associated oropharyngeal cancers increased by 1.0% pa (0.7 – 1.3) similar in males and females. Time by age analyses will be presented.

Conclusions: This analysis confirms the considerable burden of vaccine preventable HPV cancer in Australia.

P-03.31 EPITHELIAL BRIGHTNESS BY OPTICAL COHERENCE TOMOGRAPHY DISTINGUISHES GRADES OF DYSPLASIA

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Objectives: Analyze differences in the brightness of cervical epithelium on Optical Coherence Tomography (OCT) images as a distinguishing characteristic of normal, low-grade, high-grade, and cancer histologies. Methods: Women who participated in two studies of OCT were combined. All patients undergoing colposcopy or unaided Visual Inspection (VIA) were evaluated by cervical quadrant. Areas were likewise evaluated by OCT. All women had biopsies obtained from abnormal areas in any quadrant. In normal quadrants biopsies matching the OCT sites were taken at 2,4,8,or 10 o,clock at the squamo-columnar junction depending on the quadrant. Brightness of the epithelium was measured at 3 and 12 oclock and averaged together to create a normal brightness reading for each patient. Abnormal lesions were then measured for brightness; normal images were then subtracted from the abnormal image to create a difference from normal. All brightness measures were on a log scale. Mean difference from normal was used to compare brightness levels by histological grade. Two sample T-tests were used to look at differences in brightness between histological grades. Results: Histologic diagnoses were as follows 391 normal, 6 squamous metaplasia, 36 CIN II, 42 CIN III, and 8 cancer. One woman with CIN II did not have an OCT image leaving 35 CIN II for analysis. Mean brightness was 0.16, 1.56, 3.36, and 4.71 for squamous metaplasia, CIN II, CIN III, and cancer respectively. Mean brightness differed significantly between each histological grade (p-values 0.000) for the comparisons of CIN II to CIN III, CIN II to cancer, and squamous metaplasia to cancer. For the comparison of mean brightness for CIN III to cancer p=.008. Conclusions: We conclude that epithelial brightness is an important component to include in developing a mathematical algorithm to use for the diagnostic interpretation of OCT generated images of the uterine cervix.

P-03.32

RISK FACTORS FOR HIGH-GRADE CERVICAL LESIONS IN THE TATI PROJECT

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Background: 5435 women were screened by different techniques in 2001 within the TATI project in Amazonian Peru. Before gynaecological examination, women were helped to fill in an epidemiological questionnaire containing data on demographics, reproductive and sexual behaviour, screening and smoking history. Objectives: to evaluate the association between potential risk factors and high-grade cervical lesions (carcinoma in situ or worse, CIS+) in women testing positive for high risk HPV DNA. Methods: Logistic regressions (simple and multiple) were used to assess the effect of age at entry into the study, years of education, ethnicity, age at first sexual intercourse, lifetime number of sexual partners, parity, abortion, previous PAP smear, use of contraceptive methods and smoking on CIS+ among 76 cases and 572 controls (women who did not have moderate dysplasia or worse on histology), all of whom tested positive for HPV (using Hybrid Capture II). Results: In the univariate analysis, the risk of having CIS+ increased significantly with increasing age, fewer years of education and with increasing parity (p-values for trends: 0.0003, 0.0140, 0.0001, respectively). Women older than 45 years of age and those who had more than four children were at higher risk of having CIS+ (odds ratios (OR) for being age 45 or older: 3.04, 95%CI: 1.13-8.23 and for having had 4 or more children: 2.22, 95%CI: 1.05-4.69) after adjusting for other factors. There were no cases among nulliparous women. Among 133 women aged 25-29 years with parity under 3, there were only 4 CIS+ cases (3%), whereas among 100 women aged 40-49 with parity of 4 or more, 18 (18%) had CIS+, yielding an OR of 7.08 (95%CI: 2.31-21.66). Conclusions: Older age and high parity increased the risk of CIS+ in HPV positive women who participated in a comparative screening study.

HPV GENOTYPES IN VULVAR BIOPSIES IN NORTHERN TERRITORY AUSTRALIAN WOMEN

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Background: Clustering of vulvar intraepithelial neoplasia (VIN) and vulvar cancer has been documented in young Indigenous Australian women. Between 1996-2005 in East Arnhem (EA) region of the Northern Territory (NT) Australia, the age-standardised incidence of vulvar cancer amongst Indigenous women aged 0-49 years was 31.1 per 100,000 (95% CI 13.1-49.1), over 50 times higher than the national Australian rate (0.4 per 100,000, 95% CI 0.4-0.5) for the same agegroup. Objective: To examine if clustering is HPV related. Method: HPV genotypes were examined in 14 high grade VIN and 15 invasive cancer biopsies from the EA region and compared with 13 high grade VIN and 17 invasive cancer biopsies from other regions of NT. Histological diagnosed paraffin tissue biopsies were collected from 1 January 1996 to 31 December 2005 and tested for HPV genotypes using SPF10- INNO LiPA HPV test. Results: In total, 59 of 67 specimens from 60 women had assessable results. HPV genotypes included 11, 16, 26, 31, 33, 35, 39, 44, 51, 52, 53, 54, 56, 59, 66 and 82. High-risk (HR) HPV was detected in 12/14 (86%) high-grade VIN and 13/15 (87%) invasive cancer biopsies from EA region and in 12/13 (92%) high-grade VIN and 14/17 (82%) invasive cancer biopsies from surrounding regions. HPV 16 was detected in 7 (50%) high grade VIN and 7 (47%) invasive cancer EA biopsies compared with 12 (92%) high grade VIN and 11 (65%) invasive cancer biopsies from surrounding regions. There was no significant difference in detection of HR HPV in high-grade VIN or invasive cancer samples from the EA region compared with other regions of NT (p=1.0). Conclusion: No significant difference in oncogenic HPV infection between the two regions. Other environmental and genetic factors will be investigated further.

P-03.35

ARRAY CGH CHARACTERIZATION OF UTERINE CERVICAL CANCER

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Background: For the development of preneoplastic and malignant lesions of uterine cervix, involvement of human papillomavirus (HPV) is of great importance. How these viruses induce cellular transformation at a molecular level has been extensively studied, yet genetic changes recognized as complex cytogenetic alterations related to the onset of carcinogenesis still needs to be characterized. Objective: In order to identify the genomic imbalances existing in uterine cervical cancer, we used microarray-based comparative genomic hybridization (aCGH). Methods: Using BAC microarrays (GSPArray7700TM) on which 7718 Keio BAC-DNAs are spotted in triplicate, we examined copy number changes of DNA segments in uterine cervical cancer tissues. We applied the aCGH system to various stages of both squamous cell carcinomas and adenocarcinomas. We also examined the samples for the presence of various HPV types. Results: So far, we analyzed 26 tissue samples from cervical cancer patients and found that the most common segmental DNA changes were seen as gain on 1q, 3q, 5p and 20p, and loss on 11q and 6q. We confirmed that samples detected with HPV all harbored high risk genotypes. Conclusions: Recurrent gains and losses were observed. Loss found on 6q may be a novel candidate region and further analysis may lead to the discovery of novel genes important in cervical carcinogenesis.

CERVICAL DYSPLASIA IN HIV-NEGATIVE WOMEN CO-SHEDDING HPV AND EBV

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Background: Previous studies from the New Orleans HIV+ cohort have found an increase (2 fold) of cervical dysplasia in women shedding both high-risk HPV and EBV in cervical fluids. It is not known if this relationship also holds for HIV-negative women. Initial in-vitro studies have demonstrated a potential interaction between the oncogenes of HPV and EBV that does not require immune suppression or the presence of HIV. This study examines archived cervical samples HIV-negative women previously enrolled in a HPV natural history study.

Methods: Archived cervical samples (isolated DNA from cervical swabs) from 262 HIV-negative women enrolled in a colposcopy clinic were tested for the presence of EBV using a highly sensitive PCR.. HPV was detected using the reverse line blot assay and Pap smears defined using the Bethesda criteria.

Results: A total of 138 women (52%) were shedding EBV and 74 (28%) shedding high-risk HPV. HPV-only shedders were at increased risk of an abnormal Pap smear (p<.001, OR 4.11, CI 1.7-9.9) as compared to those shedding neither virus. EBV-HPV co-shedders were also at increased risk of an abnormal Pap smear (p<.001, OR 6.93, CI 2.8-17.1) as compared to those shedding neither virus. Although there were more co-shedding women with an abnormal pap smear (n=27) than those shedding only HPV (n=20), this did not reach statistical significance in this small cohort.

Discussion: There may be an increase in cervical dysplasia in HIV-negative women who co-shed HPV and EBV. However, a larger cohort will needed to be studied to confirm these findings. Such studies are underway utilized additional archived specimens and the HIV-negative women from the WIHS

P-03.38

GENOTYPING OF HPV DNA SEQUENCES FROM HIGH GRADE CERVICAL DISEASE

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Objective: Genotyping data on archival cases of cervical cancer or high grade cervical intraepithelial neoplasia (CIN3) will be useful in assessing the potential impact of HPV vaccination in the UK.

Methods: Viral DNA was extracted from wax embedded tumour blocks archived in five English Centres. All cases were initially tested by the Roche Linear Array (LA) with those cases in which no High Risk (HR) or probable HR type was detected being further analysed using the Innogenetics InnoLiPA genotyping assay.

Results: Of 168 cases found not to contain HR or possible HR types by LA 118 were found to contain such types by InnoLipa. In total HR HPV DNA was detected in 429/445 (96.4%) SCC cases, 98/102 (96%) adenocarcinoma cases, 51/52 (98%) CGIN cases and in 862/905 (95%) of CIN3 cases. The HR vaccine target types 16 and/or 18 were detected in the absence of other HR types in 316/445 (71%) 78/102 (76%) 41/52 (78.8%) and 475/906 (52.4%) respectively. HR HPV types not present in the vaccine were detected in a total of 144/599 (24%) of cancer cases compared with 387/906 (42.7%) of CIN3 cases. The most common types found in cancer cases after 16 and 18 were HPV33 (5.1%); HPV45 (3.8%); and HPV52 (3.5%). In contrast amongst CIN3 the most prevalent types were HPV 16 (57.4%); HPV33 (10.5%); HPV31 (10.2%); HPV52 (8.6%) and HPV18 (7.5%)

Conclusion: The current HPV vaccines would result in approximately 72% protection against cervical cancer and 52% protection against CIN3. The UK programme will use the Cervarix HPV vaccine and cross protection against HPV 45 and 31 has been reported for this vaccine. These types were amongst the most prevalent in SCC after HPV 16 and 18, raising the possibility that vaccination could prevent a further 5% of cervical cancers.

DIFFERENTIALLY METHYLATED GENE PROFILING BETWEEN HPV-POSITIVE AND NEGATIVE CERVICAL CANCER

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Purpose: Based on the recently accumulated reports which showed a upregulation of DNA methyltransferase 1 (Dnmt1) and an increase DNA methylation in the cells infected with the tumorigenic viruses, we hypothesized that DNA methylation is one of key mechanisms of malignant transformation mediated by HPV infection. To test this hypothesis, we sought to determine whether DNA methylation at a particular promoter is increased by HPV infection.

Materials and Methods: We performed a genomewide assessment of the methylation status of promoter regions using a microarray-based strategy, which relies solely on the cleavage of DNA with a methyl-sensitive restriction enzyme, followed by ligation and PCR. Differences in methylation between two genomes are manifested by the absence of methylated regions in the amplification products. We selected samples from advanced staged patients (stage Ib2-IIb) to minimize contamination from normal tissues. Genomic DNA from five HPV-negative and eleven HPV-positive cancer tissues were digested with the methylation sensitive enzyme HpaII. Oligonucleotide linkers were ligated to the digested fragments. Repetitive sequences were then removed by hybridization with biotin labeled Cot-1 DNA, followed by affinity capture with streptavidin magnetic particles. PCR using linker-specific primer was then used to amplify short HpaII fragments. PCR product from test (each samples) and reference DNA (a pool of all samples) were labeled with Cy5 and Cy3, respectively, and hybridized to a microarray consisting of promoters from 12,800 genes.

Results: We profiled 64 and 39 genes showing different methylation status in HPV-positive and negative cancer tissues of the human uterine cervix, respectively (p<0.05).

Conclusions: These wide-ranging profiles of differentially methylated genes should provide a basis for understanding the molecular mechanisms of HPV infection associated with carcinogenesis in human uterine cervical cancer.

P-03.40

MULTIPLICATION OF NEUTROPHIL AND MONOCYTE AS BIOMARKER FOR CERVICAL CANCER

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Background: Simple, non-aversive methods to identify cervical neoplasia and predict prognosis are needed. The purpose of this study was to investigate the clinical value of differential white blood cell (WBC) counts for the diagnosis and prognosis of cervical neoplasia.

Methods: We performed a retrospective review of 407 cervical cancers, 495 cervical intraepithelial neoplasia (CIN) patients, and 916 healthy controls at Yongdong Severance Hospital between January 2000 and December 2007. Pretreatment differential WBC counts and SCC antigen levels were recorded and the neutrophil to lymphocyte ratio (NLR) was calculated. Because pre-treatment neutrophil and monocyte counts in cervical cancers showed the potential as a tumor marker, we combined these two parameters and created a new marker by multiplying neutrophil counts by monocyte counts and then dividing by 10,000. We designated this marker MNM (multiplication of neutrophil and monocyte counts). In cervical neoplasia, the diagnostic usefulness of differential WBC counts, NLR, and MNM was evaluated in comparison with SCC antigen. The correlation between those serum markers and overall and disease-free survival was analyzed using both univariate and multivariate analyses.

Findings: MNM, with an area under the curve (AUC) of 0.703, showed a sensitivity of 53.1% and a specificity of 78.1%, which are much higher than those of NLR and SCC antigen. In a subgroup analysis according to various stages of cervical cancer, MNM detected 81 out of 136 FIGO stage IA patients (59.6%), whereas only 14 patients (10.3%) had serum SCC antigen levels above 1.5 ng/mL. On Cox multivariate analysis, MNM positivity (Hazard Ratio = 2.82 [95% CI: 0.97-8.18], P = 0.042), stage, and tumor size were independent predictors of poor prognosis.

Interpretation: Our findings suggest that pre-treatment MNM may represent a simple and cost-effective method of identifying cervical cancer, and an elevated MNM may predict an adverse outcome in cervical cancer patients.

METRONOMIC CHEMOTHERAPY COMBINED WITH ANTIGEN-SPECIFIC CANCER VACCINE INHIBITS TUMOR GROWTH

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Cancer vaccine has emerged an innovative method for cancer treatment. However, cancer vaccines alone have limited potential in treating measurable tumor burden, which highlights the importance of identifying more potent anti-cancer strategies for clinical testing. A previous study demonstrated that a chimeric DNA vaccine CTGF/E7 (connective tissue growth factor linked to a tumor antigen-Human papillomavirus (HPV) 16 E7 can generate potent E7-specific immunity and anti-tumor effects (1). This study tested immune-modulating doses of chemotherapy in combination with CTGF/ E7 DNA vaccine as a means to treat existing tumors in animals. Paclitaxel, given in metronomic sequence with CTGF/ E7 chimeric DNA vaccine, enhanced the vaccine's potential to delay tumor growth and decrease metastatic tumors in vivo. Metronomic paclitaxel chemotherapy also enhanced the efficacy of E7 chimeric DNA vaccine through the anti-angiogenic pathway and the down-regulation of regulatory T (Treg) cells rather than via direct cytolytic effects on cancer cells. This study has shown that metronomic low doses of paclitaxel are synergistic with DNA vaccine when both are given prior to vaccination in therapeutic experiments. Multiple functions have been attributed to paclitaxel, such as the inhibition of angiogenesis and suppression of Treg cells. These suggest that combined treatment with metronomic doses of chemotherapy and chimeric E7 DNA vaccine can induce more potent antigen-specific immune responses and anti-tumors, and provides the immunologic basis for further testing in cancer patients.

P-03.42

RISK FACTORS FOR CIN 2/3 IN HGSIL CASES

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Objective: This study examines medical and lifestyle factors that may predict the likelihood of having CIN 2/3 on biopsy after HGSIL PAP test.

Methods: This is a retrospective chart review of women presenting to Palmetto Health Women's Center with HGSIL PAP test from 2005 – 2007.

Results: A total of 89 charts were reviewed. 75% of the patients were African American, 17% were Caucasian, and 6% were Hispanic. Of these women, 40% had CIN 2/3 and 33% had CIN 1 on colposcopy-directed biopsies; 27% of the women with HSIL did not present for subsequent follow-up visits. Variables assessed were diabetes, body mass index, history of teen pregnancy and smoking. There were five diabetic women; three had CIN 2/3 and one had mild dysplasia. Women at the extremes of weight (BMI <18.5, underweight and >30, obese) had a two-fold greater chance of developing severe dysplasia. In our study population, smoking, a known risk factor for cervical cancer, was not associated with CIN 2/3; 64% of non-smokers had CIN 2/3 whereas 38% of the smoking women had severe dysplasia. When the non-smoking women were further stratified, a history of teen pregnancy increased the risk for developing CIN 2/3 three-fold (p value <0.05); however, teen pregnancy alone was not a risk factor for severe dysplasia.

Conclusions: Although many women have been exposed to HPV, not all these women develop cervical dysplasia or cancer, and most clear the HPV infection. This study shows that diabetes, extremes of weight and teen pregnancy may be factors associated with acquisition of severe cervical dysplasia and warrants further investigation. (Supported in part by grant # P20MD001770 from NCMHD, NIH.)

HUMAN PAPILLOMAVIRUS IN VULVAR CARCINOMA, SENTINEL NODES, PATIENTS AND PROGNOSIS.

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Background: Vulvar carcinoma is an uncommon skin cancer that mainly affects elderly women. However, recent data from the American National Cancer Institute identified vulvar cancer as 1 of 12 cancers with an increase in incidence between 1992 and 1998, and that the increase predominantly occurred in women younger than 65 years of age. Human papillomavirus (HPV) has been considered as a risk factor for development of vulvar cancer, and especially in young women.

Objectives: The aim of this project was to examine the proportion of HPV in primary tumours, as well as in sentinel nodes (SN) and correlate our findings to prognosis of the patients.

Methods: Seventy-four patients who all underwent preoperative lymph node scintegraphy with 99mTechnetium and/or blue dye to detect SN were included in the study. Presence of HPV DNA was analyzed by general PCR primers, GP5+/6+ and CPI/IIG, and HPV type was determined by sequencing or HPV type specific primers.

Results: HPV was found in 31% of the so far analyzed primary tumours and HPV-16 was the dominant type. Of the HPV positive tumours, 39% had SN containing HPV DNA, and all were HPV-16 type. Patients with HPV DNA positive tumours were younger (mean age 63.2 compared to 74.6 years, p < 0.001) and had a better disease specific survival (p < 0.05).

Conclusion: Although all patient samples have not been fully analyzed, the results indicate that patients with HPV DNA in their tumours are younger, and that presence of HPV is a prognostic favourable factor. Moreover, since 31% of the analyzed samples are HPV positive, it is likely that in the future, the upcoming vaccination program against HPV-16 and HPV-18 may be useful also against this tumour type.

P-03.45

"HPV16 E6/E7 DETECTION IN PRIMARY AND RECURRENT ENDOCERVICAL ADENOCARCINOMA".

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Background: HPV-related endocervical adenocarcinoma uncommonly metastasizes to the ovaries. Cases of iatrogenic tumor seeding to port sites following endoscopic surgery for cervical carcinomas have been rarely reported. Objectives: Aim of this presentation is to describe a case of HPV16 E6/E7 detection in primary endocervical adenocarcinoma and in ovarian, liver and port site recurrences two years after completion of surgical and radiation therapy. Methods: A 41 year old woman G0P0 presented with clinically and MRI-detected unilateral ovarian tumor (16cm) and concomitant liver and laparoscopy-related port site nodules, two years after completion of adjuvant radiotherapy to laparoscopic surgical treatment of a FIGO stage Ib2 (4.5cm) cervical adenocarcinoma with ovarian transposition, negative pelvic nodes and free surgical margins. An uneventful laparotomy was performed to debulk the disease. In addition to the pathological examination, which revealed in all neoplastic tissues adenocarcinomas similar to that of the primary tumor, specimens from ovarian neoplasm, liver and port site nodules were assessed with PCR for the presence and typing of HPV E6/E7 oncoproteins and compared with the findings in the paraffin block of the primary tumor, in order to determine whether the former were metastases or independent metachronous neoplasms. Results: In all tissue specimens from primary tumor, ovarian tumor and port site metastases HPV 16 E6 and E7 proteins were detected, whereas the liver nodule was positive only for HPV 16 E6 protein. Conclusions: Testing of specimens for HPV-type and type-specific oncoproteins could be a useful diagnostic tool and may be used as an additional marker to differentiate between recurrent or metastatic cervical carcinomas and independent neoplasms in other organs.

A OPTOELECTRONIC DEVICE IN THE SCREENING OF CERVICAL DISEASE

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Background: Cervical cancer is the second most common in female malignancies worldwide. The decreasing pattern of cervical cancer in developed countries is partially attributable to Papanicolaou (Pap) test screening. The high false negative rate of Pap test is a problem in screening system of cervical disease.

Objectives: The purpose of the study is to compare the accuracy of the TruScreen (Polartechnics, Sydney, Australia) with the pathology of cervix and to assess the sensitivity and specificity of combination with Pap test.

Methods: The study population comprised 231 women who had undergone loop electrosurgical excision procedure (LEEP) or hysterectomy at Kangnam St. Mary's hospital, Catholic university. The TruScreen was performed on women with abnormal Pap smear or patients who will undergo hysterectomy due to another gynecologic problems unrelated with cervical abnormalities. The pathology of whole cervix was used as gold standard for evaluation of accuracy of TruScreen. Results: The sensitivity of TruScreen in CIN 1 and CIN2/3 were 73.3%, 75.8% respectively. The sensitivity of Pap test combined with TruScreen in CIN 1 and CIN2/3 were 96.7%, 94.0% respectively. The specificity of TruScreen in normal cervix was 84.7%.

Conclusions: The TruScreen can be a good adjunctive test to the Pap smear to improve the accuracy in the screening test of preinvasive disease of cervical cancer.

P-03.47 BURDEN OF HPV-RELATED NON-CERVICAL ANOGENITAL CANCERS IN FRANCE

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Objective: Literature on epidemiology and management costs of human papillomavirus (HPV) related non-cervical anogenital cancers in Europe is scarce. From 40% to 90% of these cancers are associated with HPV. This study aimed to assess the medical and economical burden of anogenital (vulvar, vaginal, anal, penile) cancers in France. Methods: Data from the French national hospital database (PMSI), in which all admissions to public and private hospitals are recorded, was analysed retrospectively to assess the number of patients hospitalised for vulvar, vaginal, anal and penile cancers annually and the associated hospital costs from the healthcare payer perspective. ICD-10 diagnoses codes were used to extract all admissions for these cancers in 2006. Data for all hospital stays were extracted to assess patients' management. Radiotherapy sessions performed in the private sector, not available in the PMSI, were estimated from the SAE (Statistiques annuelles des établissements de santé) database. The mean costs of admissions were obtained from 2007 DRG (diagnosis related group) tariffs. Outpatient and daily allowance costs (paid by the healthcare payer) were based on literature review. Results: In 2006, there were 1,237, 728, 3,711, 678 patients hospitalised for vulvar, vaginal, anal and penile cancers respectively. Anal cancers were more frequent in women (69%). Total annual hospital costs were estimated at €32.6 million. Most of these costs were linked to surgical procedures. Adding outpatient and daily allowance costs led to a total annual cost of €60 million, of which 75% can be attributable to HPV. Anal cancer management accounted for 64% of the total cost. Conclusion: The costs for non-cervical HPV-related anogenital cancers are comparable to cervical cancer hospitalisation costs. Therefore prevention by prophylactic HPV vaccination may significantly reduce the number of cases and economic burden of these diseases. Further research is needed to assess the burden of precancerous lesions.

ECPV-2 DNA IN EQUINE SQUAMOUS CELL CARCINOMAS AND NORMAL MUCOSA

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BACKGROUND: Squamous cell carcinoma (SCC) represents the most common malignant tumour of the eye and the external genitals in horses. Comparable to humans, papillomaviruses have been proposed as aetiological agents of cancer in horses and recently, a novel equine papillomavirus (EcPV-2) has been identified in equine genital SCCs. However, it was not demonstrated in ocular SCCs yet.

OBJECTIVES: The first goal of this study was to determine the prevalence of EcPV-2 DNA in equine genital and ocular SCCs, genital papillomas and penile intraepithelial neoplasia (PIN) lesions. The second goal was to investigate the possibility of latent EcPV-2 infection in the genital and ocular mucosa of healthy horses.

METHODS: DNA was isolated from 45 tissue samples from genital and ocular SCCs, genital papillomas and PIN lesions and subsequently PCR with EcPV-2 specific primers was performed. For determination of EcPV-2 latency, DNA isolation and EcPV-2 PCR were performed on 131 swabs obtained from unaffected mucosa of the eye and penis or vulvovaginal region and cervix from 57 healthy horses.

RESULTS: EcPV-2 DNA was detected in all genital SCCs (17/17), genital papillomas (8/8) and PIN lesions (11/11) and in 22% of ocular SCCs (2/9). EcPV-2 DNA was confirmed by sequencing of the PCR products of two genital and one ocular SCC. In healthy horses, EcPV-2 DNA was detected in 30% (17/56) of ocular mucosa swabs, 20% (8/40) of penile swabs, 30% (5/17) of vulvovaginal swabs and 18% (3/17) of cervical swabs.

CONCLUSIONS: This study confirms the presence of EcPV-2 DNA in equine genital SCCs and is the first to demonstrate its involvement in other genital lesions and in ocular SCCs. Moreover, we demonstrated latent EcPV-2 infections in normal genital (including cervical) and ocular equine mucosa.

P-03.50

DETECTION OF BOVINE PAPILLOMAVIRUS DNA IN EQUINE SQUAMOUS CELL CARCINOMA

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Background: In humans and cattle, papillomaviruses are chiefly involved in the development and maintenance of squamous cell carcinomas (SCC). In horses, SCCs frequently affect the eyes and the genital region, thus causing considerable morbidity. Although SCCs represent a common neoplastic disease in ungulates, its aetiology is still unclear.

Objectives: Given the pathogenic association of papillomaviruses with human and bovine SCC, and the accepted causal relationship of bovine papillomaviruses types 1 and 2 (BPV-1, -2) with sarcoids and possibly other cutaneous malignancies in horses, we examined equine SCC specimens for the presence of BPV-1/-2 DNA.

Methods: Tumour tissue was collected from 4 horses affected by ocular SCCs and 7 horses bearing penile SCCs. Tissue specimens were subjected to DNA purification using DNeasy Blood and Tissue Kit (Qiagen). Subsequently, an optimized PCR was carried out from DNA isolates using two BPV-1/-2 consensus primer pairs detecting a 499 bp sequence comprising the E5 gene and a 266 bp region of the L1 gene, respectively. DNA from a sarcoid and skin of 4 healhy horses served as controls in the reaction.

Results: BPV-1/-2 E5 DNA was detected in 4/4 ocular and 1/7 penile SCCs. L1 DNA was demonstrated for 3/4 ocular and 0/7 penile SCCs. The sarcoid control scored positive for both viral genes, whereas no amplicons where obtained for negative and no template controls (sterile water), as anticipated.

Conclusion: This is the first report demonstrating BPV DNA in a subset of histologically confirmed equine SCC specimens. Given the reduced number of lesions investigated so far, this result may be accidental. Yet, it is suggestive for a possible involvement of BPV-1/-2 in the development of equine ocular SCC. Further investigations are warranted to verify this presumption.

MATERNAL TRANSFER OF ANTI-HPV ANTIBODIES FOLLOWING VACCINATION WITH GARDASIL™

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Human papillomaviruses (HPV) are DNA-based viruses, some of which infect the cutaneous and mucosal epithelium of the genital tract. HPV infection can lead to benign genital warts/papillomas and low- or high-grade intraepithelial neoplasia, as well as cervical cancer. In addition, HPV 6 and 11 can infect the respiratory tract and cause recurrent respiratory papillomatosis (RRP). RRP is characterized by benign squamous papillomas, generally non-cancerous tumors or warts that grow within the respiratory tract. Gardasil[™], a quadrivalent HPV (types 6/11/16/18) virus-like particle (VLP) vaccine, has been shown to be efficacious for the prevention of genital warts caused by HPV types 6 and 11, as well as vaginal, vulvar and cervical cancer caused by HPV types 16 and 18.

To establish whether antibodies induced by natural infection or following vaccination with Gardasil[™] cross the placenta, IgG neutralizing antibodies were measured in matched maternal and neonatal cord sera against HPV types 6, 11, 16 and 18 in the competitive Luminex immunoassay (cLIA) measure.

The results from our pilot studies show that antibody levels in maternal serum and neonatal cord blood are similar as measured in the 4-plex cLIA. These results demonstrate that Gardasil[™]-induced antibodies cross the placenta and may provide protection in neonates from acquiring HPV 6 or 11 infection or related disease. Detailed quantitative analyses will be presented.

P-03.52

COBLATION RESECTION OF RECURRENT RESPIRATORY PAPILLOMA: IMPROVED SAFETY AND EFFICACY.

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Recurrent respiratory papilloma of the airway caused by HPV6/11 results in significant morbitity with a clinical picture of multiple recurrences, frequent surgeries to keep the airway open, alteration of voice, and potential mortality if it spreads to the lungs with malignant conversion. Multiple surgical therapies have been utilized to treat this disease including laryngoscopy with forceps removal, CO2 laser, microdebrider, cryo-surgery and others. All of these have one or another shortcomings such as scarring, bleeding, potential for airway fires, inability to control the depth of tissue destruction etc. The Cobaltion Wand for removal of laryngeal papilloma has overcome many of these issues.

Coblation is an energy process and is not heat driven. it is a bipolar system whose energy ionizes saline (plasma), which contains excited particles that accelerate towards the tissue and break the tissue's molecular bonds. The tissue's molecules are broken down into simpler hydrocarbons and oxides, which are gently removed by the Wands suction. Advantages include limited depth of thermal penetration, minimal collateral tissue damage, surface temperatures 40-70 deg. C, and cut and coagulate with one handpiece. Evaluation of patients clinical results, possible immune changes, and comparison to other therapies will be discussed.

LASER CAPTURE MICRODISSECTION DETECTS HPV6 AND HPV11 IN ANOGENITAL CANCERS.

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W Quint, DDL Diagnostics Laboratory, Voorburg, The Netherlands on behalf of the RIS HPV TT study group

Background: HPV6 and HPV11 have been classified as Low Risk HPV types and are primarily responsible for benign HPV infections of the anogenital tract: Condylomata acuminata and a proportion of CIN1 cases. Conversely, relatively few HPV-6 and HPV-11 positive malignancies have been identified. The detection of HPV-6 and HPV-11 in some rare cases of genital carcinomas remains to be confirmed and deserves additional research.

Objective: To confirm the presence of exclusively HPV6 and HPV11 in Cervical, Vulva, Vagina and Penile invasive cancers.

Materials and Methods: Whole tissue sections from specimens that included HPV6 and HPV11 genotypes were selected form the close to 15.000 cases included in the ICO international survey of genital cancers. The Laser Capture Microdissection (LCM) was used for isolating specific cell populations from pathology heterogeneous tissue section. Selected cancer cells were tested by the HPV SPF10 LiPA25 version 1, Type specific E6 PCR and Sequencing. The histology diagnostic was established by a panel of pathologists.

Results: In a few cases, selected tumor cells by LCM/PCR were found to contain HPV6 or HPV11 as single infection. In another set of cases, carcinoma regions appeared to be HPV DNA negative but in the same tissue section, regions with normal epithelium were observed to contain HPV6 or HPV11.

Conclusions: HPV6 and HPV11 are solely present in cancer cells in some cases. The oncogenic potential of these low risk types remain unclear but cannot be excluded. The biological activity of these HPV types will be further investigated by HPV 6/11 mRNA analysis. The public health impact of the potential oncogenic role of HPV6/11 for primary and secondary cervical cancer prevention is likely to be marginal.

P-03.54

QUALITY OF LIFE AND UTILITY MEASURES IN JORRP

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Background: Juvenile Onset Recurrent Respiratory Papillomatosis (JORRP) is an intrusive and unpredictable condition that can have detrimental implications on the health and well-being of affected children and their families. Despite the serious and often prolonged course, there is little empirical study of quality of life, health utility, and psychosocial implications of this condition.

Objectives: 1) To measure the impact of JORRP on health-related quality of life, health utility, voice-related quality of life, and family psychosocial well-being in young people; 2) To compare these different measures, explore their intercorrelation, and their correlation with clinical disease severity.

Methods: We performed a cross-sectional study of the JORRP population at our academic tertiary pediatric referral centre. Standardized structured interviews were performed using four validated tools: the Health Utilities Index version 3 (HUI3), the Pediatric Voice-Related Quality of Life survey (PVRQOL), the Impact on Family Scale, and a Visual Analog Scale health preference measure. Clinical disease severity and demographic data were also collected.

Results: Early results demonstrate a significant impact of this disease on quality of life, and this is largely accounted for by the impact on voice together with the need for frequent surgical interventions. When data collection and analysis are complete, utility measures based on both proxy (parent) and child measures will be available.

Conclusions: This is the first study in the literature to measure and quantify the burden of JORRP on young people, using validated measures of quality of life, health utility, voice-related quality of life, and impact on the family. This information provides essential parameters for accurate modeling studies and cost-utility analysis. Specifically, this will allow future health economic analyses of HPV vaccination, to include any potential benefits gained through JORRP prevention and reduction.

COMPARISON OF HPV DNA AND MRNA LOADS IN ANOGENITAL WARTS

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Background and objectives: Anogenital warts is the commonest sexually transmitted infection (STI) in the world, and the commonest viral STI in the UK. Indeed, there were 83,745 reported first attack cases in 2006, a 3% increase from the previous year, and the trend has been relentlessly upward over the last decade. Although essentially benign, wart disease is not trivial and the estimated costs of treatment to the NHS are approximately £30 million per annum. In this study, we have analysed HPV viral loads in terms of DNA and mRNA directly from wart tissue, to elucidate the natural history of low-risk HPV infection and correlate this with the putative immune response, measured in terms of lymphocyte infiltration.

Study design and methods: Wart tissue was excised as part of treatment protocol in 30 unselected patients attending a wart treatment clinic in central London. Tissue was divided and processed immediately for DNA and lymphocytes, or processed later for RNA (frozen in RNAlater). We have performed quantitative real-time PCR for HPV L1 (DNA load), HPV E7 gene expression (mRNA load) and analysed for correlations between these and total numbers of infiltrating lymphocytes. We have tested the hypothesis that the immune response (measured in terms of total lymphocyte count) was inversely correlated with the amount of virus to be found in the wart (HPV viral loads).

Results and discussion: We present data on HPV DNA, mRNA loads and analysed for correlations with total lymphocyte counts. We discuss the significance of our findings and the implications for future work.

P-03.56

PREDICTING EFFECT OF HPV VACCINATION ON JORRP USING MATHEMATICAL MODELING

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BACKGROUND: With the licensure of a quadrivalent human papillomavirus (HPV) vaccine, many manifestations of HPV infection are now potentially preventable, including the devastating childhood disease juvenile onset recurrent respiratory papillomatosis (JORRP). Mathematical models have been used to predict the impact of vaccination on genital complications of HPV infection, but have not previously been applied to JORRP.

OBJECTIVE: Predict the impact of HPV vaccination on the incidence of JORRP using dynamic mathematical modeling. METHODS: Deterministic and stochastic population dynamic models were developed to estimate the impact of a quadrivalent HPV vaccine on JORRP incidence. The models have the flexibility to account for a variety of vaccination strategies including variable uptake and vaccination of males.

RESULTS: Mathematical models predicted a declining incidence of JORRP following implementation of HPV vaccination programs. The rate of decline was dependent on the immunization strategy, increasing with greater vaccination coverage and highest when both males and females were vaccinated. At a realistic immunization coverage of 50% of females (no males vaccinated), JORRP incidence was predicted to decrease by 1.4% per year. The model predicted an important herd immunity effect. In the long term, models predicted that HPV types 6 and 11 and JORRP could be eliminated from a population by sustained vaccination of at least 36% of girls, although this would require over 100 years at a realistic vaccine coverage rate. At lower immunization rates, the JORRP incidence declined to a new stable endemic equilibrium, but was not completely eliminated. In this scenario, vaccination of males provided incremental benefit and could tip the balance toward disease elimination. Age-structured, HPV-type specific and risk behaviour adjusted models are in development. CONCLUSION: The quadrivalent HPV vaccine is predicted to significantly decrease the incidence of JORRP, although gains will be slow at the current vaccination rates.

JUVENILE ONSET RECURRENT RESPIRATORY PAPILLOMATOSIS: ESTABLISHING A CANADIAN NATIONAL DATABASE

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Background: Accurate incidence and prevalence rates of Juvenile Onset Recurrent Respiratory Papillomatosis (JORRP) in North America are currently unavailable. Using surveys, census data and extrapolation, the prevalence of JORRP is estimated between 1.7 and 2.6 per 100 000 children in the United States. Canada represents an ideal location for the epidemiological study of JORRP and the development of population level statistics because of the publicly funded health care system and the centralization of specialized pediatric care.

Objectives: To describe the development of a National Database of JORRP in Canada.

Methods: To develop a Canadian National Database, the following milestones were achieved: 1. a case definition of JORRP was agreed to by consensus; 2. a survey of Canadian otolaryngologists was completed to determine where children with JORRP are treated; 3. the recruitment of collaborators from all pediatric health care centers in Canada; and 4. the collection and databasing of information in a standardized fashion.

Results: A case definition of JORRP was agreed upon by the Recurrent Respiratory Papillomatosis Task Force in September, 2006. Survey of Canadian otolaryngologists indicated that greater than 95% of cases were treated by pediatric otolaryngologists in pediatric academic health centers. Representation was recruited from 13 academic pediatric centers. Patient characteristics, indicators of disease severity and natural history of the disease were captured retrospectively with a standardized case report form.

Conclusions: Universal access to health care and the centralized care of JORRP patients are attributes that have enabled the development of a Canadian national database for JORRP. The database has provided population level statistics as well as provincial and regional trends. The database will provide a platform for the surveillance of new cases of JORRP and may detect a change in JORRP incidence as Human Papillomavirus vaccination programs are implemented.

P-03.58

ESTIMATING US DISEASE BURDEN OF JUVENILE RRP USING ADMINISTRATIVE DATABASES

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Background: Recurrent respiratory papillomatosis (RRP) is a histologically benign disease of viral origin caused almost entirely by HPV 6/11. JORRP can be life-threatening and consumes substantial amount of medical resources. There are few studies quantifying the burden of JORRP and therefore it is not well understood.

Objective: This study will utilize large US administrative claims databases to provide a description of the incidence and prevalence of RRP among publicly and privately insured Americans.

Methods: Disease burden will be derived from a pair of claims databases representing privately and publicly insured American children. Annual prevalence and incidence of JORRP will be described for the years 2003-2007. Potential cases will be identified using highly sensitive algorithms consisting of ICD-9 and CPT codes. A chart validation sub-study is being conducted to determine the positive predictive value (PPV) of the algorithms. The PPV will be applied to the claims-based estimates to provide a more valid estimate of burden.

Results: A preliminary analysis of the privately insured study population resulted in an average annual prevalence (2003-2006) of 4.01 per 100,000 children (range 3.76-4.43/100,000) ages 0-17 years. The final analysis will report the incidence and prevalence of JORRP stratified by age and gender with 95% confidence intervals. Results will be standardized to the United States (US) population to estimate the disease burden in the US.

Conclusion: Preliminary prevalence estimates from the current study are higher than those from published estimates which range from 0.80 to 2.59 per 100,000. However, due to the highly sensitive algorithms applied, firm conclusions should be withheld until the chart validation portion of the study is complete.

GENOMIC DIVERSITY OF THE VACCINE HUMAN PAPILLOMAVIRUS (HPV) GENOTYPE 6

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Background: Human papillomavirus (HPV) genotype HPV-6 is etiologically most commonly associated with genital warts (GW) and laryngeal papillomas (LP). Genomic variants of HPV-6, particularly those from LP, are not well characterized. A quadrivalent HPV vaccine developed recently was shown to be highly effective against GW. However, it remains unclear whether vaccine will also prevent the occurrence of HPV-6 associated LP, and whether natural variants of HPV-6 L1-capsid protein will influence the outcome of prophylactic HPV vaccination.

Objectives: To investigate and to compare genomic diversity of HPV-6 isolates from GW and LP.

Methods: Seventy-seven HPV-6 isolates obtained from tissue specimens of 45 patients with GW and 32 patients with LP were included in the study. The characterization of HPV-6 genomic variants was based on molecular analysis of LCR, E6, E2, E5 and L1 genomic regions.

Results: Among 77 HPV-6 isolates, a total of 34 HPV-6 genomic variants composed of fifteen 15 LCR, ten E6, nine E2, sixteen E5a, nine E5b, and fifteen L1 variants were identified. Twelve (15.6%) HPV-6 isolates represented genomic variants of prototype HPV-6b (6 variants), while 65 (84.4%) isolates represented genomic variants of HPV-6 "subtypes" HPV-6a and HPV-6vc (28 variants). In 64/77 (83.1%) HPV-6 isolates nucleotide alternations did not affect amino acid sequence of L1 protein, while up to two amino acid were exchanged in remaining 13 isolates. A comparison of distribution of HPV-6 variants identified in GW and LP revealed that almost two thirds of GW and almost a half of LP were caused by the same HPV-6 genomic variants.

Conclusions: Among 77 HPV-6 isolates, 34 HPV-6 genomic variants were identified. Non-prototypic HPV-6 variants predominated in GW and LP. Prophylactic vaccination with currently available quadrivalent HPV vaccine could theoretically prevent at least 77.8% and 90.6% of GW and LP, respectively, in Slovenia, which are caused by HPV-6.

P-03.60

SMOKERS HAVE A MODERATELY INCREASED RISK OF CONTRACTING GENITAL WARTS

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Background: The role of smoking as a risk factor for the contraction of genital warts is unresolved, with previous reports ranging from no effect to a five-fold increased risk among smokers compared to nonsmokers.

Objectives: To investigate whether smokers and nonsmokers differ in the risk of contracting genital warts, and whether smoking intensity also influences this risk.

Methods: A cross-section of women aged 18-45 from Denmark, Norway, Iceland and Sweden were asked to participate in the study. The survey included questions on clinically diagnosed genital warts and lifestyle. Of note, the women were asked to provide information on age-specific smoking intensity as well as the age of debut for various lifestyle parameters. A total of 58 546 women answered adequately to all questions of relevance to the present study. We used a time-dependent Cox model to investigate the risks of genital wart contraction, hence taking into consideration that individual smoking habits, as well as other covariates, may vary with time.

Results: Smokers had an increased risk of contracting genital warts compared to nonsmokers when controlling for age of first sexual intercourse, condom use, hormonal contraceptive use, pregnancy and alcohol drinking as time-dependent covariates (hazard ratio 1.23, 95% CI 1.15 – 1.32). There was an additional positive dose-response effect of smoking on the risk of contracting genital warts (hazard ratio per daily cigarette smoked 1.015, 95% CI 1.01 – 1.02).

Conclusions: This large cross-sectional study indicates that smoking moderately increases the risk of genital wart contraction.

CORRELATION OF ANAL CYTOLOGY, HISTOPATHOLOGY AND HPV IN ANAL WARTS

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Background: Condylomata acuminata of the perianal/anal anatomical region have been found to be associated with high rates of intraepithelial neoplasia (IN) and progression to anal cancer may occur. The role of anal cytology and human papillomavirus (HPV) testing to detect IN in the presence of anal warts requires further study.

Methods: Eighty three patients (63 male, 20 female) with perianal/anal condylomata acuminata underwent 101 operations for scissor excison of the lesions under general anaesthesia. HPV testing was performed prior to tissue removal.

Results: HPV positivity overall was 66.3% (male 73.4%, female 40.9%). HPV positivity was 97.1% in HIV positives compared to HIV negatives 55.6%. Cytology detected 35.3% of high-grade IN, and histology 44.1%. 60.0% of HIV positive men had high-grade IN, and 11.4% low-grade IN. A significant correlation was found between cytology and histology (% agreement = 81.2%, kappa = 0.49, 95%CI 0.29 - 0.69; P<0.0001). Cytology detected an additional 6.9% and histology 11.9% of cases of high-grade IN that would be otherwise missed.

Conclusions: High rates of IN are reported in HIV positives. Most HIV positive males with anal warts are positive for high-risk HPV in the anal canal. Recognising Histology as the gold standard, cytology under-estimates the true extent of high-grade IN. Cytology is a complementary test to histology.

CERVICAL SCREENING AND COLPOSCOPY

SESSION 04

PROGRAMME

Oral presentations

SWEDEN			
TIME	NUMBER	TITLE	ROOM
2009-05-11			
SESSION 04: CERVICAL SCREENING & COLPOSCOPY SCANIA			
11.00-11.10	O-04.01	INACCURACY OF COLPOSCOPIC-DIRECTED BIOPSY R G Pretorius	
11.10-11.20	O-04.02	NEGATIVE SCREENING NOR COLPOSCOPY PROVIDES ABSOLUTE REASSURANCE AGAINST CERVICAL CANCER P Castle, A Rodriguez, Rd Burk, R Herrero, A Hildesheim, D Solomon, M Sherman, J Jeronimo, M. Alfaro, J. Morales, D. Guillen, M. Hutchinson, S Wacholder, M Schiffman	
11.20-11.30	O-04.03	PAP SMEAR COLLECTION POTENTIATES HPV16 INFECTION IN A MACAQUE MODEL D Lowy, J Roberts, Rc Kines, H Katki, C Thompson, Cb Buck, M Bernardo, P Choyke, J Schiller	
11.30-11.40	O-04.04	THE ACCURACY OF COLPOSCOPIC BIOPSY: DATA FROM THE GARDASIL TRIALS M Stoler, A Ferenczy, D Ferris, G Perez, E Joura, R Haupt, H Sings, S Lu, P James, M Vichnin	
11.40-11.50	O-04.05	CERVIX CANCER SUBSEQUENT TO ALTERNATIVE SCREENING METHODS: PROSPECTIVE RANDOMISED STUDY P Nieminen, A Arun Pokhrel, M Hakama, L Kotaniemi - Talonen, A Anttila	
11.50-12.00	O-04.06	RISK OF CERVICAL CANCER AND MANAGEMENT OF ABNORMAL PAP SMEARS L Silfverdal, L Kemetli, B Andrae, P Sparén, W Ryd, J Dillner, B Strander, S Törnberg, S Törnberg, S Törnberg	
12.00-12.10	O-04.07	AUDIT OF THE FINNISH SCREENING PROGRAM FOR CERVICAL CANCER S Lönnberg, P Nieminen, A Anttila	
12.10-12.20	O-04.08	EXTENSION AND PROCESS PERFORMANCE OF EUROPEAN CERVICAL CANCER SCREENING PROGRAMMES G Ronco, M Van Ballegooijen, N Becker, A Chil, F Muriel, P Giubilato, J Kurtinaitis, L Lancucki, E Lynge, A Morais, M O'Reilly, P Sparen, O Suteu, M Rebolj, P Veerus, M Zakelji, A Anttila	
12.20-12.30	O-04.09	NBI COLPOSCOPY IS CRITICAL TO SELECT CIN3 FROM CIN2/3 PATIENTS T Fujii, M Nakamura, T Iwata, A Ohno, H Nishio, M Saito, D Aoki	

INACCURACY OF COLPOSCOPIC-DIRECTED BIOPSY

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Colposcopic-directed biopsy does not accurately diagnose \geq CIN 2. In ALTS, initial colposcopic impressions were not reproducible [correlation of review colposcopic impressions with initial impressions was poor (kappa=.24)] and in the Shanxi Province Cervical Cancer Screening Studies (SPOCCS), the sensitivity of colposcopic-directed biopsy for \geq CIN 2 was 55.5% to 74.7%. False-negative colposcopic impressions are associated with thin CIN 2/3 which may appear less acetowhite (in SPOCCS I, mean average thickness of CIN 2/3 from cervical quadrants with colposcopic impressions of normal (184 µm) was less than that of quadrants with colposcopic impressions of low, high, or cancer (321 µm, p<.001).

Use of an inaccurate colposcopic-directed biopsy standard results in erroneous scientific conclusions and poor clinical decisions. Examples of erroneous scientific conclusions are that CIN 2/3 is more common on the anterior cervix and that the sensitivity of acetic acid aided visual inspection (VIA) for \geq CIN 2 is 65.9%. Though 57.5% of colposcopically-detected lesions are on the anterior cervix, \geq CIN 2 is equally distributed (47.2% of \geq CIN 2 is on anterior cervix, 52.8% on posterior cervix). Though the sensitivity of VIA for \geq CIN 2 with a colposcopic-directed biopsy standard is 65.9%, with a five-biopsy standard, it is only 45.9% [inaccuracy is because colposcopy and VIA are correlated (kappa=.44)].

The inability of colposcopic-directed biopsy to exclude CIN 3 and invasive cancer causes clinicians to follow patients with a colposcopic diagnosis of \leq CIN 1 at more frequent intervals (many such women are lost to follow-up), to perform unnecessary cervical conization (which has a RR of 2.9 for subsequent perinatal mortality), and to treat CIN 2 (which may resolve without treatment).

The five-biopsy standard in which 2-mm biopsies are obtained from each cervical quadrant (even if the colposcopic impression in that quadrant is normal) followed by ECC should replace colposcopic-directed biopsy for diagnosing \geq CIN 2.

O-04.02

NEGATIVE SCREENING NOR COLPOSCOPY PROVIDES ABSOLUTE REASSURANCE AGAINST CERVICAL CANCER

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Background: At the enrollment visit of a large, population-based cohort, we applied multiple state-of-the-art cervical cancer screening methods to detect prevalent cervical cancer and to prevent subsequent cervical cancers by the timely detection and treatment of precancerous lesions. Nonetheless, cervical cancers were diagnosed during the follow-up phase of the study.

Objective: Describe the occurrences of cervical cancer in follow-up in large, population-based study of 10,049 women living in Guanacaste, Costa Rica was recruited into a natural history of human papillomavirus (HPV) and cervical neoplasia in 1993-4.

Methods: A population sample of 10,049 women living in Guanacaste, Costa Rica was recruited into a natural history of human papillomavirus (HPV) and cervical neoplasia in 1993-4. Women were screened at enrollment with 3 kinds of cytology (often reviewed more than one pathologist), visual inspection, Cervicography, and an early HPV test (Hybrid Capture Tube Test). Any positive screening test led to colposcopic referral and biopsy for diagnosis, with subsequent excision of CIN2 or worse. We retrospectively tested stored specimens for >40 HPV genotypes using a research PCR assay. We followed women typically 5-7 years and up to 11 year.

Results: Sixteen cases of invasive cervical cancer were diagnosed during follow-up. These cases resulted from: 1) six failures to detect abnormalities by cytology screening, three of which also would have been missed by HPV DNA testing even if sensitive HPV DNA detection had been used for screening; 2) seven failures of colposcopy to diagnose cancer or a precancerous lesion in screen-positive women; and 3) three failures of excisional treatment of precancerous lesions.

Conclusions: It seems unlikely that any current technologies applied once singly or in combinations in an under-screened population will be 100% efficacious in preventing incident diagnoses of invasive cervical cancer.

PAP SMEAR COLLECTION POTENTIATES HPV16 INFECTION IN A MACAQUE MODEL

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Some evidence, including data from a mouse female genital challenge model, suggest microtrauma is a necessary precondition for HPV infection. We sought to determine, in a non-human primate model, whether a cytology specimen (Pap smear) collection procedure, which disrupts the epithelium by design, renders the cervix more susceptible to HPV infection. In a series of rhesus macaques (4/group), a speculum exam was performed either with or without a cytology specimen collection procedure as it is commonly practiced in a gynecology clinic. The animals were then inoculated with HPV16 pseudovirus (PsV) expressing Red Fluorescent Protein (RFP) by instilling PsV into the endocervix and onto the ectocervix. After three days, the reproductive tracts were excised, the cervix was divided into six wedge-shaped biopsies, and five cryostat sections from each biopsy were analyzed by confocal microscopy for the number of RFP-expressing cells in the cervical epithelium. Substantial infection of the ectocervix, endocervix, and transformation zone was detected, but only in conjunction with the cytology specimen collection procedure. The mean number of infectious events per section in the PsV only group was 0.05 (95% CI 0.01, 0.18) compared to 84.3 (95% CI 45.1, 157.6) in the PsV with specimen collection procedure group. When an HPV infection inhibitor, 1% carrageenan gel, was substituted for Surgilube as the lubricant used for an internal digital exam after specimen collection, the mean number of infectious events was significantly decreased to 3.5 (95% CI 1.8, 6.9). These findings suggest that cytology screening in women might lead to a transient enhancement of susceptibility to HPV infection and that use of a carrageenan-based gel during the exam might mitigate this enhancement. Potential implications for the interpretation of prospective HPV infection studies and the rise in cervical adenocarinoma rates will be discussed.

O-04.04

THE ACCURACY OF COLPOSCOPIC BIOPSY: DATA FROM THE GARDASIL TRIALS

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Background: Abnormal pap smears prompt colposcopy. Recent large-scale trials have called the accuracy of colposcopic biopsy into question.

Objectives: We ascertained the accuracy of colposcopic biopsy within the context of 3 randomized-controlled clinical trials (FUTURE I/II/III) with pathology panel adjudication.

Methods: 760 patients aged 16-45 (placebo arm) who underwent one colposcopy within six months of their LEEP were included. The majority (92%) also had a colposcopic biopsy taken immediately before LEEP as the colposcopists' best estimate of the prevalent worst pathology. Using the final adjudicated diagnoses, the initial biopsy within 6 months was correlated with the LEEP as were the same-day biopsies.

Results: The overall perfect agreement between the initial biopsy (within six months of LEEP) and the LEEP was 42% (weighted kappa = 0.34 (95%CI: 0.29-0.39). The overall miss of CIN3/AIS was 42%. The overall agreement between the same-day pre-LEEP biopsy and LEEP was 56% (weighted kappa = 0.41 (95%CI: 0.36-0.47). The overall miss of CIN3/AIS was 66%. In both groups, there were no significant differences in the overall miss of CIN3/AIS when patients were stratified by age, # of abnormal LEEP quadrants, presence of HPV16/18 in the LEEP, or among patients with satisfactory vs. unsatisfactory colposcopy. There was a significant difference in the miss of CIN3/AIS associated with the number of biopsies (unadjusted p<0.05) within 6 months of the LEEP.

Conclusions: Colposcopic biopsy missed 42-66% of prevalent CIN3/AIS. However, subjects received the proper treatment and all biopsy/LEEP specimens were included in the analyses of vaccine efficacy. Colposcopic performance may vary depending on whether the colposcopist mindset is focused on diagnosis vs. therapy. Alternatively, biopsy may have an impact on CIN regression. These data should be taken into account when planning therapy, especially for patients in whom the biopsy is less severe than the referral cytology.

CERVIX CANCER SUBSEQUENT TO ALTERNATIVE SCREENING METHODS: PROSPECTIVE RANDOMISED STUDY

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Background: The purpose of the current study was to assess efficacy of automation-assisted screening in comparison with conventional cytology in a population-based screening programme for cervical cancer.

Methods: The study was based on a follow-up of cervical cancer and cervical intraepithelial grade 3 (CIN3) incidence among women invited in the Finnish programme during 1999-2003. The study involved 721440 ladies, one third of whom had been randomly allocated to automation-assisted screening. Information on cervical cancer and CIN3 was obtained from a linkage between screening and cancer registry files. There were altogether 2.6 million woman-years at risk, and the average follow-up time was 3.6 years with a maximum of six years.

Findings: There were no differences in the cervix cancer and CIN3 patterns between the two screening methods; RR 0.98 (95% CI 0.73-1.29) between screening arms among all invited and 1.34 (95% CI 0.75-2.40) among women tested negative. Subsequent cervical cancer risk was decreased remarkably among women tested negative in all invited age groups, in comparison with non-screened (RR 0.23, 95% CI 0.16-0.33), indicating high validity of the programme.

Interpretation: Both of the methods were valid for the screening purpose, even though the alternative method was not with a better validity. The study demonstrates that it is possible to evaluate new meth-ods for cervical cancer screening up to cancer endpoint.

O-04.06

RISK OF CERVICAL CANCER AND MANAGEMENT OF ABNORMAL PAP SMEARS

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Background: A substantial proportion of previously screened women with invasive cervical cancer have had abnormal smears earlier in life. While there is a consensus on the management of women with high-grade squamous and with non-squamous cell cytological lesions, controversies remain about the most effective management of women with low grade cytological abnormalities. In order to improve the efficiency of cervical cancer screening the reasons for why women develop invasive cervical cancer despite having had abnormal smears detected at screening deserve investigation. We conducted a nationwide population based case-control study.

Objective: To evaluate the management of women with abnormal cervical smear in terms of subsequent risk of invasive cervical cancer.

Methods: The screening histories of all invasive cervical cancer cases diagnosed in Sweden 1999-2001 and of five populationbased control women per case were reviewed. 159 cases and 258 controls under the age of 67 years had had an abnormal smear 0.5 to 6.5 years prior to cancer diagnosis. The cervical cancer risk was estimated in relation to whether the abnormal smear had been managed by repeat cytology or by histological assessment.

Results: Histological assessment of low-grade squamous cell abnormalities was strongly protective against cancer compared to repeat cytology only (odds ratio 0.46, confidence interval 0.24 to 0.89). Delaying histological assessment was also associated with a higher risk (5.65, 1.39 to 23.05 for assessment after 7-12 months). After high-grade squamous atypia, absence of any cytology or histology was a major determinant of cancer risk (12.52, 1.42 to infinitive).

Conclusions: For adequate protection against invasive cervical cancer, further assessment with histology must be recommended also for women with low-grade squamous abnormalities. Organised systems of notification of missed cases and of reinvitation to investigation could have significant cancer-preventive gains.

AUDIT OF THE FINNISH SCREENING PROGRAM FOR CERVICAL CANCER

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Background: Screening of cervical cancer has reduced incidence and mortality more than 80% in Finland. However, the incidence has increased since 1991 among women under 40 years. Several factors may affect the effectiveness; quality of cytology, screening coverage and organization. Audit studies are essential for evaluating the quality of screening. The aim was to clarify whether regular auditing could improve the test sensitivity without compromising specificity.

Methods: New cancer and CIN3 cases after preceding negative cytological testing were identified. The quality of cytological analyses was evaluated by re-reading the smears and finding out the false negative rate in Finnish screening. Six largest cytological screening laboratories with 1152481 invitations were included in this audit study. Files of cervical cancer and CIN3 cases from the national cancer registry were linked with the screening registry. Originally negative Pap-smears slides that preceded Ca or CIN3 diagnosis were found from the archives and included as 345 cases. These cases were collected from the years 1991-1999. For each case, two smears of women who had remained without Ca or CIN3 were selected as 689 controls. After blinding, re-evaluation was done using the original screening and a reference lab and an expert panel to resolve discrepancies. The cytological diagnoses were recorded using TBS2001 nomenclature.

Results: 34.7% of the case smears were originally false negatives and were diagnosed LSIL+ in re-reading, which would have led to colposcopy primarily, according to guidelines. With the same criteria 2.7% of the controls were LSIL+ in re-reading. Im the Finnish screening material only 0.8-1.0% of smears result in a referral, normally.

Conclusions: The cytological quality could be enhanced, reproducibility of cytological diagnoses is at most tolerable. However, the quality of screening is more dependent on adequate referral to colposcopy and intensified follow-up.

O-04.08

EXTENSION AND PROCESS PERFORMANCE OF EUROPEAN CERVICAL CANCER SCREENING PROGRAMMES

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Background. Monitoring cervical screening is recommended by the recently published European Guidelines for quality assurance in cervical cancer screening.

Objectives. Comparing performance indicators in different European countries.

Methods. We collected from European national or regional screening programmes the standardised tables of aggregated data and computed the key performance indicators reported in EU Guidelines.

Results. The proportion of national female population included in the target population of population-based organised screening programmes was 100% in 10 countries and 69% in one, while in 5 other countries only regional programmes, including 1 to 16% of the relevant population, were present and there was no population-based programme in the remaining. Overall some 35% of European women aged 30-60 were included in organised programmes. The percent of women invited in a screening round ranged from 19% (Slovenia, where only women not spontaneously covered women are invited) to 98% (England). Screening coverage in round varied from 10% in Cluji County, Romania to over 70% in Finland, Alsace (France), the Netherlands, England and Sweden. Referral rates to repeat cytology (ranging from 3% to 13% of screened women) or to colposcopy (ranging from 0.8% to 4.4%) and the Positive Predictive Value (PPV) of colposcopy (ranging from 8 to 69%) were strongly influenced by management protocols, in particular for ASCUS and LSIL cytology. However for example in the Netherlands both referral to repeat cytology (2.9% of screened women) and to colposcopy (1.4%) were low and colposcopy PPV was 49%. The detection rate of histologically confirmed CIN2+ ranged from 0.23% of screened women in Finland to 1.2% in Denmark.

Conclusions. We are not aware of previous studies comparing performance parameters between many European countries. Their relevance for improving the quality of cervical screening will be even larger as long as HPV testing and vaccination will be introduced.

NBI COLPOSCOPY IS CRITICAL TO SELECT CIN3 FROM CIN2/3 PATIENTS

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There have been several reports on the usefulness of narrow band imaging (NBI) in combination with magnifying endoscopy for diagnosis of small superficial esophageal cancers, Barrett's esophagus, early oropharyngeal and hypopharyngeal carcinomas.

NBI colposcopy was performed using the colposcope (OCS-500, OLYMPUS MEDICAL SYSTEMS CORP., Japan) combined with the video system (VISERA Pro system, OLYMPUS MEDICAL SYSTEMS CORP., Japan) The system enables a rapid conversion of conventional red-green-blue(RGB) images to narrow band images by pushing a button at the control panel of the video system. The illumination of NBI was composed of 415nm and 540 nm wavelength, which conformed to the absorption wavelength for hemoglobin.

Sixty-three patients diagnosed with CIN 1, 2 and 3 were enrolled at Keio University Hospital, Japan. The NBI and conventional colposcopy was compared to detect CIN2/3.

The sensitivity of the NBI colposcopy was 95.3% compared with 80.2% of conventional colposcopy. The detection rate of the CIN 3 from CIN 2/3 group was 82.6% by NBI, compared with 64.1% of conventional colposcopy. The distinctive vascularity was observed in 28.1% of CIN2/3 compared with 7.7% in CIN 1.

NBI colposocpy is superior to conventional colposcopy to detect CIN3. The vascularity is important factor to select CIN3 from CIN2/3 group. NBI colposcopy was an important instrument to decide the treatment for CIN patients.

POSTER ABSTRACTS SESSION 04

POSTER SESSION I MONDAY 10.00: ODD NUMBERS

POSTER SESSION II

TUESDAY 10.00: EVEN NUMBERS

LIQUID-BASED CYTOLOGY TESTING WITHIN U.S. AMBULATORY CARE SETTINGS, 2006

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Background: In the United States, liquid-based cytology (LBC) has rapidly replaced conventional cytology as the most commonly used method to screen for cervical cancer, despite recent evidence that LBC is twice as costly and has similar sensitivity compared to conventional cytology. Limited information is available on how use of LBC varies by insurance status, practice type, and location.

Objectives: To examine characteristics related to LBC testing in ambulatory care settings using 2006 national survey data. Methods: We examined data from 29,932 records from CDC's 2006 National Ambulatory Medical Care Survey, a national probability sample of abstracted medical records that describe visits made to office-based non-federal providers. Of these, 5,553 primary care visits occurred among non-pregnant women ages 15-64 and included cytology screening. Sample weights were used to make national estimates of annual office visits.

Results: In 2006, LBC comprised an estimated 60.7% of the 25.2 million Pap tests ordered by primary care providers. LBC was ordered more often for visits among privately insured women (10.5%) than among visits by women on Medicaid or other payment sources (4.0 and 4.5%). A logistic model of LBC use found significantly higher odds of use in practices located in urban areas relative to practices outside of metropolitan areas. The odds of LBC use were lower among larger sized practices (\geq 11 providers) and among women enrolled in Medicaid. Age, region and race/ethnicity were unrelated to LBC use in the model.

Conclusions: LBC was more commonly and preferentially used among urban practices and among women with private insurance. Given recent evidence about comparable sensitivity between LBC and conventional cytology and the higher cost of LBC, the continued use of LBC among privately insured women and practices warrants attention.

P-04.11 HPV CO-TESTING IN THE UNITED STATES AND IMPACT ON SCREENING INTERVALS

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Background: In 2003, several U.S. medical organizations recommended the use of HPV DNA testing with cytology (Pap) as an optional screening method (co-testing) for women \geq 30 years of age. Current guidelines for co-testing state that women who have negative HPV and normal Pap test results can safely increase screening intervals up to 3 years, potentially reducing utilization of limited resources. Objectives: Cervical cancer screening data from a national U.S. survey of 436 Pap test providers were analyzed to assess HPV testing and cervical screening interval practices. Methods: A cervical cancer screening supplement was collected in conjunction with CDC's National Ambulatory Medical Care Survey in 2006 using a multi-stage probability design comprised of direct patient care, office-based Pap test providers. The supplement was a nine-item questionnaire focusing on cervical cancer screening methods, use of HPV testing in screening, and clinical vignettes. A clinical vignette assessed provider intention to screen among women 30-60 years old with a history of normal Pap, a current normal Pap, and a negative HPV test. Results: While 79% of respondents (n=346) reported ever using the HPV test, only 39% were using it as a co-test with the Pap. In the clinical vignette described above, 12% of providers would recommend the next Pap test in 3 years or more, 17% in 2 years, and 60% in 1 year. Conclusions: Three years after updated guidance had been provided on HPV co-testing and appropriate screening intervals, few U.S providers were incorporating these guidelines into practice, resulting in minimal change to screening intervals and use of HPV testing. Interventions targeting both providers and patients may be necessary to address appropriate HPV test use and to decrease unnecessary testing and associated costs.

HEALTH SYSTEMS FOR HPV VACCINE INTRODUCTION IN LOW-RESOURCE SETTINGS

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Background: The WHO has noted that successful planning for new vaccine introduction in developing country settings requires an integrated and comprehensive approach that addresses individual and community factors for acceptance, institutional and infrastructure needs for appropriate and effective vaccine delivery, and the policy environment for political and financial support of the new vaccine.

Objectives: (1) To describe components of health systems and immunization financing related to future human papillomavirus (HPV) vaccine introduction; and (2) to recommend appropriate and effective strategies for HPV vaccine delivery. Methods: We performed a descriptive qualitative study that synthesized health system and immunization financing assessments performed in four countries—India, Peru, Uganda, and Vietnam. Our purposive sample of respondents included community and civic leaders, teachers/head teachers and health professionals, and national and sub-national political and technical leaders.

Results: Health interventions for 10- to 14-year-old girls were sparse or one-time events. Current immunization systems were fairly robust but needed strengthened infrastructure and human resources to manage the cold chain. Total dollars allocated for immunization had increased in recent years, but accounted for less than 1% of the total national expenditures on health. HPV vaccines could be delivered in schools, building off previous experience in provision of health services in these locations.

Conclusion: Maximum feasibility and acceptability and lowest cost for delivering HPV vaccine can be achieved by implementing through national programs based on the World Health Organization's Expanded Programme on Immunization; partnering with other sectors, especially education; strengthening existing services where needed; and using schools as the primary venue for reaching the target population.

P-04.14

SCOTTISH CERVICAL CANCER PREVENTION RESEARCH NETWORK

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Background: In Scotland, HPV vaccination of girls of 12-to 13 years started in 2008 with a 'catch up campaign' up to age 18. Scotland is in a unique position to lead on new research as 16-17 year olds will be immunised in 2008 and become eligible for cervical screening from September 2010. The formation of a cohesive National HPV Steering Group in 2006, the involvement of Health Protection Scotland, the establishment of a Scottish National HPV Reference Laboratory and excellent data linkage facilities provide a robust environment for effective collaborative research. Objectives: The main aims of the network are:

- to provide evidence to improve health outcomes in Scotland and on changes to the future national cervical screening programme
- to create a national archive of LBC samples from women attending first or second cervical screen linked to national vaccination and clinical datasets through unique CHI (Community Health Index) numbers
- to carry out HPV associated research linked to routine surveillance
- to undertake HPV testing to evaluate the performance of screening and clinical decision-making

Conclusions: We will present details of Network set-up and structures, in addition to activity, both on-going and planned.

POLICY DEVELOPMENT FOR HPV VACCINE INTRODUCTION IN LOW-RESOURCE SETTINGS

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Background: As new vaccines become available, the development and enactment of new vaccine policy regarding adoption, use, delivery, and financing is essential. Development of new vaccine policy requires ongoing translation of information between scientific communities and policymakers, comprehensive assessment of new vaccine introduction capacity, and multiple iterations of policy formulation. However, few studies present empirical data detailing this policy development process in low-resource settings.

Objectives: (1) To describe the policy environment related to human papillomavirus (HPV) vaccine introduction; (2) to identify the policy processes and key stakeholders in HPV vaccine introduction; (3) to summarize specific characteristics about HPV vaccines and their introduction that may be barriers to introduction; and (4) to recommend advocacy strategies to achieve a positive environment for cervical cancer prevention.

Methods: We performed a descriptive qualitative study of HPV vaccine policy development in four developing countries— India, Peru, Uganda and Vietnam—using an iterative, inductive, theme-based approach to data analysis. A total of 237 national policymakers, legislators, officials, and senior managers from ministries of health, finance, and planning; leaders of medical and health professional associations; cancer institutes; heads of nongovernmental organizations; and women's health advocates.

Results: While differences existed among low-income countries in specific cervical cancer, women's health, adolescent health, or immunization policy environments, we found the policymaking process itself, specific concerns related to HPV vaccines, and the information needs of policymakers for HPV vaccine introduction to be strikingly similar. Conclusions: Data on burden of cervical cancer, HPV vaccine safety and efficacy, and cost-effectiveness and vaccine affordability were top issues reported by policymakers. Advocacy strategies need to address these issues in order for HPV vaccine policy formulation and approval to be successful.

P-04.16

THE HPV-TEST REGISTRY IN THE NORWEGIAN CERVICAL CANCER SCREENING PROGRAM

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Background: The Norwegian Cervical Cancer Screening Program (NCCSP) was established in 1995. The database contains four registries: Results from PAP-smear testing (November 1991), cervical histology specimens (2002) and information on Cervical Intraepithelial Neoplasia (CIN) follow-up and treatment (1997). In July 2005 a HPV-test registry was established. NCCSP is a national co-ordinated program and receives mandatory reports from private and public pathological and microbiological laboratories, physicians and out-patient clinics.

Objectives: Early 2000 HPV-testing was performed in women on a large scale often without indication. To control the use of HPV-testing, the NCSSP advised the National Health Authorities (NHA) to implement guidelines. July 2005, the NHA stated that HPV-testing should be used in the age group 25-69 years and in triage after PAP-smear results: inadequate, ASC-US and LSIL. A tariff for HPV-testing, and a HPV-test registry was established

Method: All HPV-tests performed are reported to and registered in the NCCSP by specifications including personal identification number, name, type of test, result, genotyping and date of testing. 13 laboratories perform and report HPV-testing. Five different test-methods are in use.

Result: In the period: 1.7.2005-31.6.2008 information on 37.605 HPV-tests have been registered. In 2005 more than x% HPV-tests were performed after no or other PAP-smear results than recommended, and more than 12% of the tests were performed outside the recommended age groups. In 2008 x% of the tests are performed in accordance with the guidelines and only 2.3% tests were outside the age group 25-69. In the same period the percentage positive test results has increased from 26% to 40%.

Conclusion: According to recommendations for indication and age for HPV-testing in the NCCSP, more tests are performed in accordance with the guidelines. An evaluation of the effect of HPV-testing in triage has to await a complete evaluation, which will be performed in 2009.

SURVIVAL AFTER DIAGNOSIS OF CERVICAL CANCER, RELATED TO SCREENING HISTORY.

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Background: The Swedish organised cervical screening program invites women ages 23-60. The Nation-wide Audit performed in 2008 demonstrated lower incidence and early stage detection of cervical cancer found among participants. Objective: To compare survival rates in cervical cancer cases who participated to screening within recommended intervals and those who did not.

Methods: All cervical cancer cases 1999-2001 linked to the Swedish Causes of Death Register. Survival rates calculated for women with different screening histories, by age, histopathological type and FIGO stage and equality over strata tested with Log-Rank tests. Hazard ratios calculated in regression models to assess the relative contribution of each factor to the risk of dying from invasive cervical cancer.

Results: 52% of cases that died within fives years were diagnosed after age 65. 29% were under 65 with no smear within the recommended screening interval, 15% (19 women per year) had normal screening and 4% had an abnormal smear. Five-year survival rates in cancer cases with a smear within three years was 83% compared to 76% in cases without such a smear (Log-Rank, p=0.01, HR=1.39, 95% CI=1.02-1.88). This difference was completely explained by differences in stage distribution between the groups. Survival was not worse for adenocarcinomas than for squamous cell cancer (Log-Rank, p=0.16). Cases with a previous abnormal smear had a 5-year survival rate of 89%.

Conclusions: Participation to screening reduced mortality of cervical cancer not only by reducing the incidence but also because of detection at earlier stages. Survival was high when cancer was detected in screening participants of all ages. Half of the deaths in cervical cancer occurred in women diagnosed above the screening ages. Introduction of new screening technology needs to be paralleled by efforts to improve coverage and to identify risk groups among older women if further mortality reduction is to be achieved.

P-04.18

COMPUTER-ASSISTED THIN LAYER CYTOLOGY IMPROVES HSIL DETECTION IN ROUTINE SCREENING

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Background: A favourable contribution of computer-assisted thin layer cytology (CAS) for the detection of dysplasia has been advocated by several published trials, but its specificity has been disputed by others.

Objectives: We intended to compare the CAS approach with results from conventional Pap smear evaluation (CP) in a routine laboratory setting.

Methods: From our files of 2006/07 we compared the data from two cohorts of slides, one analysed with CAS by the ThinPrep[™] Imager (n=59047), the other diagnosed by CP (n= 57890), each from women of all age groups belonging to a low risk screening population. The diagnoses of LSIL as well as HSIL(moderate and severe dysplasia / CIS)were searched for knowing the rate of histo-logically confirmed CIN3 being 90% for CP and 94% for CAS in our institution.

Results: Mild dysplasia was found in 0.6 and 2.5% of cases for CP and CAS, respectively, moderate dysplasia in 0.1 and 0.3%, and severe dysplasia / CIS in 0.05 and 0.2%.

Conclusions: Mild dysplasia was found in 0.6 and 2.5% of cases for CP and CAS, respectively, moderate dysplasia in 0.1 and 0.3%, and severe dysplasia / CIS in 0.05 and 0.2%.

Conclusions: Our data reflects the higher sensitivity of CAS without a loss of specificity for the detection of biopsy confirmed CIN3 lesions as compared to CP. This is mainly due to the locator function by CAS, presenting immature epithelial cells with abnormal chromatin structure to the screening person for their interpretation. In our opinion, this combined approach is superior to CP evaluation and especially suited for the screening of HPV vaccinated women where the rate of Pap samples positive for dysplasia is expected to be substantially lower than in the pre-vaccination era.

FACTORS AFFECTING SCREENING FOR ANAL DYSPLASIA IN MSM.

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Background: Incidence of anal cancer in men who have sex with men (MSM) is rising. Effective screening strategies mirror cervical screening techniques, but studies demonstrate a high lost to follow-up (LTF) rate.

Objectives: Assess factors impacting follow-up screening for anal dysplasia in MSM.

Methods: Retrospective chart review identified MSM with prior anal dysplasia screening. Patients were grouped by follow-up compliance as regular follow-up (RF) ≥ 1 visit/year, LTF > 1 year since previous screening or LTF but subsequently came back for screening (LCB). Subjects answered a questionnaire either via telephone or in office assessing HPV knowledge and other factors affecting compliance.

Results: 195 MSM were enrolled (96 RF, 50 LTF and 49 LCB). RF compliant 4.75 years. LTF lost for 2.31 years. 247 LTF patients contacted. Of these, 7% subsequently scheduled a follow-up appointment. No difference seen in follow-up for age, race, years of education, insurance, HIV, average number of hours worked/week or relationship status. Mean HPV knowledge score as % of correct answers was RF 88.5%, LCB 87.5% and LTF 76.8%. Differences were significant comparing LTF with RF and LCB (p=0.0002 and p=0.003, respectively). MSM with more sex partners in the prior 6-months were 8% more likely with each additional partner to return vs. LTF (p=0.0473). MSM were significantly more likely to be RF if they had physical findings indicative of HPV, described learning they had HPV as "upsetting" or had high-grade dysplasia (RR's 4.4, 3.3 and 3.7, respectively).

Conclusions: MSM are more likely to return for anal dysplasia screening if they are knowledgeable, have physical findings of HPV, are more upset by the diagnosis or have high-grade lesions. Education and discussion are imperative to ensure adherence to screening guidelines.

P-04.20

APPLYING AN INTEGRATED STRATEGY TO ASSESS PREVENTION OF HPV-RELATED DISEASE

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Objective: The purpose is to outline the application of short-, medium- and long-term requirements of a strategy to evaluate the impact of integrated primary (HPV immunization) and secondary (cervical screening) in one Canadian province. Method: An integrated evaluation strategy (defined elsewhere) was developed in August 2008, based on literature review and consultation with scientists and epidemiologists. Options for implementation of this strategy are proposed as well as identifying benefits and challenges of application in a province that comprises 40% of the Canadian population. Results: A proposed model is presented for applying an evaluation framework (that is inclusive of primary and secondary prevention) in Ontario. Essential data requirements and database linkages for a successful evaluation strategy are delineated.

Conclusion: An applied model is presented for the first evaluation strategy that integrates primary and secondary prevention of cervical cancer and HPV-related disease. Among vulnerable populations and women who are neither screened nor immunized, customized interventions will be required to ensure that they are aware of potential risks and benefits.

This evaluation strategy may serve as a useful outline for jurisdictions in Canada and elsewhere. This new paradigm of combined primary and secondary intervention will encourage cooperation for effective evaluation of an integrated approach for control of cervical cancer and other HPV-related disease.

FTIR MICROSPECTROSCOPY: AN OBJECTIVE, POTENTIALLY AUTOMATED DISCRIMINATOR OF CERVICAL CYTOLOGY

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Introduction: Infrared (IR) absorbance of cellular biomolecules generates a vibrational spectrum, which can be exploited as a "biochemical fingerprint" of a particular cell type.

Study Objectives: Biomolecules absorb in the mid-IR (2-20 µm) and Fourier-transform infrared (FTIR) microspectroscopy applied to discriminate different cell types is evaluated

Methods: Exfoliative cervical cytology collected into LBC was examined. This consisted of cervical cytology free of atypia (i.e., normal; n=60), specimens categorised as containing low-grade changes (i.e., CIN1 or LSIL; n=60) and a further cohort designated as high-grade (i.e., CIN2/3 or HSIL; n=60). IR spectral analysis was coupled with principal component analysis (PCA), with or without subsequent linear discriminant analysis (LDA), to determine if normal versus low-grade versus high-grade exfoliative cytology could be segregated

Results: With increasing severity of atypia, decreases in spectral absorbance intensity were observable throughout the 1500 cm-1 to 1100 cm-1 spectral region; these absorbance regions were associated with proteins (1460 cm-1), glycoproteins (1380 cm-1), amide III (1260 cm-1), vasPO2 (1225 cm-1) and carbohydrates (1155 cm-1). In contrast, vsPO2 (1080 cm-1) appeared to have an elevated intensity in high-grade cytology. Inter-category variance was associated with protein and DNA conformational changes whereas glycogen status strongly influenced intra-category

Conclusions: The computational segregation of IR spectra generated using FTIR microspectroscopy has the potential to be an objective and automated approach to discriminate between normal and different grades of cervical cytology.

P-04.22

HPV VACCINE GLOBAL COMMUNITY OF PRACTICE: MEMBERSHIP AND EVALUATION

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Introduction: We recently developed an on-line HPV Vaccine Global Community of Practice (COP). Its primary purpose is to link health professionals, policy makers, and individuals to share knowledge and experience to prevent cervical cancer.

Methods: The COP was launched in June, 2008 with a global videoconference focusing on cervical cancer prevention and the role of HPV vaccines, and three weeks of on-line discussions led by experts. On-line discussion topics included: integrating HPV vaccination into current or planned cancer control programs; prioritizing different cervical cancer control strategies where budgets are limited; and social, cultural, and political issues surrounding HPV vaccine access and delivery. When joining, members were asked to indicate their country of residence, profession and reason for joining. Two months after the videoconference, an on-line survey assessed satisfaction with the videoconference and on-line discussions. Members' characteristics, reasons for joining, and satisfaction were analyzed using descriptive statistical methods.

Results: The global videoconference was conducted at nine sites and webcast. It was accessed 762 times by 147 viewers in 36 countries. There are currently 515 COP members from 90 countries. Professions represented include physician (30%), researcher/professor (27%), program manager (19%), epidemiologist (12%), and nurse/nurse practitioner (8%). The most common reasons for joining were access to resources such as guidelines and technical documents (72%), education about the role of HPV vaccines in cervical cancer prevention (60%), and sharing knowledge/experiences related to HPV vaccine delivery (56%). Among respondents to the on-line satisfaction survey (N=44), 91% were very satisfied with the content of the global forum and on-line discussions, 98% thought the on-line discussions addressed relevant issues, and 98% thought the issues discussed could be used to inform policy discussions.

Conclusions: The HPV Vaccine Global COP is a promising new mechanism for global discussion about cervical cancer prevention and HPV vaccines.

INTER- AND INTRAOBSERVER VARIATION USING P16-IMMUNOHISTOCEMICAL STAINING IN CERVICAL DYSPLASIA

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Objective: To investigate if the use of p16-immunohistochemical staining of biopsies from the uterine cervix improves the inter- and intraobserver agreement in the diagnosis of cervical squamous cell dysplasia compared with hematoxylin-eosin staining.

Materials and Methods: Cone biopsies and preceding punch biopsies from 55 patients were included in the study. From each biopsy two sections were cut and one stained with hematoxylin-eosin and one stained with p16-immunohistochemical method. Two persons reviewed the sections blinded and one of the persons reviewed the sections twice with a washout period between and blinded to all previously diagnoses. Kappa statistics were used for calculation of inter- and intraobserver agreement for both the hematoxylin-eosin stained and the p16 immunohistochemical stained sections.

Results: The interobserver agreement between person A and person B was for punch biopsies k = 0.0212 (poor agreement) when the diagnoses were based on hematoxylin-eosin stained sections and k = 0.4886 (moderate agreement) when using p16-immunohistochemically stained sections. For the cone biopsies the interobserver agreement increased from k = 0.1466 (poor agreement) based on hematoxylin-eosin stained sections to k = 0.6279 (substantial greement) based on p16-immunohistochemically stained sections.

The intraobserver agreement for punch biopsies was k = 0.3671 (fair agreement) when using hematocylin-eosin stained sections and k = 0.6299 (substantial agreement) when using p16-immunohistochemically stained sections. For cone biopsies the intraobserver agreement increased from k = 0.1145 (poor agreement) using hematoxylin-eosin stained stections to k = 0.8039 (substantial agreement) using p16 immunohistochemically stained sections.

Conclusion: The use of p16 immunohistochemical staining increases both the interobserver and the intraobserver agreement significantly compared with hematoxylin-eosin stained sections.

P-04.24

THE RHEIN-SAAR-STUDY – CONVENTIONAL CYTOLOGY VERSUS THINLAYER CYTOLOGY AND COMPUTERASSISTANCE

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Background: In spite of the legal claim for one conventional cytological smear per year since 1971 the incidence of cervical cancer in Germany is among the highest in Western Europe. Recently several studies with the use of the ThinPrep Imaging System (TIS) for computerassisted prescreening found a significant increase in sensitivity for HSIL even in comparison with manually read thinlayer smears.

Objectives: Randomised prospective study for the comparison of sensitivity, specificity and positive predictive value (PPV) of conventional cytology and thinlayer cytology (TC). In addition all TC slides were analysed with the computerassisted ThinPrep Imaging System. Study endpoint is the detection of histologically verified CIN 2+

Methods: Altogether 20.000 women attending the routine cancer prevention exam, 10.000 each randomised in two arms, should be included. TC was taken direct-to-vial. Evaluation of the TC smears was limited to cytotechnicians with at least an experience with >2000 TC slides. All women with cytological abnormalities >Pap III = ASC-H/LSIL(non-HPV-only)/ HSIL were invited for an expert colposcopy with biopsy.

Results and Conclusions: At the end of recruitment at the 17.10.2008 in 25 practices >21.000 Frauen had been included in boths arms. First results on sensitivity, specificity and PPV will be reported and discussed.

P-04.25 CYTOLOGICAL AND PATHOLOGICAL EVALUATION OF SUB-CLINICAL HPV INFECTION

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(Objectives) There are many cases having HPV-positive in DNA test but cytological normal. We try to elucidate the significance of such sub-clinical HPV infection.

(Method) Four hundred seventy-three women visited to our out-patient clinic for cancer screening, follow-up for CIN and for colposcopy. All had Pap test and hybrid capture-II test. Women who were HPV-positive or cytologically abnormal including ASC-US undertook colposcopic examination and punch-biopsy for pathological evaluation.

(Results) In the subjects having abnormal cytology or HPV positive, 58.4%, 9.4%, 23.2%, 6.1% and 2.8% were cytologically diagnosed as normal, ASC-US, LSIL, HSIL and cancer, respectively. However, colposcopy and histological examination revealed that 6.8%, 10.4%, 37.8%, 29%, 12% and 3.9% were normal, papilloma, CIN1, CIN2, CIN3 and cancer. The positive predictive value in HC-II+ for presence of lesions worse than papilloma (papilloma+) and CIN2 and the worse (CIN2+) were 99.5% and 55.3%, respectively. The sensitivity of HC-II for detecting CIN2+ was 97%, whereas that of cytology was 76%. This suggests that false positive for HPV infection is very rare in HC-II positive cases. Re-evaluation of cell morphology in Pap-test samples showed that pearl formation, epithelial plaque, parakeratosis, giant cell, koilocyte, smudge nucleation, multiple nucleation, atypical parabasal/metaplastic cells and cannon ball appearance were found more frequently in HPV-positive cases than in negative ones. Koilocyte was only seen in 19% of CIN2+ lesions. Paying attention not only to koilocyte but also other HPV-related cytological findings improved the sensitivity about 10% in Pap-test.

(Conclusion) HC-II positive means HPV-related lesions is present in somewhere of the cervix. HPV-related cytological findings other than koilocyte may be important for accurate diagnosis for CIN2 and the worse lesions.

P-04.27

VALIDATION OF NOVAPREP VIAL TEST FOR HIGH RISK HPV TESTING

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Liquid-based cytology (LBC) has been proven to be useful for analysis of cellular morphology and reflex HPV DNA testing from the same cervical scrape. The fixative used in LBC may however alter DNA preservation and render the sample inadequate for HPV DNA testing. Novaprep Vial Test (NVT) is a new LBC device which permits an automated cell spreading over slides and an automated cell suspension sampling for molecular analyses.

The objective of our study was to validate the NVT for the detection of high-risk HPV DNA with the Hybrid Capture II assay.

Seventy patients referred for colposcopy were enrolled in this study after they signed an informed consent. Two cervical specimens were harvested at the transformation zone. First, a Rovers Cervex Brush was used and placed in the NVT. The latter served for cell spreading for cytology analysis as well as for cell suspension sampling for HR-HPV testing. Second, a DNAPAP cervical sampler was used and placed in the Specific Transport Medium (STM) from Qiagen. The latter served as the gold standard for HR-HPV detection by HCII.

140 specimens were collected from 70 patients. All NVT samples were successfully processed for cell spreading. Eleven of them were however paucicellular with a number of cells < 5000 per slide. Thus these samples as well as the STM corresponding samples were excluded from analysis. Among the remaining 59 paired samples, 86% of NVT and 82% of STM were HR-HPV positive (> 1pg/mL). An overall concordance of 97% was calculated with a kappa value reaching 0.88.

The detection of HR-HPV from cervical samples placed in NVT was as efficient as that from samples placed in STM. In conclusion Novaprep Vial Test adequately preserves HPV DNA and is reliable for HR-HPV testing by HCII.

SURVEY OF KNOWLEDGE ABOUT HPV INFECTION IN HUNGARY.

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Background: Cervical cancer is one of the most prevalent gynaecological malignant disease all over the world, including Hungary. In the background of cervical cancer and the praecancerous stages stands the most common sexually transmitted viral disease: the Human Papillomavirus infection. Despite the fact that almost 100 percent of all praecancerous stages can be recognized and treated, still there are 1200-1300 newly registrated, diagnosed cervical cancer occurences every year, and nearly 500 women die in consequence of this illness in Hungary.

Objectives: The authors search for answers to some questions, such as: which are the present deficiencies and the tasks in the future of health education, and which are the main difficulties of the first and second prevention of the HPV infections.

Methods: A nationwide questionnaire organized by the authors, is in progress to assess the knowledge of certain populations (students of different ages, parents) about the prevention and the consequences of HPV infections.

Results: Comprehensive preliminary studies show that the knowledge about the HPV infection and cervical cancer in different populations is remarkably incomplete. Almost 20% of the generation concerned by the vaccination against HPV infection hadn't heard previously about the vaccine. Approximately 98% of the queried people accounted the price of the vaccine disproportionately too high correlated to domestic earnings. Additionally, the intense distrust in people in public health in Hungary is also thought-provoking.

Conclusions: Based on our results we established that parents, health education in schools, health-care workers and the media have the greatest responsibility in the primer prevention of sexually transmitted diseases. As for the future we should aim at the extensive preparation of these information mediators.

P-04.29

THE DIAGNOSTIC VALUE OF P16-IMMUNOHISTOCHENICAL STAINING IN CERVICAL DYSPLASIA

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Objective: To assess if the use of p16-immunohistochemical staining improves the agreement between the diagnoses of punch biopsies and cone biopsies compared with the traditional use of hematoxylin-eosin staining.

Materials and Methods: Punch biopsies and cone biopsies from 55 patients were cut and stained with hematocylin-eosin and immunohistochemically stained for p16. All sections were reviewed blindly by the same person. Sections from the punch biopsies and cone biopsies were reviewed separately. The dysplasie was graded CIN 1, CIN 2, and CIN 3 and the p-16-staining was scored 1-2-3 according to the height of stained cells in the spuamous cell epithelium.

Results: The agreement between the diagnoses of punch biopsies and of cone biopsies improved from 69 % using hematoxylin-eosin stained sections to 75 % using p16-stained sections.

Conclusion: There is a little, but unsignificant improvement of the agreement between punch biopsies and cone biopsies when using p16 immunihistochemically stained sections instead of using hematoxylin-eosin stained sections.

P16INK4A EXPRESSION IN MANAGING CERVICAL LESIONS WITH ABNORMAL CYTOLOGY

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Background: Cervical cancer is the second most frequent female malignant neoplasia in the world. In cervical lesions the cell cycle regulatory protein p16 has been found to be strongly over-expressed in nearly all high-grade squamous intraepithelial lesions. The national screening progamme for cervical cancer is in Romania in early stage and the vaccination of the young girls agains human papilloma virus has begun. In the same time in hospitals there is a very strong awareness for the implamentation of new methods for improving the possibility to triage the cases with worse prognosis of the cervical lessions. Objectives: Quantification of p16 positive cells in liquid-based cervical cytology (LBC) of patients with abnormal conventional cytology for the triage of cases with high grade lessions. Material and methods: Ninty-eight LBC smears (11.13%) of young and middle aged women have been selected from 880 conventional smears with abnormal cytology, in order to be analyzed by using p16INK4a (kit CINtec - ready to use, from DAKO) immuno-cytochemical staining protocol (ABC indirect tristadial method). Results: In our study, 68 cases have shown reactive or inflammatory changes, being negative for p16(INK4a). Overexpression of p16 was detected in 30 squamous intraepithelial lesions with a highgrade predominance, with nuclear and cytoplasmatic stain. The immunocytostaining was useful for cytological diagnosis, enabling to distinguish dysplastic cells from normal, reactive or metaplastic cells and to confirm positive intermediary cells from L-SIL. Conclusion: The follow-up of high grade cervical lesions by immunocytochemical assessment of p16INK4a overexpression in abnormal cervical smears gives the opportunity to select the cases for electroresection so ensuring an optimum management of cervical lesions. We intend to use too this imunocytochmical method for the follow-up programme of cases with persistent focal colposcopic lession at very young women who will be selected for colposcopic biopsies in the situation of p16INK4a overexpression.

P-04.31

VALIDITY OF TOOLS FOR CERVIX CANCER SCREENING IN KOREA

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Background: Little is compared the diagnostic validity of screening tools for cervical cancer in the primary screening in Korea.

Objectives: This study aimed to compare the accuracy of conventional Pap test, liquid-based cytology (LBC) and HPV testing in women who had routine screening in every two year.

Methods: Women who attended the two consecutive screenings for cervical cancer in two places (2005/2007 in Busan, and 2006/2008 in Suwon) were included. In the baseline study, all participants had gynecological exams including conventional Pap test and HPV testing (Hybrid Capture 2, QIAGEN). In the follow-up study, they had undergone conventional Pap test, LBC (SurePath, BD), HPV testing and biopsy under colposcopy. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of each test were calculated.

Results: We observed the slight reduction of prevalence of high-risk HPV infection from 10.3% in baseline to 8.0% in follow-up, however increase of abnormal cytology (two fold from 3.1% to 6.0% for ASCUS or worse) among 1,226 women (mean age: 49 years). Sensitivity and specificity of Pap test to detect CIN1 or worse in the F/U was 26.1% and 95.8%, respectively (for LBC 36.5% versus 93.1%, and for HPV testing 21.9% versus 93.1%). Sensitivity of Pap test, LBC and HPV testing to detect CIN2 or worse was all 100%, and the specificity was 94.3%, 91.1% and 92.2%, respectively.

The probability that woman with an abnormal cytology by Pap test has the true disease of CIN2+ would be 4.2%. PPV for CIN2+ was 4.2%, 2.7% and 3.1%, respectively.

Conclusions: Considering with higher sensitivity and specificity, LBC might be the most effective cervical cancer screening method in Korea. However, the cost-effectiveness of these screening methods should be further evaluated. Sensitivity, specificity, PPV and NPV can be used to evaluate quality of the National Cervical Screening Program.

A FOLLOW-UP STUDY OF ASC-H USING CONVENTIONAL AND LIQUID-BASED CYTOLOGY

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Background. Atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H) is a new category introduced by the Bethesda system (TBS) 2001, and their characteristics have not yet been well defined. This study was conducted to evaluate the characteristics of ASC-H using conventional cytology (CC) and liquid-based cytology (LBC). Methods. We studied 10,209 cytologic preparations (5,444 CC and 4,765 LBC) collected mostly by split sample method during July to December 2007 at the Gynecological Department of the Cancer Institute Hospital of Ariake, Japan. Cytologic characteristics were reviewed retrospectively and statistical analyses were performed using chi-square test or Fisher exact test for small numbers. Results. Rate of ASC-H was 0.86% in CC and 0.55% in LBC, with no significant difference (ns). Rate of CIN2 or more severe histology on simultaneous biopsy was 39.5% in CC and 26.1% in LBC (ns). Among ASC-H cases showing no abnormal cytology in the previous 1 year, rate of CIN2 or more severe histology on simultaneous biopsy was 47.4% in CC and 36.4% in LBC (ns), cumulative rate of CIN2 or more severe after six months was 64.7% in CC and 62.5% in LBC (ns), and rate of normalized cytology after six months was 38.9% in CC and 40.0% in LBC (ns). Among ASC-H cases detected by LBC and tested for HPV-typing (GeneSQUARE HPV Genotyping, KURABO Industries LTD, Japan), rate of CIN2 or more severe histology on simultaneous biopsy was 54.6% in HPV-positive and 0% in HPV-negative group (p=0.022), and cumulative rate of CIN2 or more severe after six months was 63.6% in HPVpositive and 0% in HPV-negative group (p=0.011). Conclusions. ASC-H characteristics after 6-month follow-up did not differ between CC and LBC preparations. ASC-H showed broad spectrum ranging from normalized cytology to invasive carcinoma. Negative oncogenic HPV result strongly suggested negative histology.

P-04.35

RISK OF UNDETECTED CIN2+ IN COLPOSCOPY NEGATIVE HPV TRIAGED WOMEN

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Background: The implementation of HPV triage for low grade cytological abnormalities requires a strategy for women who are negative on a satisfactory colposcopic examination. The most efficient action would be return to routine recall. We have analysed follow up data from a previously published English HPV Triage Pilot Study (Moss et al, BMJ 2006; 332: 83-85) to determine the safety of such an approach.

Methods: In the national pilot study, 2947 out of 5022 (58.7%) women with LSIL (borderline or mild dyskaryosis) cytology were triaged to colposcopy following HPV testing (HC2) if HPV+ve. Women who were found to have no colposcopic abnormality were followed up; some were referred directly to colposcopy and some on the basis of abnormal cytology.

Results: Out of 2947 (58.7%) women originally triaged to colposcopy, 1063 (36.1%) were found to be colposcopically negative. 518 of these women (48.7%) had documented follow up of at least 12 months. Two hundred had negative cytology, 186 had abnormal cytology, and 132 were referred directly to colposcopy without cytology. Of those with abnormal cytology there were colposcopy data on 64; 31 borderline, 18 mild, 9 moderate and 5 severe dyskaryosis. One woman with negative cytology had colposcopy. Colposcopy data were not available on 123 women; 60 borderline, 56 mild, 6 moderate and 1 severe dyskaryosis. Therefore 397 women had documented follow up of at least 12 months which included colposcopy. Of these women 6/397 (1.51%) and 13/397 (3.27%) were found to have CIN3 and CIN2/3 respectively.

Conclusion: The rate of subsequent high grade CIN amongst colposcopically negative triaged women was sufficiently low to justify return to routine recall.

SESSION 05

PENILE AND ANAL DISEASES

PROGRAMME

oral presentations

THE 25TH INTERNATIONAL PAPILLOMAVIRUS CONFERENCE MAY 8-14 2009, MALMÖ, SWEDEN

TIME	NUMBER	TITLE	ROOM
2009-05-11			
SESSIC	ON 05: PEN	ILE AND ANAL DISEASES	SCANIA
14.00-15.00	O-05.00	HPV 6/11 DISEASE – EPIDEMIOLOGY, TREATMENT, AND PREVENTION C J N Lacey	
15.00-15.10	O-05.01	ACQUISITION AND CLEARANCE OF ANAL HPV AMONG WOMEN M T Goodman, YB Shvetsov, P Thompson, LR Wilkens, BY Hernandez	
15.10-15.20	O-05.02	ANAL HPV-16 DNA VIRAL LOAD AND NEOPLASIA AMONG MSM C Wang, Q Feng, S Cherne, D Kenney, N Kiviat, Se Hawes	
15.20-15.30	O-05.03	WORLDWIDE HPV CONTRIBUTION AND GENOTYPE DISTRIBUTION IN INVASIVE PENILE CANCER C Miralles, on behalf of the HPV VVAPO study group	

O-05.00 HPV 6/11 DISEASE – EPIDEMIOLOGY, TREATMENT, AND PREVENTION

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HPV 6 and HPV 11 are the causative agents of ano-genital warts (genital warts, condyloma acuminata) and recurrent respiratory papillomatosis (RRP). HPV 6 & 11 are low oncogenic-risk HPV types, and are uncommonly found in malignant lesions. Genital warts are an extremely common sexually transmitted disease worldwide, and there is evidence for their increasing prevalence in recent years in a number of countries. Treatment options are well established, both self-administered and healthcare provider-delivered, and include anti-proliferative, immunomodulatory, and destructive therapies. RRP is a rare disease, but can be life threatening, and often requires multiple surgical procedures. Both GW and RRP cause substantial healthcare costs. A quadrivalent HPV vaccine containing HPV 6/11 VLPs has shown high levels of protection against genital warts in women. Cost-effectiveness analyses have quantified the potential benefits of population-based HPV 6/11 VLP vaccination programmes.

O-05.01 ACQUISITION AND CLEARANCE OF ANAL HPV AMONG WOMEN

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Background: The association of anal cancer with HPV infection is well established; however, little is known about epidemiology of anal HPV in healthy women.

Ôbjectives: To investigate patterns of duration and clearance of anal HPV infection in a cohort of women in Hawaii.

Methods: Viral and non-viral determinants of anal HPV acquisition and clearance were examined through a longitudinal cohort study of 431 sexually active women with incident anal HPV infection. At baseline and at 4-month intervals, interviews were conducted and cervical and anal cell specimens were obtained for HPV DNA detection.

Results: Seventy percent of women were positive for anal HPV infection at one or more clinic visits from baseline through an average 1.2 year follow-up period. The incidence of a high-risk (HR) infection was 19.5 (95% CI: 16.0-23.6) per 1000 woman-months. The most common incident HR-HPV types were HPV-53, HPV-52 and HPV-16. Baseline HR cervical HPV infection predicted the acquisition of a HR anal HPV infection (RR: 1.81; 95% CI: 1.09-3.02). Non-viral risk factors for acquiring a HR-HPV infection included younger age, greater lifetime number of sexual partners, past use of hormones, and condom use. The clearance rate for a HR anal infection was 92 per 1000 woman-months with a median duration of 150 days (95% CI: 132-243 days). The slowest clearing HR-HPV types were HPV-59 (median, 350 days) and HPV-58 (median, 252 days). Median clearance times for HPV-16 and -18, the predominant types associated with anal cancer, were 132 days and 212 days, respectively. Non-viral factors that delayed clearance of anal HPV included douching, long-term tobacco smoking and anal sex.

Conclusions: The results of this study suggest that the risk of HPV infection of the anus is as common as cervical infection among women. The majority of anal HPV infections resolve in a relatively short time.

O-05.02

ANAL HPV-16 DNA VIRAL LOAD AND NEOPLASIA AMONG MSM

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Se Hawes, University of Washington, Seattle, USA

Background: High risk HPV infection is the major risk factor for anal cancer. Little is known about HPV infection and disease progression among men who have sex with men (MSM). Multiple studies have shown that HPV viral load may be an important cofactor in cervical HPV disease progression but data is limited regarding anal HPV infection in men.

Objectives: To quantify anal HPV-16 DNA levels among HIV+ and HIV- MSM and evaluate its association with HPV-16 clearance and the development of anal intraepithelial neoplasia (AIN).

Methods: Between 1990 and 2000, MSM were recruited from clinics in Seattle and followed every three months. Anal pap smears were performed at each visit and classified according to the Bethesda system. These previously collected and frozen anal swab specimens were utilized in the current study, with HPV-16 DNA viral loads measured using multiplex real-time PCR of HPV-16 E7 copy number normalized by human cellular DNA (Ø-actin).

Results: 167 anal swab specimens, correlating to the first detection of HPV-16 infection for those patients, were analyzed. HPV-16 DNA levels ranged from 0.75 to 8.2*10^7 copies per 10^3 cells (human cell equivalent). Among the 135 men with follow-up data, 59 cleared infection. Survival analysis showed that men with a higher baseline HPV-16 viral load (> median) were less likely to clear the infection than those with lower baseline HPV-16 viral load (log-rank test: p=0.0001). The adjusted hazard ratio of HPV-16 clearance associated with each one log10 viral load increase was 0.67 (95%CI: 0.56-0.81). Higher baseline HPV-16 DNA level was also associated with the development of high-grade AIN, adjusted HR=1.42 (95% CI: 1.01-1.98). These associations were even stronger among those who entered the study as prevalent HPV-16 infected.

Conclusions: Anal HPV-16 DNA levels might play an important role in predicting the natural history of HPV infection among MSM.

O-05.03 WORLDWIDE HPV CONTRIBUTION AND GENOTYPE DISTRIBUTION IN INVASIVE PENILE CANCER

C Miralles, Catalan Insitute of Oncology, IDIBELL, Barcelona, Spain on behalf of the HPV VVAPO study group

Background: Based on the literature, around half of the penile carcinomas could be related to HPV infection but the great variability among study designs, HPV/DNA detection methods and histology classification provide uncertainty of the estimate.

Objective: The study was designed to describe the HPV contribution and genotype distribution in a large series of invasive penile cancers using a state of the art protocol.

Material and Methods: Paraffin embedded tissue blocks diagnosed as invasive penile cancers were collected from historical archives. HPV detection was done through amplification of HPV DNA by SPF-10 broad-spectrum primers PCR subsequently followed by DEIA and genotyping by LiPA25 (version 1). Samples were tested at the HPV laboratory at ICO (Barcelona, Spain). Detailed histological evaluation was done using standardized criteria with the assessment of a board of pathologists. Countries in the study include Chile, Colombia, Czech Republic, France, Greece, Guatemala, Honduras, India, South Korea, Lebanon, Mexico, Mozambique, Paraguay, Philippines, Portugal, Spain and Venezuela.

Results: The preliminary analysis included 595 penile cancer cases. Overall, 72.4% were classified as squamous cell carcinoma (SCC), non warty/basaloid 23.5% as warty/basaloid (WB) and 4.1% as mixed SCC with 30-70% of WB component. Overall HPV positivity was 31.6%, being 20.4%, 64.3% and 41.7% among each histological group, respectively. The four most common types detected as a single infection were HPV16(56.9%), HPV6(5.3%), HPV45(3.7%) and HPV35(3.2%). Any other individual HPV type showed relative frequencies below 3%. Multiple infections accounted for 11.7%.

Conclusions: The preliminary data show a strong association between histology and HPV detection. Systematic evaluation of WB component is relevant to derive HPV related tumors. The relative contribution of HPV 16 resembles that of the cervix, contrary to that observed for HPV18.

POSTER ABSTRACTS SESSION 05

POSTER SESSION I MONDAY 10.00: ODD NUMBERS

POSTER SESSION II

TUESDAY 10.00: EVEN NUMBERS

P-05.04

GENOTYPE-SPECIFIC CONCORDANCE OF ANAL AND CERVICAL HPV INFECTION

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Background. Little is known about the epidemiology of anal HPV infection among healthy women and its relation to cervical HPV infection.

Objectives. Determine the type-specific risk of acquiring 1) concurrent anal and cervical HPV infection; 2) incident anal HPV infection after a cervical HPV infection; 3) incident cervical HPV infection after an anal HPV infection.

Methods. Viral and non-viral determinants of incident anal (or cervical) incident HPV infection concurrent with or following a cervical (or anal) infection were examined through a longitudinal cohort study of 426 sexually-active women. At baseline and at 4-month intervals, interviews were conducted and cervical and anal cell specimens were obtained for HPV DNA detection.

Results. The risk of concurrent anal and cervical acquisition was higher for high-risk HPV types (RR: 1.7; 95% CI: 1.1-2.7) than for low-risk types. The risk of acquiring a cervical HPV infection after an anal infection with the same genotype was 9.6 (95% CI: 7.2-12.9); and the risk of acquiring an anal infection after a cervical infection with the same genotype was 14.4 (95% CI: 11.2-18.4). Women with a cervical HPV-16 or HPV-18 infection had a higher risk of a subsequent anal infection with the same genotype (RR: 8.2; 95% CI: 3.3-20.6; RR: 16.4; 95% CI: 1.7-160.7, respectively). Women with an anal HPV-18 infection had a risk of 21.6 (95% CI: 5.0-92.5) of a subsequent cervical HPV-18 infection.

Conclusions. The results of this study suggest that it is common for anal and cervical HPV infections to occur concurrently or consecutively. The high degree of genotype-specific concordance indicates a common source of infection, such as vaginal and anal intercourse with the same infected partner or partners, although alternate routes of transmission, including non-penetrative sexual contact involving the fingers or mouth of partners need to be explored.

P-05.05

HPV GENOTYPING IN ANAL CANCER ARCHIVED SPECIMENS IN QUEBEC PROVINCE, CANADA: 1995-2005

M S Steben, Institut national de santé publique du Québec, Montreal, Canada

E Duarte - Franco; F Coutlée; P Goggin; R Louchini; R Hadjeres; J Vouma; J Mansi

Background: Few studies have explored the clinical and epidemiologic profile of patients affected by HPV-positive or HPV-negative anal cancer, the very subject of our research.

Objectives: Estimate the prevalence of HPV infection in new cases of anal cancer;

Compare HPV prevalence and genotyping by histological type;

Examine risk factors such as smoking, HIV status

Methods: Chart review of tumour and epidemiologic characteristics of all new cases diagnosed from 1995 to 2005 in hospitals in Montreal and Quebec City with>= 5 cases;

HPV genotyping to be done for at least 200 specimens

Results: 33 eligible hospitals (selected from MED-ECHO database) provided IRB approval. Cases were identified by topography from hospitalization registries. Excluded were melanomas and patients with recurrent disease; a total of 523 charts have been audited.

Histology: 76% squamous cell carcinomas, 15% adenocarcinomas, 9% other. ICD-9: 154.2: 24%, 154.3: 31%, 154.8: 9%, 35% not found (NF). 60% were female. 30% of men were single vs 14% of women (p=0.001); median age 64 (range 25-96). 60% had ever smoked. Most often noted symptoms were bleeding (42%), pain (30%), hemorrhoid (18%) and mass (14%). Polyps were noted 10 times more often in women (0.5% vs 5.4%, p=0.003) and warts twice as much in men (8.3 vs 4.6%, p=0.07). 7% of patients had clear concomitant diagnosis of other HPV-related disease (genital warts, CIN, VIN, VaIN, AIN or PIN): 4.5% were men, 8% women; another 1% of men and 7.5% of women had procedures suggesting the presence of other HPV-related disease. 6% of charts have information on HIV status; for those with information, men were 4 times as likely to have a positive test. Genotyping results will be included in the final presentation.

Conclusions; To date this is the largest study of cases of anal cancer with HPV detection and genotyping.

P-05.06

PERIANAL BOWEN'S DISEASE IN HIV-POSITIVE MEN

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Background: Perianal high-grade anal intraepithelial neoplasia, Bowen's disease (BD), progresses to anal cancer (AC) in 2-6% of the general population. Little is known about the natural history of BD in HIV+ men who have sex with men (MSM).

Objectives: 1) To describe the characteristics of HIV+ MSM with BD and 2) to determine factors associated with progression to AC.

Methods: Patients were derived from 550 HIV+ MSM screened for anal cancer and who had proven BD. We collected information on demographics, HIV illness, HPV, lesion appearance, treatment and BD progression. Subjects who did and did not develop AC were compared.

Results: BD occurred in 36 (7%). Median (range) age, HIV duration and CD4 count were 48 (31-63), 18 (1-27) years and 327 (40-780) cells/mm3. Seventy six percent (25/30) had undetectable viral loads and 70% (21/30) were past/current smokers. Internal AIN 2/3 was found in 81% (29/36) and 100% (35/35) were positive for oncogenic HPV types [specific types (n=16): HPV-16 (n=11), HPV-18 (n=5), HPV-33 (n=9)]. Anal canal cytology showed HSIL in 11/33 (33%). Lesions were pigmented in 53%, pink in 72%, raised in 64% and ulcerated/eroded in 19%. BD was treated with imiquimod (81%), local excision (29%) and thermocoagulation (8%). A second biopsy was done in 28; 75% had persistent disease. AC developed in 14% (5/36); median (range) time from BD to AC was 2.2 (0.2-3.4) years. In two patients, this represented cancer recurrence. Factors associated with developing AC included previous AC (p=0.05), lesions with ulcerations or erosions (p=0.04).

Conclusion: Because it is common, HIV+ MSM should be screened for BD. BD is difficult to eradicate and progresses to invasive AC much more frequently than in the general population. Patients with high risk features for AC should be considered for wide excision.

P-05.07

PREVALENCE OF HPV AND AIN IN HIV+ MSM

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P Goon, University of Cambridge, Cambridge, UK

Background and objectives: Human papillomaviruses (HPV) are the aetiological agents of certain benign and malignant tumours of the skin and mucosae. The most important of these is cervical cancer, and in 2006, there was an estimated 510,000 cases with 288,000 deaths worldwide. However, in certain high-risk groups such as HIV+ men who have sex with men (MSM), the incidence of anal cancer has reached epidemic proportions. There are insufficient data at present about the natural history and prevalence of HPV in this population. We have commenced an analysis of HPV infection in the aetiology of AIN (anal intra-epithelial neoplasia) in a large population of HIV+ MSM.

Study design and methods: A prospective study recruiting patients primarily from a large population of HIV+ MSM patients based in St Stephen's centre, Chelsea and Westminster Hospital, in West London. A secondary recruiting centre will be based in Cambridge. Anal cytology will be performed, along with concurrent oral swabs and urine sampling. The presence of cytological dysplasia will necessitate high-resolution anoscopy follow-up and treatment. HPV detection from all three sites will be undertaken, along with HPV typing. PGMY PCR will be used as primary screening, with a mandatory nested PCR (PGMY-GP5+/6+) if 1° PCR is negative. Linear Array assay (Roche Diagnostics[™]) and direct sequencing will be used for HPV subtyping.

Results and discussion: Ongoing recruitment and data analyses. We expect to see multiple HPV subtypes detected from the anal canal from this population, as previously reported. Data on HPV types, infection at the different demographic sites, and the concurrent presence of anal dysplasia will be correlated.

P-05.08

DIAGNOSIS OF HIGH-GRADE ANAL NEOPLASIA - HOW GOOD ARE WE?

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N Coleman, Medical Research Council Cancer Cell Unit, Cambridge, UK

Background: Three studies now document progression of HPV-related high-grade anal neoplasia (AIN2/3) to anal squamous carcinoma (AC). AIN2/3 is treatable thus providing an opportunity to intercept the development of AC. We do not know the accuracy of current diagnostic methods of AIN2/3.

Objective: To review existing data on detection of AIN2/3.

Methods: Medical literature search and analysis of our data on detection of AIN2/3.

Results: Anal cytology is widely regarded as a screening tool for anal neoplsia. Compared to histology it has a sensitivity of 47 - 81% in the detection of at-risk individuals. However, anal cytology necessarily needs to be followed up by High-resolution anoscopy (HRA) and biopsy for definitive diagnosis. Repeated smear examination increases the sensitivity of AIN2/3 detection. Anal cytology has a much reduced sensitivity (56%) in cases where the neoplasia is limited to one quadrant of the epithelium. Although HRA directed biopsy is being considered the gold standard, its sensitivity can potentially be affected by the presence of co-existent low-grade disease. Existing data, including ours, shows that with low-grade disease, 23.7 - 51.8% have associated AIN2/3. Recent data on minichromosome maintenance proteins (MCM2) shows that it is feasible to use MCM2 as a screening tool to detect those with AIN2/3. The sensitivity of this method is comparable to anal cytology but with a higher specificity. Other methods that are in use include mapping biopsy of anal canal for histological diagnosis and digital examination. The sensitivities of these methods are not known. Factors affecting the detection of AIN2/3 include HIV positive status, volume of disease, method of screen and the expertise of the health-care worker.

Conclusion: We need to find ways to improve sensitivity and specificity in AIN2/3 diagnosis. Alternatively, we need to treat both low-grade and co-existent high-grade disease in order to eliminate AIN2/3.

EPIDEMIOLOGY OF HPV INFECTION

SESSION 06

PROGRAMME

THE 25TH INTERNATIONAL PAPILLOMAVIRUS CONFERENCE MAY 8-14 2009, MALMÖ, SWEDEN

TIME NUMBER TITLE

ROOM

2009-05-11

SESSION 06: EPIDEMIOLOGY OF HPV INFECTION				
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16.35-16.46	O-06.01	RATE OF AND RISKS FOR SECOND INFECTIONS IN YOUNG WOMEN A B Moscicki, Y Ma, S Farhat, S Shiboski		
16.46-16.57	O-06.02	HPV IN OLDER WOMEN IS ASSOCIATED WITH NEW SEXUAL PARTNERS H Trottier, S Ferreira, JCM Prado, JS Sobrinho, MC Costa, LL Villa, E Franco		
16.57-17.08	O-06.03	GENETIC VARIANTS IN HPV-BINDING GENES AND RISK FOR HPV PERSISTENCE A Hildesheim, P Gonzalez, K Yu, C Porras, M Safaeian, A Rodriguez, J Li, M Sherman, C Bratti, M Schiffman, S Wacholder, Rd Burk, S Chanock, R Herrero, S Wang		
17.08-17.19	O-06.04	ONE HPV VIRUS, ONE LESION AS DETERMINED BY LCM/PCR TECHNOLOGY W Quint, A Molijn, B Colau, M van der Sandt, D Jenkins		
17.19-17.30	O-06.05	NATURAL HISTORY OF HPV INFECTIONS AND VULVAR DISEASE DEVELOPMENT E Joura, R Insinga, H Sings, R Haupt, S Garland		

O-06.00

ASPECTS OF HPV NATURAL HISTORY THAT INFORM VACCINATION AND SCREENING

M Schiffman, Division of Cancer Epidemiology and Genetics, U.S. National Cancer Institute, USA

Longitudinal cohort studies and randomized clinical trials have clarified the natural history of HPV infection and multistage cervical carcinogenesis. Summarizing the latest available epidemiologic data, this presentation will describe the "life cycle" of different HPV genotypes with relation to risks of persistence, diagnosis of CIN3, and invasion. The epidemiologic evidence will be used to address current issues in vaccination and cervical screening programs, considering the variable resource levels of different regions.

Some examples of important natural history topics (and the prevention issues they inform):

1. The carcinogenicity of HPV types is tightly correlated with evolutionary relatedness, as detailed in the 2009 IARC classifications. (Which genotypes should be included in vaccines and screening tests?)

2. Multiple, concurrent HPV infections influence each other minimally. (Vaccination will probably not make less carcinogenic types more dangerous.)

3. Virtually any newly-detected HPV infection is benign, not just those among young women. Regardless of her age, a woman is highly likely to become DNA-negative for a new type within a few years, with no CIN3 diagnosis. (One rationale given for catch-up vaccination of mid-adult women is removed.)

4. The reassurance against risk of invasive cancer conferred by single-time HPV test lasts for a decade or more. (How often should HPV screening be performed?)

5. The increased risk of HPV16 or HPV18 positivity, compared to the pool of other types, extends to 10+ years; HPV16associated CIN3+ appears earlier than for most carcinogenic types. (How should type-specific HPV screening be used?)

6. CIN3 lesions are initially very small and easily missed by colposcopy. HPV genotype predicts risk of diagnosing CIN3+ but, taking HPV type into account, minor cytologic distinctions, histologic distinctions, and colposcopic criteria are not well-reproduced or accurate in predicting subsequent risk of CIN3+. (What is the role for these other modalities among HPV-positive women? How can colposcopic biopsy be improved?)

O-06.01

RATE OF AND RISKS FOR SECOND INFECTIONS IN YOUNG WOMEN

AB Moscicki, University of California San Francisco, San Francisco, USA Y Ma, University of California San Francisco, San Francisco, USA S Farhat, University of California San Francisco, San Francisco, USA S Shiboski, University of California San Francisco, San Francisco, USA

Background: Although HPV infections are common in young women, the rate of repeated new infection is not well documented. Some speculate that new detections reflect latent infections as women age and not "new" exposures.

Objective: to examine the rate of and risks for new HPV detection in young women. Methods: 1125 women reflecting 17,600 visits over 10 years from the ongoing UCSF Teen HPV study were examined. HPV testing is performed at 4-month intervals; sexually transmitted infection (STI) testing is annual or if symptomatic. Starting with 1st HPV detection, time to the next (2nd) visit with detection of new HPV types and then the 2nd event to time to 3rd event was calculated. Risks were determined using Cox Proportional hazard model.

Results: Persistence of any HPV detected on the initial visit highly influenced the rate of acquisition independent of whether there were multiple or single types present: 58% of women acquired a new type within 3 years if the initial infection cleared vs. 93%% if persistent. Multivariate analysis showed that persistent HPV infection, recent reported history of an STI, and number of new sex partners were independent associations (all p values <0.05). Risks for 3rd visit with new infections were similar including (p values <0.05) STI history, number of recent sex partners and persistence of a previous HPV infection.

Conclusions: Repeated new HPV infections are extremely common in young women. The association with persistence of previous HPV is likely a marker of a deficient immune response, whether naïve or HPV induced, increasing the vulnerability to infections. New infections are likely "new" exposures and not latency as marked by the associations with STIs and new sex partners. STIs are markers of "partner" risk but may also suggest that inflammation secondary to the STI.

O-06.02

HPV IN OLDER WOMEN IS ASSOCIATED WITH NEW SEXUAL PARTNERS

H Trottier, McGill University / University of Montreal, Montreal, Canada S Ferreira, Ludwig Institute for Cancer Research, São Paulo, Brazil JCM Prado, Ludwig Institute for Cancer Research, São Paulo, Brazil JS Sobrinho, Ludwig Institute for Cancer Research, São Paulo, Brazil MC Costa, Ludwig Institute for Cancer Research, São Paulo, Brazil LL Villa, Ludwig Institute for Cancer Research, São Paulo, Brazil E Franco, McGill University, Montreal, Canada

Background: There is paucity of data concerning the source of HPV infection in older women. Much of the rationale for vaccinating older women depends on understanding whether an infection in the latter represents a true new episode, acquired via sexual activity, or the re-emergence of an earlier infection that has remained latent and undetected. Objective: We analyzed infections with the same or different types that women had been exposed to in the past to assess their associations with new sexual partners at the time of the new episodes. Methods: We used long-term HPV typing data for 2462 women enrolled in the Ludwig-McGill cohort study. Hazard ratios (HR) of new or re-infections as a function of new sexual partners were calculated using Cox regression. HPV testing and typing was done with the MY09/11 polymerase chain reaction protocol. Results: First incident infection with any HPVs was associated with having new sexual partners among women of all ages (HR=2.57, 95%CI: 2.05-3.23) and among older women (≥40 years)(HR=3.10, 95%CI: 1.68-5.72). Re-infections with the same type(s) or with new type(s) were also associated with new sexual partners in all women. Using a conservative definition of clearance (at least 3 negative visits between the first and the new episode), the HR among all women for any new HPV infection with the same original type(s) was 4.69 (95%CI: 1.33-16.52) and for different type(s) was 3.31 (95%CI: 1.99-5.51). When these analyses were restricted to women 40 years and over the HR was 5.92 (95%CI: 1.65-21.25) for HPV infections with the same types and 2.94 (95%CI: 1.76-4.92) with a new type. Conclusion: New HPV infection episodes with the same or new types in older women are equally associated with new sexual partners, which suggests that HPV exposures in older women could be prevented by HPV vaccination.

O-06.03

GENETIC VARIANTS IN HPV-BINDING GENES AND RISK FOR HPV PERSISTENCE

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INTRODUCTION: HPV infrequently persists and progresses to cervical cancer. We examined host genetic factors hypothesized to play a role in determining which subset of individuals infected with oncogenic HPV have persistent infection and develop cervical cancer compared to the majority of infected individuals who will clear infection. METHODS: We genotyped 7765 tag single nucleotide polymorphisms (SNPs) from 305 candidate genes hypothesized to be involved in DNA repair, immune mechanisms and cell entry in 466 cervical intraepithelial neoplasia 3 (CIN3)/ cancer cases, 390 HPV persistent women, and 443 random controls (RC) from the 10,049-women Guanacaste Costa Rica Natural History study. We used logistic regression to compute odds ratios and p-trend for CIN3/cancer (CIN3+) and HPV persistence in relation to SNP genotypes (adjusted for age). We obtained a gene-level summary of association by

computing the minimum p-value ("minP test"). RESULTS: Gene regions statistically significantly associated with CIN3+ included 2',5' oligoadenylate synthetase genes 2 and 3 (OAS2,OAS3), sulfatase 1 (SULF1) and general transcription factor IIH, polypeptide 3 (GTF2H3) (p<0.005). From each region, the single most significant SNPs associated with CIN3+ were OAS3 rs12302655, OAS2 rs718802, SULF1 rs4737999, and GTF2H3 rs2894054 (p-trends<0.0001). The associations were also significant when comparing HPV persisters to RC; the most significant associations were HPV persisters/CIN3+ compared to RC (OAS3 p-trend=0.00006, SULF1 p-trend=0.00003, OAS2 p-trend=0.0001, GTF2H3 p-trend<0.00001). None were associated with CIN3+ when compared to HPV persisters, suggesting no association with progression to disease. We note that the associations observed were largely modest (e.g., less than two-fold).

CONCLUSIONS: We identified variants in four genes associated specifically with HPV persistence. Our results require replication but if the associations are proven causal, they might reflect modulation by these genes for risk of HPV persistence via immunological or other mechanisms.

O-06.04

ONE HPV VIRUS, ONE LESION AS DETERMINED BY LCM/PCR TECHNOLOGY

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Background: In 20-40% of CIN lesions and in 4-8% of cervical carcinoma specimens multiple HPV genotypes have been detected by PCR on DNA isolated from whole tissue sections. Whether these HPVs are causally related to CIN lesion present in the tissue is difficult to investigate by existing hybridization technologies like Insitu DNA Hybridization. Applying Laser capture microdissection (LCM) in combination with a sensitive PCR-based HPV genotyping system can significantly increase the specificity of relating HPV types to specific lesions.

Objective: To investigate in CIN lesions whether one virus or more viruses are present in one lesion.

Materials and Methods: After examination of Haematoxylin Eosin slides, selected regions were isolated by LCM (Zeiss P.A.L.M.) and were analyzed by the broad spectrum HPV SPF10 PCR/ LiPA25 HPV genotyping system version 1*. HPV genotypes detected in LCM captured lesions were compared to results in whole tissue sections in 25 CIN2+ lesions with multiple HPV types and 9 CIN 1 cases with a single HPV type.

Results: For all 25 CIN2+ cases, only one HR HPV type was present in each of the lesions examined by LCM/PCR. Types were matching HPV types detected in the corresponding whole lesion. Genotypes were identical in 100% CIN1 and in 52 % of the CIN2+ cases. In the remaining 48% CIN2+ cases, a single HPV genotype was present in each lesion, whereas analysis of the whole section had revealed multiple HPV genotypes.

Conclusion: The LCM/PCR technique is a highly accurate method providing a high resolution HPV genotyping and allows for assignment of a specific HPV type to one CIN lesion.

*SPF10 HPV LiPA 25, version 1 and SPF10 HPV DEIA are manufactured by Labo Biomedical Products (Rijswijk, The Netherlands) based on licensed INNOGENETICS SPF10 technology.

O-06.05

NATURAL HISTORY OF HPV INFECTIONS AND VULVAR DISEASE DEVELOPMENT

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Objectives: The development of vulvar intraepithelial neoplasia (VIN) after HPV infection has not been well-studied. We describe the incidence, duration, progression to VIN, and clearance of external genital HPV infections. The prevalence of 14 common HPV types (6/11/16/18/31/33/35/39/45/51/52/56/58/59) in VIN is also presented.

Methods: Data were derived from women enrolled in the placebo arm(s) of randomized double-blind clinical trial(s) of an HPV 6/11/16/18 vaccine. Testing of labial/vulvar/perineal/perianal swabs for HPV 16/18/31/33/35/45/52/58/59 occurred at day 1, and every 6-12 months through 48 months, lesions were biopsied. Biopsies and swabs were typed for HPV. Biopsies were read for endpoint determination by up to four pathologists in a blinded fashion.

Results: Of 9 high-risk HPV types examined, incident infection with HPV16 was the most common (6.0 per 100 person years). The mean time from incident infection to the development of VIN was 18.5 months (95%CI: 13.4-23.6). HPV6 or HPV11 were observed in 64.5% (20/31) of low grade VIN(1) and 29.0% (9/31) of VIN2/3, while HPV16 or HPV18 were observed in 6.5% (2/31) of VIN1 and 64.5% (20/31) of VIN2/3. Two (6.5%) VIN2/3 lesions were positive for HPV-6, with no evidence of co-infection with one of the other 13 tested HPV types. HPV16 was the most common type observed among lesions infected with multiple types (9 of 14), followed by HPV6 (7 of 14). Following the detection and treatment of incident VIN lesions, 50.0% of HPV infections cleared within 12 months.

Conclusion. A prophylactic vaccine that includes HPV-6/11/16 and 18 could potentially prevent over half of pre-cancerous vulvar lesions. Understanding the incidence and duration of HPV infection, and the risk of progression to VIN may inform preventative decisions for vulvar disease and mathematical models used in assessing the cost-effectiveness of HPV vaccination.

POSTER ABSTRACTS SESSION 06

POSTER SESSION I MONDAY 10.00: ODD NUMBERS

POSTER SESSION II

TUESDAY 10.00: EVEN NUMBERS

MULTIPLE HPV TYPES: COMPETITION OR SYNERGY?

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Background: Coinfection with multiple types of human papillomavirus (HPV) is commonly observed among women with cervical HPV infection. It has been postulated that HPV infections are fundamentally independent of each other and that competition between HPV genotypes is unlikely to exist. Elimination of certain types of HPV by vaccination may give the ultimate answer to whether certain HPV genotypes occupy a biological niche or not, and whether genotype replacement will occur. While we wait for these observations, investigations on virus interferences seem warranted. If in vivo interaction between HPV types exists, these could be synergistic or competitive. A synergistic interaction would allow one type to facilitate infection with the second and in this case a vaccine against one of the types would reduce infection acquisition of the synergistic partners. However, types that are competing against vaccine types could be predicted to increase in prevalence.

Objectives: To investigate if any two HPV types are observed more or less frequently in concurrent infections than expected.

Methods: Cervical specimens from 3558 women, were analysed for HPV DNA using a microarray (ClinicalArraysHPV, Genomica, Spain), that allows for simultaneous detection of multiple HPV infections. Standard statistical methods were used to estimate ORs.

Results: 1716 cervical specimens were HPV positive, of these, 892 specimens contained multiple HPV genotypes. By simulation it was shown that concurrent infection of multiple HPV types occurred more frequently than expected by chance. Analysis of HPV combinations showed that certain combinations were more frequently observed than expected, whereas other combinations were less so. We also show how frequently these combinations are found in relation to cervical cytology findings.

Conclusions: Our findings imply that certain HPV genotypes are dependent on the presence of other genotypes, and that examples of competition and synergy HPV genotypes in between exist.

P-06.07

APPEARANCE OF NEW HPV INFECTIONS AMONG WOMEN OLDER THAN 45-YEARS

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INTRODUCTION: HPV prevalence peaks shortly after sexual debut and declines thereafter. A second peak has been observed in some populations after age 50. Several hypotheses have been proposed to explain this phenomenon.

OBJECTIVES: To identify determinants of HPV appearance among women 45-75.

METHODS: 324 women who were HPV positive 5-6 years after enrollment into a population-based cohort in Costa Rica and 310 negative controls were invited to an additional visit 3-yrs later to obtain detailed information about sexual behavior of the women and their partners and to collect blood for lymphoproliferation assays. Participation was over 92%; after exclusions 252 cases and 265 controls were included in this analysis. ORs and 95% CIs were estimated using multivariate logistic models. HPV appearance was defined as detection of an HPV type not detected at enrollment and the period of HPV appearance as the time between the last HPV negative and the first positive result.

RESULTS: In a multivariate model, sexual behavior variables associated with risk of HPV appearance included having 2+ partners in the period of HPV appearance (OR=4.6, 95% CI=1.5-14.0) and having >1 sexual partner in their lifetime (OR=1.5, 95% CI=1.1-2.1). Reduced lymphoproliferative responses to HPV-16 VLP increased risk by 1.8-fold (95% CI=1.1-2.7). Non-significant increases in risk were observed for women who reported that their partner had other partners during the relationship. Among women who reported no sexual activity during the period of interest (54 cases and 53 controls), being in the lowest tertile of lymphorpoliferative response to PHA increased risk of HPV appearance (OR=2.2, 95% CI=1.0-8.6).

CONCLUSIONS: HPV detection in older women may reflect two distinct origins: infections acquired during recent sexual activity and reactivation of previously unrecognizable infections, perhaps due to reduced immunological responsiveness.

HPV TYPE-SPECIFIC INCIDENCE AND CLEARANCE AMONG UGANDAN YOUNG WOMEN

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Background. Knowledge on type-specific HPV infections such as acquisition and persistence of new infections as well as clearance of the virus is still inconsistent worldwide. Co-existing infection with HIV have been suggested as a modulating factor of the natural history of HPV infection.

Objectives. We performed a study of incidence and clearance of high- and low-risk type-specific HPV types in a cohort of young Ugandan women.

Methods. 380 sexually active women were followed up between February 2003 and December 2006, being tested for HPV genotyping, Syphilis, Chlamydia, Gonorrhea, HIV and pregnancy. Incidence and clearance of different HPV types were evaluated for women with at least one follow-up visit. RR's were computed using unconditional multiple logistic regression models.

Results. 155 women were free of infection at the beginning of follow-up. A total of 69 new infections were observed during follow-up, most of these with HPV 51, 56, 52, 6 and 31. HPV 16-related types were more frequently than HPV 18-related. Women infected with HIV had an elevated risk of incident infection(RR 2.8; 95%CI 0.9-8.3) with dominant HPV subtypes 33, 58, 6 and HPV 16-related. 306 prevalent infections were analysed for HPV clearance. High-risk types and HPV 16-related types cleared less than low-risk and HPV 18-related types. The highest clearance rates were observed for HPV 33, 68, 67 and 74. HIV positive women were less likely to clear infections than HIV negative women (RR 0.2; 95%CI 0.1-0.7).

Conclusions. HIV infected women seem to have a have a lower HPV clearance rate than HIV negative women.

P-06.09

EPIDEMIOLOGY OF MULTIPLE HPV INFECTIONS

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Background and Objectives: The significance of multiple HPV infections, in terms of genotype clustering patterns or impact on cervical disease, remains unclear. We investigated whether types 16 or 18 were more/less likely to be co-infected with other genotypes and assessed the impact of co-infections on CIN2+.

Methods: Sexually active women aged 18-25 years who participated in the NCI-sponsored Costa Rica HPV Vaccine Trial's pre-vaccination enrolment visit (N=5871) were analyzed. Genotyping for 25 HPVs was performed using SPF10/ LiPA. We calculated odds ratios (ORs) to assess co-infection patterns for HPV16 and 18 with 24 genotypes. Pair-specific ORs were compared with a fixed-effects pooled OR to identify genotype combinations that deviated from the underlying OR. CIN2+ risk was compared between multiple versus singly infected women after adjustment for confounders.

Results: Among HPV-positive women (42.2%, n=2478), 43.2% (n=1070) were infected with multiple types. For either HPV16 or HPV18, no significant global heterogeneity in ORs was observed for patterns of co-infection with other genotypes. HPV16 was more likely to be involved in a co-infection with HPV31 (OR=3.10; 95%CI=2.23-4.25) or HPV52 (OR=2.82; 95%CI=2.12-3.72) than with other genotypes (pooled OR=2.11). HPV18 was less likely to be involved in a co-infection with HPV31 (OR=0.34) than with other genotypes (pooled OR=2.39). Singly and multiply infected women had similar CIN2+ risk (1.56% vs. 1.59%; OR=0.98; 95% CI= 0.50-1.89). Likewise, co-infection of HPV16 with other genotypes was not associated with CIN2+ (OR=1.20; 95% CI=0.44-3.30).

Conclusions: The overall lack of heterogeneity in co-infection patterns for HPV16/18 with most other HPVs indicates that multiple infections occur at random. The similar risk of CIN2+ among singly and multiply infected women argues against synergism across co-infecting genotypes. Extensions of analyses to random-effects pooled ORs for all possible co-infection combinations across 25 genotypes and phylogenetic species will be presented.

P-06.10 TMPV1 SEROPREVALENCE STUDY IN FLORIDA MANATEES USING A VLP-BASED ELISA

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BACKGROUND: Florida manatees (Trichechus manatus latirostris) are an endangered species in the US. Although these animals are remarkably resistant to natural diseases, wart-like lesions were detected in several captive individuals in 1997. Subsequently, the etiologic agent, Tm-Papillomavirus type 1 (TmPV-1), was isolated from these lesions and characterized, representing the only papillomavirus isolated from this species to date.

OBJECTIVES: The present investigation is aimed to determine the extent of TmPV-1 infection in free-ranging and captive manatees.

METHODS: A serological assay was developed using recombinant TmPV-1 virus-like particles (VLPs) constituted by the capsid protein L1 purified from a baculovirus-insect cells expression system.

RESULTS: The pilot study showed that our L1-VLP-based immunological test was successful at detecting anti-TmPV1 antibodies in manatee sera. Subsequently, a larger seroepidemiological study was carried out on 157 individual manatees, including the species Trichecus manatus manatus sampled in Belize. An overall prevalence of 30.6% was found, with 35.0% positive sera among the captive and 29.1% among the free-ranging animals. Seropositive wild animals were found in all the sampled locations, with the highest prevalence in the Everglades (53.3%).

CONCLUSIONS: This study reveals the first evidence of TmPV1 presence in free-ranging manatees. However, 98% of tested animals did not show PV-related lesions. Conversely, a high prevalence of papillomatosis was observed among the exposed captive manatees (46.1%), suggesting that stress factors associated with captivity might either activate TmPV1 from a latent state or favor the acquisition of new infections. Nonetheless, captivity might not be the only factor, since PV-related lesions have never been observed in captive manatees prior to 1997 despite a careful monitoring. These results might have implications for future management practices of captive healthy manatees. They might help guide decisions to characterize releasability of exposed captive manatees and possibly lift current quarantine practices under appropriate circumstances.

P-06.12

REPEATED DETECTION OF HPV 16 IN YOUNG WOMEN

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Background: Once clearance is established, it has been thought that a natural immunity to the cleared HPV type develops. Consequently, repeated detection of HPV 16 after documented clearance in healthy women is thought by some to be due to re-exposure and not reactivation. If this were true, then sexual behavioral risks for repeated detection should be similar to those seen for incident infection.

Objective: to examine the rate of and risks for repeated detection of HPV 16 HPV infection in young women.

Methods: Data from the ongoing UCSF Teen HPV study was used. HPV testing is performed at 4-month intervals. STI testing for CT and GC are performed annually or if symptomatic. Repeated detection of HPV 16 was examined defined as redetection after at least 2 consecutive negative tests. Risks for re-detection were determined using Cox Proportional hazard model. Results: 322 women representing 5388 visits were examined. By 3000 days, repeat 16 detection occurred in 12.8%. Re-detection was not observed after 3116 days. 22 women developed CIN 2/3 and were exited. In multi-variate analysis, risks included current medroxyprogestserone use (HR=6.7; 95% CI 2.04-22.3), total months of oral contraceptive use (HR=1.02 per month; 95% CI 1.008-1.03) and cumulative C. trachomatis infection history (HR=4.9; 95% CI 1.9-12.8).

Conclusions: Redetection of HPV 16 was not common. Most recurrence occurred within 4-5 years after initial detection. If cleared, detection after 5 years appeared nil. The associations with repeat HPV 16 infection were all risks shown to be important in the development of cancer suggesting that these are latent infections and not new.

ASSOCIATION OF HORMONAL CONTRACEPTIVE USE AND PREVALENT HPV INFECTION

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Background & Objective: Women diagnosed with cervical cancer report longer duration and more recent use of combined oral contraceptives (COCs). It is unclear whether COC use is associated with subclinical outcomes of HPV infection prior to development of clinical disease. The objective of this study was to assess the association of contraceptive use on the risk for prevalent HPV infection in a cohort of long-term hormonal contraceptive users.

Methods: 1208 HIV negative women aged 20-37 from Thailand enrolled in a prospective study of the natural history of HPV. Baseline HPV genotype information, recency and duration of hormonal contraceptive use, sexual behavior, other STI information (CT/GC), and Pap smear diagnoses were assessed. Prevalent ratios(95% CI) assessing the association of contraceptive use on prevalent HPV infection were estimated.

Results: At enrollment, 23% and 14.7% of women were infected with any HPV or any high risk (HR)-HPV, respectively. After adjustment for age, current and past sexual risk behaviors, use of COCs for >6 years was found to be associated with an increased risk of infection with any HPV (PR:1.79 (1.14,2.83)) and any HR-HPV (PR:2.78 (1.48,5.22)) as compared to never-users. Stopping COCs >4 years vs. <6 months prior to enrollment resulted in an approximately 20% reduction in the risk of prevalent infection (PR:.76 (.54, 1.08)). No similar association was observed for recent or long duration use of progestin-only contraceptives (e.g., DepoProvera).

Conclusions: Recent, long-term COC use was associated with increased risk for prevalent HPV infection independent of sexual behavior in women without prevalent cervical abnormalities. These data suggest that early effects of COC use on the natural history of HPV infection may be involved in the etiology of cervical cancer prior to development of clinical disease.

P-06.14

TYPE-SPECIFIC HUMAN PAPILLOMAVIRUS (HPV) IN THE LOWER GENITAL TRACT

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Objectives: Compare prevalence of type-specific HPV in the endocervix to that in the upper vagina, lower vagina, perineum, and vaginal self-test.

Methods: 2,646 Chinese women aged 16 to 54 years had brush specimens collected from endocervix, upper vagina, lower vagina, perineum, vaginal self-sample and cervical cytology. 411 of 420 women with positive endocervical or self-test by Hybrid Capture 2° (HC-2) or cytology of ASC-H or worse had colposcopy and biopsies. Linear Array[®] for specific HPV types determined for all sites for 410 of 413 women havinga positive HC-2 in the endocervix or self-test a random sample of 75 of the 2,233 women that had negative HC-2 in the endocervix and self-test. HPV prevalence was calculated with and without weighting the random 75 results. Differences in unweighted HPV prevalence were tested with McNemar's. Correlation of endocervical and vaginal HR-HPV was assessed using Pearson Correlation Coefficients.

Results: Endocervical HR-HPV most accurately differentiated \geq CIN 2 from \leq CIN 1. Unweighted prevalence of HR-HPV in the endocervix (9.8%) was lower than in the upper vagina (12.1%,p<.0001), lower vagina (12.1%,p<.0001), vaginal self-test (10.9%, p=.0002), and similar to perineum (10.5%,p=.1). Correlation of endocervical HR-HPV with HR-HPV in upper vagina was 0.71, lower vagina was 0.68, perineum was 0.60, and vaginal self-test was 0.64. Unweighted prevalence of LR-HPV in endocervix (2.8%) was lower than in upper vagina (6.1%, p<.0001), lower vagina (7.3%, p<.0001), perineum (6.4%, p<.0001), and self-test (5.6%, p<.0001). Weighting only accentuated these findings.

Conclusions: HR-HPV from the endocervix (which is associated with \geq CIN 2) likely contributes to HR-HPV presence in the vagina (which was associated with \leq CIN 1). Current self-sampling likely obtains a specimen from the lower vagina and/or perineum. A self-sampling device that preferentially samples the cervix may be more sensitive and specific for \geq CIN 2.

HUMAN PAPILLOMAVIRUS SEROLOGY IN ANAL CANCER

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Background: In 2002, an estimated 30,400 adults worldwide were diagnosed with anal cancer. Approximately, 90% of anal cancers are HPV positive, with HPV16 being the most frequent type detected. This study was conducted to determine if seropositivity to other HPV types is associated with anal cancer.

Methods: A random sample of cases from our case-control study of anal cancer was included in this study. Cases were men (n=92) and women (n=142) diagnosed with squamous cell anal cancer between 1986 and 1998 in the Seattle area. Patients were ascertained through the local cancer registry and control participants (n=287) were ascertained through randomdigit telephone dialing and age matched to the cases. Participants were interviewed and blood samples were collected. HPV antibodies to L1 for HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 67, 68, and to E6 and E7 for HPV 16 and 18 were detected via a Luminex-based assay. Samples in the upper quartile of MFI values based on the HPV type- and sex-specific distribution in the controls were considered seropositive. Relative risk of anal cancer was estimated using odds ratios (ORs) and 95% confidence intervals derived from multivariate logistic models.

Results: Overall, 76.5% of cases were seropositive for HPV16L1 compared with 24% in controls. In men, anal cancer was statistically significantly (p<0.05) associated with seropositivity for HPV 16L1 (OR=11.0), 18L1 (3.3), 51L1 (5.7), 16E6 (10.7), 18E6 (2.5), and 18E7 (3.7). Seropositivity to the same antigens were statistically significantly associated with anal cancer in women (ORs of 10.3, 2.8, 1.8, 6.8, 2.2, and 2.7, respectively) along with 16E7 (OR = 1.8).

Conclusion: Among all of the high risk HPV antigens included in the assay, only seropositivity to HPVs 16, 18 and 51 were associated with anal cancer.

P-06.16

GENOTYPE DISTRIBUTION OF HPV AMONG ESOPHAGEAL CARCINOMA IN HONG KONG

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Background: The aetiological role of human papillomavirus (HPV) infection in esophageal cancer remains controversial. Studies based on different populations have reported a wide range of Formalin-fixed, paraffin-embedded tumor biopsies collected from 126 consecutive cases of histology-confirmed squamous cell carcinoma of the esophagus were tested for HPV DNA using the INNO-LiPA HPV Genotyping Extra kit that can identify 27 HPV types (HPV6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 69, 70, 71, 73, and 82).

Results: The studied subjects were all Chinese with 22 females aged 46-90 (mean: 73, SD: 13.8) yr, and 104 males aged 37-94 (mean: 69.3, SD: 11.5) yr. Altogether, 37 (29.4%) samples were tested positive for HPV DNA with 31 samples had one HPV type detected, and 6 were coinfections. Among the HPV positive cases, 26 (70.3%) harbored high-risk types, 9 (24.3%) harbored low-risk types, 2 (5.4%) were coinfected with both high- and low-risk types. The most frequently detected type was HPV16 (14/37, 37.8%), followed by HPV11 (10, 27.0%), HPV18 (8, 21.6%), HPV52 (7, 18.9%); whereas HPV6 was detected only in 2 (5.4%) samples, and HPV31, HPV33, HPV51 each in 1 (2.7%) sample.

Conclusion: HPVDNA of high-risk types were found in a substantial proportion of squamous cell carcinoma detected from southern Chinese in Hong Kong. Further studies to elucidate whether HPV is a bystander or having an aetiological role for the development of esophageal cancers is crucial, and is urgently needed in view of the availability of prophylactic vaccines for HPV.

P-06.17 "U"SHAPE OF AGE-SPECIFIC HR-HPV PREVALENCE IN CHINESE WOMEN ATTENDING HOSPITALS

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Objective: The purpose of the present study was to estimate the prevalence of high-risk human papillomavirus infection and the viral load in different age intervals, to describe the distribution of human papillomavirus prevalence in women enrolled in our hospital. Study design: We retrospectively investigated 17148 cases undergoing hybrid capture II between January 2005 and February 2007. The prevalence of human papillomavirus infection and the level of viral load were estimated in different age intervals to describe the distribution in the cases. Results: Human papillomavirus was detected in 5173 of 17148 women (30.2%), aged 17 to 79 years. The highest human papillomavirus infection prevalence appeared in the \leq 20-year interval (45.2%). After age 20, the prevalence declined rapidly and then ascended slowly from 28.5% in the 21-30-year interval to 38.0% in the \geq 61-year interval. The mean viral load of the 21-30-year interval was the lowest (271.99±499.24 relative light units/positive control) and the highest was found in the \geq 61-year interval (560.30±672.87 relative light units/positive control). Conclusions: Our study showed a "U" shape of age-specific prevalence of high-risk human papillomavirus infection occurring in women attending hospitals in Shanghai, China, similar to worldwide figures.

P-06.18

INTRATYPIC VARIANTS OF HPV 16/18/52/58, PERSISTENT INFECTION AND CERVICAL NEOPLASIA

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Human papillomavirus (HPV) 16, 18, 52, and 58 are high-risk HPV types to induce cervical neoplasia. Recent studies have documented significant associations of HPV intratypic variants with persistent viral infection and cervical neoplasia. This study aims to assess the importance of intratypic variants of HPV 16, 18, 52, and 58 in the determination of persistent HPV infection and cervical neoplasia in Taiwan. A total of 11,923 women were enrolled from seven townships in 1991-1993. The HPV DNA in their cervical cells was detected and typed by EasyChip HPV blot. A total of 807 women were infected with one or more types of HPV 16, 18, 52 and 58 at enrollment. The intratypic variants of HPVs were determined in 548 participants who had high viral load in cervical cells by the polymerase chain reaction sequencing of long control region (LCR) and E6 and E7 genes. The persistent infection of these four HPVs was determined in 264 participants who were cytologically normal at baseline examination and attended follow-up examination. Among HPV 18 variants, the Asian-American variant had a higher proportion of persistent infection (65.5%) than the European variant (7.1%) showing an odds ratio of 24.7 (95% confidence interval, 2.8-216.9). The HPV 58 prototype-like variant had a lower proportion of persistent infection (28.6%) than the HPV 58 LCR 7714 A→C variant (80.8%) showing an odds ratio of 0.10 (95% confidence interval, 0.01-0.64). Comparing with HPV16 European variant, HPV16 non-European variant was associated with an increased risk of cervical lesions (LSIL and above) at enrollment showing an odds ratio of 3.56 (95% confidence interval, 1.2-11.0). The most prevalent variants of these four HPV types were all associated with an increased risk of HSIL and cervical cancer

ROLE OF CHLAMYDIA TRACHOMATIS IN CERVICAL PRE-CANCER/CANCER AMONG HPV-POSITIVE WOMEN

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BACKGROUND: Although infections with carcinogenic HPV are a necessary cause of cervical cancer, infections are extremely common relative to cancer. It is postulated that exposure to co-factors might increase the risk of HPV-infected cells progressing to precancer and cancer. Some, but not all studies have shown Chlamydia trachomatis (Ct) to be a risk factor for cervical cancer after controlling for HPV.

OBJECTIVE: To assess prospectively, the role of Ct in cervical precancers, and type-specific HPV persistence.

METHODS: Using a case-cohort design within the Proyecto Epidemiologico Guanacaste, we identified 327 cumulative cases of prevalent (n=185) or incident (n=142) CIN2/CIN3/cancer (CIN2+) histology during follow-up, and 10% random sample of the baseline cohort (n=1100). Type-specific HPV status over multiple visits was determined using PCR amplification and reverse line-blot hybridization. Cervical Ct DNA was determined using a novel serovar-specific PCR-based Ct detection and genotyping assay (LaboBiomedicalProducts,Rijswijk, The Netherlands) on cervical samples. Corresponding plasma was used to determine Ct IgG status (Medac Ct-IgG-pElisa, Hamburg, Germany). Behavioral factors were determined from questionnaires.

RESULTS: The measures of HPV and Ct positivity were strongly associated, as expected for two sexually transmitted agents. There was not an association between Ct DNA or serology and CIN2+ among HPV DNA-positive cases and controls (OR=0.93, 95%CI=0.55-1.59 for Ct DNA-positivity; OR=1.05, 95%CI=0.70-1.56 for Ct seropositivity). Similarly, neither Ct DNA-positivity nor seropositivity increased the risk of carcinogenic HPV-persistence among HPV-positive controls, (OR=0.26, 95%CI=0.03-2.07 for Ct DNA-positivity; OR=1.17, 95%CI=0.38-3.59 for Ct seropositivity).

CONCLUSIONS: We did not find an association between Ct DNA or serology positivity and CIN2+ among HPV-positive women. In addition, we did not find any association between Ct DNA or seropositivity and type-specific HPV persistence. Our results suggest that the previous positive finding between Ct and cervical cancer could be due, in part, to confounding by HPV status.

P-06.20

LONGITUDINAL STUDY OF HPV16 VARIANTS IN ANAL AND CERVICAL SAMPLES

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Background: We investigated HPV16 variants in synchronous samples from anus and cervix, as well as longitudinal samples from anus or cervix, derived from a cohort study of HIV-infected patients.

Methods: We used a pyrosequencing method to determine HPV16 variants in stored extracts of all cervical and anal samples in which HPV16 was detected by the Roche linear array. Seven polymorphic nucleotide positions within the E6 region were analyzed to determine genotype variants in 179 anal and 43 cervical swabs from 91 different subjects.

Results: Of the 222 samples sequenced, the European variants E-G350 (35%) and Ep (32%) were most frequently detected followed by Af2 (9.9%), Af1 (9%), AA (2.3%) and As (0.9%). Fourteen samples (6.3%) contained possibly undescribed HPV16 variants and 10 (4.5%) showed multiple variants. Data from both anal and cervical specimens collected on the same date were compared in 29 instances from 25 different women. Synchronous differences were found in 10 (34.5%) instances from 9 (31.0%) women. Data from samples collected from the same anatomical site on 2 or more visits at least 6 months apart were compared in 66 instances (59 anal, 9 cervical) from 59 subjects. Longitudinal changes in the variant types detected were seen in 12 (18.6%) instances from 11 (16.7%) subjects, of which 10 were anal and 2 cervical series.

Conclusions: Variant analysis indicated multiple HPV16 infections in HIV-positive subjects, both in synchronous anal and cervical samples, as well as in longitudinal samples from each anatomic site. The incidence rate of HPV16 infections is likely an underestimate since reinfections with the same variant seem likely and would not be detected by variant analysis. It remains to be determined how reflective these data are of the general population, however intra-variant changes should be considered in HPV investigations, especially if the focus is on persistence

INTERACTIVE EFFECTS OF HPV AND COFACTORS ON CERVICAL CANCER

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Background: Human papillomavirus (HPV) has been recognized as the necessary cause of cervical cancer. Cofactors such as cigarette smoking and parity are also important risk factors for cervical cancer. This study aims to assess the joint effect of HPV infection and increased number of vaginal delivery on cervical cancer in a cohort of 10,602 women in Taiwan.

Methods: Estimates of synergy index (SI), odds ratio (OR), population attributable fraction (PAF) with their 95% confidence interval (CI) were derived from both multiplicative and additive models. The causal-pie fraction (CPF) was used to indicate the proportion of patients whose disease resulted from a particular causal pie.

Results: The age-adjusted OR (95% CI) of developing cervical cancer for the interaction between HPV infection (yes vs. no) and increased number of vaginal delivery (4+ vs. 0-3) was 17.9 (7.9- 40.8) and 11.1 (9.1-59.6), respectively, in the multiplicative and additive logistic regression models. The corresponding SIs (95% CIs) of the interaction were 3.0 (1.8-9.1) and 2.9 (1.5- 5.7). The PAF in the multiplicative logistic regression model was 72.0%, 36.0% and 72.0%, respectively, for HPV infection, increased number of vaginal delivery and their interaction. The corresponding PAFs were 68.5%, 34.3% and 68.5% in the additive logistic regression model. The sum of PAFs was greater than 100% in both models. However, the CPF in the additive logistic regression model was 34.1%, 0.0%, 34.4% and 31.5%, respectively, for HPV infection, increased number of vaginal delivery model. Both the sums of CPFs in two models equaled 100%. In conclusion, CPF may elucidate correctly the causal pies consisting independent and synergistic effects of HPV infection and increased number of vaginal delivery on cervical cancer.

P-06.22

LOW LEVEL PERSISTENCE OF HPV 16 INFECTION IN ADOLESCENT WOMEN

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Background: Most HPV infections are thought to clear after a few months. Persistent oncogenic infection, such as HPV 16, is linked to dysplasia and cervical cancer later in life. Renewed detection of a specific HPV type after apparent clearance may represent low level persistence rather than reinfection.

Objective: Describe infections with HPV 16 in a cohort of adolescent women (AW), addressing clearance and reappearance. This is a preliminary analysis of 20 AW followed for a median duration of 6.7 years (range 4.4-8.9; SD 1.2).

Methods: AW were enrolled in a longitudinal study of behavior and risk factors for STIs. A self-collected vaginal swab was obtained every 3 months, and examined for HPV by Roche Linear Array Assay (LA)(37 types). To explore low level persistence, we developed type-specific nested PCR for HPV 16 (TSN-PCR-16). Sensitivity of TSN-PCR-16 compared to LA was investigated. Long control region (LCR) amplification/sequencing experiments were designed to compare identity of an HPV 16 infection before and after apparent clearance.

Results: Median duration of 13 individual HPV 16 infections was 448 days (range 1-2853; SD 987). These infections had 35 individual periods of non-detection ≥6 months (defined by LA) followed by repeated detection. In control experiments, TSN-PCR-16 was more sensitive than LA for HPV 16. In specimens from AW, TSN-PCR detected HPV 16 during periods of non-detection with LA. For one subject, HPV 16 was detected 13 months prior to initial LA positivity and 37 months after the last LA positive specimen. Results of LCR sequencing will be presented.

Conclusions: Early findings suggest that some HPV 16 infections that appear to clear may persist at low levels. TSN-PCR will be used to detect possible low level persistence in AW with HPV 16 infections by LA.

HPV 6/11 SEROPREVALENCE AND GENITAL WART HISTORY IN THE U.S.

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Background: An estimated 1% of sexually active individuals in the United States have genital warts (GW); over 90% of these warts are reportedly caused by human papillomavirus (HPV) types 6 or 11.

Objectives: To obtain baseline estimates of HPV 6/11 seroprevalence and reported GW history among a representative sample of 14-59 year-old U.S. population.

Methods: In 2003-2004, a representative sample of 4303 sexually active 14-59 year-old participants in the National Health and Nutrition Examination Survey reported GW diagnosis history using audio computer-assisted self-interview. Participant sera were tested for HPV 6 and 11 antibodies using a multiplexed, competitive immunoassay.

Results: Overall seroprevalence of HPV 6/11 in 14-59 year-olds was 14.1%; seroprevalence was 7.3% (95% CI, 6.0-8.8) in males compared to 20.8% (95% CI, 17.9-24.0) in females, and 13.3% (95% CI, 11.4-15.5) in Non-Hispanic whites compared to 22.5% (95% CI, 17.9-27.8) in Non-Hispanic blacks. Overall, 5.5% of participants reported GW diagnosis. The percentage of diagnoses was higher in females (6.7%, 95% CI, 4.9-9.0) than in males (4.4%, 95% CI 3.4-5.7) and higher in Non-Hispanic blacks (4.4%, 95% CI, 3.3-5.8).

Among HPV 6/11 seropositive participants, 13.5% reported a history of GW diagnosis; similar in males (12.3%, 95% CI, 7.2-20.2) and females (13.9%, 95% CI, 8.7-21.4). However, among HPV 6/11 seropositive participants, a significantly higher percentage of Non-Hispanic whites reported GW diagnosis history (16.3%, 95% CI, 10.3-25.0) than Non-Hispanic blacks (10.4, 95% CI, 0.3-16.8). This racial disparity was found in both males and females.

Conclusions: Seroprevalence of GW-associated HPV is significantly higher in females than in males. Non-Hispanic blacks are more likely to have been infected with GW-associated HPV but are less likely to report a history of GW diagnosis. Reasons for this discrepancy are unclear but could include undetected disease, lower disease incidence, or non-external genital site exposure in Non-Hispanic blacks.

P-06.24

DIHYDRODIOL DEHYDROGENASE OVEREXPRESSION IS CLOSELY ASSOCIATED WITH HPV 16/18 INFECTION

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Purpose: To investigate the association of human papillomavirus (HPV) infection with the expression of dihydrodiol dehydrogenase (DDH) in uterine cervical cancer (UCC), and the relationship of overexpression of DDH with drug resistance and prognosis in UCC.

MATERIALS AND Methods: In situ hybridization (ISH) and immunohistochemistry were applied to examine pathological specimens of 145 patients with UCC. 16 different gynecological cancer cell lines were studied for HPV 16/18 and DDH expression. Drug-sensitivity assay was done for 13 different anticancer drugs.

Results: By ISH, HPV16/18 DNA was detected in 108 (74.5%) UCC cases. DDH expression determined by immunohistochemistry was detected in 81 (75%) lesions among 108 HPV-positive cases. In contrast, of 37 HPV-negative cases, DDH was only detected in 16 (43.2%) of the lesions. A significant correlation was found between DDH expression and the presence of HPV (P < 0.001), FIGO stage (P = 0.004), lymph node involvement (P < 0.001), as well as patients' survival (P = 0.002). In vitro, DDH expression was also found closely associated with HPV infection, and DDH content was proportional to cell sensitivity for cisplatin and doxorubicin. Both SKG-I and SKG-IIIa cells that had higher content of DDH were more resistant to anticancer drugs than SKG-II (P<0.001). DDH expression was decreased around 8-fold in SKG-I cells after using an shRNA method.

Conclusions: HPV infection provokes local inflammation, which can then induce DDH expression and drug resistance in UCC. The detailed biological relationship among HPV infection, expression of DDH and drug resistance, however, remains to be clarified.

ASSOCIATION OF HORMONAL CONTRACEPTIVE USE ON HPV INCIDENCE AND PERSISTENCE

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Background: Women diagnosed with cervical cancer report longer duration and more recent use of combined oral contraceptives (COCs). It is unclear whether COC use is associated with subclinical outcomes of HPV infection prior to the development of clinical disease.

Objective: The objective of this study was to assess the association of contraceptive use on the risk for incident and persistent HPV infection in a cohort of long-term hormonal contraceptive users.

Methods: 1208 HIV negative women aged 20-37 from Thailand enrolled in a prospective study of the natural history of HPV were followed every 6 months for 18 months. At baseline and follow-up age, type-specific HPV DNA, hormonal contraception use and history, sexual behavior, other STI information, and Pap smear diagnoses were assessed. The association between COC use and new HPV detection and viral clearance was assessed using Pooled logistic regression and survival analysis methods, respectively.

Results: Newly detected HPV was more common among women who reported COC use at the last visit compared to nonusers (OR: 1.42(1.05, 1.92)) which was attenuated after adjustment for age and sexual risk factors (OR: 1.27(.93, 1.74)). Women who reported current COC use during follow-up were more likely to persist in their HPV infection as compared to non-users (HR: .64 (.46, .89)) independent of sexual behavior and pap diagnosis. DMPA users were at a marginally lower risk for new detection (OR: .90 (.63 ,7.24)) and clearance (HR: .79 (.51,1.20)) as compared to non-users.

Conclusions: Current use of COCs was observed to be associated with an increased risk for persistent infection among women without cervical abnormalities during follow-up. These data suggest that early effects of COC use on the natural history of HPV infection may be involved in the etiology of cervical cancer prior to development of clinical disease.

P-06.26

LACK OF HETEROGENEITY OF HPV16-E7 COMPARED WITH HPV31 AND HPV73-E7

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BACKGROUND: More than 40 HPV genotypes infect the genital tract; however, there are substantial differences between types in their association with CIN3 and cervical cancer. HPV16 is by far most consistently associated with cervix cancer causing at lest half of all cervix cancer in the world. In addition, it is the most prevalent HPV detected and is the main target of HPV vaccines. By comparison, HPV 31 (the genotype most closely related to HPV16) is less prevalent and has a diminished association with cervix cancer compared to HPV16. Even more striking, HPV 73, while related, is only equivocally carcinogenic. The role of viral genetics in producing these differences is largely unexplored. OBJECTIVE: Our objective was to identify viral genetic sequence differences of the E7 gene of HPV 16, 31 and 73 associated with clearance, persistence, and progression.

METHODS: We randomly selected women with single HPV16, 31, or 73 infections from the Proyecto Epidemiologico Guanacaste (PEG) cohort who 1) cleared the infection within the average period of less than 1 year, 2) had type-specific persistent infection, or 3) progressed to cervical intra-epithelial neoplasia grade 2 or more severe lesions (CIN2+), however, there were no cases of HPV73 associated CIN2+ in PEG. The E7 region for all samples was sequenced.

RESULTS: For HPV16 E7, 1.7% (5/296 nucleotides) were variable, compared with 4% (12/296) of HPV31 E7, and 1.4% (4/296) of HPV73 E7. While the 12 polymorphisms in HPV31 resulted in 7 non-synonymous amino acid changes, and the 4 polymorphisms in 73 resulted in 3 non-synonymous amino acid changes, the polymorphisms in the HPV16 E7 resulted in only synonymous changes.

CONCLUSIONS: The lack of heterogeneity of HPV16 E7 oncoprotein suggests high evolutionary purifying selection that might be related to the unique properties of HPV16.

HPV INFECTION IN EUROPE

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Background: In addition to cytology-based programmes, important new methods for cervical cancer prevention are under consideration, namely human papillomavirus (HPV) vaccination and HPV testing for primary screening.

Objectives: To describe HPV prevalence in different European countries, in order to inform efforts to estimate the impact of the implementation of HPV vaccines and HPV testing in Europe.

Methods: The most recent and largest studies on population HPV prevalence in Europe were identified through MEDLINE and were summarised. A meta-analysis from an IARC database on HPV prevalence by cervical lesion grade was also used.

Results: Sixteen studies including between 897 and 46,900 women from 13, mostly Northern and Western European countries were included. Everywhere, high-risk (HR) HPV prevalence peaked before age 25 or 30 years with steady declines thereafter. For women in the 30-64-year age-range, for whom primary HPV testing is considered, age-adjusted HR HPV prevalence ranged from 2% in Spain to 12% in France. Proportions of HPV16 and 18, the two HR types prevented by current HPV vaccines, were overall 31% and 11% among all HR HPV positives. A significant correlation between the prevalence of HR HPV and high-grade intraepithelial lesions (HSIL) or worse (Pearson correlation coefficient=0.72; p=0.006) was also demonstrated. Vaccine-preventable proportions of lesions that are associated with HPV16 or 18 in Europe are: 52% of cytologically detected HSIL (prevented referral to colposcopy), 61 % of histologically confirmed cervical intraepithelial neoplasia grade 2 or 3 (prevented precancer treatments), and 76% of invasive cervical carcinoma (prevented diagnosis at cancer stage).

Conclusions: HPV burden differs between European Union countries, but the lack of data from some, notably new Member States, is of concern, especially when combined with a lack of high-quality statistics about cervical cancer incidence. This knowledge gap may be an obstacle to the prioritisation of cervical cancer prevention programmes.

P-06.28

POPULATION-BASED TYPE- AND AGE-SPECIFIC PREVALENCE OF HUMAN PAPILLOMAVIRUS IN GERMANY

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Background: Incidence and mortality rates of cervical cancer are higher in Germany than in comparable European countries despite more than 30 years of opportunistic Pap smear screening.

Objectives: Type- and age-specific human papillomavirus (HPV) distribution was investigated for the first time in Germany in a population-based sample of women before HPV vaccine was introduced.

Methods: The study population consisted of 5000 women aged 30 to 65 years and resident of the Mainz-Bingen region in Rhineland-Palatinate. The study sample was randomly drawn via population registries. Participants were invited for cervical cancer screening using conventional Pap smear and liquid based cytology. HPV infection was detected with the hc2 low and high risk probes as well as with GP5+/6+ PCR. All HPV PCR positive samples were genotyped. Women were referred to colposcopy if either cytology was classified ASCUS or worse, or if they were HPV high risk positive in the hc2 test.

Results: Prevalence of carcinogenic HPV types detected by hc2 and GP5+/6+ PCR was 6.4% and 7.1%, respectively. In total, 34 different HPV types were detected. Infection with a single HPV type was found in 84.6% of the women, while 15.4% had multiple HPV types detected. HPV 16, 56, 66, 52, 31 and 45 were the most common oncogenic HPV types in the study population. HPV 16 was found in 31.9% of cytological normal but in 54.5% of women with histological confirmed high grade lesions (CIN2+). The most common low risk HPV types were HPV jc9710, 42, 70 and 43.

Conclusions: These results give valuable knowledge for handling HPV infection in Germany, both regarding future strategies of screening and vaccination.

TYPE SPECIFIC ANALYSIS OF MULTIPLE TYPE CERVICAL HPV INFECTIONS

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Objective: To determine the frequency of multiple type infection (MTI), and determine if any HPV types are identified in MTIs more or less frequently than would be expected if MTI is independent of HPV type and based on type-specific prevalence alone.

Methods: This is a retrospective analysis of data from laboratories utilizing type specific HPV testing. Type-specific infection rates were determined. Two-type infection rates for each possible combination were calculated. The independence of the types was tested using chi-squared and Fisher's exact tests. Bonferoni adjustment was used for multiple tests; p-values less than 0.001 were statistically significant.

Results: Typing was performed on 73,563 women. 31.2% were HPV positive. 25% of these were MTIs, with type 16 present in 30.0% of MTIs. Other types in MTIs, in decreasing order of frequency, were 53, 52, 31, 66, 6, 18,11.

82.6% of MTIs involved only 2 types. The most frequent types (most notably HPV 16), were less likely to be found in MTIs than would be expected based on prevalence under the assumption of independence (p<0.001).

The α -9 specie was most frequently found in MTIs, 57.8%. For all virus species except α -9, both types in two-type infections were less likely to be from the same specie than would be expected. In contrast, when one type was from α -9 the second type was also from α -9 more frequently than expected based on type prevalence (p<0.0001).

Conclusion: MTIs accounted for 25% of HPV positive tests. HPV type 16 is involved in fewer MTIs than expected. Specie α -9 appears to have an affinity for co-infection with other α -9 types. There may be competitive and cooperative interactions between HPV types leading to less frequent involvement in MTIs than would be expected if MTI is independent of type.

P-06.30

HPV INFECTION IN 40,382 DANISH WOMEN FROM THE GENERAL POPULATION

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BACKGROUND. The Nordic countries were among the first in the world to introduce organized screening programs (the mid-1960s), but still Denmark has some of the highest incidence and mortality rates in Western Europe. A prophylactic quadrivalent (HPV6,11,16,18) and a bivalent (HPV16/18) HPV vaccine are available, but it is likely that future vaccines will contain more types.

OBJECTIVES. To assess the magnitude of potentially preventable cervical disease associated with not only HPV16 and 18 but also other HPV types such as HPV31 and 45.

METHODS. On randomly chosen days over a 3 year period, we collected consecutive liquid-based cytology samples (SurePath^{*}) from Dept. of Pathology, Hvidovre Hospital. After cytological examination, the rest of the respective samples were sent to the HPV-laboratory. HPV –testing was done by HC2 and genotyping with LiPA. The cohort was linked to the Pathology Data Bank to obtain cytological diagnoses related to the collected samples and any subsequent cytology/ histology results.

RESULTS. We included 40,482 women (14-96 years), 94% had normal cytology, 1638 (4.1%) had ASCUS/LSIL, 724 women (1.8%) had HSIL at baseline. Overall, 8,262 women (20.5%) had HR HPV types and 2,790 (6.9%) had LR HPV types. HPV16 was the most prevalent type (5.4% of all women). The next most common HPV types were HPV52 (3.9%), HPV31 (3.8%), HPV51 (3.4%), and HPV18 (2.4%). HPV16/18 was associated with 66% of CIN3 and 74% of cervical cancers, whereas HPV16/18/31/45 was found in 70% of CIN3 and in 82% of cancers (56% 16/18/31/45 alone, and 26% 16/18/31/45 together with other HR HPV types).

CONCLUSION. HR HPV is common in Danish women. Effective vaccines covering more HPV types may have a substantial preventive potential. HPV/age-relationship, HPV type distribution in women with normal cytology, ASCUS/LSIL/HSIL as well as HPV in relation to histological categories in this large cohort will be presented.

HUMAN PAPILLOMAVIRUS AND CHLAMYDIA TRACHOMATIS IN PILAGÁ INDIANS FROM ARGENTINA

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Background: Human papillomavirus (HPV) and Chlamydia trachomatis (CT) are very common sexually transmitted agents in northern Argentina. In this region inhabit a wide-spectrum of small aboriginal communities characterized by living far away from urban centers in very poor socio-economical conditions and with severe lack of appropriate facilities for primary sanitary assistance.

Objetive: The aim of this study was to determinate by PCR technique and for the first time the prevalence of CT and HPV in sexually active women belonging to the Pilaga ethnia.

Methods: A total of 227 women (10% of the total women population of the ethnia) were recruited from different aboriginal settlements between March 2005 and March 2007. Cervical exfoliated cells were collected from all subjects by sampling ecto- and endocervix using cytobrush. DNA was obtained by treating pellets with CTAB homogenization solution, extracted with chlorophorm:isoamyl alcohol method and precipitated with absolute ethanol. MY09/MY11 primers set was used for HPV detection and RFLP technique (according to Bernard et al) for HPV genotyping. CT DNA was detected using KL1/KL2 primers set.

Results: The overall prevalence for CT was 26.4% and for HPV was 48.9%. The prevalence of HPV infection was significantly higher in CT DNA positive (63.3%) than in CT DNA negative women (43.7%; OR: 2.22 / CI 95%=1.16-4.28 / p=0.009), which means a higher risk for viral infection in the second group.

Conclusion: Our data highlight a moderate but significant association between CT and HPV infections in female samples studied and the need for a better knowledge of the current impact of sexual transmitted infections, other than those included in the official notification requirements in Argentina; this may contribute towards a better management of physicians in their gynecological practices, particularly when assisting vulnerable populations.

P-06.33

TYPE-SPECIFIC HPV PREVALENCE AND CYTOLOGICAL OUTCOMES CORRELATIONS IN NUNAVUT, CANADA

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BACKGROUND: The Department of Health and Social Services in Nunavut, Canada is considering evidence to inform HPV vaccination and cervical cancer prevention. Preliminary data suggested a suboptimal match between circulating HPV types in Nunavut and types in the quadrivalent HPV vaccine. Nunavut has a young, mainly Inuit population of 30,000 that is sparsely distributed across 2 million square kilometers in 28 communities.

OBJECTIVES: 1) Determine the prevalence and distribution of HPV types in Nunavut and correlate type-specific infection with cytological outcomes as part of a surveillance program. 2) Provide evidence for decision-making regarding implementation of HPV immunization in Nunavut.

METHODS: Residual cervical specimens from routine Pap testing were tested for 45 HPV types using our in-house Luminex genotyping assay. A unique identifier linked HPV results to cytology. Type-specific HPV prevalence was analysed and correlated with abnormal cytology.

RESULTS: Results from 1116 women suggest a 34.1% prevalence of any HPV type, and a 24.4% prevalence of at least one oncogenic type. Prevalence of the vaccine types 16, 18, 6 and 11 were 7.3%, 2.1%, 1.6% and 0.7% respectively. HPV types 31 (4.3%) and 45 (2.3%) were more prevalent than type 18 in this population. 82.9% of women with low-grade squamous intraepithelial lesions (LSIL) and 100% of women with high-grade squamous intraepithelial lesions (HSIL) were infected with one or more oncogenic HPV types. The proportion of vaccine types HPV16 and/or HPV18 in women with LSIL and HSIL were 34.3% and 66.7%, respectively.

CONCLUSIONS: There is a high prevalence of HPV infection among women in Nunavut. HPV16 is the most common type, followed by HPV31. The proportion of oncogenic vaccine types is greatly increased in women with abnormal cytology. Results to date have informed evidence-based decision making regarding HPV immunization in Nunavut.

ORAL HUMAN PAPILLOMAVIRUS INFECTION IN HEALTHY INDIVIDUALS: A SYSTEMATIC REVIEW

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Introduction: Human papillomavirus type 16 (HPV16) is the cause of a subset of head and neck squamous cell carcinomas. We therefore aimed to determine the prevalence of oral HPV16 infection among healthy adults from the published literature.

Methods: We systematically reviewed and abstracted data from published studies that evaluated oral HPV infection in healthy, cancer-free subjects. We determined the pooled prevalence of HPV16, carcinogenic HPV, and any HPV and plan to evaluate study and laboratory methods as sources of variability in these estimates. Analysis of individual-level data (e.g.: gender, age, behavior) was not possible as such data were often not available in the literature.

Results: Sixteen eligible studies were identified; 1.8% (95% confidence interval [CI] 1.3-2.4%; range in individual studies: 0.2 to 14.0%) of 2495 healthy subjects had an oral HPV16 infection, 4.1% (95% CI 3.4 - 4.7; range in individual studies: 0.2 to 18.3%) of 3702 subjects had carcinogenic HPV infections, and 5.0% (95% CI 4.4 - 5.9; range in individual studies: 2.6 to 20.7%) of 3331 subjects were positive for any HPV. Analyses by specimen collection and laboratory methods are on-going.

Conclusions: A small but noteworthy proportion of healthy individuals are infected with HPV types known to cause cancer in the oral region; these data argue for large, well-designed studies aimed at understanding the natural history and epidemiology of oral HPV infection.

P-06.35 SEXUAL BEHAVIOUR IN AUSTRALIA/NZ AND IMPACT ON HPV VACCINATION

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Background: Characterisation of sexual behaviour is important for estimating the effectiveness of HPV vaccination programs in different settings.

Objectives: To characterise age of debut and annual partnerships in adults in Australia and New Zealand (NZ), and to examine the effect of any differences on the predicted impact of HPV vaccination.

Methods: Surveys of sexual behaviour published in the period 1990-2008 were reviewed. Age at sexual debut was compared between the two countries using a binary logistic regression analysis, and partnerships in adults were modelled using polytomous logistic regression. Covariates for each model included country, gender, age and year of survey. Predictions of the impact of HPV vaccination were based on a previously developed dynamic simulation model.

Results: Compared with Australians, NZ adolescents were significantly more likely to debut at each single year of age between 13-16 years (odds ratio 2.9-1.5). In contrast, differences between the two countries in the number of partnerships experienced by adults were not significant (P=0.5). Based on the differences in age of debut, the predicted prevalence of oncogenic HPV in 10-14 year old females was 2% in Australia and 9% in NZ. Assuming similar coverage in the two countries and routine vaccination of 12 year old females with catch-up performed in older females, vaccination will reduce the incidence of HPV 16 by 56% in Australia and 53% in NZ by 2010. However, if routine vaccination were to be delayed until age 15, the reduction would be 43% in Australia and 39% in NZ by 2010.

Conclusion: In both countries, routine vaccination starting at 12 years will be substantially more effective than delaying starting until 15 years. Although NZ adolescents debut earlier than their counterparts in Australia, the relative reductions in HPV incidence are expected to be comparable in the two settings.

HUMAN PAPILLOMAVIRUS IN WALES (UK): FROM PREVALENCE TO DISEAS

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Background: Few studies have investigated HPV infections associated with cervical cancer and the prevalence of HPV types in the same population. This represents a gap in our understanding of the aetiology of HPV infection and carcinogenesis. Whether an HPV infection will lead to cancer depends on many variables, including HPV type, host immunogenotype, age, genetics, epigenetics, and behavioural and environmental factors. The presence of cytological screening may also alter the spectrum of HPV types identified in cervical cancers. Many of these variables are population specific, so to achieve a coherent picture of HPV associated carcinogenesis requires studies in defined and stable populations, from prevalence to invasive disease.

Objectives: The objective of this study was to apply a single HPV typing methodology to both a national sample of cervical cancers, and a substantial unselected screening population, and so provide a coherent picture of HPV infection and disease in a cytologically well screened population.

Methods: The GP5/6+ PCR enzyme immunoassay was used to identify specific HPV types in 10,000 consecutive anonymous screening samples and in 453 archival cervical cancers (operated 2000-2006). Information on patient age, social deprivation, cytology and histology was also obtained. The results of these analyses were combined in a case control analysis of the risk of infection with specific HPV types.

Results: This analysis showed that the Odds Ratios (ORs) for cervical cancer associated with specific HPV types change dramatically with age. The OR for HPV16 and 18 in women aged >29 were 166.3 (95% CI 118.2–233.8) and 38.2 (95% CI 22.7–64.0) respectively; the OR for other oncogenic types were in general at least 10 fold less (ranging from 2.0 to 13.8 in the same group).

Conclusions: These results would support genotype specific testing for HPV16 and 18 in women aged >29 years.

P-06.37

ASSOCIATION OF POLYMORPHISM-1082 A/G (IL-10) WITH HUMAN PAPILLOMAVIRUS CERVICAL LESIONS

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Background: Women with cervical lesions caused by HPV infection have a higher chance of carrying genetic polymorphisms associated to the elevated production of cytokines, such as interleukin 10 (IL-10). Studies suggest that the individual capacity of producing more elevated levels of IL-10 may be related to the development of cervical cancer. Objectives: To verify the association of different degrees of cervical lesions with alleles observed at the promoter region (-1082) of the IL-10 gene in women infected by HPV. Methods: A case-control study was carried out including 84 cases and 211 controls. Cases were defined as women HPV positive (PCR) and with abnormal cervical histopathology results. Controls were women at same age groups as cases, HPV negatives and with normal cervical cytology results. ARMS-PCR was used to identify the IL-10 polymorphism. Results: The genotypic frequency observed in the group of patients was 0.12 (AA), 0.29 (AG), 0.59 (GG); the allelic frequency was 0.26 for "A" and 0.74 for "G". In the control group, the genotypic frequency was 0.22 (AA), 0,49 (AG), 0.53 (GG); the allelic frequency was 0.47 for "A" and 0.53 for "G". There was a significant difference between the studied groups for both, allelic and genotypic frequencies (p<0.0001). In the present study, the prevalence of high risk HPV (HR-HPV) infection was 23.6% for HPV-16, 15.3% for HPV-31 and 9.7% for HPV-18. Women positive to HPV-16, HPV-18 and HPV-31 presented the allele "G" in 94%, 86% and 73% of the samples, respectively. Also, 86% of HSIL and 60% of LSIL carry the "G" allele. Conclusion: The results suggest that the allele "G" seems to be more expressed in patients with HR-HPV infections and also with HSIL.

INCIDENCE AND PREDICTORS OF HPV-6/11/16/18 INFECTION IN YOUNG NORWEGIAN WOMEN

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Background: Long-term efficacy evaluations of quadrivalent HPV-6/11/16/18 vaccine are ongoing in Nordic countries. Regional data are needed for historical comparison.

Objectives: To report on incidence and predictors of HPV-6/11/16/18 infection.

Methods: A 4-year prospective observational study enrolled Norwegian women aged 16-24 years from 1998-2000. HPV-6/11/16/18 DNA PCR of 3 genital tract sites and serology testing was performed every 6 and 12 months, respectively. Sociobehavioral and clinical data were collected every 6 months. HPV incidence was studied for each HPV type, and for 6 or 11, 16 or 18, and all 4 types among women HPV DNA PCR- and seronegative for the type(s) at baseline. Cumulative incidence was estimated using the Kaplan-Meier method. Baseline and time-varying covariates were evaluated in Cox models; 2-sided testing at 5% significance level.

Results: Of 898 volunteers enrolled, 860 had HPV PCR results at entry and during follow-up. Four-year cumulative incidences (95% confidence interval) were 20.0% (17.1-23.4%), 2.2% (1.3-3.5%), 25.0% (21.7-28.8%), 13.6% (11.3-16.4%), 21.5% (18.5-25.0%), 30.4% (26.7-34.5%), and 37.8% (33.6-42.3%), for HPV-6; 11; 16; 18; 6 or 11; 16 or 18; and all four types, respectively. Multivariable models indicated that being single, having no regular partner, a greater number of lifetime partners, sexual intercourse \leq 4 times/mo, and Chlamydia PCR positivity predicted increased risk of HPV-6, 11, 16, or 18 incidence. Despite overlapping information in marital/partner status and sex frequency, these covariates remained significant independent predictors. Drinking, smoking, and age at first intercourse were predictive in univariate, but not multivariable models. Risk factors were generally consistent in HPV type-specific analyses.

Conclusions: Factors measuring new partners and Chlamydia infection were associated with risk of incident HPV-6/11/16/18 infection. Incidence was low for HPV-11, but high for HPV 6, 16, and 18, indicating potential for HPV vaccination to have significant impact in this population.

P-06.39

HPV TESTING IN CYTOLOGICALLY NEGATIVE WOMEN ATTENDING AN OUTPATIENT CLINIC

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BACKGROUND: A substantial number of high-grade cervical lesions are missed by cytological testing. Adding hrHPV testing to cytology improves the efficacy of cervical screening in a population based screening setting, but it remains to be elucidated whether this also holds for an outpatient population.

OBJECTIVES: To study the additive value of hrHPV testing in a population of women visiting the gynaecological outpatient clinic of a university medical centre.

METHODS: Cervical samples of 1166 women who underwent cervical cytology testing in the gynaecological outpatient clinic of the VU University Medical Center in Amsterdam, between January 1st and July 1st 2007, were also tested for hrHPV using the GP5+/6+-PCR test. History and follow-up data were retrieved passively from the Dutch nationwide network and registry of histo- and cytopathology (PALGA) and the hospital information system and actively through recall of patients. In this abstract we focus on women with normal cytology and a positive hrHPV test.

RESULTS: Of all included women 1016 (87.1%) had normal cytology, of whom 153 (13.1%) were hrHPV positive (median age 34.0 years, range: 18-80). Until now, cytological and/or histological follow-up data are available for 81 (53.0%) of these women. 40 Women (26.1%) were passively followed-up of which in eight cases histology was available. The histological abnormalities comprised three CIN1, two cGIN2 and one CIN3 lesion. 41 Women (26.8%) were actively followed-up of which in 26 cases histology was available. The histological abnormalities in this group comprised one CIN1, two CIN2 and two CIN3 lesions.

CONCLUSION: These data suggest that a clinical population with normal cytology is at risk for significant cervical lesions if the hrHPV test is positive.

PREVALENCE OF DIFFERENT HPV TYPES AND ESTIMATION OF PROGNOSTIC RISK-FACTORS

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Objectives: To evaluate the prevalence and risk factors of HPV

among women who attended the gynecological outpatient clinics of three hospitals of Athens.

Methods: AMPLICOR HPV TEST and LINEAR ARRAY HPV GENOTYPING TEST (LA) (ROCHE) were used for the detection HPV DNA. The data analysis was based on LA Test. Multiple logistic regression was used to identify independent prognostic factors for HPV positivity.

Results: The overall prevalence of HPV DNA was 49.1% (95% CI: 43.5%, and 54.7%). The prevalence (95% CI) of high risk HPV types was 30.3% (25.3%, 35.7%).Predominant types were HPV-6 (12.2%) and HPV-16 (10.0%). From multiple logistic regression analysis, the risk of HPV infection was higher among women with multiple lifetime sexual partners (>3 vs. 1: OR=3.1, 95% CI: (1.5, 7.2)), Pap smear findings (LGSIL/HGSIL vs. negative: OR=2.8, 95% CI: (1.2, 6.5)), presence of warts(yes vs. no: OR=3.0, 95% CI: (1.5, 6.3)) and no history of deliveries (no vs. yes:OR=2.6, 95% CI: (1.0, 6.7)). The risk of HPV infection from carcinogenic genotypes was higher among younger women (OR for 1-year increase in age=0.93, 95% CI:(0.89, 0.98)), among women with multiple lifetime sexual partners (>3 vs. 1:OR=2.9, 95% CI: (1.2, 7.2)) and Pap smear findings LGSIL/HGSIL vs.

negative:OR=5.6, 95% CI: (2.2, 14.5).

Conclusions:

The prevalence of HPV in our study group was 49.1%. Prognostic risk factors for the detection of any HPV were the presence of genital warts, multiparity, the LGSIL/HGSIL finding in Pap-smears and the absence of births while the age was an additional prognostic risk factor for high risk types.

P-06.42

HPV TYPES AND OTHER RISK FACTORS OF CIN IN KOREA

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Purpose: HPV types 16 and 18 are clearly involved in the etiology of cervical cancer and HPV-16/18 vaccine was already introduced into clinical settings. To evaluate the associations of HPV types and other associated cofactors with CIN, we performed a hospital-based, prospective case–control study in the Catholic University of Korea.

Methods: A total of 190 women were examined gynecologically and information on risk factors was obtained by personal interview with a standardized, pre-tested questionnaire. These study was divided into 80 women without CIN pathology at the St Paul's Hospital and 110 women diagnosed by CIN at the Kangnam St. Mary's hospital. Cervical cytology was prepared with a liquid-based smear and HPVs were diagnosed and typed by the HPV DNA Chip. Odds ratios were calculated by simple logistic regression test to assess each risk factors associated with CIN and after that, multiple logistic regression test was done.

Results: The most frequet HPV types were HPV-16, HPV-58, HPV-51, HPV-35 in the CIN group. The odds ratios of three major risk groups on phylogeny as follow; group A9 (HPV-16, HPV-31, HPV-33, HPV-35, HPV-52, HPV-58) for CIN was 108.6 (95% confidence interval [CI]: 34.7-340.1, p <0.05) and most closely related to CIN. The odds ratio of group A7 (HPV-18, HPV-39, HPV-45, HPV-59, HPV-68) and A6 group (HPV-53, HPV-56, HPV-66) were 18.7 and 30.6 (95% CI: 9.7-96.4, p <0.05). Among the variables, early sexual debut, unmarried state and low parity were significantly associated with CIN by simple logistic regression test (p <0.05) but mode of delivery, menarche age and smoking did not have significant relations.

Conclusions: As has been shown, HPV A9 group infection was the most important risk factors of CIN. In clinical characteristics, such as early sexual exposure, unmarried state and low parity were significant cofactors in CIN women.

TYPE SPECIFIC PREVALENCE OF ONCOGENIC HPV TYPES IN TELEMARK, NORWAY.

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In Norway HPV testing is used for secondary screening where cytology is equivocal, unsatisfactory or LSIL. HPV testing and repeat cytology is performed 6 months after the precipitating cytological diagnosis. HPV positive women are rescreened after a further 6 months; a new finding of the same HPV type is regarded as presumptive persistence requiring colposcopic follow-up.

In the period July 2003 - October 2007 1470 cervical samples from the Telemark region in Southeastern Norway were tested and genotyped for oncogenic HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 using L1 consensus PCR and reverse lineblot (the PapType 13 test). The most prevalent type was HPV16 (16%), followed by HPV31 (8%), HPV18, 33, 39, 45 and 51 (4.5 - 6%), HPV 35, 52, 56, 58 and 59 (2 - 3%) and HPV68 (1.1%). Prevalence of all types declined with age, with the exception of HPV51, 58 and 59. Prevalence declined with age for all types except HPV types 51, 56 and 58. 61% of infections persisted for at least six months; 39% resolved or were cured.

P-06.44

SEROPREVALENCE OF HUMAN PAPILLOMAVIRUS 16 INFECTION AMONG TEENAGERS IN CHINA

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A population-based seroprevalence study of human papillomavirus (HPV) 16 infection was carried out among two groups with teenagers in Shanxi Province, P. R. China during 1998-1999. Group 1 included 235 boys and 239 girls in Senior Middle School of Xiangyuan County. The mean age was 16.5 years. The HPV 16 seroprevalence was 4.3% and 10.5% among boys and girls, respectively. Group 2 included 322 boys and 360 girls in Taiyuan University. The mean age was 20.0 years. The HPV 16 seroprevalence was 6.5% and 7.2% among boys and girls, respectively. In one year, follow-up was carried out to all the boys and girls in both groups. The HPV 16 seroprevalence was 6.4% and 10.0% among boys and girls in Senior Middle School of Xiangyuan County, 7.8% and 6.1% among boys and girls in Taiyuan University, respectively. These results indicated that most of Chinese people, both men and women, started to carry HPV 16 infection persistently if they obtained the infection during their teenage period.

HLA CLASS II IS ASSOCIATED WITH CERVICAL INTRAEPITHELIAL NEOPLASIA

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Background: Previous studies suggested that continuous infection of oncogenic human papillomavirus (HPV) is necessary for progression of cervical HPV lesions, and host immune response to HPV is thought to be a very important factor for prognosis of the lesion.

Objectives: To evaluate the role of variation in human leukocyte antigen (HLA) expression in cervical cancer pathogenesis.

Methods: A total of 76 Japanese women who had histologically diagnosed cervical intraepithelial neoplasia (CIN) 1 or 2 were followed-up for more than six years. HPV detection and typing were performed by a GP5+/GP6(+)-PCR-based method. Human leukocyte antigen (HLA) DQ and DR expressions were examined by Terasaki-NIH standard method. The patients underwent cytological and colposcopic examinations every four months. We defined a progressive disease as a lesion that developed CIN 3. According to the findings during follow-up, the 76 patients were classified into three groups with progressive, persistent, and regressive disease. Relative risks (RR) of progression were estimated by χ 2test.

Results and Conclusions: There was progression to CIN 3 in 21 patients (27.6%). Oncogenic HPVs detected in 18 of 21 patients with progressive disease. The patients with HLA DQ6 or DR9 were 3.86 times more likely to progress to CIN 3 than those without these types of HLA (p=0.0082). DQ3 was associated with a decreased risk of progression (RR=0.325, p=0.018). In patients with DR4, HPV 16 positive CIN were significantly rare (RR=0.824, p=0.048), and lesions with HPV 52 or 58 were 5.77 times more likely to remain (p=0.018).

Certain types of HLA have a strong influence upon the prognosis of cervical HPV lesions. Geographical difference of prevalence of HPV may depend on the difference of frequency of HLA.

P-06.46

VACCINE-RELATED HPV GENOTYPES IN INVASIVE CERVICAL CANCER IN ASTURIAS (SPAIN).

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BACKGROUND: Prophylactic vaccines against Human Papillomavirus type 16 and 18 are already available for public use in Spain. HPV vaccination will reduce the number of women who require colposcopy, biopsy and treatment for precancerous cervical lesions and cervical cancer. However, there is limited information as to the distribution of HPV genotypes in women with cervical cancer in Asturias (north of Spain). OBJETIVE: To determine the most common HPV types in Asturias to be able to evaluate the impact of implementing HPV vaccines in this population. METHODS: 234 paraffin embedded tissue blocks of invasive cervical cancer samples diagnosed between 1998 and 2008 in all hospitals in Asturias were included. The Human Papillomavirus detection and genotype was determined by SPF-10 broad-spectrum primers PCR subsequently followed by DEIA and genotyping by LiPA25 (version 1). In each sample, the human b-globin target gene determination (209 bp fragment) was used as a guide for specimen adequacy. The detection and typing was performed at the facilities of ICO (Catalan Institute of Oncology), Barcelona, Spain. RESULTS: HPV/DNA was detected in 207(88.5%) of the 234 cervical cancer samples analyzed. Single infections were identified in 191 samples (92.3%) of the HPV positive cases and multiple infections in 16(7.7%). The five most common types detected as single types were HPV16 (60.9%), HPV18 (6.8%), HPV45 (4.8%), HPV33 (4.3%) and HPV31 (3.9%). HPV16, 18 and both types combined accounted for 74.9% of the HPV positive cases. CONCLUSION: The introduction of an efficacious HPV16/18 vaccine could potentially prevent the occurrence of 68% of cervical cancer cases and up to 76% of the cases if full cross-protection against HPVs 31 y 45 is assumed.

ROLE OF HPV INFECTION IN THE OUTCOME OF IN-VITRO FERTILIZATION

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Background: Sexually transmitted infections are of major concern in the field of reproductive medicine. Even though human papilloma virus (HPV) is one of the most common genital infections, little is known as to a possible relationship of HPV and in-vitro fertilization (IVF).

Objective: To evaluate the prevalence of HPV infection in infertile couples in relation to parameters and outcome of IVF. Method: A total of 104 couples (women: mean age 34.7 years; men, mean age 38.0 years), undergoing infertility treatment were evaluated. Causes of infertility were female (24.1%), male (58.6%), couple (6.8%) and idiopathic infertility (10.5%). Parameters of IVF examined were age of the women, number of oocytes, number of mature oocytes, number of embryos transferred and pregnancy rate. Cervical samples and semen were collected before oocyte harvesting and examined for HPV presence by using the HPV INNOLiPa assay (Innogenetics). Women were treated with standard ovulation induction protocols; pregnancy was defined as the presence of fetal heart beat. The chi-square test and adjusted odds ratio (OR') with confidence interval (95%CI) from a logit model were used for data analysis.

Results: HPV infection was diagnosed in 18 (17.3%) women and 8 (7.7%; p=0.04) men, with a total of 22 (21.1%) couples HPV infected. HPV-16 and HPV-66 were the most prevalent types. No difference was evident between HPV-positive and HPV-negative couples in terms of causes of infertility and IFV parameters of (p=0.20). Pregnancy was obtained in 7/22 (31.8%) HPV infected and 24/82 (29.2%) uninfected couples (p>0.05). HPV detection was not associated with IVF failure (OR'=0.91; 95%CI:0.28-2.79); failure of treatment was independently associated with age of the women (OR'=3.08; 95%CI:1.11-8.53) and number of embryos transferred (OR'=0.26; 95%;CI:0.07-0.91).

Conclusions: Preliminary data from the present study would not indicate any association between HPV infection and IVF outcome. Analysis of additional couples is currently underway.

P-06.48

POLIMORPHISM-1082 A/G IL-10: ASSOCIATION WITH HPV INFECTION AND EPIDEMIOLOGICAL CORRELATES

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Background: The genetic polymorphisms of IL-10 have been implicated in the susceptibility for neoplasic development. In HPV infections, IL-10 may be associated to the elimination of the virus or to its persistence and progression to cervical lesions. Objectives: To verify the frequency of the polymorphism present at the promoter region (-1082) of the IL-10 gene, relating such findings with social and behavioral characteristics of the studied population. Methods: A case-control study was carried out including 84 cases and 211 controls. Cases were defined as women HPV positive (PCR) and with abnormal cervical histopathology results. Controls were HPV negatives women with normal cervical cytology results. ARMS-PCR was used to identify the IL-10 polymorphism. Results: Cases were, on average, younger than controls [36 years (17-72) vs. 44 (16-73)], they had the first sexual intercourse at younger age[18 years (11-31) vs. 19(13-39)], and also, they had a higher number of sexual partners [6(1-90) vs. 3 (1-10)]. There was a significant difference between the groups regarding the variables age, age at first intercourse, number of sexual partner (p<0.0001). Among cases, it was observed a significant association between the "GG" genotype and LSIL. It was also observed a significant association between women with HPV infection who had more than 2 deliveries with the presence of allele "G" (AG and GG), p=0.04. There was a significant difference between the studied groups for both, allelic and genotypic frequencies (p<0.0001). Conclusion: The results suggest that the allele "G" seems to be more expressed in patients with higher number of deliveries, HPV infections and also with LSIL.

INTERLEUKIN-6 AND IL-10 IN WOMEN WITH PERSISTENCE OF DNA-HPV INFECTION

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Background: Studies suggest that the individual capacity of producing more elevated levels of IL-10 may be related to the development of cervical cancer. Objective: To identify the role of Interleukin-6, IL-10 and epidemiological correlates in women with persistence of DNA-HPV. Methods: Nested case–control study that enrolled 88 women in Porto Alegre city, southern Brazil. Cases were women persistently infected with HPV(n=34), and controls were asymptomatic women negative for HPV DNA(n=54). Face-to-face interviews, cervical specimens and blood samples were collected at enrolment. The outcome was persistence HPV infection. A multiple logistic regression analysis was performed to estimate adjusted odds ratios with 95% confidence intervals.

Results: The analysis showed that age at first intercourse below 20 years (OR=19.65; CI95% 2.43-68.85), four or more sexual partners during lifetime (OR=5.67; CI95% 1.28-24.99), women with a previous altered Pap smear (OR= 10.17; CI95% 1.80-57.33), being single (OR= 12.94; CI95% 2.43-68.85) and IL6 3.106 pg/ml were associated with persistence of HPV infection. IL-10 was not associated with HPV persistence. Conclusion: These results support the sexual transmission of HPV and suggest that persistent infections with the same high risk-HPV type are more likely to progress towards cervical neoplasia. Some other risk factors, like IL-6 levels, may have additional prognostic value.

P-06.50

MULTIPLE HIGH-RISK HPV INFECTION IN CERVICAL INTRAEPITHELIAL NEOPLASIA

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Objectives; To evaluate the incidence and pattern of multiple high-risk HPV infection in patients with histologically verified cervical intraepithelial neoplasia (CIN).

Methods; This prospective study included 158 consecutive patients who underwent loop electrosurgical excisional procedure (LEEP) due to abnormal papanicolaou smear. All patients underwent HPV test with commercialized HPV DNA chip (MyHPV chip*, MyGene corp., Seoul, Korea) just before LEEP. The incidence and pattern of multiple HPV infection were analyzed.

Results; A total of 106 patients had CIN (CIN 1 in 39, CIN 2 in 15, CIN 3 in 63) and 9 patients had invasive cervical cancer. Of 106 patients with CIN, 98 patients had high-risk HPV infection and the most common 3 types of high-risk HPV were 16, 18, and 58 in order of frequency. Mutiple high-risk HPV infection was found in 44 patients (45%) (double infection in 35, triple infection in 8, and quadruplet infection in 1). The rate of multiple high-risk HPV infection was not different by CIN severity.

Conclusion; Multiple infection was frequently found in patients with histologically verified CIN. However, it was not related to the CIN severity.

HPV DISTRIBUTION AND PREVALENCE IN BRUSSELS WOMEN BEFORE HPV-VACCINATION PROGRAM

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Data regarding HPV prevalence and type distribution in women are necessary to assess the potential impact of the HPV vaccines and this before implementation of large-scale vaccination programs. Therefore, we conducted in Brussels an agestratified epidemiological study to determine the prevalence of HPV infection in samples with different grades of cervical neoplasia as determined by cytology and histology.

We determined the HPV prevalence in women, aged 25-65 years, enrolled between January 2006 and December 2008. We collected > 1000 liquid-based cytological and histological samples with a normal cytology or a diagnosis of ASCUS, LSIL, HSIL and cervical cancer. PGMY09/11 consensus PCR revealed that HPV could be detected in \pm 9 % of the women with a normal cytology. The HPV prevalence increased as the severity of the cervical lesion also increased (\pm 30.5 % in ASCUS, \pm 54.5 % in LSIL and > 90 % in HSIL samples).

Among HPV positive samples and independently of cytological diagnosis, HPV-16 was the most common type (\pm 23 %). Although less frequent, HPV-31, -51 and -52 were relatively common (prevalence range: \pm 11.5 – 15.5 %). HPV-18 was found in \pm 8.5 %. Overall, HPV-16 or -18 is responsible for \pm 61 % of cervical cancer cases. One could conclude that the anti HPV-16 and -18 quadrivalent and bivalent vaccines that are now available will prevent around 60 % of the cervical cancers in Belgium.

P-06.52

SEROPREVALENCE OF HPV16 INFECTION AMONG NEWLY MARRIED COUPLES IN CHINA

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A seroprevalence study of human papillomavirus (HPV) 16 infection was carried out among 96 pairs of newly married couples before and one year after their marriage in Changzhi City, Shanxi Province, P. R. China. The mean age was 25.0 years for husbands and 23.4 years for wives. Before their marriage, the HPV 16 seroprevalence was 7.3% and 8.3% among husbands and wives, respectively. One year after their marriage, follow-up was carried out to all the huabands and wives. The HPV 16 seroprevalence increased to 9.4% and 12.5% among husbands and wives, respectively. These results indicated that most of young Chinese people obtained new infection of HPV 16 after they just started to have the sexual life.

HPV-TYPE PREVALENCE IN CENTRAL AND SOUTHERN ITALY IN HEALTHY WOMEN.

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Background: In Italy the HPV vaccine is free for twelve years old girls and mass vaccination campaigns are starting now in all the Italian regions. The Italian Ministry of Health financed a set of studies aimed at describing the pre-vaccine epidemiology of HPV.

Objective: to describe the type specific prevalence of HPV infection in the general population in central and southern Italy.

Methods: about 4000 consecutive women aged 25-64 years attending to 10 cervical cancer screening programmes in 5 Italian regions have been tested for HPV infection with HCII low and high risk probe, HPV detection was done through amplification of HPV DNA by GP5+/GP6+ primers PCR subsequently followed by genotyping with RLB. The HPV-negative samples were retested by LIPA (version2, Innolipa, Innogenetics) and with E6/E7 HPV type-specific primers. The HPV-positive samples but un-typed were sequenced.

Preliminary results on 3128 women: the prevalence of high and low risk HPV infection is 9.8% and 3.6% respectively. The prevalence is higher in the 25-30 years old, 23.7% and 12.5% for high and low risk respectively, and decreases constantly with age reaching the 2.5% and 2.5% for high and low risk respectively at 60-64.

The preliminary results from genotyping shows that the most frequent types are: HPV16 (17.3%), HPV31 and 66 (15.4%), and HPV56 (11.5%). The prevalence of co-infections was 1.5%.

Conclusions: despite southern Italy has a lower cervical cancer incidence than norther Italy, the prevalence in general population of high risk infection is slightly higher. This suggests a situation in rapid transition probably due to different coverage of screening and to sexual behaviours changes.

P-06.54

HPV-TYPES UNDERLYING CIN2, CIN3, AIS, INVASIVE CERVICAL CANCER IN ITALY

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Background: in 2008 the Italian Ministry of Health launched the HPV mass vaccination for the 11-years old girls. As a propedeutic action the Ministry financed a set of studies aimed at assessing the pre-vaccine HPV epidemiology. All the studies used the same methodology.

Objective: to identify the HPV types underlying Cervical Intraepithelial Neoplasia (CIN) grade 2, 3, Adenocarcinoma In Situ (AIS) and invasive cervical cancer in central and southern Italy.

Methods: the electronic databases of the pathology units of eight centres in six Italian regions (Tuscany, Sicily, Sardinia, Lazio, Abruzzo and Campania) were collected. All the CIN2-3, AIS and invasive cervical cancer were identified. The glandular lesions (invasive and in situ), the non-Italian cases and the invasive cancers were all sampled, while only half of CIN2-3 were sampled. Paraffin embedded cases were collected from historical archives according to a standardized protocol. HPV detection was done through amplification of HPV DNA by GP5+/GP6+ primers PCR subsequently followed by genotyping with RLB. HPV-negative samples were retested by LIPA (version2, Innolipa, Innogenetics) and with E6/E7 HPV type-specific primers. Un-typed HPV-positive samples were sequenced. Samples were tested in two laboratories with common quality controls.

Results and conclusions: Totally 1000 cases were selected, 60% were retrieved. Preliminary date indicate that the three most common types detected in cervical cancer were HPV16, HPV18 and HPV45, while in non-invasive lesions (Cin2-3) the most common type detected in cervical cancer was HPV16, followed by HPV18, 31, 33. We found 4,5% and 8,6% HPV co-infection in invasive and in CIN2-3 lesions respectively (all with HPV16).

HPV16 and HPV18 are consistently the most common HPV types in cervical cancer and CIN2-3 in Italy, followed by HPV45, 31 and 33.

PREVALENCE AND ASSOCIATED FACTORS TO HPV-16 IN SOUTHERN BRAZIL

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Background: Cervical cancer is the second type of cancer more frequent among women and is responsible for more than 230 thousand deaths each year. Its incidence in less developed countries is twice the one observed in developed countries. Human papillomavirus (HPV) infection is a necessary condition for the development of cervical cancer, being the most frequently observed the HPV-16 and HPV-18. Objective: To verify the prevalence of HPV-16 in relation to other HPV types and to identify the factors associated with such infection. Methods: A cross-sectional study was performed enrolling 1461 non-symptomatic women in the city of Porto Alegre, southern Brazil. Women answered a standardized questionnaire, which include questions about demography, reproductive and behavioral factors. A cervical sample was collected to perform a HPV-DNA test (PCR) using primers My09 (5'-CGT CCA/C AAA/G GGA A/TAC TGA TC-3') and My11 (5'-GCA/C CAG GGA/T CAT AAC/T AAT GG-3'), and specific primers for HPV types. Multiple Logistic Regression was performed to verify the factors associated to HPV-16 infection. Results: The mean age and mean age at first intercourse were 42 ± 14 and 19 ± 4 , respectively. About 83% of the women had at least 1 delivery, the majority (73%) reported only 1 partner and 71% had at least 3 partners during their life. A total of 17% had never performed a Pap smear. The overall HPV prevalence was 25.3%, being the HPV-16 the most frequent type observed (21%), followed by the HPV-31 (14%). The HPV-16 infection associated factors were having a partner with genital condiloma (OR=3.55;95%CI:1.18-10.6) and having an abnormal Pap test (ASCUS or more)(OR=4.30;95%CI:2.09-8.85). Conclusion: The results suggest that having a partner with infections by low risk HPV types may facilitate the development of HPV-16 in women. Also, it was observed an association HPV-16 with cytological findings reinforcing the relevance of a screening program.

P-06.56

HPV PREVALENCE AND DISTRIBUTION IN NORTH OF ISRAEL

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Background: Many studies describe the distribution of HPV infections in women with ASC-US, CIN 1or CIN 2-3 lesions.

Objectives: To describe the distribution of HPV types in Haifa district in women with ASC-US on Pap smear or CIN 1, CIN 2-3 on cervical histology.

Methods : We conducted a multicentral prospective evaluation of HPV typing in all consecutive patients referred to the colposcopic clinics .

HPV viral sequences were identified by nested PCR using L1 consensus primers that enable the amplification of the majority of HPV and sequence analysis.

Results: 867 samples were evaluated of these, 487 (55.9%) were HPV positive. HPV 16 was the commonest virus reaching 26.2% of the cases, multiple HPV types were found in 10.9%, HPV 66 in 10.1%, HPV 6 in 6.4% of the evaluated women. The incidence of HPV 18 and HPV 11 was low (2.5%, 1.4% respectively) 46.5% of the 213 ASC-US cases were found to be positive for HPV. 27% were positive for HPV 16, and 14%, were positive for HPV 66,.

In 106 women with CIN 2-3 HPV 16 was found in 42.4% ,HPV 31 in 11.9% , HPV 18 in 2.5% and 2% were negative for HPV.

43% of the women with CIN1 were positive for high risk HPV types.

Conclusions: In the Northern part of Israel HPV 16 is the most prevalent virus, but multiple HPV infections and HPV 66 is found in 10.9% and 10.1% respectively.

HPV typing may have a role in the triage of women with ASC-US or CIN 1.

HPV vaccine has a long term potential to prevent approximately 45% of CIN 2-3 lesions in the Israeli population.

A LONGITUDINAL STUDY OF HPV INFECTION IN ADOLESCENT WOMEN

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Background: Our prior studies showed nearly all sexually active (SA) adolescent women (AW) became HPV-infected over 27 months. We performed a study with longer follow-up to provide insight into behaviors and other factors influencing HPV detection and persistence.

Objectives: Describe initial findings of HPV infection in AW followed for seven years.

Methods: AW, ages 14-17, were enrolled in a longitudinal study. Subjects were interviewed quarterly for behavioral risk factors, and a self-vaginal swab for HPV was obtained. All swabs were examined for HPV by the Roche Linear Array Assay for 37 types. We report findings from the 20 AW with the longest follow-up.

Results: At enrollment, average age was 14.9 years (range 14-16; SD 0.8). Fourteen subjects were SA initially (all 20 by study end), many with multiple partners. Mean duration of follow-up was 6.9 years (range 4.4-8.9; SD 1.2). A mean of 26.2 (SD 4.1) swabs per subject were obtained; 95.5% were adequate based on β -globin positivity. All subjects had at least one quarterly swab positive for any HPV and at least one oncogenic type. Mean number of types per subject was 12.9 (range 5-21; SD 4.8). Mean number of oncogenic types was 7.6 (range 2-13; SD 3.0). The most frequently detected oncogenic types were HPV 16, 59, and 66; the most common non-oncogenic type was HPV 6.

Conclusions: Frequent and prolonged testing found an extremely high prevalence of HPV (both oncogenic and nononcogenic) in this SA cohort of AW. Further analysis will provide insights into the influence of behavioral factors and other STIs on HPV detection and persistence.

P-06.58

HPV AND FERTILITY: MORE QUESTIONS THAN ANSWERS

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Background: The negative impact of sexually transmitted infections (STI) on fertility has long been recognized. However, the exact role of the most frequent STI, human papillomavirus infection, is not well understood.

Objective: We will present a review of the published data on the potential impact of HPV infection on various aspects of reproductive health.

Results: Despite the high prevalence of HPV infection in reproductive age couples and anecdotal reports of its deleterious effects, there is a paucity of data on the impact of HPV infections on fertility outcomes. Most studies are small. Definition of HPV infection is heterogeneous. Most studies focus on populations undergoing infertility treatments. HPVs can be found in fresh semen and in washed sperm cells and express oncogenes. Some have reported an increase in asthenospermia, while others have observed an increase in velocity. HPV infection may reduce pregnancy rates following in vitro fertilization treatment and may increase sponteneous abortion risk. However, some investigators have found no effect of HPV infection on reproductive health indicators.

Conclusion: Larger studies including proper comparison groups and relying on valid HPV detection techniques are urgently needed to better understand the impact of HPV infection on fertility.

CENTERS FOR DISEASE CONTROL AND PREVENTION

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Background: Monitoring the impact and effectiveness of HPV vaccine in the U.S. will allow evaluation of the duration of protection, health disparities in implementation, and potential changes in HPV epidemiology. The sustainability of a vaccine program may depend on measuring outcomes of the program. We describe initial plans to comprehensively monitor HPV vaccine impact in the U.S.

Objectives: To describe HPV vaccine monitoring activities on biologic and clinical outcomes in the U.S.

Methods: We describe the background on decision-making for outcomes to target for surveillance, describe the plans to monitor these principal outcomes, describe the status of the evaluations, and the challenges encountered to date.

Results: To measure impact of the HPV vaccine, we targeted the outcomes of type-specific HPV infection, genital warts, cervical cell abnormalities, and HPV-associated cancers. Type-specific HPV prevalence is currently monitored by self collected vaginal swabs in a national population based survey. Genital warts will be monitored through a network of STD clinics and billing data. Projects to monitor precancers have been initiated, some of which will include typing of lesions and central histology review through a web-based virtual slide archive. Use of billing data to track precancers and other HPV related outcomes are being investigated. Finally, existing cancer registries will provide a framework for monitoring cervical cancer, precancers, and other HPV related cancers, as well as HPV types contributing to cancers.

Conclusions: A wide range of surveillance activities have been initiated in the U.S. to measure the impact of the HPV vaccine on select clinical outcomes. We anticipate that the variety of these activities targeting different outcomes will allow for a comprehensive evaluation of the impact of the HPV vaccine in a dynamic environment. Baseline feasibility evaluations to determine long term viability for specific monitoring programs will be useful.

P-06.60

GENITAL HPV CIRCULATION AMONG YOUNG AND MATURE WOMEN IN ALBANIA

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Background: HR-HPV types prevalences may vary in different regions. Although the precise description of viral types circulation is a key feature in screening and prevention campaigns, accurate data are only available about a limited number of areas all over the world, mostly concentrated in western Europe and north America

Objectives: To provide data about HR types circulation among young and mature women in Albania for the organization of dedicated screening campaigns and vaccine programmes.

Methods: young women (aged 18-30) and mature women (above 30) living in the Tirana and Durres districts (Albania) asking for routine gynaecological counselling were invited to participate to the study. Patients yielding written informed consent with no other reason for seeking medical advice and no history of neoplastic disease were enrolled. Patients where administered full gynaecological visit and cervico-vaginal exfoliated cell samples were taken for virological and cytological assay. Histological evaluation and colposcopy were administered according to the gynaecological judgement based on current criteria. HPV detection was performed by MY09/MY11 amplification followed by a nested amplification with the GP5+/GP6+ primers. Viral typing was revealed by direct PCR products sequencing. Young and mature patients were analysed separately and the results compared with data of other geographical areas type

Results: a total of 400 women were evaluated for viral infection and for cytological alteration. The global Viral types prevalences among Albanian people as well as the prevalences among young and mature women will be reported and discussed

Conclusions Cervical cancer prevention and screening is a major concern for European governments and population. This work aims to outline the HR HPV circulation in Albania, a country interested by an intense migratory efflux and soon expected to gain an increasingly relevant position in European trade and peoples exchange.

THE ASSOCIATION OF HPV AND CHLAMYDIA TRACHOMATIS IN CERVICAL NEOPLASIAS

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Background: The significance of the association between the human papillomavirus (HPV) and other sexually transmitted infections in the development of cervical neoplasias has been investigated and the more consistent data have pointed to an association with Chlamydia trachomatis (CT). Objective: The objective of this study was, therefore, to evaluate positivity for CT in HPV-positive women with cervical intraepithelial neoplasia (CIN) and cervical carcinomas. Methodology: A total of 126 women referred to the Santa Casa de Misericórdia in Goiás, Brazil because of an abnormal cervical smear were included in the study. HPV DNA was detected by polymerase chain reaction and genotyping by reverse dot blot hybridization. Positivity for CT was evaluated using ELISA for the detection of IgG antibodies. Results: The total prevalence of HPV infection was 85.7% (108/126), and 94.4% of these cases (102/108) were related to high-risk oncogenic types. Multiple infections represented 50% (51/102) of all cases. Positivity for CT was 26.2% (33/126). Thirty women (23.8%; 30/126) tested simultaneously positive for CT and HPV and in 96.7% of these cases (29/30) these infections constituted a high-risk oncogenic HPV type. Of these 30 women, 13.3% (4/30) tested negative at histology, while 76.6% (23/30) tested positive for CIN and 10% (3/30) were found to have invasive carcinomas. The most prevalent HPV type in the CT-positive women was HPV 16 (53.33%; 16/30) and in these women, final diagnosis revealed cervicitis in 12.5% of cases (2/16), CIN in 68.7% of cases (11/16) and invasive carcinoma in 12.8% of cases (3/16). Conclusions: A diagnosis of neoplasia is more common than a non-neoplastic diagnosis in women who test positive for HPV and CT.

P-06.62

PREVALENCE OF HUMAN PAPILLOMAVIRUSES AND TYPES IN DIFFERENT AGE GROUPS

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Cervical carcinoma is the tenth leading cause of mortality among women in Hungary. A multicentre study was performed in 1997. The average HPV prevalence in women was 17.6%. The prevalence of HPV in a carcinoma screening programme was 43.4%.

In 4 age groups, (I) 12-17, (II) 18-24, (III) 25-34 and (IV) 35-45-year-old women were examined by bimanual, colposcopic, cytological and molecular genetic methods. A short questionnaire was completed by these subjects. HPV PCR, was introduced for the detection of HPV. In the positive cases, the HPV types were determined by linear array (Roche). Parallel cytological examinations were performed with HPV diagnostic methods.

220 (28.0%) of the 788 samples were positive with the PCR method, and 568 (72.0%) were negative. A higher prevalence of HPV was detected by PCR in comparison with the results of the earlier survey where nucleic acid hybridization was used. The highest HPV prevalence by age 35%, was detected in the 18-24-year-old group. The highest HPV prevalence by education 50%, was detected in the lowest educated patient group (<8 years in elementary school). An increasing number of life time partners increased the prevalence of HPV. In the present study, types 58, 35 and 33 were most common in age groups I and II, while types 16, 58 and 35 were most frequent in age groups III and IV.

The higher prevalence may be explained by the enhanced sensitivity of the amplified method. The PCR and the linear array procedure can detect 37 types, but the hybridization method only 18 HPV types. Many carcinoma-prevention screening programmes in Hungary are free of charge. However, statistical surveys have demonstrated that screening programmes among women are not effective. A compulsory vaccination program against HPV infection could possibly decrease the mortality rate from cervical carcinoma among Hungarian women.

RESEARCH ON HPV INFECTION AND ANTIBODY IMMUNITY OF KOREAN WOMEN

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Purpose: Human papillomavirus (HPV) infection is estimated to be the most common sexually transmitted infection. Baseline population prevalence and seroprevalence data for HPV infection in Korean women would be useful. The purpose of this study was to estimate the prevalence and seroprevalence of HPV-6, -11, -16, and -18 infections in a population of Korean women.

Materials and methods: The study population consisted of Korean women aged 20 to 59 years who were interviewed and completed the structured questionnaires eliciting information related to sociodemographic characteristics. Vaginal swab specimens were analyzed for HPV DNA tests by Hybrid Capture[®] 2 assay and type-specific HPV DNA test. Serum samples were tested for specific neutralizing antibodies to HPV-6, -11, -16, and -18 by Merk and Co Inc., using a multiplexed competitive Luminex[®] assay with antibody levels reported in milli-Merk units per milliliter (mMu mL-1).

Results: The overall HPV prevalence was 16.6% among Korean women aged 20 to 59 years (n=800).

HPV-6/-11 prevalence was 5.0% and HPV-16/-18 prevalence was 14.1% with the highest prevalence of 10.0% and 40.0% among women aged 20 to 29 years, respectively. The HPV seroprevalence was 4.1% for HPV-6, 2.3% for HPV-11, 5.5% for HPV-16, 4.7% for HPV-18, and 9.2% for any of the four types.

Conclusions: HPV infection is common in Korean women. This study indicated that the burden of prevalent HPV infection in Korean women was highest among those aged 20 to 29 years. In addition, although seroprevalence may underestimate cumulative exposure to HPV, it seems to be a useful molecular epidemiologic tool to assess HPV infection.

P-06.64

HPV TYPE DISTRIBUTION IN DANISH WOMEN WITH HIGH-GRADE DYSPLASTIC LESIONS

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Background: The distribution of HPV types in high-grade precancerous lesions and Adenocarcinoma in situ (AIS) have been shown to differ by geographic region. In Europe, current data on HPV-type distribution are incomplete and the study methodology differs between studies. It is therefore important to collect data on the HPV-type distribution using a standardised protocol across Europe.

Objectives: The objective of this cross-sectional, retrospective study was to assess the distribution of HPV-types in women diagnosed with high-grade intraepithelial lesions and AIS in several European countries, including Denmark. Results from a parallel study on HPV types in invasive lesions are presented separately.

Methods: Archived cervical samples collected from a sample of Danish women \geq 18 years were tested in DDL diagnostic laboratory (Voorburg, The Netherlands) using the PCR-SPF10 LiPA25 version 1 methodology* that detects 14 oncogenic HPV types (HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) and 11 low-risk HPV-types (HPV-6, 11, 34, 40, 42, 43, 44, 53, 54, 70 and 74).

Results: Of the 290 cervical samples collected in 2005 and 2006, 276 tested HPV-positive. The most frequent histological diagnosis was CIN3 (225/276; 81.5%). A single HPV-type was found in 77.9% (215/276) of the women. In the majority of single infected women, the most frequent HPV-types were HPV-16(54.0%), HVP-33 (13.5%), HPV-31 (10.7%), HPV-18 (7.9%) and HPV-52 (4.7%). Multiple HPV types were found in 21.4% (59/276) of the women, with HPV-16 being the most frequent type detected

Conclusions: These data are consistent with previous studies in Europe where HPV-16 has been identified as the most frequent type in precancerous lesions. A complimentary parallel study on invasive cancer confirms that HPV-16 and 18 are the most prevalent types for HPV vaccination.

*Labo Biomedical Products (Rijswijk, The Netherlands) based on licensed INNOGENETICS SPF10 technology.

HUMAN PAPILLOMAVIRUS AND RISK FACTORS IN WOMEN FROM NORTHERN ARGENTINA

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Background: Epidemiological factors may affect HPV infection onset and course. Objectives: To investigate the HPV prevalence and risk factors in women from Northern provinces of Argentina with high cervical cancer incidence estimates. Methods: 728 women were included: 155 from Formosa, 258 from Tucumán, 84 from Tafí del Valle and 231 from Misiones. Colposcopy and Pap smear (PAP) were performed. HPV type, age, economic and educational status, onset of sexual intercourse, stable partner, Nº of partners, Nº of children and pregnancies, smoking, STD history, contraceptive use were assessed. Results: Age range: 14-78, mean: 34.9 ± 12.0; 81% of women were poor or indigent. PAP results: 91.1% were normal and 1.8% showed cervical lesion (non cancerous). HPV was detected in 35.95% of women, 56.32% corresponded to HPV-AR. HPV prevalence differed between centers; the highest being in Misiones and Tafi (50.2% and 44.05% respectively), where HPV-AR prevalence was also the highest: 27.3 and 25% respectively. The infection peak corresponded to the < 25 year group. The HPV prevalence in Formosa was : HPV 16: 25.7%; HPV 58:14.3%, and types 35, 39, 52, 59, and 66: 5.7% ea. In Tucumán, HPV 16 was 23%; HPV 31: 11%, HPV 53: 8.2% and types 11, 45 and 6: 5.5% ea. In Tafi, HPV 16 was 33.4%, followed by types 31, 58 and 6: 8.3% ea. In Misiones, HPV types 6, 56 and 33 were the most prevalent. Among significant risk factors in the 4 centers' population were identified: Nº of partners OR 1.8 for 3-4 partners (CI 1.2-2.6) and OR 4.9 (CI 2.9- 8.5) for 5 and more partners; smoking OR 1.4 (CI 1.0-2.0). Conclusions: The HPV infection peak in the <25 years group confirms previous data. The study contributes for the prevention program design and the understanding of the natural history of HPV infection in the region.

P-06.66

HPV11 AND HPV16 INFECTION OF HUMAN MAMMARY EPITHELIAL CELLS

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Human papillomavirus (HPV) infections have been postulated as causative agents of breast cancer. The data are contentious, but some studies show HPV DNA in significant percentages of breast cancer and mammillar samples. Although HPV16 E6 and E7 can immortalize normal human mammary epithelial cells (HMEC) in vitro, it is unclear whether HPVs can naturally infect breast epithelium and promote tumorigenesis. As HPVs demonstrate a strict tropism for squamous epithelium, we do not expect HPV infections of HMEC to be productive. We have found that transfected HPV genomes are transcriptionally active in MCF10A and 76N+tert HMEC lines. HPV11 and HPV16 genomes can be persistently maintained in MCF10A cells over ≈25 population doublings. We recently determined that HPV16 virions are capable of infecting MCF10A cells. Therefore, we are in the process of investigating whether HPV16 or HPV11 can infect HMEC, including primary mammary cells. Our overall goals are to understand how HPVs infect HMEC and whether genomes can be persistently maintained in HMEC, either episomally or via integration. We are currently delineating the entry route of HPV16 and HPV11 in HMEC and in natural host human keratinocytes. We plan to determine if the infections induce signals and proliferative activities that might promote mammary epithelial cell transformation.

HUMAN PAPILLOMAVIRUS GENOTYPES IN CERVICAL SAMPLES FROM URUGUAYAN WOMEN.

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Background: Cervical cancer is one of the most important causes of death, particularly in developing countries, including Uruguay, representing a major public health problem. Although in our country a screening program of cervical cancer has been implemented in the last few years, no study about the presence and distribution of HPV in Uruguay has ever been reported.

Objectives: to investigate the distribution of HPV genotypes in Uruguayan women from Montevideo. Methods: we analyzed 205 citologically and histologically diagnosed (Bethesda, 2001) samples: 167 were Low grade SIL (L-SIL), 37 High grade SIL (H-SIL), and 1 was an adenocarcinoma; 82 normal control samples were also included. The presence of HPV DNA was detected by PCR using MY09/MY11 consensus primers and positive samples were genotyped by RFLP. Beta globin PCR was included as internal control. Results: Overall, HPV DNA was detected in 24.7 % of women. In L-SIL, H-SIL, and adenocarcinoma samples, the rates observed were 25% (42/167), 43% (16/37) and 100 % (1/1), respectively. Similarly, HPV DNA was detected in 12 (14.5%) out of 82 control women. In all cases, HPV 16 was the genotype most commonly found (55%, 44 %, 100 % and 33 % of genotyped samples, in L-SIL, H-SIL, adenocarcinoma, and control samples, respectively). Conclusions: This report represents the first study carried out in Uruguay about HPV distribution. Notably, the HPV prevalence rate in SIL was low, particularly in those categorized as LSIL. In agreement with almost all studies reported elsewhere, we found out that also in Uruguay HPV 16 is the most widely distributed genotype; however, HPV 18 was no detected. The results reported in this study might represent useful information in determining the impact of HPV vaccine program implementation.

P-06.68

PREVALENCE, CLEARANCE, INCIDENCE OF HPV INFECTIONS IN WOMEN 18-24.

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Background Cervical cancer is a most common cancer in women worldwide but there are limited epidemiological data on HPV infection in young women in Italy.

Objectives Aim of this study was to obtain information on young population genotype prevalence, clearance and incidence of HPV infections.

Methods We carried out a long-term follow-up study on young women, living in Tuscany, to evaluate the prevalence of single or multiple infection and the correlation with sexual behaviour. Participants provided information about: socio-demographic characteristics (age, gender, university attended, work, reproductive and menstrual factors, cigarette smoking) and sexual history (age at first intercourse, age of the first partner, number of sexual partners, number of sexual partner of the partner, use of condom or other contraceptive). Eight thousand women aged 18-24 years were randomized in regional achieves and received an invitation letter with the request to participate in the study.1066 women were enrolled and underwent HPV-test. If HPV-HR was negative we asked to repeat HPV after 12 months, or every 6 months, if HPV-HR was positive, over a period of 3 years.

Results and conclusions This study showed a prevalence of HPV-HR and LR of 19,32% and 10,04% respectively. Among HR types, HPV16 (8,54%), 31 and 56 (2,44%) and 51 (2,06%) were the most frequently detected, whereas, among LR types, HPV11 (4,13%) and 6 (3,85%) predominated.

In the crude analysis an association between use of condom and HPV positivity was found with decreasing OR in women never using condom or always using. In the multivariate analysis two determinant were relevant: number of lifetime sexual partner and number of previous partners of partner.

This information should be important to evaluate new future prevention strategies, and to better understand the epidemiology of HPV infection across different population.

SESSION 07

EXPERIMENTAL THERAPEUTICS

PROGRAMME

Oral presentations

THE 25TH INTERNATIONAL PAPILLOMAVIRUS CONFERENCE MAY 8-14 2009, MALMÖ, SWEDEN

TIME	NUMBER	TITLE	ROOM	
2009-05-11				
SESSION 07: EXPERIMENTAL THERAPEUTICS K				
08.30-08.41	O-07.01	IMPACT OF TUMOR-INFILTRATING HPV-SPECIFIC T-REGULATORY CELLS IN CERVICAL CANCER PATIENTS Sj Piersma, Jm van der Hulst, Es Jordanova, Gg Kenter, Sh van der Burg		
08.41-08.53	O-07.02	CLEARANCE OF HPV IN VIN: NORMALIZATION OF IMMUNOCOMPETENT CELL COUNTS L Blok, A Terlou, A Kleinjan, M Van Seters, C Heijmans - Antonissen, I Beckmann, M Van Beurden, T Helmerhorst		
08.53-09.05	O-07.03	IMMUNE THERAPEUTIC STRATEGIES FOR PREINVASIVE HPV DISEASE CL Trimble		
09.05-09.16	O-07.04	HPV PSEUDOVIRIONS AS VEHICLES FOR GENETIC IMMUNIZATION AND GENE THERAPY RC Kines, BS Graham, J Nicewonger, TR Johnson, J Roberts, CB Buck, D Lowy, J Schiller		
09.16-09.27	O-07.05	REQUIREMENTS FOR EFFECTIVE IMMUNOTHERAPY OF CHRONIC VIRAL INFECTION IN SKIN GR Leggatt, J Zhong, S Mattarollo, X Liu, R De Kluyver, S Fiorenza, J Zhou, U Hadis, N Warif, A Rahimpour, J Broom, I Frazer		
09.27-09.38	O-07.06	T-CELL IMMUNITY OF THERAPEUTIC VACCINATED PATIENTS CORRELATES WITH CLINICAL RESPONSES MJP Welters, GG Kenter, APG Vloon, MJG Löwik, TMA Berends - van der Meer, F Essahsah, JW Drijfhout, ARPM Valentijn, LM Fathers, J Oostendorp, GJ Fleuren, CJM Melief, SH van der Burg		
09.38-09.49	O-07.07	HPV16 IMMUNE ESCAPE IS INDUCED BY THE MINOR CAPSID PROTEIN A. Raff, L Fahey, D Da Silva, Wm Kast		
09.49-10.00	O-07.08	COMBINED THERAPY WITH AD-LIGHT AND HPV16E6E7-VRP INDUCES SIGNIFICANT TUMOR REGRESSION S Kanodia, D Da Silva, T Karamanukyan, L Bogaert, YX Fu, WM Kast		

O-07.01

IMPACT OF TUMOR-INFILTRATING HPV-SPECIFIC T-REGULATORY CELLS IN CERVICAL CANCER PATIENTS

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The majority of cervical cancers are caused by HPV-16 and -18. The E6 and E7 proteins of HPV are essential in cellular transformation and are expressed in both pre-malignant and advanced cervical lesions.

The overall objective of this study is to comprehend the contribution of the adaptive immune system in the anti-tumor response. This was assessed by analysis of infiltration, specificity and function of tumor-infiltrating lymphocytes (TIL).

In a group of 59 patients we analyzed TIL infiltration by staining for CD3, CD8, CD57, and FoxP3. For specificity and functional assays, HPV-specific T-cells were isolated from metastatic lymph nodes and tumor tissue from 74 patients in a different group. T-cell clones were isolated from positive TIL and LN cultures and fully characterized for type, specificity and function. Functional assays included both anti-CD3 and antigen-dependent suppression assays on both CD4 and CD8 T-cells.

Stratification according to lymph node status revealed a significantly stronger CD8 T-cell tumor infiltration and higher CD8/T-regulatory cell (Treg) ratio in the group of patients with a better prognosis. Analysis of freshly isolated TIL resulted in the detection and isolation of HPV E6 and E7 specific CD4+ T-cells in at least half of HPV-16 or -18 positive patients. HPV-specific CD4+ Treg clones were found to suppress proliferation and cytokine (IFN γ , IL-2) production by responder CD4+ and CD8+ T-cells. This capacity to suppress depends on their activation by cognate HPV antigen and on close range interactions.

Low CD8/Treg ratio is a significant independent unfavorable prognostic factor in cervical cancer patients. Frequently, the tumor is also infiltrated with HPV-specific CD4+ and CD8+ T-cells which can potentially attack the tumor. The infiltrating T-cells also include HPV-specific Tregs which are able to suppress the reactivity of neighboring T-cells. Their presence may explain the failure of the immune system to control HPV-induced tumors.

O-07.02

CLEARANCE OF HPV IN VIN: NORMALIZATION OF IMMUNOCOMPETENT CELL COUNTS

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Recently our group reported on an RCT in which a non-invasive new treatment for usual-type VIN was successfully used. Treatment with the immune-response modifier imiquimod resulted in a complete response in 35% of patients and represented a significant reduction in lesion size for an additional 46% of patients. Furthermore, after treatment with imiquimod, in 58% of patients, HPV virus could no longer be detected at the lesion. In the current investigations numbers of immunocompetent cells before and after imiquimod treatment are compared between patients that cleared, and did not clear the HPV infection.

In usual-type VIN, before and after imiquimod treatment, numbers of immunocompetent cells in epidermis and dermis, and the presence or absence of HPV virus, were assessed. The following markers were used: CD1a+: immature dendritic cells (DCs)/Langerhans cells, CD207+: immature DCs expressing Langerin, CD208+: mature DCs, CD123+/ CD11-: plasmacytoid DCs, CD94+: Natural Killer cells, CD14+: monocytes, CD4+: T-helper cells, CD8+: cytotoxic T-cells, CD25+/HLA-DR+: regulatory T-cells; and CD68+: macrophages. Furthermore, RT-PCR was used to identify the 14 most prevalent hrHPVs.

In usual-type VIN patients numbers of several immunocompetent cells in the dermis (CD208+, CD123+/CD11-, CD4+, CD8+, CD25+/HLA-DR+, CD68+) and in the epidermis (CD14+) were significantly increased in comparison to healthy controls. Upon clearance of HPV after imiquimod treatment, interestingly, upregulated cell numbers returned to control values in dermis and epidermis indicating normalization of the local immune response due to success of imiquimod treatment. In usual-type VIN lesions where HPV was not cleared; upregulated cell counts did not return to normal values, indicating failure of treatment. Intriguingly, among the HPV-cleared patients, clinically there were some that showed partial response to imiquimod treatment. These patients are currently reinvestigated.

In conclusion, clearance of HPV after imiquimod treatment of usual-type VIN results in normalization of numbers of immunocompetent cells at the lesion.

O-07.03

IMMUNE THERAPEUTIC STRATEGIES FOR PREINVASIVE HPV DISEASE

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Background: We have established a prospective cohort of CIN2/3 subjects who are followed for a 15-week window prior to conization. No subject has had progression of disease in this timeframe. In fact, 25% of HPV16+CIN2/3 undergo complete regression in this window. Detectable CD8+ T cell responses to E6 and E7, measured in longitudinally obtained peripheral blood specimens, do not predict lesion outcome. Immunologic parameters at the lesion site are associated with disease.

Objectives: We sought to elicit E7-specific T cell responses in subjects with HPV16+CIN2/3.

Methods: We carried out a phase I dose-escalation assessment of pNGVL4a-sig/E7/HSP70, in subjects with HPV16+CIN2/3. Based on maturing immunologic data, we have opened a DNA-prime, vaccinia-based boost with and without imiquimod protocol for this patient cohort.

Results: DNA vaccination alone is safe, feasible, and well-tolerated. Detectable immune responses to vaccine antigen were identified in the highest dose cohort, but were not significantly higher than those we identified in our unvaccinated cohort. However, lesional CD8+ infiltrates were higher in vaccinated subjects than in unvaccinated subjects. Lesions which have failed to regress over the study window have provided an opportunity to study the phenotype and functional polarization of lesion-associated immune cell subsets, as well as characteristics of the lesions themselves that could mitigate either access or function of localized CD8+ T cells.

Conclusions: Although preinvasive HPV disease is associated with localized immune cell infiltrates, localization and access are not sufficient to eliminate disease in our brief study window, suggesting that factors intrinsic to the lesions themselves mitigate the ability of immune cells to eliminate established CIN2/3 in a 15-week timeframe. Strategies to enhance lesional access and function of effector cells will be discussed.

O-07.04

HPV PSEUDOVIRIONS AS VEHICLES FOR GENETIC IMMUNIZATION AND GENE THERAPY

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We are evaluating HPV pseudovirions (PsV) as gene delivery vehicles for genetic immunization. Preliminary experiments indicated that a systemic IgG response could be elicited against red fluorescent protein (RFP) after a single dose of RFPexpressing PsV was instilled intravaginally into a nonoxynol-9 pre-treated mouse. Using Respiratory Syncytial virus (RSV) M/M2 as model antigens, we discovered that intravaginal (Ivag), but not intramuscular (IM), immunization with HPV PsV elicited strong mucosal and systemic B-cell and CD8+T-cell responses. In contrast, both IVag and IM adenoviral (rAd5) genetic immunization elicit strong responses, similar to those induced by IVag HPV PsV. Because of the disparities noted between immunization route and gene delivery vehicle, we examined the level and kinetics of antigen expression using luciferase as the encapsidated reporter gene. IVag delivery of rAd5 and HPV PsV, and IM immunization with rAd5, all show an initial peak in protein expression between days 1 and 2 post-administration. Gene expression in the vaginal tract became undetectable by day 7 in both the rAd5 and HPV immunized groups, whereas the rAd5 IM group dropped to low levels but have remained steady over time. By contrast, luciferase expression after IM delivery of HPV PsV was delayed, and levels have slowly increased over 5 months. We conclude that the female genital tract can serve as a potent inductive site for both T and B cell responses, provided antigen is transiently expressed in wounded keratinocytes. The ability of HPV PsV to generate strong mucosal and systemic B and T cell responses suggests that PsV expressing HPVE6/ E7 or antigens from HIV or HSV would be interesting vaccine candidates. Additionally, the long-term gene expression in the muscle, in the absence of an immunological response, may make HPV PsV an attractive vehicle for gene therapy applications.

O-07.05 REQUIREMENTS FOR EFFECTIVE IMMUNOTHERAPY OF CHRONIC VIRAL INFECTION IN SKIN

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Background: Persisting infection of epithelial surfaces with some human papillomaviruses conveys significant risk of epithelial cancers. Immunosuppression increases the risk of persisting infection, but the mechanisms by which cell mediated immunity clears infection, and the reasons why it sometimes fails to, are unclear.

Aim: To use a murine model in which skin expressing antigen as a transgene in keratinocytes is grafted to an immunocompetent host to define mechanisms for clearance of keratinocytes expressing non-self protein.

Results: Some non-self antigens invoke rejection of skin grafts expressing the antigen from a keratin promoter, while others including the two transforming proteins of HPV, E6 and E7, do not. Rejection requires CD8 T cells, is enhanced by CD4 T cells even in an antigen experienced host, and is significantly enhanced by local inflammation. Promoters of T cell activation, and of antigen presentation, and some cells of the innate immune system enhance priming to new antigen, and rejection of grafts from an antigen experienced host. Anti-inflammatory cytokines constitutively expressed in skin, and some cells of the innate immune system, suppress graft rejection, and their inhibition or removal enables rejection of E7 expressing grafts.

Conclusions: The fate of keratinocytes expressing non-self antigen is determined by a balance between pro- and antirejection regulatory forces in skin, and the balance affects not only priming to new antigen, but the effectiveness of effector cells generated by previous priming or by immunisation. Appropriate manipulation of the local immunological environment may therefore assist immunotherapy targeted at HPV infected epithelium.

O-07.06

T-CELL IMMUNITY OF THERAPEUTIC VACCINATED PATIENTS CORRELATES WITH CLINICAL RESPONSES

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Background: The development of HPV16-induced cancers is associated with failure of T-cell immunity, lack of T-cell homing and local presence of HPV16-specific regulatory T cells. Recently, we reported the ability of a HPV16 E6 and E7 synthetic long peptide (SLP) vaccine to restore HPV16-specific T-cell immunity in cervical cancer patients. Subsequently, this vaccine was shown induce complete clinical responses (CR) in 45% of patients with HPV16+ VIN3.

Objectives: To assess the relationship between vaccine-induced T-cell immunity and clinical responsiveness.

Methods: A combination of IFNg-ELISPOT, multiplex cytokine assays, proliferation assays and multi-parameter flow cytometry were deployed to delineate the presence, magnitude, breadth, type, polarization and function of HPV16-specific T-cell responses both in the blood as well as in the vaccination site or lesion, as the latter will reveal the migratory capacity of T-cells.

Results: All patients showed vaccine-induced T-cell responses in one or more of the assays used. Comparison of the strength of the response, defined as the product of the magnitude and breadth of T-cell reactivity, revealed a significantly stronger IFNg (and other cytokines)-associated, proliferative CD4+ T-cell response as well as a broader response of CD8+ T cells in the group of patients with a CR compared to the patient with no clinical benefit. Notably, type 1 and 2 cytokine responses peaked after the first vaccination and this correlated with clinical outcome. At the vaccination site, HPV-specific T cells were more frequently found in CR patients than in those with no CR.

Conclusions: The clinical efficacy of anti-tumor vaccines may be determined by their capacity to induce strong and broad multi-functional responses to tumor antigens. Only assays, designed to measure the magnitude, breadth and multi-functionality of immune responses will reveal correlates of effective T-cell responses in the immunotherapy of cancer.

O-07.07

HPV16 IMMUNE ESCAPE IS INDUCED BY THE MINOR CAPSID PROTEIN

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Background: Persistent high-risk human papillomavirus (HPV) infection of the cervical epithelium is causally associated with the generation of cervical cancer. Previous work has demonstrated that HPV, despite being a foreign antigen, does not activate the antigen presenting cells at the site of infection. This represents an immune escape mechanism that halts the further activation of the adaptive immune system.

Objectives: Our objective was to determine which HPV protein is responsible for the induction of this immune escape mechanism.

Methods: We incubated Langerhans cells with either HPV16 L1 virus-like particles (VLP) or HPV16 L1L2 VLP and assessed various phenotypic and functional characteristics. We determined expression of surface activation markers via flow cytometry, cytokine and chemokine secretion using Bio-Plex assays, and migration with a CCL21-directed migration assay. The ability of HPV16 exposed Langerhans cells to initiate an HPV16-specific T cell response was determined by an in vitro immunization assay. Finally, signaling pathways were examined by western blot analyses.

Results: We demonstrate that Langerhans cells exposed to the minor capsid protein L2 in HPV16 L1L2 VLP are not phenotypically or functionally mature. However, HPV16 L1 VLP significantly induce activation of Langerhans cells.

Conclusions: Our data suggest that the HPV16 minor capsid protein L2 plays an active and specific role in the induction of the immune escape of HPV through the manipulation of Langerhans cells. This novel function is the first immune modulating interaction attributed to the HPV minor capsid protein L2 and is a significant step forward in our understanding of the mechanism of HPV immune escape. The minor capsid protein L2 now represents an attractive HPV immunotherapeutic target.

O-07.08

COMBINED THERAPY WITH AD-LIGHT AND HPV16E6E7-VRP INDUCES SIGNIFICANT TUMOR REGRESSION

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Background: The importance of T-cell mediated immunity in clearing human papillomavirus (HPV)-induced tumors is well established. It is also known that lymphotoxin-beta receptor (LTBR) signaling plays an important role in the formation of lymphoid structures, where T-cells are primed. LIGHT, a ligand for LTBR, establishes lymphoid-like tissues inside tumor sites and recruits naïve T-cells into the tumor. We have previously shown that therapy with recombinant adenovirus carrying LIGHT (Ad-LIGHT) induces expansion of HPV-specific T-cells.

Hypothesis: We hypothesized that boosting HPV-specific T-cells, generated by Ad-LIGHT therapy, with HPV16E6E7-Venezuelan Equine Encephalitis Virus Replicon Particles (HPV16E6E7-VRP) can eradicate HPV-induced tumors.

Research Design: To test efficacy of combined therapy with Ad-LIGHT and HPV16E6E7-VRP, B6 mice were challenged with HPV16-expressing C3 tumors. On day 14 and 17 after tumor challenge, Ad-LIGHT or Ad-Control particles were injected intra-tumorally at 10^10 virus particles per mouse. On days 24 and 31 mice were subcutaneously injected with PBS or 10^7 HPV16E6E7-VRP. Tumors and spleens were harvested on day 38 to measure T-cell responses. Additional mice were maintained to monitor tumor growth and survival.

Results: FACS with HPV16 E7(49–57) tetramers and IFNg ELISPOT analysis revealed that Ad-Control treated mice had no functional E7(49–57)-recognizing T-cells. Mice treated with Ad-LIGHT and HPV16E6E7-VRP had stronger functional tumor-specific T-cell responses as compared to mice treated with either Ad-LIGHT or HPV16E6E7-VRP alone. All mice treated with either Ad-Control or HPV16E6E7-VRP alone died by day 47 whereas 70% of mice treated with Ad-LIGHT and 100% of mice treated with Ad-LIGHT and HPV16E6E7-VRP survived. On day 60,10% of mice treated with Ad-LIGHT and HPV16E6E7-VRP were tumor-free.

Conclusions: Our data show that combined therapy with Ad-LIGHT and HPV16E6E7-VRP induces functional tumor-specific T-cells that eradicate large well-established tumors in 50% of mice.

POSTER ABSTRACTS SESSION 07

POSTER SESSION I MONDAY 10.00: ODD NUMBERS

POSTER SESSION II

TUESDAY 10.00: EVEN NUMBERS

P-07.10 THERAPEUTIC IMMUNISATION WITH CRPV-VLP INDUCES REGRESSION OF ESTABLISHED PAPILLOMAS

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There is overwhelming evidence that persistent infection with high-risk human papillomaviruses (HR-HPV) is the main risk factor for invasive cancer of the cervix. Due to this global public health burden, two prophylactic HPV L1 virus-like particles (VLP) vaccines have been developed. While these vaccines have demonstrated excellent type-specific prevention of infection by the homologous vaccine types (high and low risk HPV types), no data have been reported on the therapeutic effects in people already infected with the low-risk HPV type. In this study we explored whether regression of CRPV-induced papillomas could be achieved following immunisation of out-bred New Zealand White rabbits with CRPV VLPs. Rabbits immunised with CRPV VLPs had papillomas that were significantly smaller compared to the negative control rabbit group (P<0.05). This data demonstrates the therapeutic potential of PV VLPs in a well-understood animal model with potential important implications for human therapeutic vaccination for low-risk HPVs.

P-07.11 SAFETY AND IMMUNOGENICITY OF HSPE7 AND POLY-ICLC IN CIN PATIENTS

D Da Silva, University of Southern California, Los Angeles, USA PCR Emtage, Nventa Biopharmaceuticals Corporation, San Diego, USA J Weber, H Lee Moffitt Cancer Center, Tampa, USA WM Kast, University of Southern California, Los Angeles, USA

Background: Heat shock proteins (Hsp) have been utilized in preclinical models as vaccine components for infectious disease prophylaxis and cancer therapy. Animal studies showed that heat shock protein Hsp65 fused to the HPV 16 E7 antigen (HspE7) resulted in the generation of E7-specific humoral and cell-mediated immune responses. Poly ICLC, a TLR3 agonist, is a broad inducer of innate immunity and is being developed as a vaccine adjuvant and antitumor agent. Objectives: The primary objective of this study was to demonstrate safety and tolerability of concomitant administration of HspE7 and Poly ICLC in women with CIN. Secondary objectives included the evaluation of immunologic parameters to characterize the immune response against HspE7.

Methods: A multicenter, nonrandomized, open label, Phase 1 study was performed. Four patient cohorts were immunized with escalating doses of Poly ICLC on a fixed dose of HspE7 subcutaneously, once every 4 weeks for three cycles. Immunological monitoring was performed on peripheral blood prior to the first immunization and 7 days after each administration of HspE7 and Poly ICLC. Samples were analyzed for cellular immune responses by interferon gamma ELISPOT and T cell proliferation, antibody responses and cytokine responses.

Results and Conclusions: HspE7 and Poly ICLC were determined to be safe and well tolerated in all cohorts. Immunologically, patients demonstrated significant changes in antibody responses to HspE7 with all showing an increased antibody titer to the immunizing antigen. Serum cytokine responses were not consistently detected upon drug administration. T cell responses demonstrated a dose response with Cohort 1 showing no responses and Cohorts 2-4 demonstrating clear HPV 16 E7 antigen specific immune responses. In conclusion, the Phase 1 demonstrated that the combination of HspE7 and Poly ICLC is safe, tolerable and immunogenic in humans; showing antibody and T cell responses to the immunizing antigen.

P-07.12

TARGETING ENDOSOMAL-LYSOSOMAL COMPARTMENT LEADS TO BOTH MHC-I-II PRESENTATION OF ANTIGEN

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The understanding of antigen presentation pathways by the major histocompatibility complex (MHC) molecules may facilitate the development of effective vaccines. We have previously developed a molecular approach that directly routes a model antigen, human papillomavirus type 16 (HPV-16) E7 into the endosomal/lysosomal compartments by linking it to the sorting signal of the lysosome associated membrane protein type 1 (LAMP-1) (Sig/E7/LAMP-1). We showed that mice vaccinated with recombinant vaccinia encoding Sig/E7/LAMP-1 resulted in increased number of E7-specific CD4+ T cells as well as E7-specific CD8+ T cells. In order to determine if the enhancement in the CD4+ and CD8+ T cells was due to the direct presentation of E7 antigen through the MHC class II and I presentation pathways to directly activate E7-specific CD4+ and CD8+ T cells, we infected human B lymphoblastoid cell lines (B-LCLs) with Sig/E7/LAMP-1 vaccinia and incubated the infected cells with either the HPV-16 E7-specific CD4+ or CD8+ T cells. Our data suggest that B cells infected with Sig/E7/LAMP-1 vaccinia were capable of activating E7-specific CD4+ and CD8+ T cells. Our data suggest that targeting of E7 antigen to the endosomal/lysosomal compartments led to enhanced MHC class I and II presentation of E7 antigen.

P-07.13

CERVICAL CANCER IMMUNOTHERAPY EMPLOYING PLANT VIRUS- AND PLANT- DERIVED SEQUENCES.

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Background. The practice of prophylactic Human papillomavirus (HPV) vaccination (Gardasil, Cervarix) needs cost improvements being currently unaffordable in developing countries, where it would have greatest benefit. On the other hand, therapeutic vaccination to cure the cervical cancer 500,000 cases diagnosed every year is still seeking for safe, efficacious and cost-effective formulations. Objectives. Plant- and plant virus-derived sequences are innovative antigen immunological 'promoters' and can create the pro-inflammatory environment necessary for the clearance of persistent HPV infection and related cancers without implying the safety concerns for clinical use existing for other currently tested immune response modifiers. Main goal of our project is to devise a safe strategy, initially based on DNA immunization, for enhancing the poor HPV antigen immunogenicity. Methods. We fused the Potato Virus X coat protein gene (PVX-CP) with a mutagenized HPV16 E7 sequence (E7GGG-CP). To further increase E7 protein immunogenicity, a secretory construct of the E7GGG sequence was made by N-terminal fusion with a plant-derived signal sequence. Results and Conclusions. The fusion with the PVX-CP sequence enhanced the E7-specific humoral and cell-mediated immune responses when administered to mice. After challenge with tumor-inducing E7-expressing cells, vaccinated mice were protected from either cancer establishment or progression. Further, in a heterologous prime-boost regimen, priming with E7GGG-CP DNA vaccine and boosting with a plant-purified E7GGG fusion with an engineered bacterial carrier (LicKM) resulted in 100% tumor rejection. Data from these studies indicate that effective therapeutic vaccines can be accomplished exploiting the potential of plant or plant viral sequences. Moreover, heterologous administration schedules for future bedside application of HPV-related human therapeutic vaccines are possible. We are currently investigating other plantderived sequences with immunological features, that might lead to improved vaccines (DNA or protein) of relevance against HPV.

P-07.14 ORTHOTOPIC MOUSE MODEL OF HPV16 ASSOCIATED ORAL CANCER.

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Head and neck cancers are the eighth most common tumour in the world. Head and neck squamous cell carcinoma accounts for over 90% of all head and neck cancers. Unfortunately, the mortality rates for this disease have not improved in the past 40 years despite advances in the delivery of treatment and in surgical reconstruction. A quarter of these tumours is associated to HPV infection and, in the majority of cases, to the HPV 16. Preventive vaccines are introduced in the clinical practice but only for women and the high costs render these vaccines not affordable for low-income countries. Thus therapeutic intervention must be developed and pre-clinical models are needed. Tumours developed from TC-1 cells are currently the most often used mouse model of HPV16-induced tumours in vaccine testing. This model suffers of several limitations: it is not orthotopic and does not allow to study the spread of cancer cells to produce tumours that mimic histopathologic growth as in the head and neck cancer patient, including invasion into surrounding tissues and spread to lymph nodes that is one of the primary determinants of outcome for patients. Moreover, understanding the process of lymph-node metastases in this disease is essential for improving prognostic capabilities and for designing rational therapeutic strategies. Therefore a mouse orthotopic model of oral cancer expressing the E7 antigen of HPV 16 has been developed and tested for challenge of different protein- or DNA-based therapeutic vaccines. AT-84 cell lines have been engineered to express the HPV16 E7 gene and implanted in the oral pavement of C3H mice by an external route to simplify the experimental procedures. This pre-clinical model of HPV associated oral cancer appears to be useful in study immunotherapeutic intervention in conditions resembling those of the human patients, including local invasion and distant metastases.

P-07.15

DEVELOPMENT OF A THERAPEUTIC HPV-11 DNA VACCINE

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Objectives/Hypothesis: HPV-6 and -11 are associated with anogenital warts and Recurrent Respiratory Papillomatosis. Although a prophylactic vaccine has been developed which protects against infection with HPV, therapeutic vaccine is still needed for patients with established HPV infection and HPV-associated lesions. In this study, we aim to identify HPV-11-specific CTL epitopes and develop an HPV11 E6/E7 DNA vaccine.

Methods: We constructed a DNA vaccine encoding the HPV-11 E6 and E7 fusion proteins. C57BL/6 mice were vaccinated with DNA encoding the HPV-11 E6/E7 proteins, and splenocytes were incubated with each of the overlapping peptides spanning either the HPV-11 E6 or E7 protein. The frequency of E6/E7 peptide-specific CD8+ T cells was then analyzed by intracellular cytokine staining assay. The in vivo antitumor effects were characterized using an E6/E7 expressing tumor model generated by transducing a murine cell line, TC-1, with a retrovirus expressing the fusion of HPV-11 E6/E7.

Results: C57BL/6 mice vaccinated with HPV-11 E6/E7 DNA vaccine generated strong E6(aa41-70) peptide-specific CD8+ T cell responses. These E6(aa41-70) peptide-specific T cells could be activated by TC-1 cells expressing HPV11 E6/E7, indicating that the peptide can be endogenously processed. No significant E7 peptide-specific T cell responses were observed. Further characterization of this E6aa41-70 peptide-specific T cell response revealed that E6(aa44-51) is the minimal CTL epitope and is restricted by H2-Kb. In addition, HPV-11 E6(aa44-51)-specific CD8+ T cell responses generated by this DNA vaccine are capable of controlling the growth of HPV-11 E6/E7-expressing tumors.

Conclusions: We have successfully developed DNA vaccine that targets the E6/E7 genes of HPV-11. In addition, we have identified an E6-specific H-2Kb-restricted CTL epitope, which will be useful for the development of quantitative CD8+ T cell immunological assays. We have also established an HPV-11 E6/E7 expressing tumor model, which will serve as an excellent model for future vaccine development.

P-07.16 SEROLOGICAL RESPONSE TO AN HPV16 E7 BASED THERAPEUTIC VACCINE

K Van Doorslaer, Albert Einstein College of Medicine, Bronx, USA LL Reimers, Albert Einstein College of Medicine, Bronx, USA Y Studentsov, Albert Einstein College of Medicine, Bronx, USA M Einstein, Albert Einstein College of Medicine, Bronx, USA RD Burk, Albert Einstein College of Medicine, Bronx, USA

Purpose: Infection with oncogenic human papillomaviruses has been linked to the development of cervical neoplasia and cancer. The exclusive expression of E7, a viral oncogene, in infected cells makes this protein a specific target for immunotherapy. We recently reported the results of a trial in women with cervical carcinoma-in-situ using HspE7, a protein vaccine consisting of full length HPV16 E7 linked to a heat shock protein from M. bovis. The stimulating effects of HspE7 on specific cytotoxic T lymphocytes have been demonstrated in vitro and in (pre-)clinical trials. The induction of a B-cell response by HspE7 and its association with clinical outcome is unknown, and is the purpose of this study.

Experimental design: We measured the serum IgG levels against HPV16 E7 and HPV16 and -18 VLPs using a multiplexed Luminex based assay in 57 women with CIS who received the HspE7 vaccine.

Results: Vaccination with HspE7 results in a modest, yet maintained increase in HPV16 E7 specific IgG levels. While not significant, increased HPV16 E7 IgG levels appear to be correlated with a positive therapeutic effect. Women who were previously treated for recurrent disease (by LEEP) had significantly higher HPV16 E7 IgG levels compared with subjects without recurrent disease (p=0.01). In women with recurrent disease, higher IgG levels correlated with complete pathological response.

Conclusions: This study suggests that IgG levels could potentially be used as a marker for response to a therapeutic vaccine. Further translational investigations of the 'priming' of local immune responses using extirpative procedures should be explored.

P-07.17 BPV-1 L1 EXPRESSION IN E. COLI.

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Papillomavirus are associated with different carcinogenic processes in human and other animals. In cattle, Bovine Papillomaviruses (BPV) are associated with malignant neoplasias of the upper gastrointestinal tract and urinary bladder. These viruses have a genome of approximately 7900 bp of double-stranded DNA, with at least nine potential reading frames, divided between the early (E) and late (L) regions. The E region encodes the replication and transcription regulatory proteins, E1 and E2, and the transforming proteins E5, E6 and E7, which are associated with the uncontrolled proliferation and loss of differentiation of the infected cells. The L region corresponds to the structural proteins L1 and L2 that assembles into the viral capsid during the maturation process. In order to improve the development of a prophylactic vaccine for BPV-1 targeting L1 protein, our aim was to clone the BPV1 L1 gene and expression in E. coli. BPV-1 L1 was amplified by PCR using upstream primer including a SauI restriction site and the downstream primer including a KpnI site. L1 codon sequence of BPV-1 was cloned into pGEM-T (Promega), excised with SauI and KpnI enzymes, and subcloned in pET (Novagen) for protein expression. BPV1 L1 gene. The detection of L1 expression indicated that the model is appropriate that can be used for further L1 gene expression for further vaccine development.

P-07.18

MODE OF CRPV DNA INFECTION HELPS TO EXPLAIN INTER-LABORATORY DISPARITIES

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BACKGROUND: The cottontail rabbit papillomavirus (CRPV) model is the only animal model available to study the full life cycle of papillomavirus-induced disease from initiation of infection to malignant progression. Our laboratory has used this model system for many years, as have several other laboratories throughout the world. Results from the different laboratories have not always been in agreement.

OBJECTIVES: In order to explain the inter-laboratory disparities, we hypothesized that the differences might be explained by 1) The different methods used to create papillomas in the animals and/or 2) The use of different CRPV viral strains.

METHODS: We performed two experiments. In the first, we used our highly efficient delayed scarification technique to study infection profiles for three viral strains in common use throughout the world as well as their E8 ATG mutants. In the second, we infected animals with the gene gun, also on pre-scarified sites. Gene gun delivery of DNA is the other commonly used infection technique.

RESULTS: Infections resulting from our scarification technique produced papillomas on 100% of the sites and growth was uniform. On the other hand many sites infected via gene gun did not produce papillomas, especially for the E8 mutant, and those papillomas that did appear, grew at highly variable rates. Regressions were common.

CONCLUSIONS: Mode of DNA delivery but not strain of virus contributes to differential inter-laboratory results. We suggest that the delayed scarification technique provides more consistency and reproducibility, is much less expensive than the gene gun, and does not "prime" anti-viral immune responses which can confound experimental results.

P-07.19 RESULTS OF CIGB-228 VACCINATION IN PATIENTS WITH HGSIL

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Human papillomavirus type 16 (HPV 16) infection has been linked to the development of cervical and anal dysplasia and cancer. One hallmark of persistent infection is the synthesis of the viral E7 protein in cervical epithelial cells. The expression of E7 in dysplastic and transformed cells and its recognition by the immune system as a foreign antigen make it an ideal target for immunotherapy. In animal models we have shown that a vaccine CIGB-228 consisting of restricted HLA-A2 HPV16 E7 peptide adjuvated in very small size proteoliposomes (VSSP) is capable of eradicating malignant TC-1 HPV16+ tumours in mice. We have now assessed the safety, immunogenic and clinical therapeutic effect of this vaccine in a Phase I Trial in humans. At the time of these report 7 patients with high grade epithelial lesions (HGSIL) comprised patients with NICII/III and CIS were injected subcutaneously 4 times at week interval by s.c. injection of the CIGB-228. Immunological monitoring was performed by the analysis of blood samples, drawn before and after each vaccination. Overall the vaccine was well tolerated. Apart from moderate swelling and redness of the skin at injection site, the vaccination caused fever for a short period. Overall, no toxicity > grade II was observed. Vaccination resulted in the induction of strong HPV16--specific T-cells by IFNg-ELISPOT after vaccinations compared to prior to vaccination. Clinical therapeutic effect at 3 month follow-up could be measured in 7 patients: histological complete response occurred in 4 out of 7 patients, partial response in 1 out of 7 patients and 2 patients were without any disease progression. These data suggest that our CIGB-228 vaccine is safe, induces an immune activation and shows clinical response in patients.

SESSION 08

VIRUS LIFE CYCLE

PROGRAMME

ROOM

K1-3

THE 25TH INTERNATIONAL PAPILLOMAVIRUS CONFERENCE MAY 8-14 2009, MALMÖ, SWEDEN

TIME NUMBER TITLE

2009-05-11

SESSION 08: VIRUS LIFE CYCLE

11.00-11.11	O-08.01	LOW- AND HIGH-RISK HUMAN PAPILLOMAVIRUS E7 PROTEINS REGULATE P130 DIFFERENTLY A Roman, L Barrow - Laing
11.11-11.22	O-08.02	HPV E7 INDUCES G2 ARREST FOLLOWING S-PHASE IN DIFFERENTIATED KERATINOCYTES N.S. Banerjee, L.T. Chow, N.J. Genovese, H.K. Wang, T.R. Broker
11.22-11.33	O-08.03	HPV-31 REQUIRES ATM ACTIVITY FOR REPLICATION IN DIFFERENTIATING CELLS C Moody, L Laimins
11.33-11.44	O-08.04	GENOMIC INSTABILITY INDUCED BY THE HIGH RISK HUMAN PAPILLOMAVIRUSES M Ustav, T Laos, H Isok - Paas, E Ustav, M Kadaja
11.44-11.55	O-08.05	ROLE OF L2 CYSTEINES IN INFECTION AND NEUTRALIZATION BY RG-1 R Roden, S Jagu, B Karanam, P Day, R Gambhira
11.55-12.06	O-08.06	HPV16 L2 INTRAMOLECULAR DISULFIDE BOND IS CRITICAL FOR INFECTIVITY M Ozbun, S Campos
12.06-12.17	O-08.07	TISSUE TROPISM OF CANINE PAPILLOMAVIRUS IS DETERMINED INVIVO AND KERATINOCYTE-INDEPENDENT H Yuan, D Zhou, M Marko, J Wang, R Tucker, X Liu, R Schlegel
12.17-12.28	O-08.08	TOWARDS AN UNDERSTANDING OF PAPILLOMAVIRUS LATENCY USING THE ROPV MODEL G Maglennon, P McIntosh, J Doorbar

O-08.01

LOW- AND HIGH-RISK HUMAN PAPILLOMAVIRUS E7 PROTEINS REGULATE P130 DIFFERENTLY

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Human papillomaviruses (HPVs) are divided into high-risk (HR) and low-risk (LR) types based on their oncogenic potential. HPV16 and 18 are considered HR types and can cause cervical cancer. HPV6 and 11 are classified as LR and cause benign genital warts. Viral proteins of both HR and LR HPVs must be able to facilitate a replication competent (S-phase) environment. The E7 proteins of LR and HR HPVs are responsible for maintenance of S-phase activity in infected cells. HR E7 proteins target all pRb family members (pRb, p107 and p130) for degradation. LR E7 does not target pRb or p107 for degradation, but we have shown that it targets p130 for degradation. We suggest that degradation of p130 is critical to completion of the viral life cycle. Therefore, we have been investigating the mechanism by which E7 mediates p130 degradation. We asked whether HPV16 and HPV6 E7 could affect p130 localization. Nuclear/cytoplasmic fractionation was performed using HPV6 E7 and HPV16 E7 transduced human foreskin keratinocytes. Data generated by nuclear/cytoplasmic fractionation showed that HPV16 E7, but not HPV6 E7, may relocalize or retain p130 in the cytoplasm. Immunofluorescence was performed as a confirmatory approach and similar results obtained. Leptomycin B, a specific inhibitor of nuclear export, did not affect 16E7-mediated relocalization of p130. Dysregulation of p130 localization by HPV16 E7 may lead to enhanced degradation. In contrast, the half-life of p130 was decreased in the nucleus in the presence of HPV6 E7 but not HPV16 E7. p130 was stabilized by proteasome inhibitor treatment. Therefore, p130 may be targeted for degradation in the nucleus by HPV6 E7. Elucidating the mechanism of p130 degradation may identify potential targets for preventing degradation of p130, thereby restoring cell cycle/differentiation control and aborting the virus replication cycle.

O-08.02

HPV E7 INDUCES G2 ARREST FOLLOWING S-PHASE IN DIFFERENTIATED KERATINOCYTES

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Background: Human papillomavirus E7 gene induces S-phase re-entry in post-mitotic, differentiated keratinocytes in squamous epithelium. We recently developed a novel system which produces high titers of infectious HPV-18 virus in organotypic cultures of primary human keratinocytes (PHKs). In this system, after S-phase, the differentiated cells were arrested in G2, as revealed by abundant cytoplasmic cyclin B1. It was in these cells viral DNA amplification initiated (Wang, Duffy, Broker, and Chow. 2009. Genes & Dev. in press). Objectives: We wish to identify the viral gene responsible for G2 arrest in differentiated keratinocytes. Methods: We probed by in situ methods and western blots host proteins that regulate G2-M transition in PHK raft cultures. Results: In the productive HPV-18 raft cultures, cytoplasmic cdc2 was also induced relative to control PHKs. However, cdc25c, which activates cdc2 by removing inactivating phosphates on T14 and Y15, was itself induced in an inactive form, being phosphorylated at S216. We further showed that retrovirus expressing HPV-1, HPV-11, or HPV-18 E7, each of which induces S phase re-entry in differentiated keratinocytes, also induced elevated cytoplasmic cyclin B1, cdc2, and the inactive, phosphorylated cdc25c. E7 mutations unable to induce S phase re-entry did not upregulate these host proteins. In addition, the Wee1 kinase, which causes inhibitory phosphorylation of cdc2, was upregulated by E7 relative to control PHK raft cultures. Conclusion: These observations demonstrate that E7 induces G2 arrest following S phase re-entry in the differentiated keratinocytes, providing an opportunity for viral DNA amplification.

O-08.03 HPV-31 REQUIRES ATM ACTIVITY FOR REPLICATION IN DIFFERENTIATING CELLS

HPV-51 REQUIRES ATM ACTIVITT FOR REPLICATION IN DIFFEREN

C Moody, Northwestern University, Chicago, USA

L Laimins, Northwestern University, Chicago, USA

Background: The ATM and ATR protein kinases are key regulators of the DNA damage response. Upon damage, numerous downstream targets of these kinases are activated, allowing for DNA repair while blocking replication of damaged DNA. Many viruses, such as Adenovirus and SV40, interact with and/or affect components of the cellular response to DNA damage. Objectives: We wanted to determine whether activation of ATM and its downstream target Chk2 play any role in the HPV-31 life cycle. Methods: HPV-31 positive human foreskin keratinocytes (HFKs), as well as normal HFKs, were grown in high calcium medium to induce the differentiation-dependent phase of the viral life cycle. Confocal fluorescence microscopy, Western Blot analysis and Southern blot analysis were utilized to examine the activation of DNA damage repair proteins during the course of the viral life cycle. Results: We found that keratinocytes stably maintaining HPV-31 epsiomes exhibited activation of ATM-dependent repair proteins throughout the viral life cycle, in both undifferentiated and differentiated cells. In addition, we observed the assembly of activated ATM, Chk2 and H2AX into nuclear repair foci in HPV-31 positive cells. Upon differentiation, the number and size of the foci dramatically increased compared to undifferentiated cells. Using a specific inhibitor of ATM (KU-55933), we found that ATM kinase activity is not required for episomal maintenance in undifferentiated cells. However, inhibition of ATM activity in differentiating HPV-31 positive cells significantly impaired viral genome amplification, indicating that ATM activity is necessary during the productive phase of the viral life cycle. Conclusion: Overall, these results indicate that rather than circumventing the DNA damage response, HPV commandeers this pathway to facilitate the viral life cycle. Currently, experiments are underway to define which cellular repair proteins are necessary to promote HPV replication in differentiating cells.

O-08.04 GENOMIC INSTABILITY INDUCED BY THE HIGH RISK HUMAN PAPILLOMAVIRUSES

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In the HPV cancer cells, "high risk" HPVs are found in most of the cases integrated into the cellular genome. The integrated subgenomic HPV fragments express oncoproteins E6 and E7 at the elevated levels necessary to maintain the transformed state and carry the functional origin of replication. We have previously shown that HPV replication proteins E1 and E2 induce bidirectional re-replication from the integrated HPV origin, which leads to the rearrangements within the locus of integrated HPV. In the current study, we demonstrate that the E1 and E2 dependent initiation of the DNA replication from the integrated HPV origin follows the "onion skin"-type replication mode leading to the generation of the heterogeneous population of replication intermediates, including linear, branched, open circular and supercoiled plasmids as detected by two-dimensional neutral-neutral gel-electrophoresis. We show by immunofluorescence analysis that the DNA repair/ recombination centres are assembled at the sites of integrated HPV replication, which recruit viral and cellular replication proteins, MRE complex, Ku70/80, ATM, Chk2, and to some extent ATRIP and Chk1 (S317). We also demonstrate that the synthesis of histone yH2AX - the hallmark of the DNA double strand breaks - is induced and the Chk2 is activated by phosphorylation in the HPV replicating cells. All these changes suggest that the replication intermediates are actively processed by the cellular DNA repair/recombination machinery. Finally, the FISH analysis reveals that the de novo crosschromosomal translocation can occur in the cells where the re-replication of integrated HPV could be induced. Most importantly we demonstrate that the same changes take place under the physiological conditions in the HeLa and SiHa cells where HPV16 and HPV18 replicate episomally. We conclude that the presence of HPV replication origin in the cellular genome contributes to the formation of the genomic instability commonly seen in HPV-associated cancer cells.

O-08.05

ROLE OF L2 CYSTEINES IN INFECTION AND NEUTRALIZATION BY RG-1

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BACKGROUND: The L2-specific monoclonal antibody RG-1 neutralizes HPV16 and HPV18. The RG-1 epitope is not fully accessible until after virions have bound to the cell surface and L2 is cleaved by furin. RG-1 recognizes L2 residues 17-36 that include two cysteine residues conserved between HPV genotypes. Vaccination with this epitope in the appropriate context induces neutralizing antibodies in rabbits, and protects mice from experimental viral challenge. OBJECTIVES: To determine the role of two cysteines and other conserved residues within this epitope during infection and neutralization by the RG-1 antibody. METHODS: Cyclized or reduced peptides encompassing residues 17-36 were tested for binding to RG-1 by ELISA. Mutations to alanine or serine, or deletion of conserved residues within the RG-1 epitope were introduced into full length HPV16 L2 and tested for their impact upon binding by RG-1 in Western blot. HPV16 pseudovirions incorporating mutant L2 were prepared and their uptake and infectivity compared to wild type. RESULTS: RG-1 bound equivalently to HPV16 L2 peptides 17-36 with or without an intact C22-C28 disulphide bridge in ELISA. RG-1 also bound to HPV16 L2 13-31, but not to 9-28 or 25-44. Mutations K20A, C22A, C22S, C28A, C28S, or P29A prevented RG-1 binding to HPV16 L2 by Western blot, whereas Y19A, K23A or Q24A had no impact. Deletion of residues 17-29 or 27-36 from HPV16 L2, or point mutation of either C22 or C28 to alanine or serine eliminates both viral infectivity and reactivity with RG-1, but does not compromise virion assembly. Despite their lack of infectivity, HPV16 pseudovirions containing C22A or C28A mutant L2 bind to cell surfaces and expose the 17-36 region on the virion surface as for wild type virions. CONCLUSIONS: Two adjacent cysteines within HPV16 L2 play a critical role in infection, and are recognized by RG-1.

O-08.06

HPV16 L2 INTRAMOLECULAR DISULFIDE BOND IS CRITICAL FOR INFECTIVITY

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Minor capsid protein L2 performs an indispensable but uncharacterized role in human papillomavirus infection. A neutralizing B cell epitope has recently been mapped to the N-terminus of HPV16 L2, residues 17-36. Here we examine the redox state of Cys22 and Cys28 two highly conserved cysteines located within this epitope. Denaturing/non-reducing gel analysis and thiol labeling experiments of wild type and cysteine mutant HPV16 virion particles strongly support the existence of a buried intramolecular C22-C28 disulfide bond. The disulfide was confirmed by tandem mass spectrometry (MS/MS) of L2 protein from non-reduced virions. Single C22S and C28S and the double C22/28S mutants were non-infectious but had no defects in cell binding, endocytosis, or trafficking to lysosomes by 8h post infection. During infection with L2 mutant particles, there was a marked decrease in L2 levels compared to wild type L2-containing virions, suggesting a failure of mutant L2/genome complexes to exit the endo/lysosomal compartment. Previous work has suggested that the furin-dependent exposure of the 17-36 epitope and subsequent interaction of this region with an unknown receptor is necessary for egress from the endo/lysosomal compartment and infection. Identification of the C22-C28 disulfide suggests that reduction of this disufide bond may be necessary for exposure of 17-36 and HPV16 infection.

O-08.07

TISSUE TROPISM OF CANINE PAPILLOMAVIRUS IS DETERMINED INVIVO AND KERATINOCYTE-INDEPENDENT

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Canine oral papillomavirus (COPV) preferentially infects the oral mucosa of dogs whereas Canis familiaris papillomavirus type 2 (CfPV2) infects footpads and interdigital regions. We have compared the ability of these viruses to replicate in three distinct tissue sites in vivo and ex vivo. Consistent with its known natural tropism, CfPV2 was incapable of inducing papillomas on oral mucosa. However, when oral and footpad biopsies were infected ex vivo with CfPV2 and xenografted into SCID mice, CfPV2 induced tumors that contained high-titer CfPV2, indicating that CfPV2 can replicate ex vivo in oral mucosa equivalent to that observed in footpad tissue. Similarly, COPV, although capable of inducing oral tumors in vivo, failed to induce papillomas on genital mucosa. However, when biopsies of dog oral mucosa or genital mucosa were infected ex vivo and implanted into SCID mice, large tumors were produced from both tissues. Histologically these lesions were equivalent and L1 capsid protein was detected by immunohistochemistry. Moreover, infectious virus particles were isolated from both of these lesions. Finally, COPV could also induce productive tumors using biopsies of footpad tissue. Together, these data indicate that tissue tropism for the canine papillomaviruses can be bypassed ex vivo, suggesting that viral replication and tumor formation is independent of keratinocyte origin but rather is regulated by local in vivo factors.

O-08.08

TOWARDS AN UNDERSTANDING OF PAPILLOMAVIRUS LATENCY USING THE ROPV MODEL

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Evidence from disease states induced by human papillomavirus (HPV) infection would suggest that papillomaviruses persist in tissues following immune-mediated lesion regression in the absence of clinical signs of disease. Such infections may later be reactivated to form a productive infection. Studies in animals have demonstrated the ability of viral DNA to persist following lesion regression and the ability of viral RNA to be detected following induction of an asymptomatic infection (caused by low level virus inoculation) without any lesion formation. Rabbit oral papillomavirus (ROPV) closely mimics the life-cycle of low-risk HPVs. Following experimental infection of the tongue in New Zealand White rabbits, small papillomas appear macroscopically within 2 weeks and grow until 4-5 weeks with complete regression by 8 weeks. Using this model, we have detected ROPV DNA and spliced RNA transcripts up to 22 weeks post-regression in the absence of macroscopic and microscopic signs of disease using real-time PCR. Following a rapid and large decline in ROPV DNA and RNA during lesion regression, there follows a more gradual but persistent decline. mRNA encoding sequences within the E1/E2/E6/E7 ORFs is detectable during this asymptomatic period but no evidence of a productive infection as determined by E4 and L1 immunofluorescence is apparent. Our results show that persistence of ROPV following regression is associated with a gradual loss of viral DNA. Despite evidence of viral activity as determined by mRNA detection, there is a failure of the virus to complete its life-cycle. It is likely that the immune system is central to maintaining ROPV in an asymptomatic state. We are examining this by observing reactivation of the virus following immunosuppression, and by analysis of the regression sites by laser capture microscopy.

POSTER ABSTRACTS SESSION 08

POSTER SESSION I MONDAY 10.00: ODD NUMBERS

POSTER SESSION II TUESDAY 10.00: EVEN NUMBERS

P-08.09

TISSUE-SPANNING REDOX GRADIENT-DEPENDENT ASSEMBLY OF NATIVE HUMAN PAPILLOMAVIRUSES

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Recent research utilizing pseudovirions (PsV) suggests that papillomavirus capsids can undergo a redox-dependent conformational change that takes place over the course of many hours. This conformational change is characterized by resistance to proteolysis, chemical reduction, and the appearance of a more orderly capsid structure via TEM. The possibility that native virions (NV) may also undergo a redox-dependent "maturation" event was examined in the context of the complete papillomavirus life cycle utilizing organotypic "raft" cultures. Human foreskin keratinocytes infected with HPV16 were grown for 10, 15, and 20 days with the intent to expose virions to the highly oxidizing environment of the cornified envelope for extended periods of time. Hematoxylin and eosin staining of raft tissue depicts a continual build-up of the cornified layer at days 15 and 20 while the basal layer retains its original width, suggesting that virions assembled by day 10 would eventually collect in the cornified layer. Western analyses of Optiprep-purified viral lysates confirm an increase in the oxidative state of 15 and 20 day tissues as cellular keratin becomes increasingly dense over time. Correlating with the oxidative state of the tissue, 20-day virions are more resistant to chemical reduction and endonuclease-treatment, have a higher infectious titer, and are more susceptible to neutralization via anti-L2 "external loop" Abs than 10 and 15-day virions. These results suggest that maturation of virions takes place over the course of many days within tissue. Further, treatment of organotypic cultures with various redox-altering compounds such as: GSH, GSSG, and seleniumbased reagents modulate the phenotypes listed above. Further research into the redox-dependent assembly and maturation mechanisms of NV will be presented.

P-08.10 HEAT SHOCK PROTEIN 70 FUNCTIONS IN HPV VIRION PRODUCTION

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Heat shock proteins (HSPs) are induced by many stressors and have numerous cytoprotective activities wherein they interact with diverse protein substrates to assist in folding to prevent misfolded or otherwise damaged protein molecules. HSPs also function in protein transportation between cellular organelles. HSPs, including inducible HSP70 (HSP70i), are involved in the replicative cycles of many different viruses, but relatively little is known about the role of HSP70i in PV life cycle activities. Cell-free studies implicate HSP70i in viral genome replication with E1 and E2 and in capsid assembly and disassembly for PV and polyomaviruses. Herein we analyzed HSP70i's role in the HPV infectious life cycle in the natural host cell. We used the organotypic (raft) epithelial tissue culture system to recapitulate the full viral life cycle of the carcinogenic viral type, HPV31. Upon heat shock of HPV31 infected epithelial tissues, we find high and sustained expression of HSP70i coincident with enhanced HPV genome replication and virion production. Whereas there is no effect on L1 capsid protein expression levels, HSP70i colocalizes with and enhances L1 localization to the nucleus of differentiated cells. Adenovirus-mediated gene transfer was used to study the direct effects of HSP70i in naturally HPVinfected differentiating raft tissues and in monolayer cells expressing HPV L1 and L2 capsid proteins. Results were similar to those in heat-shocked tissues. In monolayer cells, HSP70i increases genome replication and promotes cytoplasmic to nuclear relocalization of L1 without obvious effects on L1 expression levels. Results indicate that HSP70i augments HPV genome replication and the production of infectious virions. We are currently focused on determining the mechanisms by which HSP70i impacts the specific HPV31 life cycle activities, including PV genome replication, L1/L2 localization, and capsid assembly.

P-08.11

ROLE OF THE E4 CYCLIN-BINDING MOTIF IN HPV18 REPLICATION

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Some HPV E4 proteins have been shown to perturb the keratin cytoskeleton in overexpression systems; an event that is linked to sequestration of cyclins. Tethering of cyclin B1 in the cytoplasm is important for G2 arrest by HPV16, although the relevance of this in infections is unknown.

We have identified a cyclin-dependent kinase (cdk)-bipartite consensus recognition motif that consists of a cdkphosphorylation site (Thr23) and a cyclin-binding (Cy) motif consisting of two overlapping RXL motifs (43-RRL-45; RXL-1, 44-RLL-46; RXL-2) in HPV18 E1^E4. Mutations generated within the motif show that the Cy-motif is required for interaction with cyclins A, B and their kinase partners. The Cy-motif is also essential for cdk1/2 phosphorylation of Thr23. Overexpression of HPV18 E1^E4 in epithelial cells causes sequestration of cyclin-cdks into the cytoplasm and this is dependent on the Cy-motif, but not Thr23.

Full-length E1^E4 functions support HPV18 genome amplification in human foreskin keratinocytes (HFK). Therefore to determine whether the bi-partitie motif is involved in viral DNA amplification we constructed HPV18 mutant genomes containing a disrupted motif and established cell lines in multiple HFK donors. Neither viral genome establishment or maintenance replication was dependent on the integrity of this motif. Induction of viral DNA amplification following suspension of cells in methylcellulose was not dependent on the cdk-phosphorylation site, T23. However, while we observe amplification of genomes containing mutations within the Cy-motif (RXL-1 and RXL-2), the level of amplification is reduced in RXL-1 cells, but not those containing RXL-2 mutant genomes. Our data therefore suggests that an association between E4 and cyclins is not essential for viral DNA amplification, although it may have a role in facilitating efficient replication in differentiated cells. Further investigation of the affect of these mutations on vegetative HPV18 functions is ongoing.

P-08.12

E1^E4 CONTRIBUTES TO HPV GENOME AMPLIFICATION BUT IS NOT ESSENTIAL

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The E1^E4 protein is abundantly expressed during productive infection and coincides with the onset of viral genome amplification. It reorganizes cytokeratin network and induces cell-cycle arrest at G2/M. Several groups' work suggests that full length E1^E4 protein from high-risk HPV types 16, 18, 31, and CRPV contributes to efficient genome amplification in the late stages of the virus life cycle. Absence of E1^E4 in CRPV inhibits viral genome amplification. Loss of full length E1^E4 in HPV18 and 31 disrupt virus late functions in differentiated primary human keratinocytes. Different truncations of 16E1^E4 protein have different effects on the activation of viral late function in differentiated NIKS, a spontaneously immortalised human keratinocyte cell line. However, mutational analysis in HPV11 fails to demonstrate any biological activity for E1^E4 in the productive stage of virus life cycle in differentiated hTERT immortalized human keratinocytes. Thus data is contradictory and the role of E1^E4 in natural infection is unclear. To further elucidate the role of E1^E4 in viral genome amplification, we established a number of HPV16 and 18 genome wild type (WT) or E1^E4 knock-out (E4KO) containing NIKS populations and cell lines. When grown in monolayer culture, there was no significant difference in growth rate between WT and E4KO cells. Southern blotting and QPCR revealed however that genome amplification was delayed rather than abolished in the E4KO cells following methylcellulose (MC) treatment, and that the full productive cycle can be supported in organotypic raft culture in the absence of E1^E4 expression. These data are consistent with the idea that E1^E4 enhances replication efficiency and life-cycle completion rather than being essential for these events. Differences in the timing of analysis may underlie the different results reported in the literature. The nature of the accessory functions provided by E1^E4 is currently under investigation.

P-08.13 PRODUCTION AND CHARACTERIZATION OF INFECTIOUS VIRUS OF HPV16 AFRICAN VARIANTS

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HPV16 variants containing intratype variations in its genome are defined as containing less than a 2% difference in the sequence of the L1 major capsid protein as compared to the prototype genome of a German isolate of the European family of HPV16 variants. The variants are classified into the geographical regions from which they originate. Epidemiological data demonstrate a connection between certain variants and an increased risk of cervical cancer. Interestingly, studies show that infection by HPV16 variants of African origin persist longer in African American women whereas European variants persist longer in white women. This suggests that sequence variations could have an effect in the biological behavior of the virus. In addition, HPV infection appears to be prevalent in both men and women but the occurrence of cancers associated with HPV infection is greater in women suggesting that gender specific factors may impact the viral life cycle. Variant HPV16 genomes from the African type 2 branch were isolated from clinical samples by rolling circle amplification (RCA). Following electroporation stable cell lines from human foreskin keratinocytes (HFKs) and human cervical keratinocytes (HCKs) maintaining episomal variant HPV16 genomes have been selected by immortalization. The organotypic "raft" culture system was used to produce native virions from these cells. Three different HPV16 variants were analyzed to determine if the variants differ in activity compared with the prototype. Preliminary data suggest higher viral titers are obtained from human cervical keratinocytes compared with foreskin kerationcytes suggesting the influence of gender specific factors. The HPV16 variants are being sequenced to identify major regions of sequence divergence. Data will be presented correlating sequence variations to differences in viral replication and the effect of the host on the viral life cycle.

SESSION 09

VIRAL GENOME REPLICATION AND ANTIVIRALS

PROGRAMME

ROOM

THE 25TH INTERNATIONAL PAPILLOMAVIRUS CONFERENCE MAY 8-14 2009, MALMÖ, SWEDEN

TIME NUMBER TITLE

2009-05-11

SESSION 09: VIRAL GENOME REPLICATION AND ANTIVIRALS K1-3 ANALYSIS OF PAPILLOMAVIRUS INITIATION OF DNA REPLICATION 15.00-15.30 O-09.00 A Stenlund, S Schuck 16.00-16.11 O-09.01 DRUG-LIKE COMPOUNDS THAT INHIBIT HPV-16 E6 E Androphy, J Baleja, J Cherry 16.11-16.22 O-09.02 HPV-11 E1 RELOCALIZES MRE11 AND NBS1 TO REPLICATION CENTERS L.T. Chow, L.L. Dao, J.H. Yu, T.R. Broker MITOGEN-ACTIVATED PROTEIN KINASES ARE CRUCIAL FOR HPV DNA 16.22-16.33 O-09.03 AMPLIFICATION J.H. Yu, T.R. Broker, B.Y. Lin, H.K. Wang, L.T. Chow 16.33-16.44 O-09.04 TRANSCRIPTION FACTOR REQUIREMENT FOR E2 MEDIATED PLASMID SEGREGATION T Silla, A Männik, M Ustav PAK3 INHIBITORS IDENTIFIED BY HIGH-THROUGHPUT-SCREENING AS THERAPEUTICS FOR HPV-ASSOCIATED CANCERS 16.44-16.55 O-09.05 K Hellner, A Baldwin, J Xian, R Stein, M Glicksman, K Munger 16.55-17.06 O-09.06 RHO KINASE INHIBITION INCREASES KERATINOCYTE PROLIFERATION AND HPV DNA REPLICATION A McBride, S Chapman DIFFERENTIAL METHYLATION OF HPV16-URR DURING EPITHELIAL 17.06-17.17 O-09.07 DIFFERENTIATION AND NEOPLASTIC TRANSFORMATION S Vinokurova, M Reuschenbach, C Sutter, F Kommoss, D Schmidt, M von Knebel Doeberitz COX-2 AND PGE-2 - TARGETS IN RECURRENT RESPIRATORY 17.17-17.28 O-09.08 PAPILLOMAS B. Steinberg, R. Wu, A. Abramson, M Shikowitz

ANALYSIS OF PAPILLOMAVIRUS INITIATION OF DNA REPLICATION

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Preparation of a DNA template for initiation of DNA replication requires the sequential utilization of activities that recognize the origin of DNA replication (ori), melt the DNA duplex, unwind the DNA template and recruit and load replication factors. The biochemical basis for these events is poorly understood largely because of the high degree of complexity of the machineries involved in these processes. In papillomaviruses, all of these activities reside in one or a few polypeptides providing a system with low complexity for study.

The first of these steps is carried by the E1 initiator and the E2 transcription factor, which together form a complex on the ori (E12E22). The second step, template melting, is carried out by a double trimer (DT) of E1 utilizing a particular structure feature, a -hairpin in the E1 helicase domain. The DT is a direct precursor for the E1 double hexamer (DH), which has helicase activity and unwinds the ori DNA. We have analyzed the transition from the DT to the DH using genetic and biochemical approaches. By following the transition from melting to helicase formation over time, we can now provide a description of the changes in template structure that accompany template melting and helicase loading. We have also performed a complete surface mutagenesis of the E1 helicase domain and we have identified 10 mutants that arrest at specific points in the DT to DH transition. Paradoxically, although these mutations do not affect the formation of the starting complex (DT) or of the hexameric helicase, they affect the transition between these two points. These mutations define a new E1 interaction surface, which is conserved between papillomavirus E1 proteins, but not with the initiator proteins from the polyomaviruses.

O-09.01 DRUG-LIKE COMPOUNDS THAT INHIBIT HPV-16 E6

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The high-risk human papillomavirus E6 proteins that cause cervical cancer bind to the ubiquitin ligase E6AP and mediate p53 ubiquitination and degradation. E6AP and other cellular proteins encode a specific amino acid motif that is recognized by E6. In addition, E6 proteins bind to a diverse set of cellular PDZ factors through a C-terminal domain factors that is not involved in E6AP or p53 association. We have pursued a small molecule approach to identify inhibitors of E6 and potential antiviral therapy. Using structure-based methods to select compounds that mimic the conserved E6 binding motif, we have identified chemicals that inhibit E6 binding to E6AP and block p53 degradation. We also designed and implemented a high-throughput screen of a chemical diversity library and isolated another series of E6 inhibitory compounds. These chemical entities are being tested in cell-based models and preliminary data demonstrate inhibition of E6-dependent p53 degradation. Based on preliminary structure-activity relationships (SAR), we have identified three classes of compounds. Development of highly active, high potency E6 inhibitors may be useful in the pharmacologic treatment of HPV infections and malignancies.

HPV-11 E1 RELOCALIZES MRE11 AND NBS1 TO REPLICATION CENTERS

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Background: a number of DNA viruses encode proteins that cause the degradation and relocalization of the MRN complex to the viral replication centers. These interactions are crucial to viral DNA amplification. The MRN complex, composed of Mre11, Rad50, and Nbs1 proteins, is the main repair complex for double-stranded DNA breaks. MRN localizes to sites of DNA damage, leading to ATM activation, cell cycle arrest, and DNA repair. Objectives: We investigated whether MRN played a role in HPV DNA replication. Methods: We examined the localization of MRN in primary human keratinocytes into which an HPV ori-containing plasmid was cotransfected with an operon encoding HPV-11 GFP-E1 and E2 proteins necessary for ori-specific replication. Fluorescence microscopy, site directed mutagenesis, and biochemical analyses were conducted. Results: we showed that Mre11 and Nbs1, but not Rad50, were recruited to the HPV replication centers that were positive for GFP-E1 and RPA. Recruitment of MN required active ori replication but did not lead to degradation of either protein. Mre11, but not Nbs1, was co-immunoprecipitated with E1 from cell lysates. An E1 protein mutated in a putative Mrell binding motif abolished co-immunoprecipitation with Mrell and the recruitment of MN to the replication centers. However, this mutant form of E1 supported ori replication as efficiently as the wild type protein. The recruitment of MN did not lead to ATM activation at the replication centers, as revealed by yH2AX localization. Moreover, as with other DNA viruses, some of the replication centers were juxtaposed to a subset of PML bodies. This distribution was independent of MN. Conclusions: Unlike the other DNA viruses, the recruitment of MN by the E1 protein to the HPV replication centers is not essential for viral ori-specific replication in this transient replication assay and this recruitment does not activate ATM.

O-09.03

MITOGEN-ACTIVATED PROTEIN KINASES ARE CRUCIAL FOR HPV DNA AMPLIFICATION

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Background: Human papillomaviruses (HPVs) are prevalent pathogens that infect cutaneous or mucosal epithelia. There are no drugs that can reliably treat HPV infections. The productive phase of HPV infection takes place only in differentiated keratinocytes. Our lab has recently achieved robust HPV-18 production in organotypic (raft) cultures of primary human keratinocytes. Viral DNA replication is dependent on the ori and the virus-encoded ori binding protein E2 and replicative helicase E1. All other replication machineries and substrates are supplied by the host cell. Our previous study has shown that HPV-11 E1 is phosphorylated by mitogen-activated protein kinases (MAPKs) to enable efficient nuclear import and viral ori replication and that the MAPK docking motifs and substrates are conserved among E1 proteins of HPVs and BPV-1. Objectives: we wish to determine whether MAPKs are also crucial for HPV-18 DNA amplification in PHK raft cultures. Methods: We examine HPV-18 DNA amplification in PHK raft cultures in the presence of MAPK inhibitors and that of an E5 mutant genome. Results: ERK- or p38-specific inhibitors effectively inhibited the wild type viral DNA amplification without affecting host DNA replication, nor squamous differentiation. An HPV-18 E5 knockout mutant had the same phenotype as inhibitor-treated raft cultures harboring the wild type genome. Conclusions: We propose that E5 is responsible for MAPKs activation in differentiated keratinocytes to facilitate E1 nuclear import crucial for viral DNA amplification. We also propose that E1 nuclear import can be an effective platform to screen for small molecular weight chemicals that can inhibit HPV DNA replication.

TRANSCRIPTION FACTOR REQUIREMENT FOR E2 MEDIATED PLASMID SEGREGATION

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Several eukaryotic DNA viruses maintain their genomes as extrachromosomal multicopy nuclear plasmids in proliferating host cells. Such episomal maintenance is characteristic of latent infection of the Bovine papillomavirus type 1 (BPV1), Epstein-Barr Virus (EBV) as well as for Kaposi sarcoma associated Human herpesvirus type 8 (HHV8). For the BPV1 and two members of the gammaherpesvirus family – EBV and HHV8 - an effective segregation of viral genomes into daughter cells and nuclear retention during mitosis is mediated through a single viral protein serving as a molecular linker, which attaches viral genomes to the host mitotic chromosomes. These linker proteins are E2 for BPV1, LANA for HHV8 and EBNA-1 for EBV. The BPV1 E2 protein is well characterized and it has been shown that N-terminal transactivation domain is responsible for chromatin association. Additionally, this domain interacts with the E1 protein and carries transactivation function. For LANA of HHV8 it has been shown that first 22 amino acids from N-terminus are required for chromatin attachment. We have generated a series of E2 pointmutants and number of chimerical E2 proteins where we have replaced E2 transactivation domain with different domains from the analogous proteins of herpesviruses. Analysis shows that segregation/partitioning function is dependent on several domains, including specifically chromatin attachment domain and transcription activation domain. We shall present the data demonstrating the role of each and every domain in assuring the segregation/partitioning function to the viral genome at the different steps of the cell cycle and we would discuss the mechanism of segregation/partitioning provided by the viral proteins in the case of these viruses.

O-09.05

PAK3 INHIBITORS IDENTIFIED BY HIGH-THROUGHPUT-SCREENING AS THERAPEUTICS FOR HPV-ASSOCIATED CANCERS

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High-risk human papillomaviruses (HPVs) are etiologic agents of cervical carcinomas. Due to viral genome integration during malignant progression, only the HPV E6 and E7 oncoproteins remain consistently expressed in the tumors. To determine whether HPV oncoprotein-mediated cellular network perturbations can be harnessed to identify novel therapeutic targets, an shRNA high-throughput kinase screen was performed. 33 kinases were identified that are essential for the survival of cervical cancer cell lines, but not essential for normal epithelial cells. Further analyses revealed that one of these kinases, PAK3, a p21/Cdc42-activated protein serine/threonine kinase, becomes essential as a direct consequence of p53 tumor suppressor inactivation by HPV16 E6 expression. Depletion of the p53 tumor suppressor by RNA interference in primary epithelial cells is sufficient to confer sensitivity to PAK3 inhibition. Therefore, PAK3 serves an excellent target for anti-cancer therapy. To identify potential inhibitors, we developed an enzyme assay to screen a small molecule library containing 125,000 compounds in a high throughput setting, using homogeneous time resolved fluorescence resonance transfer technology. The screen identified 580 compounds that result in >70% inhibition of PAK3 activity. These "hits" were reconfirmed using triplicates of 10-fold dilutions of the initial screening concentration. 137 compounds proved to be potent inhibitors of PAK3. Accordingly to their potency profile and chemical structure, 16 are currently being evaluated as leads for the development of anti-neoplastic agents.

RHO KINASE INHIBITION INCREASES KERATINOCYTE PROLIFERATION AND HPV DNA REPLICATION

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Human papillomaviruses replicate persistently in specific types of cutaneous or mucosal epithelia. HPVS will only replicate in keratinocytes and, in the laboratory will only undergo their complete life cycle and generate progeny virus in the tissue culture equivalent of a stratified epithelium. This process requires establishing stably replicating viral DNA genomes in primary human keratinocytes and culturing them in three-dimensional skin equivalents. For the most part, such studies have used "high-risk" HPVs because the E6 and E7 oncoproteins of these viruses are able to immortalize the host keratinocytes. However, keratinocytes will often senesce before cells containing stably replicating "low-risk" HPV genomes can be selected and the organotypic culture established. We show here that inhibition of the ROCK (Rho kinase) pathway increases proliferation and immortalizes primary human keratinocytes. The cells are maintained in a basal-like state and thus provide the ideal environment for establishment of HPV infection. ROCK inhibited cells have a greatly increased ability to support viral DNA replication of both "low risk" and "high risk" HPV genomes and will be invaluable in studying the life cycles of a wide range of HPVs.

O-09.07

DIFFERENTIAL METHYLATION OF HPV16-URR DURING EPITHELIAL DIFFERENTIATION AND NEOPLASTIC TRANSFORMATION

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Background. Replication and expression of HPV-genomes depends on the differentiation status of the epithelial host cells. Progression of acute to transforming HPV-infections is triggered by the expression of the E6 and E7 genes in basal cells. Objectives. Methylation of the viral genome may affect regulatory features that control transcription of the viral genome. Here, we analyzed the methylation pattern of HPV16 URR during epithelial differentiation and neoplastic transformation and analyzed how changes in HPV URR methylation affect the viral oncogene expression. Methods. HPV16 DNA isolated from laser-microdissected epithelial cells with different degrees of differentiation was analyzed by bisulfite genomic sequencing (BGS). Subsequently, we compared the methylation pattern of the HPV16 URR in HSIL lesions with adjacent infected normal epithelium using p16INK4a as biomarker for HPV transformed epithelial cells. Results. The p97 promoter in basal cells of low grade lesions contained unmethylated CpGs. In contrast, the HPV16 promoter in differentiated superficial cells was methylated, including CpGs within E2 and SP1 binding sites. In the enhancer region 4 CpGs within NF1 and TEF1 binding sites (CpG) were heavily methylated in the basal cells but showed a lower degree of methylation in the more differentiated cells. The 5' LCR region including 2 CpGs within the distal E2 binding site (E2BS1) was hypomethylated in all differentiation stages. In the majority of high grade squamous intraepithelial lesions (9/10, 90%), consistent methylation of E2BS1 was observed. Methylation of E2BS1 leads to 4-6 fold activation of the early p97 HPV16 promoter. Conclusions. These data underline the hypothesis that the methylation state of the viral genome is substantially changed depending on the degree of epithelial differentiation, thereby mediating the differentiation dependent expression signature of HPV genomes. Methylation of the E2 binding site 1 activates the p97 promoter and allows for uncontrolled high level of viral gene expression.

COX-2 AND PGE-2 - TARGETS IN RECURRENT RESPIRATORY PAPILLOMAS

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Recurrent respiratory papillomas are pre-malignant tumors of the airway caused by HPV 6/11, that overexpress the EGFR and the GTPase Rac1, have enhanced PI3K and NF-kB activity with decreased levels of IkB-b but not IkB-a, and express COX-2 and its product PGE-2. Exogenous PGE-2 increases papilloma cell proliferation while celecoxib, a selective COX-2 inhibitor, reduces proliferation and increases apoptosis. A pilot clinical trial of celecoxib has shown significant efficacy in treatment of the disease, and a large double blinded placebo-controlled study is in progress. Using cultured papilloma cells, we are studying signal transduction pathways that contribute to COX-2 induction, and asking whether the same pathways are activated by PGE-2 in a positive feedback loop.

The p21-activated kinase (Pak) family members are serine/threonine kinases that can be activated by Rac and related GT-Pases. Pak1 and Pak2 (Pak1/2) phosphorylation is markedly increased in papillomas tissues and cultured papilloma cells compare to normal laryngeal epithelium. Knockdown of Rac1 with specific siRNA reduced the levels of phospho-Pak1/2, and knockdown of either Pak1 or Pak2 partially reduced expression of COX-2. Knocking down Pak 1/2 with siRNAs increased levels of IkB-b in papilloma cells, and reduced nuclear localization and abundance of NF-B. Our studies suggest that the EGFR activates parallel Rac1 to Pak1/Pak2 to NF-kB, and Rac1 to p38 MAPK pathways, and that both pathways contribute to COX-2 expression in respiratory papillomas. Initial studies show that PGE-2 activates at least some of the same pathways involved in COX-2 expression. These pathways could potentially be effective therapeutic targets for HPV-induced tumors. Laboratory studies that are part of the celecoxib clinical trial will ask whether these same pathways are affected in vivo, and address possible mechanism(s) of efficacy. An update on the status of the trial will be provided.

POSTER ABSTRACTS SESSION 09

POSTER SESSION I MONDAY 10.00: ODD NUMBERS

POSTER SESSION II

TUESDAY 10.00: EVEN NUMBERS

THE STUDY OF STABLE REPLICATION OF HUMAN PAPILLOMAVIRUSES

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Papillomaviruses have evolved systems to either overrun the cellular defence to replicate their small genome multiple times per cell cycle or to mimic the cellular DNA replication by replicating it approximately once per cell cycle. Most of our knowledge about the episomal maintenance during latent infection has been accumulated from studies of the BPV1, adequate information about the HPVs is limited. The long-term stable maintenance of BPV1 replicon is dependent on the E1 and E2 proteins linked with non-covalent attachment of the viral genomes to host chromatin, efficient partitioning and nuclear retention during mitosis. The E2 protein tethers the genome by binding to the multimeric E2 binding sites identified as Minichromosome Maintenance Element.

We developed stable cell lines constitutively expressing the E1 and E2 proteins in different cell lines, including human keratinocytes. The expression vectors for viral replication proteins of HPV11, HPV16 and HPV18 has been engineered based on the accumulated knowledge about the mRNA structures for effective expression of the E1 proteins. The promoters driving expression of the E1 and E2 have great importance for establishing the stable cell lines. We used the U2OS cells for transduction with the expression vectors for E1 and E2 proteins and used the stable cell lines in the complementation and replication assays. The stability of expression and compartmentalization of replication proteins was analysed by immunofluorescence using confocal microscopy and flow cytometry. The inheritance of different episomal replicator-based plasmids for HPV-11, -16, -18 were studied using G418 selection in these cell lines along with involved cellular control mechanisms to determine components that are necessary and sufficent for stable episomal replication, maintenance and segregation/partitioning.

The major objective of this study has been to identify the trans-factors and cis-elements involved into stable maintenance function of the HPVs and find the mechanism of establishment of latent infection.

P-09.10

ADENO-ASSOCIATED VIRUS TYPE 2 INDUCES APOPTOSIS IN HPV INFECTED KERATINOCYTES

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Background: Seroepidemiological studies showed that cervical cancer patients exhibit antibodies to AAV2 less frequently than matched controls, suggesting that AAV2 plays a "protective" role against cancer development. In HPV/AAV2 coinfected cultures, AAV2 targeted the p21WAF1 CDK inhibitor for accelerated proteosome mediated degradation. In contrast, AAV2 infection of primary human keratinocytes (HK) resulted in upregulated p21WAF1 protein levels, which was previously correlated with growth arrest in fibroblasts. Since in normal cells, p21WAF1 protein levels are decreased in preparation for S phase entry and progression, our observations appeared to contradict the role of AAV2 as a tumor suppressive parvovirus.

Objectives: To delineate potential mechanisms of AAV2 suppression of HPV oncogenesis, we characterized downstream consequences of AAV2 infection in actively cycling HPV infected cells.

Methods: We used the CIN-612 9E cervical cells which maintain episomal genomes of HPV31b. As controls, we used HK cells. Monolayer cell cultures infected with AAV2. Control and AAV2 infected cell samples were collected over a period of 7 days.

Results: AAV2 infected HPV cells culminated in apoptotic cell death, as determined by DNA laddering and caspase-3 cleavage. Cell death was correlated with increased percentage of cells with S phase DNA content concomitant with AAV2 Rep protein expression and diminished E7 oncoprotein levels. AAV2 regulated S phase entry was characterized by upregulated total pRb protein levels, displaying both hyper- and hypophosphorylated forms, increased p21WAF1, p16INK4 and p27KIP1 protein levels and increased CDK2 associated kinase activity. In contrast, primary human keratinocytes infected with AAV2 failed to express Rep proteins or undergo apoptosis.

Conclusions: AAV2 regulates death of HPV positive cells by targeting cell cycle pathways which promote breach of the G1/ S check-point, which further connect to pathways of apoptosis. AAV2 regulation of apoptosis in a HPV specific manner could be utilized for developing novel therapeutics for cervical cancer treatment.

ACTION OF THE HIV PROTEASE INHIBITOR LOPINAVIR AGAINST HPV

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Objective: High-risk types of the human papillomavirus are a major cause of human cancer. Many viruses, including HPV, inappropriately activate the proteasome to degrade cellular proteins that are detrimental to viral replication. Previous studies have shown that HIV protease inhibitors such as Lopinavir can inhibit this ability. Lopinavir has proved effective against the SARS virus, we have previously shown that it may also have the potential to treat HPV infections and we now report new observations on its prospective mode of action as an antiviral agent.

Methods: Proteins from Lopinavir treated and control HPV16+ve SiHa cervical carcinoma cells were extracted and labelled with Cy3 and Cy5 fluorophors. These were then used to immuno-probe a Panorama Xpress 725 antibody-microarray (Sigma) in order to investigate the effects of lopinavir on cellular protein expression.

Result: Of the 725 proteins represented on the microarray, 3 had decreased expression and 48 had increased expression following lopinavir treatment. Predictably, apoptosis related proteins such as survivin, p53 and annexin V were upregulated but most significant was the observation that RNase L was elevated by treatment with Lopinavir. This increase in RNase L protein has been confirmed by Western blotting whereas RT-PCR showed that lopinavir did not alter RNase L mRNA levels when compared to untreated cells.

Conclusion: RNase L is an interferon-inducible antiviral protein that is known to cleave viral RNA in infected cells. Our data is the first to indicate that the antiviral activity of Lopinavir may be due, in part, to stabilisation of the RNase L protein. We are currently confirming and validating this effect with siRNA mediated silencing of RNase L in HPV16 positive cells. This should establish a novel mode of action for lopinavir in relation to HPV and potentially to other viruses.

P-09.13

SCFV FRAGMENTS AGAINST HPV16 PROTEINS AND CHARACTERISATION OF THEIR ACTIVITIES

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We investigated different approaches based on antibodies in single-chain format to counteract and study the functions of different HPV16 proteins.

Anti-E7 scFvs. The scFvs 32,43,43M2 and 51 against the 16E7 oncoprotein, selected by phage-display, were previously characterized for their reactivity and stability. The most stable 43M2 and 51 were shown to inhibit HPV16-positive SiHa cell proliferation when expressed in the nucleus and the endoplasmic reticulum, by a retroviral system. The scFvs epitope mapping was performed by Biacore and immunoassays using several 16E7 point and deletion mutants. The results show that the anti-E7 scFvs bind the E7 N-terminus but not the C-terminus. The binding of scFvs 32 or 51 to E7 is mutually exclusive with that of scFv43 or 43M2. Immunoassays results suggest that scFv43 and 43M2 bind to the 16E7 pRb-binding site, implying that the mechanisms underlying their antiproliferative activity are different with respect to scFv 32 and 51. Experiments are in progress to evaluate the scFvs efficacy in counteracting the E7 oncogenic activity in a mouse model.

Anti-E6 scFv. The scFvI7 against the 16E6 oncoprotein was selected by IAC technology by Dr Visintin(SISSA,Italy). Its intracellular expression was characterized in SiHa cells by immunofluorescence. Its effect on p53 protein, the main E6 target, was evaluated and the results showed an increased intracellular level of p53.

Anti-HPV16 pseudovirions scFvs. Six scFv antibodies against the HPV16 pseudovirions, reconstructed in Dr Schiller's Lab (NIH,USA), were selected by Phage Display Technology. The selected scFvs were shown to recognise the recombinant protein 16L1 in immunoassays in vitro. One aim was to individuate the best candidates to be expressed as "intracellular antibodies" to study viral assembly, a crucial step for virus maturation. Furthermore, the scFv neutralizing capacity is being evaluated to choose candidates for in situ drugs blocking HPV virus infections and their sexual transmission.

P-09.14 TUMOR CELL SELECTIVE CYTOTOXICITY INDUCTION BY AN PLANT PREPARATION

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The plant Brucea javanica has shown impressive efficacy for treating various diseases including cancer. However, the mechanism by which B. javanica acts is poorly understood. Numerals attempts have been made to identify active ingredients from the organic extract of B. javanica but the results were disappointing. We have established tissue culture assays to study the effects of B. javanica on cervical cancer cells. Our results demonstrated that the water soluble but not organic extract from B. javanica is selectively toxic to cervical cancer cells but not normal control cells. Induction of apoptosis by B. javanica appears to be a possible mechanism by which it kills cancer cells. The effect of B. javanica is not limited to cervical cancer as it also kills several other cancer cells. Interestingly, a significant increase of p53 protein level was observed in these apoptotic cells. Our studies indicated that both p53-dependent and p53-independent activities contributed to herb-induced cell death. Using Liquid chromatography (LC)/mass spectrometry (MS), a number of fractions from an extract of the herbal preparation were collected and demonstrated to have cell toxicity. These results imply that further studies with B. javanica may lead to the development of novel anti-cancer drugs.

P-09.15

INHIBITION OF THE EGF-RECEPTOR PREVENTS IMMORTALIZATION BY HPV

C Woodworth, Clarkson University, Potsdam, New York, USA D Jette, Clarkson University, Potsdam, New York, USA A Mohammed, Clarkson University, Potsdam, New York, USA M Moses, Clarkson University, Potsdam, New York, USA K Scalia, Clarkson University, Potsdam, New York, USA D Stevens, Clarkson University, Potsdam, New York, USA E Vandermark, Clarkson University, Potsdam, New York, USA Background: The HPV E6 and E7 oncogenes are selectively r

Background: The HPV E6 and E7 oncogenes are selectively retained and expressed in most cervical carcinomas, and expression of E6 and E7 is sufficient to immortalize normal human epithelial cells. However, most HPV infections do not progress to malignancy, indicating that additional cofactors are important. Cervical epithelial cells express multiple epidermal growth factor-related mitogens, and the epidermal growth factor receptor (EGF-R) is often over expressed in cervical carcinoma. EGF-R function can be selectively inactivated by small molecule kinase inhibitors that block ATP binding and autophosphorylation of the receptor on tyrosine.

Objectives: We investigated whether one EGF-R inhibitor, Erlotinib, could prevent immortalization of human cervical epithelial cells by the HPV-16 E6/E7 oncoproteins.

Methods: Cells were cultured from normal cervix and infected in vitro with HPV-16 E6 and E7 genes using recombinant retrovirus vectors. Cultures were treated with Erlotinib (0.001 to 1.0 micro molar) for 8 weeks and the number of cultures that became immortal was determined. Results: Erlotinib inhibited immortalization of human cervical cells by HPV E6/E7 in a dose-dependent manner. The drug stimulated apoptosis selectively in cells that expressed the HPV-16 E6/E7 oncoproteins. Cells expressing only E7 were more susceptible than those expressing E6 or E6/E7. Erlotinib induced senescence in a subpopulation of E6/E7-expressing cells that did not undergo apoptosis. Erlotinib did not appear to block HPV gene expression.

Conclusions: These results suggest that inhibition of the EGF-R prevents immortalization by stimulating apoptosis or senescence in cells that express HPV-16 E6 and E7. Since immortalization by HPV-16 E6/E7 is an important early event in cervical carcinogenesis, the EGF-R may be a potential target for therapy.

PATHOGENICITY OF CDV-RESISTANT SIHA CELLS IN AN ATHYMIC MICE MODEL

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Both in vitro and in vivo data provide evidence of a strong activity of cidofovir (CDV, HPMPC), against HPV. Several studies have shown the potential use of CDV to treat HPV infections by either topical administration or local injection. However the mechanism of action of CDV against HPV-induced proliferation remains to be elucidated.

SiHa cells (HPV-16 positive) were subcultured in increasing concentrations of CDV until they became resistant to the compound. A model where HPV-positive transformed cells are grown as xenograft in athymic nude (nu/nu) mice was used to study the pathogenicity of the CDV-resistant (CDVr) SiHa cells. An intermediate passage (#51) and a high passage (#202) of the CDVr cells, as well as wild-type cells, were tested. Adult nu/nu mice were inoculated subcutaneously with 2x10e6 cells of each cell type and tumor size was recorded weekly. Animals inoculated with the intermediate passage CDVr cells developed the smallest tumors, while mice injected with the high passage CDVr cells presented the biggest and fastest growing tumors. Only animals inoculated with the SiHa wild-type cells presented a wasting syndrome. Animals were sacrificed and different organs as well as the tumors were recovered and processed for histology at several timepoints post-inoculation of the cells. Significant splenomegaly was observed in animals inoculated with wild-type and CDVr high passage cells. The cells from the spleens were isolated and changes in the population of immune cells between control mice and mice inoculated with the different tumor cells were investigated by flow cytometry analysis and immunostaining. Preliminary data showed significant divergences between control mice and animals inoculated with the different tumor cells.

The use of this mice model to characterize the tumorigenic potential of the CDVr cells might lead to a better understanding of the resistant phenotype and of the mechanism of action of CDV against HPV.

P-09.17 GENE PROFILING IN CDV-TREATED AND CDV-RESISTANT HPV-TRANSFORMED CELL LINES

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Cidofovir (CDV, HPMPC), is an acyclic nucleoside phosphonate analogue with antiviral activity against a broad spectrum of DNA viruses, including HPV. However, to date there is not much insight into the mechanism of action of CDV against HPV. Three different cervical carcinoma cell lines harboring integrated HPV-16 (SiHa and CaSki) or HPV-18 (HeLa) together with one HPV-negative cell line (HaCaT) were subcultured for more than 50 passages in increasing concentrations of CDV until they became resistant to the compound. These CDV-resistant (CDVr) cell lines offer an interesting tool to investigate the mode of action of CDV against HPV and the mechanism(s) responsible for the resistant phenotype. The relative gene expression of selected sets of human genes was analyzed in the four different cell lines by RT²-PCR arrays (SABiosciences). The tested arrays cover over 400 genes related to cancer, drug resistance and metabolism, tumor metastasis, DNA damage and signalling pathways. Different conditions were tested for each cell line: untreated wild-type cells (control), wild-type cells treated with CDV (50 µg/ml) for 4 days, intermediate passage (#50) CDVr cells and high passage (#100) CDVr cells. Relative gene expression was obtained by fold change calculations compared to control cells. IGF, MMP1 and TEK were identified as upregulated genes in the four different CDV treated cells, suggesting a potential role for these genes in the mechanism of action of CDV. Additionally, changes in gene expression between CDVr cells and the corresponding wild-type cells were consistently observed in the intermediate and high passage CDVr cells. On the other hand, differences in gene expression among the various CDVr cell lines were noted. These results allowed the identification of sets of genes potentially involved in either the mode of action of CDV or the mechanism(s) related to CDV-resistance. To extend this study, experiments with microarrays are currently ongoing.

ANTIPROLIFERATIVE EFFECTS OF MIS ON CERVICAL CANCER

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Objective: MIS is well known to act as a regulator of female reproductive function. After the expression of MIS type II receptor in cervical neoplasia was conformed, and the growth inhibition of cervical cancer cells by administration of highly purified MIS was sstudied.

Materials and Methods: This study included 20 paraffin-fixed and 18 fresh cervx and cancer tissues. RT-PCR was used for MISR II mRNA expression, and in situ hybridization and immunohistochemistry were used to observe expression of MISR II mRNA and MISR II protein. The effect of MIS on the viability of the cervical cancer cell was tested using human recombinant MIS, and MTT assay was performed. Flow cytometry was used to evaluate the cell cycle distribution and annexin-V-FITC staining method was performed to demonstrate the apoptosis.

Results : Expression of MISR II mRNA on cervix and carcinoma was confirmed by RT-PCR. Expression of MISR II was observed on all normal cervix and carcinoma tissues. The strongest expression was shown on cervical squamous carcinoma, followed by normal cervical tissues and adenocarcinoma tissues. No significant difference in expression of MIS II protein and MISR II mRNA between normal cervix and carcinoma tissues.

MTT assay showed the negative correlation between the MIS exposure time and the viability of cervical cells (P=0.008). The changes in cell cycle distribution after MIS exposure demonstrated that S and G2M phases were decreased, G0G1 and sub G0G1 phases were increased. Annexin-V-FITC staining showed cellular apoptosis after MIS exposure in cervical carcinoma cells. There was a negative correlation between cellular survival and MIS exposure time.

Conclusion: MISR II is present on normal cervical and cervical carcinoma tissues, and MIS shows receptor-mediated antiproliferative capability on cervical cells in vitro. MIS may be used as a biological modifier or therapeutic modulator on MISR II-expressed tumors in future.

P-09.19

TERAMEPROCOL EFFICACY STUDIES IN HPV INFECTION MODELS

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Background: Tetra-O-Methyl Nordihydroguaiaretic Acid (terameprocol), a plant derived lignan with Sp1 promoter inhibition properties and anti-HPV effects via inhibition of the p97 promoter and anti-cancer effects via Cdc2 inhibition and G2/M arrest of dividing cells. Terameprocol is formulated as a vaginally applied ointment for use in CIN and there is existing excellent safety data in clinical trials Phase I using once-a-week dosing.

Objective: To demonstrate the efficacy of topical terameprocol against HPV in vitro and in vivo.

Methods: In vitro screening: A431 cells were plated at 2 x 105 cells/well in 6-well cluster dishes. Replicate aliquots of HPV-11 were added to each well representing an MOI of 150 particles per cell. Titrations of terameprocol were added 48 hrs after addition of virus and incubated a further 72 hrs. Cell cultures were harvested, lysed with Trizol reagent (GIBCO/BRL) and RNA prepared. QRT-PCR was conducted to quantitate the proportion of viral E1^E4 transcripts (measures EC50) and a cellular reference RNA for the TATA-binding protein (measures CC50).

In vivo screening: The CRPV rabbit model was used to determine efficacy of termeprocol 2% ointment and 2% terameprocol in DMSO. We induced skin papillomas using purified CRPV viral DNA placed onto scarified rabbit skin. Dosing regimes include a preinfection, early infection, and late infection treatment start times.

Results: In vitro: we found good efficacy of terameprocol against HPV-11 infection, with an EC50 of 0.4 ug/ml and a CC50 of 5.0 ug/ml with a Selectivity Index of 12.5. Based on these encouraging results, terameprocol is now in vivo studies and results will be forthcoming.

Conclusion: Terameprocol demonstrates encouraging anti-HPV activity in vitro and we plan to continue with in vivo experiments pending the success of which Clinical trials Phase I/II will be planned.

PKC INHIBITORS ENHANCE HDAC INHIBITOR ANTI-TUMOUR ACTIVITY IN CARCINOMA CELLS

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BACKGROUND: Persistent infection by HR-HPV and continuous expression of E6/E7 viral oncoproteins are associated with inactivation of cellular death pathways. Histone deacetylase inhibitors (HDACi) by modifying the acetylation state of histones, induce growth arrest of cervical carcinoma cells as well as apoptosis through an E2F-mediated process with induction of the pro-apoptotic p73 and isoforms. On the other hand, Staurosporine (ST), a protein kinase inhibitor (PKCi), and its analog UCN-01 exert anti-proliferative effects and induce p53-dependent apoptosis in HeLa and CaSki cells. OBJECTIVES: To increase carcinoma cell sensitivity to apoptosis by activating different death pathways in presence of HDACi and PKCi. METHODS: HeLa and CaSki cells were treated by HDACi (sodium butyrate or trichostatine A) or PKCi (ST or UCN-01) or both. Percentage of cells with depolarized mitochondria membranes and percentage of cells with fragmented DNA, as well as amount of pro-/anti-apoptotic proteins were studied. In vivo, CaSki xenografted nude mice were treated intraperitonally with each compound alone or in combination. Tumour growth was followed up. RESULTS: Cotreatment of cervical-derived cancer cells by HDACi and PKCi blocked cell cycle progression in G1 phase and led to a significant increase of apoptotic cells. It also enhanced synergistically the expression of p53, p73 as well as of p21 and Bax while it decreased the anti-apoptotic protein (Bcl-2, Bcl-XL) expression. As a consequence, caspase 3 and PARP cleavage was greater in cells treated with HDACi plus PKCi in comparison with cells treated with single agent. In vivo data show that each compound, NaB or UCN-01, administered to xenografted mice, was able to inhibit tumour growth and cotreatment was more efficient than monotreatments. CONCLUSION: This preclinical study with the combinative use of HDACi and PCKi should produce proof of principle paving the way for the subsequent clinical evaluation for treatment of cervical cancer.

P-09.21

ORGANOTYPIC EPITHELIAL RAFT CO-CULTURES TO EVALUATE SELECTIVITY OF ANTI-HPV AGENTS

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The acyclic nucleoside phosphonate (ANP) analogues display a broad spectrum of activity against a range of DNA viruses and retroviruses. In addition to their antiviral activity, several of the ANPs exhibit cytotoxicity towards proliferating cells, including HPV-positive cells.

selectivity of PMEG [9-(phosphonylmethxyethyl)guanine] and its prodrug cPrPMEDAP The [9-(2phosphonylmethoxyethyl)-N6-cyclopropyl-2,6-diaminopurine] compared to cidofovir (CDV, HPMPC) against HPV has been evaluated in organotypic epithelial raft co-cultures of primary human keratinocytes isolated from neonatal foreskins and the cervical carcinoma cell line SiHa (HPV-16 positive). In control untreated co-cultures, rafts showed regions with dysplastic morphology, normal epithelium and areas with mixtures of both types. In contrasts, rafts that were treated with PMEG, cPrPMEDAP and CDV showed areas of fully differentiated normal epithelium and destruction of the tumor cells. Inhibition of SiHa cell proliferation in the rafts by the different ANPs was concentration- and time-dependent. The AxioVision image analysis program (Zeiss) was used to quantify the area and the number of both tumor and normal cells. Four fields per raft were analyzed and mean values were calculated. When rafts were allowed to differentiate for 3 days and medium containing serial dilutions of the compounds were added for 7 subsequent days till the cultures were fixed, 50% of tumor cells were quantified in untreated control cultures. Rafts treated with PMEG at concentrations of 5, 2, 0.5 and 0.2 µg/ml had, respectively, 0%, 15% 37% and 26.1% of tumor cells while those treated with cPrPMEDAP presented, respectively, 8%, 9.6, 21, and 40% of tumor cells. Doses of 20, 5 and 2 µg/ml of CDV decreased the percentage of tumor cells to, respectively, 1, 6, and 47%.

This system is currently being used to evaluate the selectivity of these anti-HPV agents against other HPV-positive cell lines [i.e. CK-1 (HPV-33), CaSki and W12 (HPV-16), HeLa (HPV-18)].

CIGB-300, A NOVEL PEPTIDE EXHIBITING ANTITUMOR PROPERTIES IN CERVICAL MALIGNANCIES.

¹S Perea, ¹O Reyes, ¹I Baladron, ¹D Bacardi, ¹Y Perera, ¹JL Marcelo, ¹M Cruz, ¹C Valenzuela, ¹O Mendoza, ¹P Lopez, ¹K Cosme, ¹G Gil, ¹V Besada, ¹E Lopez, ²H Farina, ¹I Hernandez, ¹M Solares, ¹A Santana, ¹A Diaz, ¹C Lopez, ¹LJ Gonzalez, ¹H Garay, ¹Y Puchades, ³R Gomez, ²D Alonso, ¹L Herrera, ¹B Acevedo

¹CIGB, Havana, Cuba; ²Quilmes University, Buenos Aires, Argentina; ³ELEA Laboratories, Buenos Aires, Argentina

Exploitation of protein kinases as cancer therapeutic targets is continuously growing and its clinical validation becomes a reality as therapies with some specific inhibitors have showed clinical benefit in cancer patients. CK2 phosphorylation event is a promising target for cancer therapeutics as it is involved on cell malignant transformation, protection against apoptosis, and other cancer related events. CK2 is a serine-threonine kinase frequently deregulated in many human tumors. Likewise, the phosphorylation of HPV-16 E7 oncoprotein has been also linked to the capacity of these DNA tumor viruses to transform epithelial cells. Here, we hypothesized that a peptide binder to the CK2 acidic domain in the HPV E7 could exhibit anti-cancer properties in vitro, in tumor animal models and in cancer patients. By screening a random cyclic peptide phage display library, we identified the CIGB-300, a cyclic peptide which abrogates the CK2 phosphorylation by blocking recombinant substrate in vitro. Interestingly, synthetic CIGB-300 led to a dose-dependent antiproliferative effect in a variety of tumor cell lines and induced apoptosis as evidenced by rapid caspase activation. Importantly, CIGB-300 elicited significant antitumor effect both by local and systemic administration in murine syngenic tumors and human tumors xenografted in nude mice. Finally, we performed a First-in-Human clinical trial with a dose-escalation of CIGB 300 in patients with High Squamous Intraepithelial Lesions (HSIL). The peptide was found to be safe and well tolerated in the dose range studied. Likewise, signs of clinical benefit were identified after the CIGB-300 treatment as evidenced by significant decrease of the tumor lesion area and histological examination. Our results provide an early proof-of-principle of clinical benefit by using an anti-CK2 approach in cervical malignancies. Furthermore, this is the first clinical trial where an investigational drug has been used to target the CK2 phosphorylation domain.

SESSION 10

CELLULAR IMMUNOLOGY, BASIC SCIENCES

PROGRAMME

ROOM

THE 25TH INTERNATIONAL PAPILLOMAVIRUS CONFERENCE MAY 8-14 2009, MALMÖ, SWEDEN

TIME NUMBER TITLE

2009-05-12

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ADVANCES IN MUCOSAL HPV IMMUNOLOGY

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HPV 16 infects the epithelial layer of the cervical mucosa. Langerhans cells (LC) are the resident antigen-presenting cells at the site of infection and are responsible for initiating an immune response against HPV. However, LC exposed to HPV do not induce an HPV specific T cell immune response and our data indicate that the HPV L2 protein is responsible for that. The molecular mechanism that is involved in this immune escape entails the activation of the PI3-kinase signaling pathway and the suppression of the MAP-kinase signaling pathway. Candidate HPV receptors on LC may be PGE2 receptors as they are linked to a similar signal transduction pathway and indeed ligands for these receptors block HPV from interacting with LC. Demonstrating that Toll-like receptor 7 (TLR7) and TLR8 are expressed on LC; we hypothesized that their ligands would activate LC exposed to HPV 16 and lead to the induction of HPV16 specific T cells. Surprisingly LC are not activated when exposed to HPV16 and treated with Imiquimod (TLR7 agonist). However, LC exposed to HPV16 and treated with the compound 3M-002 (TLR8 agonist) are activated and initiate an HPV16 specific T cell response. These data strongly indicate that 3M-002 is a promising therapeutic compound for treatment of HPV infections and HPV induced lesions. This compound can potentially be added to therapeutic vaccine regimens aimed at inducing HPV specific T cells. Our data also show that the infiltrating capacity of such T cells can be boosted by the forced expression of the molecule LIGHT in HPV induced lesions.

O-10.01 ERADICATION OF VIN3 LESIONS BY VACCINATION WITH LONG PEPTIDES

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Therapeutic vaccination with a synthetic long peptide (SLP*) vaccine mediated the eradication of established human papilloma virus type 16 (HPV16)-positive tumors in mice and controlled wart growth and latent virus infection in rabbits persistently infected with cottontail rabbit papilloma virus. Subsequent phase I/II studies with an HPV16 SLP* vaccine, consisting of 13 long peptides covering the HPV16 E6 and E7 antigens, in patients with advanced HPV16-positive cervical cancer, revealed that this vaccine was safe and highly immunogenic. The purpose of the current study was to test the clinical efficacy of this HPV16 SLP* vaccine in HPV16-induced high grade vulvar intraepithelial neoplasia (VIN3), a premalignant epithelial disorder, spontaneous regression of which occurs in less than 2% of patients and in which recurrence after standard treatment is high.

In a phase 2 trial, 20 women with VIN3 were vaccinated three times sc in the limbs with a mix of the HPV16 E6 and E7 synthetic long peptides formulated in Montanide ISA-51. The endpoints were objective clinical responses, defined as reduction of at least 50% in lesion size (partial response) or complete regressions, and HPV16-specific T-cell responses, determined before and after vaccination.

The vaccine was safe, as no side effects exceeding CTC grade 2 were observed. At 3 and 12 months after the last vaccination an objective response was observed in 12/20 (60%) and 15/20 (75%) patients respectively. Nine of them showed a complete and durable regression of the lesions at 12 months. The strength of the vaccine-induced HPV16-specific T-cell response was significantly higher in the group of patients with a complete regression of their lesions as compared to non-responders.

This study shows that in women with VIN3 objective clinical responses can be achieved by therapeutic vaccination with synthetic long peptides that is able to induce effective HVP16-specific T-cell responses

HUMAN PAPILLOMAVIRUS TYPE-8 EARLY PROTEINS DEREGULATE IMMUNE HOMEOSTASIS IN SKIN

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Human papillomavirus type 8 (HPV8), a cutaneous genus beta HPV type, has skin carcinogenic potential.

Investigating HPV-associated skin tumors from Epidermodysplasia verruciformis patients, we observed that Langerhans cells responsible for epithelial immunosurveillance were strongly reduced, potentially allowing viral persistence. In contrast, lesional skin was highly infiltrated by inflammatory immune cells, predominantly macrophages and mast cells. Both cell types are known to support skin carcinogenesis by providing chronic inflammatory responses that sustain epithelial hyperproliferation.

Mice expressing the HPV8 early region under the keratin14 promoter spontaneously develop skin tumors. Of note, in these mice we detected a very similar immune deregulation as in human lesions.

To investigate a direct impact of viral proteins on immune dysregulation, primary human keratinocytes were retrovirally engineered to express HPV8 early proteins. Subsequent analysis revealed that the HPV8 E7 oncoprotein specifically interferes with mRNA and protein expression of the Langerhans cell attracting chemokine MIP-3alpha. In contrast, the HPV8 E6 oncoprotein strongly enhanced expression of the macrophage attracting chemokine MCP-1.

In summary, our data demonstrate for the first time that HPV8 actively deregulates immune homeostasis in skin: it suppresses immunosurveillance, a prerequisite for viral persistence, and promotes chronic inflammation, an important promoter of skin tumorigenesis.

O-10.03 DIRECT LONGITUDINAL COMPARISON OF T-CELL RESPONSES TO PROPHYLACTIC HPV VACCINES.

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Objectives: The two prophylactic HPV vaccines differ. The quadrivalent vaccine Gardasil contains virus-like particles (VLP) of HPV types 6,11,16, and 18 adjuvanted with aluminium salts. Cervarix contains VLP of types 16 and 18 together with the TLR 4 stimulating adjuvant AS04. For both vaccines phase II/III clinical trials showed comparable efficacy in protection from infection with vaccine-type HPV and dysplasia. T helper cells are important for memory, recall, and anamnestic responses. Only few reports on cellular immune responses to the prophylactic HPV vaccines are available to date.

Methods: In a longitudinal study in 36 vaccinated individuals blood samples before, after each vaccination, and one year following the first vaccine dose will be analysed. Immuneresponses analysed are antibody titers to HPV-L1 by multiplex serology and specific T cells by flow cytometry ex vivo. T cell frequencies and differentiation are determined for L1 and E6/E7 antigen-specific memory CD4 T cells identified by intracellular staining for CD4, CD154, IL-2, IL-4, and IFN-γ.

Results: An interim analysis of immunogenicity until month 7 is done. The responses to the two vaccines are compared according to antibody titres and T cell parameters. While antibody titres differ in favour of Cervarix, especially for HPV18 L1, the T cell responses are comparable for both vaccines.

Discussion: To date clinical efficacy of both HPV vaccines is comparable. Any difference in immunogenicity of the two vaccines is interesting for the evaluation of sustained immunity. This is the first direct and company-independent comparison of the immunogenicity of both vaccines.

THE ROLE OF TOLL-LIKE RECEPTORS IN HPV-16 PERSISTENCE

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Background: Although the mucosal immune response is thought to be critical in controlling HPV, little is understood about the exact mechanisms involved. Objectives: We investigated the association between TLR expression and HPV persistence or clearance in young women with incident infections with oncogenic HPV types 16, 18, or 51, or non-oncogenic HPV type 6.

Methods: Messenger RNA expression of TLR1, TLR2, TLR3, TLR4, TLR6, TL 7, TLR8, and TLR9 was measured by quantitative RT-PCR using endocervical specimens, collected before and at viral acquisition, in a cohort well characterized for HPV infections. Wilcoxon rank sum test was used to compare the change in expression from no- to- incident infection in women who subsequently cleared infection with those who did not.

Results: HPV 16 infections that persisted were significantly associated with dampened expression of TLRs 2, 3, 7, 8 and 9 (p=0.04, p=0.01, p=0.01, p=0.03, and p=0.03 respectively). No associations were found for HPV 18, 51 or 6. Controlling for HPV16 persistence using ordinary least squares, change in TLR7 expression was inversely correlated with change in IL-4 (estimate= -0.44, p=0.03) and IL-13 (estimate= -0.92, p=0.001); change in TLR8 expression was inversely correlated with change in IL-13 (estimate= -0.75, p=0.03); and change in TLR9 was positively correlated with change in TNF (estimate= +0.72, p=0.007).

Conclusions: This study suggests that one of the mechanisms by which HPV 16 interferes with innate immune responses is through dampened TLR expression in the cervical mucosa. As downregulation of the TLRs was associated with increased Th2 cytokines, we suggest that the observed downregulation may be part of a viral immune evasion mechanism that acts to limit the local Th1 cytokine response; thus conferring a survival advantage for the pathogen. Understanding the mechanism involved in this decreased expression could lay the foundation for new therapies.

O-10.05

TUMOR ASSOCIATED MACROPHAGES REGULATION OF ADAPTATIVE IMMUNITY

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CD4 Th1 response against HPV antigens is associated to infection and precursor lesion clearance, while regulatory T cell response has been associated to tumor progression. Recently, researchers have found that increasing numbers of tumor associated macrophages are correlated to higher grade lesions. Tumor associated macrophages and myeloid derived CD11b+Gr1+ cells have a role in angiogenesis and tumor evasion in many tumor models. In order to better characterize the role of the macrophage population infiltrating HPV16 associated tumors, we are using the TC-1 mouse model.

We observed that approximately 13% of total TC-1 tumor cells in mice are F4/80+CD11b+CD45+ macrophages and 2% are Gr1+CD11b+CD45+. We also observed expansion of myeloid populations CD11b+F4/80+, by 2 fold, and CD11b+Gr1+, by 3 fold, in the spleens of tumor bearing mice. Macrophages isolated from tumors display 3 fold more basal Arginase I activity than peritoneal macrophages and are resistant to treatment with LPS/IFN to induce iNOS II activity. Tumor isolated macrophages and CD11b splenocytes from tumor bearing mice induce IL10 and Foxp3 expression in CD8 lymphocytes. CD11b+ splenocytes also induce apoptosis on CD8 T cells and inhibit antigen driven proliferation, indicating an effect on T cell response suppression.

Depletion of tumor associated macrophages with clodronate liposomes delays tumor growth, allows a specific CD8E749-57 T cell population to expand in the spleen, and promotes tumor infiltration by lymphocytes, some of which specific for the E749-57 epitope.

Altogether, our results suggest that the macrophage population infiltrating HPV16 associated tumors in mice has a role in triggering suppressor mechanisms that facilitates tumor growth. Depletion or manipulation of this population may increase the efficiency of protocols of therapeutic vaccines against HPV associated lesions

EP2R AND EP4R: CANDIDATE RECEPTORS FOR HPV16 ON LANGERHANS CELLS

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Background: High-risk human papillomaviruses (HPV) infect the epithelial layer of the mucosa where Langerhans cells (LC) are the primary resident antigen presenting cells. LC do not induce an HPV-specific immune response due to the early activation of PI3K.

This comprises an HPV immune escape mechanism. The receptor(s) that HPV16 binds to on LC has yet to be identified. It is well documented that E-Prostanoid receptors, EP2R and EP4R, are essential in regulating the maturation and function of LC.

Objective: Our objective is to identify the receptor(s) that HPV16 binds to on LC that induces the signal transduction cascade, which drives the immune escape. We are specifically investigating EP2 and EP4 prostanoid receptors (EP2R and EP4R) as HPV16 receptors on LC.

Methods: We compared the signal transduction cascades initiated by HPV16 in LC to that of the signal transduction cascades initiated by Prostaglandin E2, the natural ligand of EP2R and EP4R by western blot analyses. To determine if EP2R and EP4R are receptors for HPV16 on LC we blocked the receptors with the Prostaglandin E2 and agonists or antagonists specific for each receptor and assessed the ability of HPV16 to bind to LC.

Results: Our findings show that the signal transduction cascades of EP2R and EP4R initiated by Prostaglandin E2 and HPV16 are strikingly similar. Both Prostaglandin E2 and HPV16 induce early up-regulation of PI3K while down-regulating MAPK activation. Additionally, we demonstrate that an EP2R agonist (Butaprost) and EP2R and EP4R antagonists (AH6809 and AH23848, respectively) greatly hinder the interaction between HPV16 virus-like particles and LC. Extending these results, we also show that Prostaglandin E2 significantly blocks HPV16 virus-like particles from binding to LC.

Conclusions: These data imply that HPV16 binds to both EP2R and EP4R on LC, thereby initiating a human immune escape route of HPV16.

POSTER ABSTRACTS SESSION 10

POSTER SESSION I MONDAY 10.00: ODD NUMBERS

POSTER SESSION II

TUESDAY 10.00: EVEN NUMBERS

HLA CLASS-I AND KIR GENOTYPES ASSOCIATE WITH HPV-INDUCED CERVICAL CANCER

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Background: Persistent high risk HPV infections cause about half a million cervical cancer cases yearly throughout the world, with the highest incidence occurring in developing countries. Vaccines against HPVs are available that aim to prevent cancer development in future generations. However, host-pathogen interactions are evolutionarily dynamic with genetic variability contributing to selection of survival mechanisms for both. Attempts to elucidate the mechanisms involved in HPV induced cancer are still necessary.

Objectives: The MHC complex is a reasonable target for the study of genetic susceptibility to HPV because of the role of class I HLA molecules in antigen presentation and modulation of innate immune responses, and the ability of HPV to regulate HLA expression.

Methods: Samples from case-control studies from Latvia and Mozambique were used with the hypothesis that the distribution of HLA-C genotypes encoding the amino acids involved in engaging killer immunoglobulin-like receptors (KIR) on NK cells, distribute differently between individuals with HPV-induced cervical cancer and healthy controls. Likewise, the distribution of KIR genes was compared between these groups, as well as genotype combinations between HLA-C and KIR.

Results: The genotype determining the amino acid at position 66 of HLA-C associates with HPV infection in a sample of 126 cases and 109 control individuals from Mozambique while the genotype for the amino acid 80 associates with cervical cancer. We also observed a significantly reduced prevalence of KIR2DS1 genes among cases. In a sample of 301 cases and 227 controls from Latvia we observed that genotype combinations between HLA-C and KIR genes that may lead to reduced effector cell activation were more prevalent among HPV infected individuals. A similar trend was observed for cancer cases from Mozambique.

Further exploration of the HLA class I / KIR interaction in HPV infection and HPV-induced cervical cancer is certainly warranted.

P-10.08

DEEPLY INFILTRATING HPV-INDUCED CERVICAL TUMORS INDUCE NON-BENEFICIAL T-CELL RESPONSES.

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Objective: Detailed analyses of HPV-specific immunity in a large group of patients with HPV-induced (pre)malignant genital lesions in relation to HLA-types and prognostic factors.

Methods: Patients were HLA typed and HPV16/18 E6 and E7 specific T-cell immunity was assessed by proliferation in combination with cytokine bead array using freshly isolated PBMC and by HPV-specific CD4+CD25+Foxp3+ regulatory T-cell analyses. The results were analyzed in relation to known disease-related HLA types (DR7, DR13, DR15/DQ06), invasion-depth of tumor, tumor size and lymph node status.

Results: In total 161 HLA-typed patients, 119 with cervical cancer (CxCa), 26 with CIN3 and 16 with VIN3, were analyzed. CxCa patients expressing the HLA-DR13 haplotype were underrepresented as compared to the Dutch population (18% vs. 28%; p=0.014), whereas HLA-DR7 was overrepresented in patients with HPV16+ CxCa (32% vs. 19% p=0.006) and HPV16+ CIN3 (27%). In 31 (36%) of 87 cases, from whom blood could be tested, a proliferative response to HPV16/18 E6/E7 was detected. Notably, only a minority of these responses were associated with IFN γ production (9/31) or other cytokines. The presence or absence of HPV-specific immunity was not associated with HLA-type, tumor size nor with LN status. However, the depth of invasion was associated with the presence of HPV-specific CD4+CD25+Foxp3- (activated) T-cells (p=0.03) and HPV-specific CD4+CD25+Foxp3+ regulatory T-cells (p=0.04) were found in the group of patients displaying HPV-specific proliferation, indicating that an infiltrating tumor can induce both helper as well as regulatory CD4+ T-cells. Indeed, the detection of both types of CD4+ T-cells were significantly correlated (p=0.01).

Conclusions: Infiltration of the surrounding normal tissue by cervical tumors is associated with the induction of a detectable HPV-specific proliferative response which comprises both HPV-specific dysfunctional CD4+ T-helper cells and CD4+ regulatory T-cells.

PD-L1 EXPRESSION CONFERS SURVIVAL BENEFIT TO PATIENTS WITH CERVICAL CARCINOMA

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Background: PD-L1 and PD-L2 are ligands for PD1, which is expressed on activated effector T-cells and regulatory T-cells (Tregs). Ligation of PD1 suppresses T-cell function.

Objectives: To investigate the expression of PD1 on tumor-infiltrating CD8+, CD4+ and CD4+Foxp3+ T-cells as well as its ligands PD-L1 and PD-L2 at the surface of tumor cells in relation to overall survival of a group of 115 cervical cancer patients.

Methods: Three-color fluorescent immunohistochemistry was used to study the number and phenotype of tumorinfiltrating T-cells and standard immunohistochemistry to assess PD-L1 and PD-L2 expression.

Results: Expression of PD-L1 was observed in 22 of 115 tumors and PD-L2 was found in 34 cases. Since PD-L1 and PD-L2 alter the function of PD1-expressing T-cells, we studied their expression in the context of tumor-infiltrating T-cells. The expression of PD-L1 was associated (p=0.022) with higher intraepithelial infiltration by CD4+Foxp3+Tregs. No correlation was observed with PD-L2. Interestingly, while patients with high numbers of Tregs and PDL1-negative tumors displayed poor survival, patients with PD-L1+ tumors and high numbers Tregs showed a better survival (p=0.098), similar to that of patients with low numbers of Tregs. PD-L1 expression did not impact on the survival of patients with low numbers of Tregs. PD1-expression was found on Tregs as well as on CD4+ and CD8+effector cells, suggesting that PD-L1 interaction with PD1 may incapacitate Tregs. Notably, increased survival was not due to a lower number of PD1+ effector cells in PD-L1+tumors. However, PD-L1+ tumors contained more PD1+ Tregs (p=0.074), suggesting that ligation of PD1 especially affects Tregs. To prove this we used PD1+ Treg clones and showed that ligation with soluble PD-L1 resulted in loss of function of these Tregs.

Conclusions: PD-L1 expression at the surface of cervical cancer cells may incapacitate tumor-infiltrating PD1+ Tregs and enhance survival.

P-10.10

REGULATORY T-CELLS AND PROGRESSION OF PRECURSOR LESIONS OF CERVICAL CANCER

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Background: Host immune response seems to play a role eliminating the Human Papillomavirus infection and preventing progression to cancer. In cervical cancer, a low density of T cells is associated with an increased risk of relapse. The characterization of TILS in cervical cancer may be helpful for the design of strategies to predict progression or for immunotherapies. Objectives: to evaluate the relationship between the type, location and number of CD4+, CD8+ and CD25+ Foxp3+ (T regs) with the progression of precursor lesions and prognosis of cervical cancer. Methods: paraffin sections (4um) from 120 women from Colombia, diagnosed with CINI, CINII, CINII and invasive cervical cancer (30 each histological grade) were used for immunohistochemical double staining of CD4, CD8 and CD25/Foxp3 cells. The number of cells in 10 hpf (high power fields) was counted in epithelium and stroma. The Kruskal-Wallis test was applied for the analysis of the differences on the numbers of infiltrating cells among the histological grades and Mann-Whitney test for the analysis of the differences of number of infiltrating cells between the locations (stroma vs. epithelium). Results: From 77 cases analyzed 17 were CINI, 26 CINII, 17 CIN III and 17 invasive cancers. Independent of histological grade and location, the median number of CD8+ was higher than the median number of CD4+ cells. We found a higher median number of T regs in the stroma (p=0,003) and epithelium (p=0,0006) of invasive cervical cancer. In addition, the CD8+/Tregs was higher in the stroma of cervical cancer than in CIN III (p=0.009) but the CD4+/T-regs cells ratio was higher in CINIII than in cervical cancer (p=0.0122) Conclusions: The number of T regs, the increase of CD8+/T regs cells ratio and decrease of CD4/T-regs cells ratio seem to predict the progression to invasive cancer.

SEX HORMONES AFFECT IMMUNE RESPONSE TO HPV16 AMONG HEALTHY WOMEN

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Background: Exposure to estrogen and progesterone in the form of combined oral contraception (COC) increases the risk of cervical cancer, potentially through modulation of the host immune response. HPV 16 virus-like particles (VLP) are a potent inducer of the cellular immune response. The effect of reproductive hormones on immune responses to HPV 16 VLPs has not been assessed.

Objective: The effects of estrogen and progesterone alone and in combination on proliferation and cytokine response to HPV 16 VLPs in peripheral blood mononuclear cells (PBMC) were measured in 20 normal healthy donors.

Methods: PBMCs from normal healthy donor women were stimulated in vitro with HPV 16 VLP (2.5ug/ml) in the presence of 17 β -estradiol (E2) or progesterone (P4) administered either alone or in combination. Lymphoproliferation was assessed through colorimetric measurement of formazan salt. In a preliminary sub-sample (n=3), cytokine production (IL-2, TNF- α , IL-8, IL-1 β , IFN- γ , IL-10, IL-6, IL-1ra, IL-4, IL-5, IL-17) was measured using a multiplexing bead-based assay. Student's t-test was used to compare differences across hormone treatment groups. Exploratory factor analysis was used to identify potential groups of cytokines differentially regulated by hormone treatment.

Results: E2 and P4 either alone or in combination resulted in a significant (15% + /-2%) decrease in lymphoproliferation relative to untreated controls (p<0.01). Factor analysis revealed two distinct clusters that were differentially regulated by hormones and defined as mediating proinflammatory (IL-1 β , IL-8, IL-2) and anti-inflammatory (IL-6, IL-5, IL-1ra) immune responses.

Conclusions: This pilot study reveals reproductive hormones alone or in combination reduce the proliferative capacity in human PBMCs stimulated with HPV 16 VLP in vitro. Reduced lymphproliferation may be caused by differential regulation of inflammatory cytokine production. Further testing is currently being performed to validate these preliminary findings.

P-10.12

SKIN TEST IN THERAPEUTIC VACCINATION TRIAL IN WOMEN WITH CIN.

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Background: The detection of cellular immunity to HPV is elaborate in vitro.

Objectives: Skin testing as easy method to monitor a vaccination trial was evaluated.

Method: We have used a synthetic peptide-based HPV16-specific skin test to detect cellular immune responses to HPV16 E7 in the course of a therapeutic vaccination trial in women with high grade CIN.* The patients mono-infected with HPV16 received 3 times either the HPV16 L1E7 chimeric virus-like particle vaccine (n=24) or placebo (n=12). The women were intradermally challenged at week 0, 12, 24 with 5 overlapping peptides covering HPV16 E7 as previously described.**

Results: DTH reactions to E7 were detected in 5/12 (42%) complete histological responders, in 3/9 partial responders (33%) with more than 50% lesion size reduction and in 2/15 (13%) non-responders. In 4 patients with high score (>7mm) DTH reactions 3 (75%) exhibited histological response and interestingly, in the non-responder HPV51 was detected in addition during follow up. When excluding this patient from analysis, statistics revealed a significant association between high score DTH and histological response (p=0.03). Four of the 10 positive skin test reactions were already measured at the baseline evaluation before vaccine application. DTH measured in week twelve of the study was most likely induced in 5 women by vaccination, but also developed spontaneously in 1/3 histological responders in the placebo group.

Conclusions: The skin test was safe as no adverse events were observed. The association of DTH with response suggests that in situ detection of HPV16-specific T-cells migrating into the skin is practicable in vivo. In comparison, there was imperfect match between regression and the cellular Th1 or CTL immune response measured in the circulating T cell pool in course of this vaccination study in vitro.

*Kaufmann A. et al. Int. J. Cancer 2007;121:2794.

**Höpfl et al. Lancet 2000;356:1985.

DYSFUNCTIONAL HPV16-SPECIFIC T-CELL RESPONSE AFTER SURGERY AND VIRAL PERSISTENCE

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PURPOSE: To characterize HPV16 E6- and E7-specific T-cell immunity in patients with high-grade squamous intraepithelial lesions (HSIL).

EXPERIMENTAL DESIGN: Peripheral blood mononuclear cells isolated from 38 patients with HPV16+ HSIL were used to determine the magnitude, breadth, and polarization of HPV16-specific T-cell responses by proliferation assays and cytokine assays. Furthermore, HSIL-infiltrating T cells isolated from 7 cases were analyzed for the presence of HPV16 E6-and/or E7-specific T cells, phenotyped, and tested for the specific production of IFN-gamma and interleukin-10 as well as for their capacity to suppress immune responses.

RESULTS: HPV16-specific T-cell responses were absent in the circulation of the majority (approximately 60%) of patients who visit the clinic for treatment of a HPV16+ HSIL lesion. Notably, HPV16-specific T-cell reactivity was predominantly detected in patients returning to the clinic for repetitive treatment of a persistent or recurrent HPV16+ HSIL lesion after initial destructive treatment. The majority (>70%) of these HPV16-specific T-cell responses did not secrete proinflammatory cytokines, indicating that most of the subjects, although in principle able to mount a HPV16-specific immune response, fail to develop protective cellular immunity. This notion is sustained by our observation that only three HSIL-infiltrating T-cell cultures contained HPV16-specific T cells, one of which clearly consisted of HPV16 E7-specific regulatory T cells. CONCLUSIONS: The presence of HPV16-specific T cells with a non-Th1/Th2 cytokine and even suppressive signature in patients with HSIL may affect the outcome of vaccine approaches aiming at reinforcing human papillomavirus-specific immunity to attack human papillomavirus-induced lesions.

P-10.14

KILLER IMMUNOGLOBULIN-LIKE RECEPTOR (KIR) ASSOCIATION WITH CERVICAL DYSPLASIA.

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Background: E5 and E7 proteins encoded by HPV act to reduce MHC class I expression. These mechanisms have evolved to evade the action of cytotoxic T cells. Natural Killer (NK) cells remove virally infected and tumour cells through recognition of decreased MHC class I and may play a part in removal of cervical dysplastic cells. KIR are a polymorphic group of receptors on NK cells that recognise polymorphic MHC class I. Some KIR are activating while others are inhibitory and the balance between these signals determine target cell cytolysis.

Objectives: Determine KIR association with cervical dysplasia and with HPV genotype in dysplasia.

Methods: KIR gene frequencies of Western Australian (WA) women with a history of HSIL (n = 147) were compared to the WA population (n = 189). Women with current HSIL (n = 21) were segregated into those infected with HPV 16/18 and non-HPV 16/18. KIR types were determined by PCR-SSP, HPV genotypes by PCR and DNA sequencing.

Results: KIR2DL2 and KIR2DS2 were significantly decreased in the patients with a history of HSIL compared to the control group (p = 0.046 and 0.049 respectively). There was a significant decrease in the frequency of KIR3DL1 in HSIL samples infected with non-HPV 16/18 compared to those infected with HPV 16/18 (p = 0.0475).

Conclusion: This study suggests that KIR2DL2 and KIR2DS2 may have a protective role in HSIL/cancer. KIR3DL1 was found in high frequency in the WA population (93.7%) and in a similar frequency in HSIL infected with HPV 16 or 18. However, the low frequency of KIR3DL1 in non-HPV 16/18 infected HSIL indicates that women lacking KIR3DL1 may be more susceptible to cervical dysplasia when infected with HPV other than 16/18. This may explain why HPVs other than 16 and 18 cause only a small percentage of dysplasia/cancer.

ASSESSING POTENTIAL HPV16 HLA-A2-RESTRICTED CD8 T-CELL RESPONSES

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Background: Induction of a specific cell-mediated immune response will aid future HPV vaccines and therapeutics. Objectives: Our laboratory employs two HLA-A2.1 transgenic preclinical models (HHDII mice and HLA-A2.1 transgenic rabbits) to aid our pursuit of new HPV HLA-A2-restricted epitopes

Methods: HHDII mice were immunized twice with either HPV16E7 49-57 or HPV16E7 82-90 peptides and the CTL responses assessed via tetramer staining and interferon-gamma intercellular cytokine staining for specificity and functionality. HLA-A2 transgenic rabbits were vaccinated with a DNA vaccine containing either HPV16E7/49-57 or HPV16E7/82-90 epitopes and then challenged with an epitope-modified cottontail rabbit papillomavirus (CRPV) containing the same epitope inserted at the end of the CRPVE7 gene. Protection was determined by papilloma presence or absence and papilloma size.

Results: Peptide immunizations of HHDII mice with either HPV16E7 49-57 or HPV16E7 82-90 peptides stimulated epitope-specific CTLs in spleen cells of the mice. Gene gun vaccination of the HLA-A2 rabbits with the HPV16E7/82-90 DNA vaccine followed by challenge with the epitope-modified CRPV DNA containing the same inserted epitope resulted in complete protection from infection. However, no protection from infection was achieved in the HLA-A2 rabbits vaccinated with the HPV16E7/49-57 DNA vaccine followed by challenge with the epitope-modified CRPV DNA containing the same epitope containing the same epitope.

Conclusions: These data point to the incomplete symmetry between the HHDII mouse model and the HLA-A2 rabbit models for assessment of HLA-A2-restricted HPV epitope responses as measured by in vitro induction versus in vivo protection.

P-10.17

CD1D IS DOWNREGULATED IN HPV-ASSOCIATED LESIONS BY HPV E5 PROTEIN

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[Background] CD1d, MHC class1 like molecule interacting with NKT and NK cells, plays a role in both innate and adaptive immunity to various microbes. CD1d downregulation has been reported in microbes occurring genital persistent infection, including HSV, HIV, and Chlamydia trachomatis. This is thought to be a mechanism of immunoevasion for the microbes. Our immunohistochemical study revealed CD1d downregulation in human papillomavirus (HPV)-associated lesions (unpublished data).

[Objective] Purpose of this study is to address mechanism by which CD1d is downregulated in the HPV-associated lesion. We here focused on HPV E5 protein since E5 is reported to affect on function of classical MHC molecules.

[Methods] CD1d gene was transduced stably into C33a cell, a HPV-negative human cancer cell line (C33a/CD1d). FLAGtagged HPV 6 or 16 E5 genes were transduced transiently into C33a/CD1d or CD1d-bearing vaginal epithelial cells by retrovirus system. Alterations in CD1d expression were assessed by RT-PCR, western blotting, and immunostaining. Cell-surface CD1d was examined by flow cytometry. To see mechanism of CD1d alteration, the E5-positive cells were treated with proteasome inhibiter MG132.

[Results] CD1d mRNA level did not alter in E5-positive cell. Western blotting and Immunostaining revealed CD1d was downregulated clearly at protein level by gene transduction of either HPV16 or 6 E5. The downregulation was observed in both C33a/CD1d and vaginal cell lines. Flow cytometry confirmed that cell-surface CD1d decreased in E5-positive cells. CD1d was rescued by MG132 treatment, indicating CD1d was degraded by proteasomal proteolysis.

[Conclusions] HPV E5 caused CD1d downregulation at post-transcription level regardless of the HPV genotype. HPV could co-opt cellular proteolytic pathway for quality control to degrade CD1d. CD1d downregulation by E5 may be a novel mechanism by which HPV evade attack by immune cells and attain persistent state.

MUCOSALLY DELIVERED PEPTIDES PRIME STRONG IMMUNITY IN HLA-A2.1 TRANSGENIC RABBITS

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Background: Our previous studies have demonstrated that DNA vaccine delivered intracutaneously by gene-gun but not as intramuscular injection can stimulate strong protective and therapeutic immunity in rabbits. Recent studies showed that peptides delivered by the mucosal route also stimulated local and systemic immune responses.

Objectives: Because mucosal delivery is much easier to apply and the costs are significantly less when compared to genegun delivery systems, we investigated whether mucosally delivered peptides can prime immunity in our cottontail rabbit papillomavirus (CRPV) infection model.

Methods: An HLA-A2.1 restricted CRPV E1 peptide (E1/303-311, MLQEKPFQL) that showed complete protection when used as a DNA vaccine was used for this study. E1/303-311 fused at the carboxyl-terminal of TT helper motif (QYIKANSKFIGITEL) or PADRE [AK(X)VAAWTLKAAA] were used as mucosal immunogens. HPV16E7/82-90 (LLMGTLGIV) fused with either TT helper motif or PADRE was used as negative control for peptide vaccine. HLA-A2.1 transgenic rabbits were immunized with each of the fusion peptides (100 µg) together with 25µg CpG2007 (TCGTCGTTGTCGTTTTGTCGTT) by the ocular and intranasal route for three immunizations. A final booster of half dose (about 6µg) of corresponding epitope DNA vaccine was administered 3 days after CRPV DNA challenge.

Results: Significantly smaller papillomas were found in rabbits immunized with both TT or PADRE fused CRPVE1/303-311 peptides when compared with TT or PADRE HPV16E7/82-90 peptides. 18/20 and 15/20 CRPV DNA challenge sites in rabbits immunized with TT or PADRE fused CRPVE1/303-311peptide vaccines regressed respectively by week 8 after DNA challenge. 7/20 and 8/16 challenge sites in rabbits immunized with TT or PADRE fused HPVE7/82-90 regressed. Conclusions: We conclude that mucosal peptide immunization could be combined with a single DNA vaccination to prime protective immunity in rabbits.

P-10.19

DNA VACCINATION AGAINST HPV16 E7 ONCOPROTEIN COMBINED WITH TLR AGONISTS

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Vaccination with naked DNA is a rapidly developing immunization method. It induces both humoral and cellmediated immunity, but the level of immune responses is often low when compared with traditional vaccines. Various strategies have been developed to increase the efficacy of DNA vaccines including utilization of immune adjuvants. Intramuscular (i.m.)injection and intradermal (i.d.) delivery by a gene gun are the most frequently used methods of DNA application. However, the effect of immunostimulatory substances was mostly examined in the mix with a DNA vaccine inoculated intramuscularly. In our work, we tested the influence of adjuvants on DNA vaccination performed by i.d. gene-gun application. First, we evaluated the effect of the mix of toll-like receptor 9 (TLR9) and TLR3 agonists, CpG oligodeoxynucleotide ODN1826 and polyI:C, respectively, on immunization with the plasmid pBSC/E7GGG.GUS producing a modified form of the HPV16 E7 protein. While the addition of ODN1826 and polyI:C into immunization cartridges did not markedly change vaccination efficacy, intraperitoneal (i.p.) injection of the mix considerably inhibited immune reactions. Then, we compared the effect of ODN 1826 and polyI:C given i.p. either alone or in the mix on the day of pBSC/E7GGG.GUS delivery or two days before or after this delivery. Furthermore, we tested the topical administration of the TLR7 ligand imiquimod. However, none of the modifications enhanced the efficacy of the pBSC/E7GGG.GUS plasmid. We rather recorded the inhibitory effect of TLR agonists on gene-gun DNA immunization almost irrespective of the day of their administration.

INNOVATIVE VLP-BASED PLATFORM FOR THE DEVELOPMENT OF HPV16 THERAPEUTIC VACCINE

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Exogenous virion-associated antigens have been shown to efficiently induce cross-presentation in dendritic cells. We recently characterized a HIV-1 Nef mutant (Nef7) showing a virion-incorporation of about 100-fold higher compared to that of the w.t. counterpart in lentiviral and retroviral particles. The Nef7 acts as a cargo molecule for a wide array of molecules. We characterized non-replicating genomeless VLPs incorporating high levels of the fusion proteins Nef7-E6 and Nef7-E7. The analysis of purified VLPs demonstrated that the fusion protein incorporation compared with that of the HIV-1 CAp24 protein was approximately 1:5. The lentiviral particles containing E6 and E7 were inoculated in C57BL6 mice at different doses (5-15 g of HIV-1 CAp24/mouse). The VLPs induced a HPV-specific cellular mediated immune response. However, only a weak anti-E6 or anti-E7 antibody response was observed in all the animals. The tumour protection after challenge with TC-1 tumour cells was evaluated on groups of mice immunized with VLPNef7, VLPNef7-E7 and VLPNef7-E6, alone or in combination, using the vaccine schedule of 3 boosters or a prime-boost regimen. The mice immunised with VLPNef7-E7 showed the higher percentage of tumour protection (40-60%) compared to the animals immunised with VLPNef7-E6 (10%). Even animals immunised with VLPNef7 showed a 10-20% tumour protection, suggesting a stimulation of the innate immunity by the particles. Cytokine secretion profile of the VLP-stimulated splenocytes also indicated the induction of innate and adaptive immune responses. The protection from TC-1 cells challenge correlated with E7-specific IFN- immune response. This was consistent with the idea that exogenous lentivirion-associated antigens undergo efficient cross-presentation only for the E7. The exploitation of Nef7 antigen-bearing lentiviral or retroviral particles, could represent a promising approach for the development of an innovative vaccination strategy against HPV-16 associated cancer.

P-10.22

ROLE OF IL10 IN HPV16 ASSOCIATED TUMORS IN C57B/6 MICE

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Cytokines and chemokines are key molecules involved in local immune response against tumor development. Moreover, a great variety of these molecules are produced by tumor cells leading to the recruitment of leukocytes associated to tumor progression. Due to the importance of local immune response against tumor establishment we proposed to evaluate the expression of cytokines, chemokines and their receptors involved in the inflammatory response in HPV associated tumors.

TC-1 cells were inoculated subcutaneously in C57B/6 mice and tumor growth kinetics was followed. We observed that TC-1 tumors were infiltrated mainly by CD45+CD11b+F4/80+ cells (72% of infiltrate/CD45+), and virtually no lymphocytes. CD45+ cells were sorted from the CD45- population and both cell types were used for RNA expression analysis. We evaluated the expression of 84 genes by Real-Time PCR array (SuperArray). Our results showed the high expression of IL-10 by the infiltrate. Additionally, we observed TGF β expression, another cytokine related to tolerance towards tumors. We also found CCL2 and CCL5 expression by tumor cells and the absence of CCL3. To explore the biological significance of IL-10 expression, we proposed to investigate tumor progression in IL-10-/- mice. By comparing tumor growth in wild type and IL-10-/- mice, we observed a slower progression of tumor growth in IL-10-/- mice. In our study, FACS analyses showed the majority of infiltrating leukocytes were macrophages (F4/80+ cells). Interestingly the levels of these cells were reduced in IL-10-/- mice (40%). On the other hand, we observed an increase of TCD8+ and TCD4+ lymphocytes infiltrate in IL-10-/- mice while they were rarely found in wild type.

These data suggest that IL-10 play a role in local immune adaptive response regulation. Moreover, some chemokines produced by tumor cells may be important regulators of leukocyte recruitment in this tumor model. Further investigations are being conducted to confirm these observations.

NATURAL AND IN VITRO PRIMED T CELL REACTIVITY AGAINST P16INK4A

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Background: The tumor suppressor p16INK4a is consistently overexpressed in HPV-transformed cervical precancer lesions and invasive carcinomas, whereas in normal tissues barely any p16INK4a expression is detectable. Since we could find spontaneous humoral immune response against p16INK4a and tumor infiltrating T cells reactive against a HLA-A*0201 restricted p16INK4a peptide in cervical cancer patients, immune tolerance towards this autoantigen seems to be not fully sustained. Thus p16 INK4a might be an interesting target for active immunotherapeutic approaches in cervical cancer patients.

Objectives: In this study we aimed at mapping of reactive p16INK4a epitopes for in vitro initiated and spontaneous cellular immune responses against p16INK4a in cervical cancer patients.

Methods: T cells from an HLA-A*0201 positive healthy donor were repeatedly stimulated in vitro with autologous dendritic cells pulsed with seven overlapping 30mer peptides covering the complete p16INK4a sequence. Peptide specific reactivity was assessed by interferon-gamma ELISpot assays. In addition, spontaneous T cell reactivity in 21 cervical cancer and precancer patients was measured against the p16INK4a peptides by IFN-gamma ELISpot assays. Cytotoxicity of patient-derived T cells was demonstrated by lysis of HLA-matched peptide-loaded B cells.

Results: After day 28, T cells of the healthy donor showed ELISpot reactivity against 3 out of the 7 long overlapping p16INK4a peptides. In contrast no reactivity was seen on day 0. Interestingly, out of the 21 patients, 5 showed weak spontaneous ELISpot reactivity against the same 30mer peptide. Cytotoxicity against HLA-matched peptide-loaded B cells could be demonstrated for one patient.

Conclusions: Our finding that naïve T cells can be primed with p16INK4a peptides and that activated T cells reactive against the same p16INK4a peptide can be found in cervical cancer patients without any evidence for autoimmune reactions further suggests that immune responses against p16INK4a may constitute an important aspect of the immune surveillance of high grade HPV-induced lesions.

P-10.24 CYTOMETRIC CYTOTOXICITY ASSAYS FOR DETECTION OF RARE SPECIFIC T CELLS

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Background: For detection of T cells from clinical samples, sensitive and cell sparing functional assays are required for analysis of T cell responses. Methods: We developed two highly sensitive, non radioactive, functional, cytometry-based cytotoxicity assays for the determination of very low frequencies of antigen specific CTL. The Plasmid Transfection Fluorolysis Assay (PTF-A) detects polyspecific T cell reactions against a proteasomal degraded full length protein presented by plasmid transfected target cells (K562-A2, BLCL, EL-4). The Epitop Loading Fluorolysis Assay (ELF-A) detects cytotoxic T cell reactions induced by minimal peptide epitope loaded target cells (T2, B-LCL).

Results: Comparison of the ELF-Assay with the standard Chromium Release Assay revealed that less than 1/10 of antigen specific T cells (CMV pp65 495-503, IMP MP1 58-66) were needed to detect a specific lysis. Median kill by 3-10 specific T cells amounted at 80% specific lysis underscoring the sensitivity of the ELF-Assay. Validation of the PTF-Assay showed that the immunodominant epitops were very efficiently processed out of the entire antigen in K562-A2 and B-LCL resulting in significance comparable to the ELF-Assay. Further we were able to detect low numbers of HPV16 E7 specific T cells in mice vaccinated with our p14 vaccine by a very accurate lysis of HPV16 E7 expressing EL-4 target cells.

Currently we are trying to adapt our method for the detection of p16 reactive CTLs in blood samples from HPV positive donors.

Conclusion: We developed a versatile, practicable and very sensitive assay for direct measurement of cytotxicity triggered by polyspecific or monospecific T-cells against any antigen of interest.

ORAL ADMINISTRATION OF POLY-GAMMA-GLUTAMATE INDUCES TLR4- DEPENDENT ANTITUMOR EFFECT

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Previously, we reported that the oral administration of high molecular mass poly-gamma-glutamate (gamma-PGA) isolated from Bacillus subtilis sp. Chungkookjang induced antitumor immunity but the mechanism underlying this antitumor activity was not understood. In the present study, we found that application of high molecular mass gamma-PGA induced secretion of tumor necrosis factor (TNF)-alpha from the bone-marrow-derived macrophages (BMDMs) of wild type (C57BL/6 and C3H/HeN) and Toll-like receptor 2 knockout (TLR2-/-) mice, but not those of myeloid differentiation factor 88 knockout (MyD88-/-) and TLR4-defective mice (C3H/HeJ). Production of interferon (IFN)-gamma-inducible protein 10 (IP-10) in response to treatment with gamma-PGA was almost abolished in C3H/HeJ mice. In contrast to LPS, gamma-PGA induced productions of TNF-alpha and IP-10 could not be blocked by polymyxin B. Furthermore, gamma-PGA-induced Interleukin-12 (IL-12) production was also impaired in immature dendritic cells (iDCs) from MyD88-/- and C3H/HeJ mice. Downregulation of MyD88 and TLR4 expression using small interfering RNA (siRNA) significantly inhibited gamma-PGA-induced TNF-alpha secretion from the RAW264.7 cells. Gamma-PGA-mediated intracellular signaling, such as the activations of c-Jun N-terminal kinase (JNK), p38 kinase, nuclear factor (NF)-kappa B, and interferon regulatory factor-3 (IRF-3) were markedly inhibited in C3H/HeJ cells. The antitumor effect of gamma-PGA was completely abrogated in C3H/HeJ mice compared with control mice (C3H/HeN) but significant antitumor effect was generated by the intratumoral administration of C3H/HeN mice-derived iDCs followed by 2,000 kDa gamma-PGA in C3H/HeJ. These findings strongly suggest that the antitumor activity of gamma-PGA is mediated by TLR4.

P-10.26 THE ROLE OF REGULATORY T CELLS IN ALPHAVIRUS-BASED IMMUNIZATIONS

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In a previous study we demonstrated that in patients with (pre)malignant cervical lesions so-called regulatory T cells (Tregs) are increased (Visser J et al., Clin Exp Immunol, 2007). This immunosuppressive state may hamper the efficacy of immunotherapeutic vaccines.

In the present study we examined the role of Tregs on an immunotherapeutic strategy against HPV-induced cervical cancer. This immunization is based on a vector system derived from an alphavirus, the Semliki Forest virus (SFV). This vector encodes for the HPV oncoproteins E6 and E7 (SFVeE6,7). We determined the effect of the in vitro and in vivo depletion/inactivation of Tregs on the efficacy of SFV-based immunizations and the effect of Tregs on cytotoxic T cell (CTL) responses in immunized mice.

The in vivo Treg-depletion/inactivation (using anti-TGF-beta+anti-IL-10 or anti-CD25 treatment) combined with SFVeE6,7 immunizations did not enhance the frequency of HPV-specific CTLs nor their cytolytic activity. This observation was confirmed by in vitro cultures of spleen cells depleted from Treg cells, where no further increase in target cell lysis was noticed. Moreover using a newly established and optimized micro-CTL assay we showed that Treg cells are able to suppress HPV-specific CTLs expansion in vitro only at very high spleen to regulatory T cells ratios. The cytolytic activity of HPV-specific CTLs in vitro was not affected by the presence of Tregs.

In conclusion, decreasing the level of Tregs in vitro and in vivo does not further enhance the efficacy of SFVeE6,7 immunizations. As literature suggests that Tregs may play a more prominent role in tumour-bearing animals we will, in the near future, also study the role of Tregs under these conditions.

This research was supported by an Ubbo Emmius grant from the graduate school GUIDE, University of Groningen.

DC VACCINE USING HPV16 E7 CONJUGATED TO CHOLERA TOXIN

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Background: Human papillomavirus (HPV), especially HPV16 and 18, is associated with an increased risk of developing cutaneous and mucosal papillomas and dysplasias, and the virus is a necessary cause for development of invasive cervical cancer.

Objectives: We wanted to evaluate whether cholera toxin (CT) as a carrier/adjuvant can enhance the T-cell immune responses to a cancer antigen in an in vitro model of therapeutic dendritic cell (DC) vaccination in women with cervical dysplasia caused by HPV.

Methods: Immature or mature monocyte-derived DC from patients were pulsed with the HPV16 antigen E7, either alone or conjugated to CT. The antigen-pulsed DC were incubated with autologous CD4+- and CD8+ T-cells and the proliferative responses and cytokine production were measured.

Results: E7-pulsed DC induced neither T-cell proliferative responses nor Th1 cytokine secretion. CT-conjugation of E7 significantly improved the capacity of pulsed DC to activate antigen-specific T-cell proliferation. The CT-E7-pulsed DC also produced significantly more of the Th1 cytokine IL-12. Furthermore we observed that DC pulsed with CT-conjugated E7 from HPV16 caused a HPV genotype cross-reactive recall response in T-cells from women currently infected with HPV types other than HPV16.

Conclusions: These data show the potential of using CT-conjugated antigens for human DC vaccination, and indicate that DC pulsed with CT-conjugated E7 from HPV16 could represent a new strategy in the treatment of women with HPV-induced cervical disease.

P-10.28

ENHANCING DNA VACCINE POTENCY BY CO-ADMINISTRATION OF ALLOGENEIC MHC-CLASS-I DNA

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Intramuscular administration of DNA vaccines can lead to the generation of antigen-specific immune responses through cross-priming mechanisms. We propose a strategy that is capable of leading to the destruction of antigen-expressing cells may enhance cross-priming, and thus, result in improved antigen-specific immune responses. Therefore, in the current study, we evaluated immunologic responses elicited through electroporation mediated intramuscular administration of a DNA vaccine encoding calreticulin (CRT) linked to HPV-16 E7 (CRT/E7) in combination with DNA expressing HLA-A2 as compared to CRT/E7 DNA vaccination alone. We found that the co-administration of a DNA vaccine in conjunction with a DNA encoding an allogeneic MHC molecule could significantly enhance the E7-specific CD8+ T cell immune responses as well an antitumor effects against an E7-expressing tumor, TC-1 in C57BL/6 tumor-bearing mice. Furthermore, a similar enhancement in E7-specific immune responses was observed by co-administration of CRT/E7 DNA with DNA encoding other types of allogeneic MHC class I molecules. This strategy was also applicable to other antigenic systems, such as ovalbumin. Further characterization of the injection site revealed that co-administration of HLA-A2 DNA led to a significant increase in the number of infiltrating CD8+ T lymphocytes as well as CD11b/c+ antigen presenting cells. Furthermore, the E7-specific immune responses generated by intramuscular co-administration of CRT/E7 with HLA-A2 DNA was reduced in HLA-A2 transgenic mice. Thus, our data suggest that intramuscular co-administration of DNA encoding allogeneic MHC class I can further improve the antigen-specific immune responses as well as antitumor effects generated by DNA vaccines through enhancement of cross-priming mechanisms.

LOCAL CYTOKINES PRODUCTION LEVEL IN HPV-INFECTED WOMEN.

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The purpose. To study level of local cytokines production in cervical mucose in women with cervical HPV – infection before and after interferon immunotherapy.

Materials and methods: I group- 22 HPV positive patients with cervical pathology(LSIL and HPV positive). The control group - 14 HPV -negative women (groups were comparable). ELISA was used to estimate the level of proinflammatory cytokine TNF- α and anti-inflammatory IL-10 in cervical mucus (pg / ml). All patients from the I group received the b-interferon (1 mln EU in one suppository) intravaginal per day, within 10 days.

Results. The significant increasing of the TNF- α level in HPV-positive group in comparison with HPV-negative (accordingly, 108.5+ 9,4 and 57.5+ 6.2, p <0.005) was found. The level of IL-10 in cervical mucus in the I group was significantly lower (35.0+ 4.4 and 67.5+ 4.9, p <0.002). Follow-up within 3 months after interferon therapy has shown the improvement of a colposcopic picture in 11 (50%) patients (reduction of AWE).

The level of IL-10 increased (up to μ o 61.3±13.3, p=0.08), the level of TNF- α decreased (to μ o 60.6±7.2, p<0.01). As a result the level's ratio production of crucial proinflammatory and anti-inflammatory cytokines (TNF- α / IL-10) significantly decreased from 3.1±0.8 to 1.0±0.5 (p<0.01).

The cytokines level in 3 months has been investigated also among 9 patients of control group. No significant changes in their dynamics was revealed.

Conclusion. The clinical manifestation of cervical HPV- infection is defined by expressed «proinflammatory» character of local cytokines production (increased TNF- α). In half cases the b-interferon treatment improved the clinical condition of patients and it was accompanied by some positive changes in cytokine synthesis.

P-10.30

HLA CLASS II POLYMORPHISMS AND HIGH-RISK HPV-ASSOCIATED CERVICAL INTRAEPITHELIAL NEOPLASIA

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The association between HLA class II polymorphisms and cervical cancer was inconsistent in previous reports. In this longitudinal study, we examined the importance of HLA class II polymorphisms in the development of high-risk HPV-associated cervical intraepithelial neoplasia. A total of 392 women who were cytologically normal with the infection of HPV 16, 18, 52 or 58 at enrollment were included in this study. The HLA class II (DRB1, DQA1, and DQB1) polymorphisms were determined by sequence-specific oligonucleotide probes. The newly developed squamous intraepithelial lesion (SIL) and cervical cancer were ascertained through computerized linkage with Nation Cervical Neoplasia Registry and National Cancer Registry, respectively, until 2006. The Cox proportion hazards model was used to estimate hazard ratio (HR) and 95% confidence interval (CI) for each HLA class II polymorphism after adjustment for other risk factors. Persistent infection was defined as having the infection with same high-risk HPV type at both enrollment and follow-up examinations. The persistent infection of high-risk HPV types was significantly associated with an increased risk of SIL or cervical cancer. Among women with HPV 16 infection, DQB1*06 genotype was associated with an increased risk of SIL with a multivariate-adjusted HR (95% CI) of 2.5 (1.5-4.3). Among women with HPV18 infection, DRB1*0403 genotype was associated with a increased risk of SIL, but the association not statistically significant. Both DRB1*08 and DQB1*04 were associated with a 4- to 5-fold risk of SIL in women with HPV52 infection. These findings suggest that HLA class II alleles may play a role in the development of SIL in women infected with high-risk HPVs.

HLA CLASS II POLYMORPHISMS AND PERSISTENT INFECTION OF HIGH-RISK HPVS

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Persistent infection of oncogenic HPV types such as HPV 16, 18, 52 and 58 is a risk predictor of cervical cancer. However, only a small proportion of women with HPV infection are affected with persistent infection and consequent cervical cancer. The highly polymorphic human leukocyte antigens (HLA) may interact with HPV to result in its persistent infection. This study aimed to elucidate the association between HLA class II polymorphisms and persistent infection of high-risk HPVs. A total of 392 women who were cytologically normal with the infection of HPV 16, 18, 52 or 58 at enrollment were included in this study. The HLA class II (DRB1, DQA1, and DQB1) polymorphisms were determined by sequence-specific oligonucleotide probes. Persistent infection was defined as having the infection with same high-risk HPV type at both enrollment and follow-up examinations. Allele or haplotype frequencies were compared between participants with and without persistent infection using Pearson's X2 test or Fisher exact test. Logistical regression model was used to estimate odds ratios (OR) of persistent HPV infection with 95% confidence intervals (CI) for HLA class II polymorphisms after adjustment for other risk factors including lifetime sex partners, menopausal status, and high viral load (over 1000 copies per 50ng) at enrollment. Both DRB1*DR2 and DQA1*0102 were associated with an increased risk of persistent HPV 16 infection showing multivariate-adjusted ORs (95% CIs) of 2.5 (1.5-4.3) and 2.2 (1.5-3.6), respectively. While DRB1*04 and DQA1*0501 were associated with an increased risk of persistent HPV 18 infection, DRB1*09 was associated with a decreased risk of persistent HPV 18 infection. The DQB1*03 was associated with an increased risk of persistent HPV52 infection. These findings suggest that HLA class II genotypes may be involved in the persistent infection of high-risk HPV types.

P-10.32

LAUNCH VECTOR SYSTEM: A NOVEL PLATFORM FOR HPV16-E7-BASED THERAPEUTIC VACCINES

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Background. A prophylactic vaccine against HPV is now available but, due to the long latency period between infection and onset of cancer, the benefits of prophylactic vaccination will not be visible for decades. Thus, a therapeutic vaccine, targeting already infected individuals, is also required. The E7 oncoprotein from HPV is an attractive candidate for anti-cancer vaccine development. Objectives. The E7 antigen can be expressed in numerous systems, so it is essential to determine which system offers higher benefits for production. The system of choice ideally would produce the safest and most active material at the lowest cost. No one system will likely be ideal for all proteins. Practical considerations for each individual recombinant protein produced will determine the choice of production system. Methods. We have utilised accelerated production platform in plants that is applicable for expressing a broad range of monomeric and multimeric proteins, including vaccine antigens. This is accomplished by using launch vectors that enable the use of non-genetically modified plants for target production. Combination of launch vectors and non-genetically modified plants creates a highly competitive production platform that brings a new concept to biomanufacturing. Results and Conclusions. In this study we engineered the HPV16 E7 coding sequence as a fusion to -1,3-1,4-glucanase (LicKM) of Clostridium thermocellum and produced this fusion antigen at high yields in Nicotiana benthamiana plants using the pBID4 launch vector. Target antigen was purified and evaluated in a mouse model of cancer for its potential as a therapeutic vaccine candidate. The vaccine induced E7-specific IgG and cytotoxic T-cell responses inhibiting tumor development following challenge with an E7-expressing tumor cell line. These data demonstrate the potential of this platform for producing effective human therapeutic vaccines in plants.

CTLS GENERATED BY SUBOPTIMAL EPITOPE IMMUNIZATION IN HLA-A2.1 TRANSGENIC MICE

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Background: Our previous studies have reported one HLA-A2.1 restricted cottontail rabbit papillomavirus (CRPV) E1 epitope (CRPVE1/303-311, MLQEKPFQL) as a DNA vaccine stimulated strong protective immunity to CRPV infection in HLA-A2.1 transgenic rabbits and strong immune responses in HLA-A2.1 transgenic mice (HHD) following peptide immunization.

Objectives: We wanted to test if a suboptimal epitope could stimulate CTL response using strong stimulus.

Methods: CRPVE1/303-311 was modified to generate two suboptimal epitopes (MVQEKPFQL, identified as CRPVE1/304V) and (PLQEKPFQL, identified as CRPVE1/303P). These two epitope peptides were tested for binding affinity and also immunogenicity by peptide immunization in HLA-A2.l transgenic mice (HHD).

Results:CRPVE1/304V and CRPVE1/303P showed significantly lower affinity to A2 molecules at lower concentration when compared to the wild type CRPVE1/303-311 peptide. Spleens harvested from these two modified epitope immunized mice were stimulated in vitro with either CRPVE1/304V or CRPVE1/303-311 peptide pulsed mouse dendritic cells. CRPVE1/304V immunized animals were able to stimulate specific CTLs to wild type E1/303-311 epitope but not to CRPVE1/304V after in vitro stimulation. CRPVE1/303P failed to generate specific CTLs following stimulation with both CRPVE1/304V and wild type CRPVE1/303-311 peptides. Interestingly, wild type CRPVE1/303-311 epitope also failed to generate any specific CTLs to the CRPVE1/304V epitope stimulation.

Conclusions:We conclude that the stimulus used for in vitro stimulation plays an important role in induction of specific CTLs from peptide immunized animals.

P-10.34

RAPID NF-KAPPA B-DEPENDENT UP-REGULATION OF ICAM-1 BY HPV16-E6 AND -E7

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The intercellular adhesion molecule-1 (ICAM-1) and its receptor, the lymphocyte function-associated antigen-1 (LFA-1), play a crucial role in the formation of immune synapses. Successful immune synapse formation is critical for the adhesion and killing ability of innate and adaptive immune effector cells, such as natural killer (NK) cells or T cells. Earlier studies by different groups showed a significant ICAM-1 up-regulation in situ in high-risk human papillomavirus (HPV)-induced high-grade cervical intraepithelial neoplasias (CIN), in which the two viral oncoproteins E6 and E7 are highly expressed. Furthermore, it was described that ICAM-1 expression is up-regulated in vitro in HPV16-E6E7-immortalized human epithelial cells. Yet, these studies did not elucidate the mechanism underlying the observed ICAM-1 up-regulation in high-risk HPV-E6 and -E7 expressing cells. Here, we describe that E6 and E7 of high-risk HPV together rapidly (after 48h) induced ICAM-1 expression in their natural host cells, primary human keratinocytes. ICAM-1 was up-regulated in HPV16-E6E7-expressing keratinocytes both on protein and mRNA level compared to vector control (VC)-expressing keratinocytes. This up-regulated ICAM-1 expression in HPV16-E6E7 expressing keratinocytes led to enhanced conjugate formation with LFA-1-expressing immune effector cells.

INTERLEUKIN 18 (IL-18) AND ITS RECEPTOR (IL-18RA) IN CERVICAL CARCINOGENESIS

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Background. The host response to malignant tumors is a primary function in cellular immunity, modulated by IL-18 and other cytokines. Objective. To determine mRNA expression of IL-18 and the alpha chain of its receptor (IL-18Ra) in premalignant lesions and cervical cancer (CC). Methods. Two-hundred and twenty-five cDNA samples were analyzed; 192 cases of premalignant lesion or CC, diagnosed by histopathology and distributed into grades: a) low grade squamous intraepithelial lesion (LSIL) n=119, b) high grade squamous intraepithelial lesion (HSIL) n=38 and c) CC n=35. Thirtythree cervical scrapes were included from cytologically normal women (CN). IL-18 and IL-18Ra mRNA expression was detected by RT-PCR. As positive control for IL-18 we used RNA from HeLa cells and for IL-18Ra RNA from peripheral blood mononuclear cells from a patient with Chronic Granulocytic Leukemia. Results. Forty-five percent of women with CN and 48.5% with CC expressed IL-18 mRNA. In LSIL, 27.7% of the samples and in HSIL 23.7% expressed this message. We found significant differences in the median level of IL-18 mRNA expression between CN and LSIL (p=0.028) and between LSIL and CC (p=0.042). Of the 225 samples, 0.9% of the CN expressed IL-18Ra. Sixty percent of the samples were HPVpositive/IL-18 mRNAnegative and 25% were HPVpositive/IL-18 mRNApositive. Of the HPV 16positive samples, 71% were negative for IL-18 mRNA. Conclusions. These findings suggest a negative modulation of the Th1 response, favoring carcinogenesis. It is possible that HPV, through its oncoproteins, blocks IL-18 expression or interferes the signaling of this cytokine by blocking IL-18Ra, diminishing the cellular immune response and favoring viral pathogenesis and carcinogenesis.

P-10.36

HPV16 L1-E2/L1-E1^E4 CHIMERIC CAPSOMERS AS PROPHYLACTIC AND THERAPEUTIC VACCINES.

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Background. Current prophylactic vaccines against Human Papillomavirus (HPV) types 16 and 18 do not exhibit therapeutic effects on women that have been already infected. A combined prophylactic and therapeutic vaccine, which prevents new infections and at the same time clears current ones, is most desired. E2 and E1^E4 are viral proteins playing central roles during the viral life cycle. Objective. Generate HPV16 L1-E2 and L1-E1^E4 fusion proteins to produce chimeric capsomers able to induce humoral and cellular immune response, and antitumoral activity. Methodology. Different fragments from the HPV16 E2 gene and the complete E2 and E1^E4 genes were cloned C-terminally to GST-L1 gene in the pGex vector. Resulting plasmids were transformed into Rosetta Escherichia coli (E. coli) and induced to express fusion proteins that were purified by GST-trap column followed by gel filtration. Fusion proteins were analyzed by sucrose gradient sedimentation followed by western blot, electron microscopy, and ELISA. These proteins will be used to immunize groups of C57/BL6 mice to evaluate the induction of humoral (detected in serum by ELISA anti-L1) and cellular immune responses (detected by ELISPOT anti-IFN from spleenocytes) against fusion proteins; and finally, to evaluate their capacity to reduce or inhibit tumor growth in mice. Results. We generated constructs L1 N10+2 C29-E2N1, L1 N10+2 C29-E2H, L1 N10+2 C29-E2C and L1 N10+2 C29-E1^E4, carrying fragments of E2 gene (corresponding to amino, hinge and carboxi-terminal domains) as well as full length E1^E4 gene product. Fusion proteins were expressed and purified from Rosetta E. coli, obtaining protein yields ranging from 330 g/l to 160 g/l. Sucrose sedimentation gradients and electron microscopy showed that fusion proteins form heterogeneous aggregates, and ELISA assays indicated the presence of neutralizing epitopes; we are currently evaluating their immunogenicity in vivo, using a mouse model. Conclusions. We have produced and performed the initial analysis of fusion proteins containing L1-E2 and L1-E1^E4 from HPV type 16.

SNP'S ANALYSIS IN IL-10 GENE PROMOTER IN HPV INFECTED WOMEN

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Background. It is well known that IL-10 and TGF–b1 are highly expressed locally in patients with pre-malignant lesions and cervical cancer and that may induce a local immunosuppression. HPV E2 protein, which is involved in HPV gene expression, it is also able to trans-activate the human IL-10 gene expression throughout HPV E2 recognition site-like into IL-10 regulatory region. Objective. To identify the SNP's in the regulatory region of the IL-10 gene, and to analyze the SNP located in the site of recognition of the E2 protein of VPH in the promoter of IL-10 gene of the cellular line SiHa and in women with and without infection by VPH. Methods. An exhaustive search of reported SNP's was realized in the regulatory region of the IL-10 gene in the GenBank, and the public bases available. We analyzed through sequencing the presence of the SNP corresponding to the site of recognition of the E2 protein of VPH in the promoter of IL-10 gene of the cellular line SiHa and in women with and without infection by VPH were determined. Results and Conclusions. 57 SNP's in the promoter of the IL-10 gene have been reported, of which 30 are validated. The SNP (C/G) (rs3001100) reported in -4203 in the HPV E2 recognition site-like into IL-10 gene regulatory region has not been validated. We found allele C in this position of the promoter of IL-10 in SiHa cells and in women with and without infection by VPH. We hypothesized, that the presence of this SNP in the regulatory region of IL-10 gene may increases the affinity of the HPV E2 protein, suggesting a possible mechanism by which oncogenic HPV induce IL-10 gene expression during cervical cancer development, which represents a HPV strategy to evade the host immune response and favor viral persistence.

P-10.39

G-308A TNF-A POLYMORPHISM AND TNF-A MRNA IN CERVICAL CANCER

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Background. In persistent infections with high-risk types of human papillomaviruses (HR-HPV), the development of invasive cervical cancer (CC) may be associated with TNF-a expression, related to -308A allele of TNF-a polymorphism Objectives. To determine if the G-308A TNF-a polymorphism is associated with mRNA expression in the cervical epithelium from cytologically normal women (CN), with squamous intraepithelial lesion (SIL) or CC and to evaluate the association between the G-308A TNF-a polymorphism and the risk for CC. Methodology. We studied 100 women with CN, 47 with LSIL, 38 with HSIL and 33 with CC. In all samples HPV DNA was detected by PCR using MY09/MY11 and GP5+/GP6+ primers. Viral type was determined by RFLP's or sequencing. The G-308A TNF-a polymorphism was analyzed by PCR-RFLPs. The TNF-a mRNA was detected by RT-PCR. Results. Eighty-four percent of cases with LSIL, 79% with HSIL, 100% with CC and 54% of women with CN were HPV-positives. HR-HPV types were the most frequent in patients with SIL and CC; HPV-16 was found in the 71.4% of LSIL, 56.7% of HSIL and 72.7% of CC. Eighty-four percent of women with CN, 70.2% of LSIL and 81.6% of HSIL showed genotype GG at -308 region of TNF-a. Fifty-one percent of the cancer tissues showed genotype GA. Proportion of patients that expressed TNF-a mRNA increased in parallel with the severity of the lesion (17.1% in LSIL-36.4% in CC). AA women have 1.2 times the likelihood of express TNF-a mRNA. The relative risks of incidence for CC was 8-fold higher in women with genotype GA at -308 region of TNF-a(OR= 9, IC95%= 1.6-8.9). Conclusions. G-308A polymorphism was found to be associated with mRNA TNF-a expression in the cervical epithelium and this SNP appears to be associated with an increased risk for the development of CC

COST-EFFECTIVENESS AND MODELLING STUDIES OF HPV VACCINATION

SESSION 11

PROGRAMME

THE 25TH INTERNATIONAL PAPILLOMAVIRUS CONFERENCE MAY 8-14 2009, MALMÖ, SWEDEN

TIME NUMBER TITLE ROOM 2009-05-12 SESSION 11: COST-EFFECTIVENESS AND MODELLING OF HPV VACCINATION **SCANIA** MODELLING THE EPIDEMIOLOGICAL IMPACT OF HPV VACCINES: THE 11.00-11.35 O-11.00 IMPACT OF ASSUMPTIONS ON RECOMMENDED STRATEGIES G P Garnett, I Baussano COST-EFFECTIVENESS OF HPV-16/18 VACCINATION IN A COUNTRY WITH 11.35-11.46 O-11.01 EFFECTIVE SCREENING. T Westra, R Rogoza, N Ferko, H Tamming, M Drummond, T Daemen, J Wilschut, M Postma 11.46-11.57 O-11.02 HPV-16/18 VACCINATION IN SPAIN. HEALTH AND ECONOMIC IMPLICATIONS ON SCREENING. M Diaz, J.J Kim, J Ortendahl, M O'Shea, L Bruni, E Ferrer, F Bosch, S de Sanjose, S.J Goldie MODELING THE IMPACT OF HPV 16/18 VACCINATION ON PAP TEST ABNORMALITIES IN THE UNITED STATES 11.57-12.08 O-11.03 D Datta, H Weinstock, La Koutsky, K Hsu, Er Unger, J Shlay, P Kerndt, K Ghanem, M Hagensee, H Chesson VIRAL TRANSMISSIBILITY AND NATURAL IMMUNITY INFERRED FROM HPV TRANSMISSION MODELLING 12.08-12.19 O-11.04 JA Bogaards, M Xiridou, H de Graaf, VM Coupé, CJLM Meijer, J Wallinga, J Berkhof 12.19-12.30 O-11.05 DYNAMIC MODELING OF HPV VACCINE EFFECTIVENESS: IMPACT OF PARTNERSHIP FORMATION N Van de Velde, M Brisson, MC Boily

O-11.00

MODELLING THE EPIDEMIOLOGICAL IMPACT OF HPV VACCINES: THE IMPACT OF ASSUMPTIONS ON RECOMMENDED STRATEGIES

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Evidence that HPV 16 and 18 vaccines were effective at preventing infection with these viruses and the associated early stages of cervical cancer development, led to the rapid introduction of HPV vaccination in many developed countries. In some cases health economic models showed that vaccination was cost effective. The cost effectiveness of HPV vaccination in such models do depend upon the biological assumptions in the model that determine population level effectiveness, but results were universally favourable. Unfortunately, similar consensus is not possible on the incremental effectiveness of different vaccination strategies, such as vaccinating older women, running catch-up vaccination programmes, vaccinating boys and altering the targeting of vaccination. Here, the assumptions about patterns of sexual behaviour, acquisition of naturally derived immunity, and progression and regression of lesions alter the findings. Generally, the harder HPV is to control the more benefits will derive from additional efforts in vaccination. For example, the higher transmission probability required to generate the observed HPV-16 prevalence, if we assume that there is naturally derived immunity, makes it harder to eliminate HPV-16 through vaccination of girls alone and makes the vaccination of other groups more beneficial. Models can be fitted to observed HPV, lesion and cervical cancer prevalence and incidence data to narrow down the range of biological assumptions that are reasonable. In addition, deriving the outcomes associated with the different assumptions allows extremes of effectiveness to be explored. Assumptions about the coverage and effectiveness of screening programmes and how these will change over time play a major role in determining the importance of vaccination programmes, with the paradox that the less effective screening is the more cost effective vaccination campaigns will be.

O-11.01

COST-EFFECTIVENESS OF HPV-16/18 VACCINATION IN A COUNTRY WITH EFFECTIVE SCREENING.

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Background: Cervical cancer (CC) is one of the most common cancers among women worldwide. Despite an effective CC screening programme with high coverage in the Netherlands, 600-700 new CC cases are diagnosed annually. As CC is caused by persistent infection with oncogenic-HPV, implementation of an HPV-16/18 vaccine has been predicted to reduce the CC burden.

Objective: To estimate the costs and benefits of the addition of HPV-16/18 vaccination to CC screening in the Netherlands.

Methods: In order to calculate the incremental cost-effectiveness ratio (ICER) for vaccinating Dutch girls against HPV, a Markov model that simulates the transition between different health-states was calibrated to Dutch incidence and mortality data. The model followed a cohort of 100,000 Dutch girls during their life-time. For the base-case analysis the following assumptions were made: 100% vaccination of 12-year-old girls, 95% vaccine efficacy against HPV-16/18, vaccine price of €100/dose, no booster vaccination needed, and cross-protection against the HPV-types 31 and 45, at 50% and 90% respectively. Probabilistic sensitivity analysis and univariate sensitivity analysis were performed to estimate the robustness of the simulated base-case ICER.

Results: Adding HPV vaccination to the Dutch CC screening programme would annually cost \notin 31.5 million while saving \notin 11.5 million as a result of a reduction in CC screening and treatment costs. Besides the cost offsets, HPV vaccination is predicted to save 2907 life-years resulting in a discounted ICER of \notin 22,700 per life-year gained (\notin 18,500/QALY). Sensitivity analyses showed the ICER was most sensitive to discount rate, vaccine price and waning immunity. The probabilistic sensitivity analysis showed the robustness of the estimated base-case ICER.

Conclusion: Despite the existence of a highly effective cervical cancer screening programme in the Netherlands, the addition of prophylactic cervical cancer vaccination of pre-adolescent girls is predicted to be cost-effective, supporting the evaluation by the Dutch Health Council.

O-11.02

HPV-16/18 VACCINATION IN SPAIN. HEALTH AND ECONOMIC IMPLICATIONS ON SCREENING.

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Background: Spain is already implementing HPV-16/18 vaccination. Opportunistic screening (annual/biennial) covers roughly 80% of 18-65 year–old women, but is insufficient in older women, of low socioeconomic level, and in rural areas. Objectives: To assess the health and economic impact of HPV-16/18 vaccination on current screening practices in Spain. Methods: A multi-HPV type microsimulation model was calibrated to epidemiologic data from Spain utilizing likelihood-based methods and used to estimate cervical cancer outcomes, such as lifetime risk of cancer, life expectancy, lifetime costs and clinical information. Cost-effectiveness analysis was performed using a subset of good-fitting parameter sets. Strategies included: 1) screening over age 25, varying frequency and screening test; 2) HPV-16/18 vaccination of girls at age 11 combined with screening. Parameter uncertainty was evaluated using deterministic and probabilistic sensitivity analysis. Costs from Spain were expressed in 2005€.

Results: Assuming lifelong vaccine immunity, screening with cytology alone was less effective and cost-effective than all strategies combining vaccination and screening. For girls vaccinated at age 11, starting cytology screening with HPV triage at age 30 every 5 years costs $27,700 \in$ per year of life saved (YLS). This strategy would be considered cost-effective based on the per-capita GDP threshold. For unvaccinated women, cytology screening with HPV triage every 5 years starting at age 30 to at least age 65 costs 19,300 (YLS), and reduces the number of cytologies and colposcopies by 75% compared with cytology alone. Results were sensitive to vaccine efficacy, duration of immunity, screening behavior post-vaccination, and cost of vaccination.

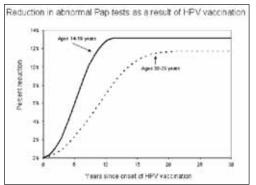
Conclusions: Although Spain is a low-risk country for cervical cancer, inequitable screening coverage and overuse of cytologies make screening inefficient. For adolescent girls, high vaccination coverage, and an organized screening for all women starting at age 30 every 3-5 years incorporating HPV triage represents the best balance between costs and benefits.

O-11.03

MODELING THE IMPACT OF HPV 16/18 VACCINATION ON PAP TEST ABNORMALITIES IN THE UNITED STATES

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Background: Mathematical modeling can provide estimates of the potential impact that HPV 16/18 vaccination will have on the prevalence of Pap test abnormalities over time.

Methods: We used age specific prevalence of HR-HPV types and Pap test abnormalities on females aged 14-29 years attending family planning clinics in 6 US cities as part of the HPV Sentinel Surveillance Study, 2003-2005, to create a simple model of the impact of HPV vaccination against types 16/18. Assumptions for the model included 1) 100% vaccine efficacy, 2) lifelong duration of vaccine protection, 3) 50% vaccination coverage of 12-year-old girls and 4) 15% of females 13 to 26 years of age would receive catch-up vaccination each year. Coverage rates were assumed to increase linearly over a five year phase-in period. The abnormal Pap test category included ASC-US, ASC-H, LSIL, HSIL, AGC, and AIS results. Frequencies of abnormal Pap tests were calculated according to presence or absence of HR-HPV types 16/18. The reduction in the probability of an

abnormal Pap test was a function of the percentage of females in that age group who had received HPV vaccination before that year. The impact of HPV 16 and 18 vaccination on the probability of an abnormal Pap test was applied in estimating the reduction in abnormal Pap tests. No adjustments were made for herd immunity or Pap abnormalities due to LR-HPV types.

Results: Among 3457 females, 15% of Pap tests were abnormal and prevalence of HPV types 16 and/or 18 was 9%. The projected reductions in abnormal Pap tests among females aged 14-19 and 20-29 years are shown in the figure below. Conclusions: Our model estimates 13% (14-19 year olds) and 7% (20-29 year olds) reductions in abnormal Pap tests among females attending family planning clinics in the US within 10 years of vaccine introduction.

O-11.04

VIRAL TRANSMISSIBILITY AND NATURAL IMMUNITY INFERRED FROM HPV TRANSMISSION MODELLING

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VM Coupé, VU University Medical Centre, Amsterdam, The Netherlands
CJLM Meijer, VU University Medical Centre, Amsterdam, The Netherlands
J Wallinga, National Institute of Public Health and the Environment, Bilthoven, The Netherlands
J Berkhof, VU University Medical Centre, Amsterdam, The Netherlands

Background: Transmission models are increasingly being used to asses the cost-effectiveness of HPV vaccination. The viral transmission probability and duration of natural immunity are only weakly identified in those models.

Objectives: We aim to provide estimates of transmission probability for 14 high-risk HPV (hrHPV) types and infer a role for natural immunity in explaining the observed type-specific prevalence through modelling.

Methods: We developed a dynamic model for sexual partnership formation, parameterized through a cross-sectional survey on sexual behaviour among teenagers, adolescent and adults. HPV transmission was included by allowing for two stages of infection, as informed by observations on hrHPV type-specific clearance in cervical screening: an initial phase characterized by high clearance and a subsequent persistent phase. We constructed ranges for the type-specific HPV progression and clearance rates together with ranges for viral transmission probability, duration of natural immunity and sexual contact parameters. The endemic equilibrium for each parameter combination was obtained by numerical approximation and compared to the age-dependent prevalence of 14 hrHPV types found in a large population-based screening trial (POBASCAM).

Results: In total, we considered ~10,000 parameter combinations. The data strongly favour long-lasting natural immunity: models in which natural immunity wanes within 10 years following clearance always conflict the observed hrHPV prevalence, whereas models that assume lifelong immunity can easily be fitted to the data. Viral transmission probability is positively correlated to the duration of natural immunity, and may differ substantially between hrHPV types.

Conclusions: Our study supports the notion of long-lasting natural immunity in HPV infection, strengthening the usual implicit assumptions in HPV transmission models. Transmission probability may differ substantially between hrHPV types, although all types are highly transmissible.

O-11.05

DYNAMIC MODELING OF HPV VACCINE EFFECTIVENESS: IMPACT OF PARTNERSHIP FORMATION

N Van de Velde, Laval University, Quebec, Canada *M Brisson,* Laval University, Quebec, Canada *MC Boily,* Imperial College, London, United Kingdom

Background: Current dynamic models of HPV infection assume that sexual partnerships are sequential and instantaneous (no duration) and therefore transmission occurs instantaneously. Partnership duration has been shown to influence the spread of other sexually transmitted diseases.

Objectives: To examine the impact of explicitly modeling partnership formation and dissolution (i.e. duration) on the population-level effectiveness of HPV vaccination.

Methods: We developed a stochastic individual-based dynamic model of sequential partnership formation and dissolution, and HPV transmission (16,18,6/11,31/33/45/52/58 and other-HR). The modeled population is characterized by age, gender, 4 sexual activity levels and HPV type-specific infection status. We compared the predictions of two models: Model 1, which takes into account duration of partnerships, and Model 2, which does not. For each model, we identified multiple fitting parameter sets using the same sexual behavior and epidemiological data. The outcomes compared include: 1) goodness-of-fit, and 2) reduction of age/type-specific HPV incidence in the vaccinated and unvaccinated populations over time. Main strategies investigated were vaccination of: 1) girls, 2) boys and 3) catch-up. For each strategy, we varied: 1) age at vaccination, 2) coverage by sexual activity level, 3) vaccine duration and 4) efficacy.

Results: Given its greater flexibility, Model 1 fitted a wider range of age-specific sexual behavior data more adequately than Model 2 (age at sexual onset, number of partners in the last year, duration of partnerships, proportion in a stable relationship and rate of partner change). The influence of these differences on the impact of vaccination will also be highlighted.

Conclusion: Complexity should only be added to models if significant gains can be made to the robustness or validity of predictions. HPV models which explicitly include partnership formation and duration capture important aspects of sexual behavior that influence the age of HPV acquisition and may therefore affect predictions of the impact of vaccination.

POSTER ABSTRACTS SESSION 11

POSTER SESSION I MONDAY 10.00: ODD NUMBERS

POSTER SESSION II

TUESDAY 10.00: EVEN NUMBERS

DYNAMIC MODELING OF HPV VACCINE EFFECTIVENESS: IMPACT OF MODEL STRUCTURE

N Van de Velde, Laval University, Quebec, Canada *M Brisson,* Laval University, Quebec, Canada *MC Boily,* Imperial College, London, United Kingdom

Background: Conflicting results have been obtained from mathematical modeling studies of HPV vaccine effectiveness and cost-effectiveness. Given the difference in the structure of the different models used to represent HPV transmission and natural history, it is important to understand which structural assumptions have the greatest influence on model predictions.

Objectives: To examine the impact of the following structural assumptions on population-level HPV vaccine effectiveness: 1) herd immunity (static vs. dynamic models), 2) natural immunity (SIR vs. SIS models) and 3) grouping of HPV-types.

Methods: We developed a stochastic individual-based dynamic model of HPV transmission and infection (HPV-16,18,6/ 11,31/33/45/52/58 and other-HR). The modeled population is stratified by age, gender, 4 levels of sexual activity and HPV type-specific infection status (susceptible, infected and immune). Our general model can represent the baseline structure (dynamic, SIR, 5-type model), and the desired structural assumptions mentioned above. For each structural assumption, we identified multiple fitting parameter sets using the same sexual behavior and epidemiological data. We compared predicted reduction in age/type-specific HPV incidence in vaccinated and non-vaccinated populations. Strategies investigated included vaccination of: 1) girls, 2) boys and 3) catch-up. For each strategy, we varied: 1) age at vaccination, 2) coverage, 3) waning vaccine efficacy functions (step, constant, sigmoid) and 4) efficacy.

Results: We will highlight, for each vaccination strategy and vaccine characteristic, which structural assumptions have a significant impact on model predictions of HPV vaccine effectiveness.

Conclusion: Models should be as parsimonious as possible given the research question investigated. Deciding the level of model complexity required is a central part of infectious disease modeling. Quantifying the impact of different model structures on HPV vaccine effectiveness under a wide range of vaccination scenarios will help modelers better understand the consequences of their assumptions, and help decision makers better understand the reasons underlying conflicting results from HPV models.

P-11.07

UNVACCINATED BACKPACKERS WILL SPREAD GENITAL WARTS IN AUSTRALIA

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DG Regan, National Centre for HIV Epidemiology and Clinical Research, Sydney, Australia **B Donovan**, National Centre for HIV Epidemiology and Clinical Research, Sydney, Australia

Background: International backpackers are relatively sexually active, and travel in large numbers to Australia each year. Approximately 80% of young Australian women are being vaccinated with a quadrivalent vaccine, which protects against the genital-wart-causing HPV 6 and 11 types. However, many backpackers are only receiving a bivalent vaccine, which provides no protection against these types.

Objectives: To estimate the burden of genital wart cases amongst Australians that is attributable to unvaccinated backpackers.

Methods: The following assumptions were used to build a two-strain model of HPV 6 and 11 transmission within and between the backpacker and local populations: locals who have sex with backpackers are young and relatively sexually active; only unvaccinated individuals become infected; and infected people clear HPV over months and are never reinfected.

We used latin hypercube sampling to analyse uncertainty and sensitivity. The model outputs were the proportions of the local population who have been infected with HPV 6/11.

Results: For most combinations of parameters, when backpackers were absent, the relatively high local vaccine coverage eliminated HPV 6/11 from the local population. When unvaccinated backpackers were introduced, on average 15% of the local population became infected between the ages of 16 and 27. The most sensitive parameters were the mean duration of infection and the partner change rate of backpackers.

When backpackers were present but vaccinated, the number of local cases was reduced by between 10% and 40%; the high sexual activity of backpackers meant that the infection could not be eliminated.

Conclusions: The mass vaccination of females will substantially reduce HPV 6/11 transmission, and will thereby protect unvaccinated females and males from genital warts. A highly active unvaccinated group will undermine these benefits.

ESTIMATING POPULATION IMPACT OF QUADRIVALENT HPV TYPES-6/11/16/18 VACCINE IN MALAYSIA

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Background: the quadrivalent (6,11,16,18) HPV vaccine was approved in Malaysia for prevention of cervical cancer, vulvar and vaginal pre-cancers, and genital warts in women. Objective: To assess the health and economic impact of the quadrivalent (6,11,16,18) HPV vaccine in Malaysia. Methods: A published mathematical model of the transmission dynamics of HPV infection and disease was adapted to Malaysia. Model inputs were obtained from public data sources, published literature, and clinical-trial databases. We evaluated scenarios including routine vaccination of females at age 12 along with two alternative, temporary, female catch-up programs: (1) ages 12-14 and (2) 12-24,. The vaccine coverage rates were 90% for the routine and 50% for the catch-up vaccination programs. We evaluated all vaccination strategies in combination with current cervical cancer screening practices. Results: The most effective strategy was vaccination of females by age 12 along with the temporary female coverage up to age 24. Relative to current standard of care (screening only), this vaccination strategy reduced the incidence of HPV 6/11/16/18-related genital warts-female, genital warts-male, cervical intraepithelial neoplasia (CIN) grade 1, CIN 2/3, and cervical cancers by 93% (265,599 cases), 91% (265,740 cases), 81% (79,982 cases), 81% (323,404 cases), and 63% (74,247 cases), respectively over 100 years. The incremental costeffectiveness ratios of the three vaccination strategies were MYR23,558 (US\$7,230) (dominated), MYR23,295 (US\$7,150), and MYR 23,423 (US\$7,189), per quality adjusted life year (QALY) gained for the routine vaccination strategy at age 12 and the routine plus catch-up vaccination strategies at ages 12-14 and 12-24 respectively. Conclusions: In Malaysia, vaccination with a quadrivalent (6,11,16,18) HPV vaccine can reduce the incidence of cervical cancer, CIN, and genital warts at a cost per QALY ratio within the range typically regarded as cost-effective.

P-11.09

IMPLICATIONS OF IMPROVED SCREENING SENSITIVITY FOR SCREENING AND VACCINATION POLICY

J Peto, London University, London, UK

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Improvements in training and technology have increased the sensitivity of cytology, causing marked changes in the observed natural history of HPV and cervical neoplasia. The incidence of diagnosed lesions is proportional to screening sensitivity and underlying incidence in young women, but if untreated cases persist but are often missed by screening an increasing proportion in older women will have developed many years earlier. When sensitivity (or coverage) improves the incidence rate will rise at all ages but then fall in older women, as prevalent lesions will be progressively detected and treated. This pattern was seen in national CIN3 incidence rates in Britain following the introduction of the national screening programme in the 1980s. The marked fall in the moderate or worse cytology rate between the first and second screening rounds of the ARTISTIC HPV screening trial, from 1.9% in round 1 to 0.4% in round 2, was presumably due at least partly to this effect, as staff were trained in LBC when it was introduced when the trial began. There was some overcalling of cytology following the introduction of LBC, but the round 2 vs round 1 OR for moderate or worse cytology was still 0.43 (95% C1 0.18, 1.00) after adjusting for age and previous screening history and excluding the first 18 months of recruitment. Long-term follow-up with modern cytology and HPV testing will be needed to establish the underlying natural history, but it seems likely that CIN3 is usually initiated during initial HPV infection but remains detectable for many years. This would have several important implications. The screening interval could safely be extended and a single negative HPV test might justify HPV vaccination at any age, although the public health benefits of early HPV16/18 vaccination in countries with modern screening have probably been exaggerated.

MODELING ANAL/PENILE CANCER IN HPV VACCINATION - A SYSTEMATIC REVIEW

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Among the HPV-associated malignancies, anal cancer accounts for 12% and penile cancer 3% of incident cases in the U.S. Although the international incidence of penile cancer varies greatly from region to region, substantial evidence on the increased incidence of anal malignancies is mounting. There is also tremendous interest in prevention of HPV related disease in men through vaccination. The purpose of this review is to develop parameters that can be used in decision analytic models on HPV-related anal and penile cancers based on the totality of evidence.

Methods: We performed a literature search to identify relevant publications in the PubMed database. We included studies with (a) have non-concurrent data from human subjects; (b) possess at least two of three key measurements (HPV, precancer, and cancer); and (c) were published in English, prior to August 1, 2008. Furthermore, we conducted a citation review and further additions by expert opinion. Systematic review was performed as per the methods recommended by the Cochrane Collaboration.

Results: Among the 607 anal precancer/cancer articles, 35 articles met the inclusion criteria. Among the 512 penile precancer/cancer articles, 12 articles met the inclusion criteria. Four additional anal articles were identified through citations/expert opinion. Within the 39 anal articles, 14 of the 26 treatment studies reported individual level data for metaanalysis. The monthly rates of recurrence following precancer treatment ranged from 0% to 42% across the 14 studies. The median annual rate is 33% and the annual rate for the combined sample is 22%.

Conclusion: Decision analytic models provide a framework for formulation of vaccination policies, incorporating the totality of evidence. This systematic review summarizes the evidence on HPV and anal and penile cancers to inform economic evaluations, specifically male vaccination against papillomavirus (MVP).

P-11.11 FUNDING COLLABORATIVE NON-COMMERCIAL POLYVALENT HPV VACCINE DEVELOPMENT AND TESTING

J Peto, London University, London, UK

A polyvalent vaccine that protects against most carcinogenic human papillomavirus types and is so cheap that it can be offered worldwide to women (and preferably to men as well) will be developed eventually, but this may take many years. The delay in developing such a vaccine is a crucial parameter that was ignored in the cost-benefit analyses that have persuaded the UK and governments in many other developed countries to commit enormous resources to purchasing HPV16/18 vaccines for young girls. A reduction of 5 years in this delay could (1) reduce government expenditure on vaccines in developed countries by several billion euros; (2) greatly reduce their expenditure on cervical screening and treatment; and (3) prevent about 2 million cervical cancer cases and a million deaths worldwide. A cost-benefit analysis that took account of this delay would thus show that European governments could reduce their combined expenditure on vaccination and continued screening of their own populations by billions of euros through such an initiative.

European and other governments that are already paying for HPV16/18 vaccines should therefore establish collaborative scientific and funding arrangements with this aim on purely financial grounds. Options might include organising independent international clinical trials of new vaccines, funding the optimisation and scale-up of vaccine manufacture, and buying out some patents to make them generic. Most small and many large companies working on HPV vaccines would welcome a large agreed price or a patent-sharing agreement for a promising development on HPV vaccines or adjuvants that had not yet been tested in a large trial. Large non-commercial trials can be relatively inexpensive within a framework such as the UK National Health Service where neither medical staff nor patients are paid to participate.

EXPECTED IMPACT OF HPV VACCINATION IN FRANCE

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F Aubin, Service de Dermatologie, CHU Saint Jacques, Besançon, France
C Clavel, CHU REIMS, IFR 53, Laboratoire Pol Bouin, Hôpital Maison Blanche, Reims, France
R Dachez, Laboratoire LCL, Paris, France
J Gondry, Service de Gynécologie-Obstétrique, CHU d'Amiens, Amiens, France
X Carcopino, Service de Gynécologie-Obstétrique, CHU Nord, Marseille, France

Objective: To assess the expected impact in France of a quadrivalent HPV 6/11/16/18 vaccine on the occurrence of genital HPV-induced lesions in women.

Methods: 4 large French multicenter studies assessing the HPV type-specific prevalence in invasive cervical cancer (ICC), CIN2/3, low-grade squamous intraepithelial lesions (LSIL) and external acuminata condylomata (EAC) in women were recently published (EDiTH I to IV studies respectively). These studies based on 400-500 cases each (256 for EAC) and using the same centralized genotyping methodology, provided relevant data for assessment of potential vaccination benefit. A markov model based on these HPV prevalence data and on a quadrivalent vaccination of 14 years old girls as recommended in France, was performed to assess the number needed to vaccinate (NNV) defined as the number of women who would need to be vaccinated to prevent an HPV-related event during their lifetime. The expected annual number of cases which could be prevented by this vaccination was also calculated.

Results: The EDiTH studies reported an HPV 6/11/16/18 prevalence of 82% in ICC, 64% in CIN2/3, 34% in LSIL and 83% in EAC. Using a theoretical vaccine efficacy of 100%, we estimated that 130 young women need to be vaccinated to prevent a case of cervical cancer, 17 for a case of CIN2/3 and 13 for a case of genital warts. The model also indicated that immunization of 80% of a 14 year-old cohort of 370 000 girls could prevent 2 495 CC, 17 985 CIN2/3, 8 004 LSIL, and 22 531 EAC cases in France.

Conclusions: HPV quadrivalent vaccination would thus substantially reduce the burden of female genital lesions in France.

P-11.13 EVALUATION OF VACCIANTION AGAINST CERVICAL CANCER

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BACKGROUND: The recent introduction of vaccines against human papilloma virus (HPV) creates hope of a more powerful protection against cervical cancer in the future. Cytological screening and HPV vaccination are partly complementary and partly overlapping measures to prevent cervical cancer and it is crucial to optimise their combined effects.

OBJECTIVES: To evaluate the effectiveness and cost of different human papilloma virus (HPV) vaccination strategies in Sweden, taking into account incidence reduction via the cervical cancer screening programme. We will investigate the prevalence and natural history of HPV infection in the population and evaluate how HPV infection contributes to the development of cervical intra-epithelial neoplasia (CIN) and progression to invasive cervical cancer.

METHODS: The dynamics of disease progression is captured by a differential equation model using methods with roots in control systems theory. This model will serve as a background to determining optimal joint screening and HPV vaccination policies. This method has been used in epidemiological settings to allow study of larger systems than would be feasible with traditional approaches, such as Markov simulation, and allows partially observed transitions. This model is stochastic, which means that we also get precision measures such as confidence intervals of the estimates.

RESULTS: A national cervical cancer screening register has been constructed and will be used in the simulation studies. Voluntary registrations of administered HPV-vaccinations are currently being collected at the Swedish Institute for Infectious Disease Control. The mathematical model is being fit to data on cervical cancer from the Cancer registry, and will be extended to incorporate the dynamics of HPV-infections.

CONCLUSIONS: This project intends to introduce new and powerful modelling methods to epidemiology and science. It will also provide knowledge and operative tools to optimise the performance of a combined strategy for screening and vaccination against cervical cancer in Sweden.

HPV TYPE-SPECIFIC NATURAL HISTORY MODELS IDENTIFIED THROUGH SIMULATION MODELLING

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Background: In order to generate simulation models of cervical pre-neoplastic and neoplastic disease, we previously used a single, age-specific matrix of progression, regression and stasis rates between high-risk HPV infection, CIN1, CIN2, CIN3 and invasive cervical cancer. This natural history model essentially reflects a weighted average of the characteristics of all oncogenic HPV types. However, evidence suggests that HPV 16 and HPV 18 will exhibit a more aggressive progression pathway through the natural history stages compared to the average effect of other oncogenic types. In order to model the effects of current HPV vaccination programs, we were required to specify separate natural history models for vaccinated and unvaccinated HPV types.

Objectives: To refine the existing natural history model into separate natural history models for oncogenic HPV types included in current generation vaccines (HPV 16 and HPV 18), and other high risk HPV types.

Methods: We calibrated three separate natural history models to reproduce observed type-specific HPV prevalence and cancer incidence. The new, type-specific natural history models were functions of our original, all-type natural history model, and the form and parameter values of these functions were determined through an iterative process of calibration and specification.

Results: We identified a set of natural history models which reproduced the calibration targets for HPV 16, HPV 18, and other high-risk HPV types. Our specified natural history models for HPV 16 and HPV 18 are substantially more aggressive than other high-risk HPV types.

Conclusions: The prevalence of specific HPV types in cytologically normal women and in invasive cervical cancer can be used to specify type-specific natural history models. The type-specific models have widespread application in the estimation of the impact and cost-effectiveness of HPV vaccination with first-generation prophylactic vaccines.

P-11.16 IMPACT OF FEMALE HPV VACCINATION ON MALES IN AUSTRALIA

K Canfell, Cancer Epidemiology Research Unit, The Cancer Council New South Wales, Sydney, Australia M Smith, Cancer Epidemiology Research Unit, The Cancer Council New South Wales, Sydney, Australia J Brotherton, National HPV Vaccination Program Register, Victorian Cervical Cytology Registry, Melbourne, Australia JB Lew, Cancer Epidemiology Research Unit, The Cancer Council New South Wales, Sydney, Australia RV Barnabas, HIV Vaccine Trials Network, Fred Hutchinson Cancer Research Center, Seattle, USA RJ Walker, Cancer Epidemiology Research Unit, The Cancer Council New South Wales, Sydney, Australia

Background: A national publicly-funded vaccination program against HPV commenced in Australia in 2007. Vaccination is being delivered to females aged 12-26 years in the catch-up phase until 2009.

Objective: To investigate the extent to which males will benefit from the existing public vaccination program in females in Australia, and to assess the incremental impact of also vaccinating males.

Methods: Building on previous work, we performed a mathematical modelling analysis of HPV transmission and vaccination in the Australia, using estimates of current coverage rates in females. Early data from the program show coverage of approximately 75% in 12 year old females, with lower rates in older ages.

Results: The existing public program targeting only females is expected to result in a reduction in the age-standardised incidence of HPV 16 of 83% in females and 56% in males by 2050. The addition of routine vaccination of 12 year old males with catch-up in 12-15 year olds would increases the overall reduction in HPV 16 incidence by 2050 to 88-94% in females and 68-82% in males by 2050, under a range of feasible coverage assumptions for males. The existing program in females only is expected to result in long term reduction in male cancers of the head and neck, anus and penis of approximately 18%, which would increase to between 22-27% if males were also vaccinated.

Conclusions: The current public vaccination program will benefit males as well as females, by substantially and rapidly reducing HPV 16 infections in Australia. Even under the most optimistic coverage assumptions for male vaccination, at least two thirds of the potential maximal benefit to males will be achieved via the existing public program in females.

SESSION 12

VIRAL ATTACHMENT AND ENTRY

PROGRAMME

THE 25TH INTERNATIONAL PAPILLOMAVIRUS CONFERENCE MAY 8-14 2009, MALMÖ, SWEDEN

TIME NUMBER TITLE

ROOM

2009-05-12

SESSION 12: VIRAL ATTACHMENT AND ENTRY				
14.00-14.30	O-12.00	THE COMING OF AGE OF EXPERIMENTAL HPV INFECTIONS M A Ozbun		
14.30-14.40	O-12.01	INITIAL STEPS DURING IN VIVO PAPILLOMAVIRUS INFECTION P Day, K Johnson, Rc Kines, J Roberts, C Thompson, D Lowy, J Schiller		
14.40-14.50	O-12.02	CYCLOPHILIN-B MEDIATES CONFORMATIONAL CHANGES OF HPV16 L2 DURING INFECTIOUS ENTRY M Bienkowska - Haba, H.D. Patel, M. Sapp		
14.50-15.00	O-12.03	CYCLOPHILINS FACILITATE INFECTIOUS ENTRY OF HPV16 RM Schowalter, DV Pastrana, DE Anderson, P Day, CB Buck		
15.00-15.10	O-12.04	TEMPOROSTRUCTURAL REARRANGEMENTS OF THE HUMAN PAPILLOMAVIRUS L2 EXTERNAL LOOP M Conway, C Meyers		
15.10-15.20	O-12.05	COMPREHENSIVE CELL BIOLOGICAL ANALYSIS OF HPV-16 ENTRY INTO HOST CELLS M Schelhaas, P Day, J Schiller, A Helenius		
15.20-15.30	O-12.06	CLATHRIN- AND CAVEOLIN-INDEPENDENT ENTRY OF HPV16 VIA TEMS C Lambert, G Spoden, K Boller, L Florin		

O-12.00

THE COMING OF AGE OF EXPERIMENTAL HPV INFECTIONS

M A Ozbun, Department of Molecular Genetics and Microbiology, The University of New Mexico School of Medicine, Albuquerque, USA

Carcinogenic HPVs have been propagated in the laboratory for ≈ 17 years, and currently there are multiple means for obtaining infectious PV virion stocks for experimental infections. Processes dependent upon epithelial differentiation include natural warts, host tissue xenografts in rodents, and cultured organotypic (raft) tissues. These options are not amenable for every PV genotype, and yields are variable and dependent upon each specific viral genotype. Generally, cutaneous PV lesions yield the highest virion levels; virion yields of mucosal genotypes decrease as oncogenic potential increases. Differentiation-dependent approaches are limited to encapsidation of replication-competent viral genomes, and often only genomes conferring a growth advantage (i.e., immortalization) to the cells wherein they replicate. Although pure virion stocks can be obtained for many high-yield cutaneous PV genotypes (BPV1, CRPV, HPV1), only low-purity crude virion stocks have been reported for carcinogenic HPVs (types 16, 18, 31) typically via organotypic epithelial tissue propagation.

Recently, advances in methods for PV virion production independent of epithelial differentiation permit the isolation of high-titer, high purity viral stocks from virtually any PV genotype for which genomes are available. Techniques are based upon the ability of transiently expressed L1+L2 capsid proteins to self-assemble and efficiently package local DNA molecules from 5- to 8-kb. In addition to the high-yield and purity of such virion preparations, the approach provides the ability to produce infectious particles encapsidating any viral or pseudoviral (i.e., reporter) genome, regardless of ability of the DNA to replicate. These virus particles share many structural and functional similarities with differentiation-induced virions; however, some questions remain about the absolute physiological likeness among differentially derived virion stocks.

The goal of this presentation is to discuss the apparent advantages and disadvantages among the varied means of virion production, to demonstrate the known likenesses, and to outline potential disparities that have yet to be tested.

O-12.01 INITIAL STEPS DURING IN VIVO PAPILLOMAVIRUS INFECTION

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The host factors required for in vivo infection have not been investigated for any papillomavirus. Using a recently developed murine cervicovaginal challenge model, we previously reported that HPV pseudovirions preferentially bind to the basement membrane (BM), in contrast to their binding directly to cells or extracellular matrix (ECM) during in vitro infection. Here we have extended the in vivo analysis by first determining that unlike ECM binding, BM binding was dependent upon heparan sulfate proteoglycans (HSPGs) for HPV16, HPV31, and HPV5, as treatment of the genital tract with heparinase III prior to pseudovirus instillation significantly inhibited infection of all three types by greater than 90%, and clearly inhibited pseudovirion attachment to the BM, as well as to cell surfaces. Furthermore, while bound to the BM, HPV16 pseudovirions underwent a conformational change that exposed an L2 cross-neutralization epitope, a process that occurs preferentially on the cell surface in vitro. In addition, as previously shown in vitro, HPV16 pseudovirions whose L2 had been precleaved by furin, a cellular protease required for exposure of the L2 epitope and infection, can bypass the HSPG requirement for in vivo attachment, as heparinase III-treated vaginal tracts did not prevent furin-precleaved virions from binding robustly to the cell surface.

Thus, there are noteworthy differences, as well as some similarities, between HPV pseudovirion infection in vivo and in vitro. In vivo, HSPG are the primary attachment factors for HPV16, HPV31, and HPV5, in contrast to some reports for in vitro infection. The results with furin-precleaved pseudovirions are consistent with a dynamic model of in vivo infection in which furin cleavage, which may occur on the BM, allows transfer of virions from HSPG moieties to a second receptor that is present on the cell surface.

O-12.02

CYCLOPHILIN-B MEDIATES CONFORMATIONAL CHANGES OF HPV16 L2 DURING INFECTIOUS ENTRY

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Following binding to the primary attachment receptor heparan sulfate proteoglycans (HSPG), human papillomavirus type 16 (HPV16) particles undergo conformational changes affecting the major and minor capsid proteins, L1 and L2, respectively. This results in exposure of the L2 N-terminus, reduced affinity to HSPG, transfer to uptake receptors, and infectious internalization. We report here that target cell cyclophilin B (CyPB), a member of the peptidyl-prolyl cis/trans isomerase family, is required during two distinct steps in HPV16 infection. Cell surface CyPB mediates conformational changes in L2. Inhibition of cell surface-resident CyPB prevented exposure of the L2 N-terminus and blocked HPV16 and HPV18 infection by inducing noninfectious virus internalization. Mutation of the N-terminal CyP binding site yielded exposed L2 N-terminus in absence of active CyPB and bypassed the need for cell surface CyPB. However, this mutant is still sensitive to CyPB inhibition and requires CyPB, likely in the endocytic compartment, for completion of infection. Possible mechanisms of action for CyPB after internalization will be discussed.

O-12.03 CYCLOPHILINS FACILITATE INFECTIOUS ENTRY OF HPV16

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With the goal of identifying cellular factors important for the infectious entry of HPV16, a virion-bound photoactivatable crosslinking reagent was used to isolate virion-interacting cellular proteins under denaturing conditions. Following L1 immunoprecipitation, two peptides corresponding to human cyclophilin B were identified by mass spectrometry. One of the two cyclophilin B peptides was covalently modified in a manner consistent with crosslinker attack, suggesting a possible direct interaction between L1 and cyclophilin B. Cyclophilin B is a secreted chaperone that, like other cyclophilins, mediates peptidyl-prolyl cis/trans-isomerization of client proteins. Cyclosporin A (CsA) inhibits the isomerase activity of cyclophilins, and this drug displayed dose-dependant inhibition of HPV16 pseudovirus infection, with an IC50 of approximately 2 µM. Microscopic studies suggest that CsA does not exert major effects on the early stages of the infectious entry process, such as virion surface-exposure of L2 epitopes or initial endocytic internalization. Interestingly, BPV1, CRPV, as well as HPV31 (a close relative of HPV16) were discovered to be relatively resistant to the effects of CsA. To analyze the source of the CsA susceptibility of HPV16, we created chimeric HPV16/31 pseudoviruses that contained the L1 of one type and the L2 of the other type. Inhibition assays using the L1/L2 chimeras suggested the L1 protein is predominantly responsible for the relative resistance of HPV31 to CsA. Thus, a cyclophilin chaperone activity, possibly provided by cyclophilin B, appears to facilitate the infectious entry of HPV16 via effects on L1.

O-12.04 TEMPOROSTRUCTURAL REARRANGEMENTS OF THE HUMAN PAPILLOMAVIRUS L2 EXTERNAL LOOP

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Human papillomavirus (HPV) capsids are comprised of 72 pentamers of the major capsid protein L1. Recent cryoelectron microscopy images of HPV16 PsV suggest that each pentamer of L1 can be occluded with a monomer of the minor capsid protein L2. Further research suggests that an N-terminal external loop of L2 exists which can be the target of neutralizing and cross-neutralizing antibodies. We have previously shown that native virions (NV) exploit a tissue-spanning redox gradient which facilitates multiple assembly/maturation events in the context of the complete papillomavirus lifecycle. Importantly, effective neutralization of HPV16 via anti-L2 "external loop" Abs is dependent on the maturation state of the virion, suggesting that the L2 external loop changes conformation over time. Here, we show that N-terminal L2 cysteine residues: Cys22 and Cys28 are dispensable for infectivity, and actually enhance infectivity when substituted for Ser. Neutralization profiles using a panel of anti-L2 antibodies are also altered in mutant virions in comparison to wild-type. Specifically, mature Cys22,28Ser virions are resistant to neutralization, mature Cys28Ser virions are partially susceptible to neutralization, and mature Cys22,28Ser virions are resistant to neutralization. Analysis of the inner conical hollow of an HPV16 L1 pentamer (Bishop et al. 2007) identified Cys145 as a potential L1 residue that may interact with Cys22 or Cys28. Further research into the potential temporostructural rearrangements of the L2 external loop will be presented.

O-12.05 COMPREHENSIVE CELL BIOLOGICAL ANALYSIS OF HPV-16 ENTRY INTO HOST CELLS

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Endocytosis/pinocytosis is the major route used by viruses to enter cells. For decades, it has been thought that pinocytosis is mainly comprised of clathrin-mediated endocytosis. However, current research indicates a complex network of diverse ongoing and triggered pathways. The entry pathways of viruses involve clathrin-coated vesicles, caveolae, macropinosomes, or further still poorly characterized pathways.

The existing literature describes the entry of Human papillomavirus type 16 (HPV-16) into various different cell lines to occur by clathrin and non-clathrin pathways. To comprehensively analyze the cell biological features of HPV-16 entry into human epithelial cells, we compared HPV-16 pseudovirion (PsV) entry to Semliki Forrest Virus, Simian Virus 40, and Vaccinia Virus, which enter cells by clathrin-mediated endocytosis, caveolar endocytosis, and macropinocytosis, respectively. To probe the cell biological requirements of virus entry, we used various methods of pertubations (e.g. drug inhibition, siRNA silencing, overexpression of dominant negatives). Our analysis included infection data, i.e. GFP expression after plasmid delivery by HPV-16 PsV, in combination with electron, immunofluorescence, and video microscopy. In total, 48 inhibitors, 70 siRNA targets, and 20 dominant negative proteins, were tested.

The obtained data indicated that HPV-16 entry into HeLa and HaCaT cells was clathrin-, caveolin-, cholesterol-, and dynamin-independent, and that entry did not occur by macropinocytosis. In contrast, HPV-16 made use of a potentially novel endocytic pathway that was dependent on actin dynamics, tyrosine kinase signalling, and led the virus to late endosomes/lysozomes.

O-12.06

CLATHRIN- AND CAVEOLIN-INDEPENDENT ENTRY OF HPV16 VIA TEMS

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Background: Since many viruses exploit the endocytic machinery of their host cells for infectious entry they are valuable tools to investigate the complex mechanisms of endocytosis. It has become clear that viral uptake often takes place via clathrin- or caveolar-mediated endocytosis. However, recent studies suggest the existence of additional, less characterized invasion routes.

Objectives: The aim of this study was to characterize the endocytic mechanism that is used by human papillomavirus (HPV) type 16.

Methods: Different cell lines were infected with HPV16 pseudovirions (PsV) and the association of virions with defined cellular marker proteins during the invasion route was analyzed by live cell imaging, immunofluorescence-, and electronmicroscopy. Furthermore, specific endocytic mechanisms were inhibited by the expression of dominant-negative mutants or siRNA mediated knockdown and the effect on infectivity was tested.

Results: PsV showed no association with clathrin or caveolin at the cell surface and intracellular compartments. Accordingly, inhibition of clathrin- or caveolin-mediated endocytic mechanisms had no inhibitory effect on HPV16 infectivity. Infectivity was even enhanced when clathrin- and caveolin- mediated endocytosis was inhibited simultaneously or the function of the large GTPase dynamin was blocked. However, we detected specific colocalization of virions with tetraspanin-enriched microdomains (TEMs) on the plasma membrane and in endocytic vesicles. Treatment of cells with tetraspanin-specific antibodies and siRNA inhibited HPV16 cell entry and infection.

Conclusions: Our data showed that cellular uptake of HPV16 occurs by a mechanism of clathrin- and caveolin-independent endocytosis. The morphological and functional significance of tetraspanins suggests that these molecules are involved in a novel endocytic pathway. Since tetraspanins have the capacity to form TEMs by multiple intermolecular interactions with various transmembrane molecules, they may define specific microdomains that contain secondary receptor molecules for HPV16-invasion.

POSTER ABSTRACTS SESSION 12

POSTER SESSION I MONDAY 10.00: ODD NUMBERS

POSTER SESSION II

TUESDAY 10.00: EVEN NUMBERS

P-12.07

HPV58 CAPSID BINDING TO KERATINOCYTES AS DEFINED BY MONOCLONAL ANTIBODIES

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Background: Human Papillomavirus (HPV 58) is a high-risk HPV type associated with progression to invasive genital carcinomas.

Objectives: To determine the neutralizing and binding characteristics of a newly developed set of monoclonal antibodies (Mabs) to HPV58 particles. The Mabs were used to test binding profiles of HPV58 virus like particles (VLPs) to human HaCaT cells, and to the extracellular matrix (ECM) secreted by these cells.

Methods: We developed six Mabs that bind to conformational epitopes on HPV58 L1 VLPs. Three Mabs were selected for more in-depth study, 58J6, 58D10 and 58F3. HPV58 L1 VLPs, pseudoviruses (pSVs) (58 L1, L2 and a pseudogenome) and 'quasiviruses' (58 L1, L2 and an HPV11 genome) were utilized in these studies.

Results: All six Mabs showed different capacities for binding to HPV58 VLPs and pSVs as determined by ELISA assay. They neutralized HPV58 pSVs as assessed by pre and post attachment neutralization. HPV58 'quasiviruses' were used to measure infection and neutralization in HaCaT cells as assessed by QRT-PCR. We then utilized the Mabs to show that HPV58 L1 VLPs bind to HaCaT cells, and partially co-localized with the ECM protein, Laminin 5 (LN5) secreted by these cells. We determined that unlike HPV11 VLPs, HPV58 VLPs bind to LN5 and to other unknown receptor(s) in the ECM. Unique properties of the antibodies were discovered when VLPs were incubated with Mabs prior to VLP attachment to HaCaT cells and ECM.

Conclusion: Our findings suggest that HPV58 utilizes multiple binding sites on HaCaT cells and ECM. These Mabs provide a unique set of tools to study the binding and internalization properties of a previously untested high-risk HPV type and the opportunity to compare these characteristics with other HPV types.

P-12.08

CLEAVAGE OF NATIVE HPV16 L2 N-TERMINUS BY FURIN

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Studies using pseudovirions (PsV) have implicated L2 in early events of the viral life cycle including the entry of virions into the cell and escape of the viral genome from endosomes after viral uncoating. In HPV16 PsV, L2 is cleaved by furin proprotein convertase at a consensus site in the N-terminal region. Inhibition of infection by the use of a furin inhibitor suggests that cleavage by furin is necessary for infection. Furthermore, pre-cleavage of L2 in immature PsV by furin enhances infectivity in furin-deficient cells. We were interested in whether native virions produced under physiologically relevant conditions of differentiating host tissue would show the same dependence on cleavage by cellular furin to undergo a successful infection. Human foreskin keratinocytes infected with HPV16 were cultured to produce infectious virus. Infection of HaCat (keratinocyte cells) as well as CHO (Chinese hamster ovary cell lines), was dependent on cleavage by furin. Pre-treatment of virions with furin in vitro, as well as furin treatment post-attachment of virions to the cell surface, enhanced infectivity in furth deficient cells. Establishment of stable cell lines maintaining genomes lacking the furin consensus site in L2 will provide further insight to the role of cellular furin in HPV infection. Infection of primary cells will be used to confirm the physiological importance of cellular furin in HPV infection. Dependence of furin by immature and mature native virions will be presented in addition to the necessity of furin cleavage by HPV18, HPV31, and HPV45 virions.

P-12.09

MONOCLONAL ANTIBODY SENSING CELL ATTACHMENT-INDUCED SHIFT IN HPV16 L1 CONFORMATION

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Increasing evidence suggests that following binding to primary attachment receptors, heparan sulfate proteoglycans, L1 and L2 undergo conformational changes. Whereas the shift in HPV16 L2 conformation is rather well defined and involves the exposure of the N-terminus on the capsid surface, the changes in L1 conformation are not well characterized yet. Here we describe a neutralizing monoclonal antibody (NmAb) that is specific for the L1 conformation induced by cell attachment. Binding of virions to extracellular matrix (ECM) did not induce reactivity with this NmAb. Rather, the conformational shift required direct interaction with cells and an extended incubation at 37°C. However, the antibody can induce the change in unfixed HPV16 particles. The shift occurs prior to L2 conformational change and does not require cell surface CyPB activity, which catalyzes rearrangement of the L2 N-terminus. This antibody will provide a valuable tool to characterize the sequence of events occurring on the ECM and cell surface. It will also allow us determining if receptor engagement can induce global conformational shifts of the viral capsid.

P-12.10 STUDIES OF HPV 16 PSEUDOVIRION E1^E4 INTERACTION.

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Human papillomaviruses are the cause of nearly all cervical cancers and HPV 16 carries a high risk for malignant progression. In low grade HPV16 cervical lesions the viral capsid proteins, L1 and L2, are expressed in the uppermost layers of the epithelium where assembly of infectious virions occurs. L1 is found only in cells containing the HPV E1^E4 protein and we have found that L1 and E1^E4 are found in a similar location after nuclear degeneration. E1^E4 accumulates to high levels in the upper layers of the epithelium and we have recently shown that its ability to form amyloid-like fibrils contributes to its accumulation.

Given that the capsid proteins are observed only in regions of the epithelium having high levels of E1^E4 we sought to assess whether E1^E4 associates with the viral structural proteins. Using GST pull downs we have found that the E1^E4 protein associates with both L1 and L2. In addition, when expressed in tissue culture, E1^E4 levels are elevated in the presence of L1 and L2 suggesting that E1^E4 is stabilized on binding to the viral capsid proteins. These results indicate that L1 and L2 contribute to maintaining levels of E1^E4 in the upper layers of the epithelium.

Our results also suggest that E1^E4 associates with assembled capsid, since E1^E4 co-sediments with pseudovirions. The relevance of this association has been determined using a combination of assays to monitor various aspects of pseudovirion formation and function. Our results indicate a functional interaction between the HPV 16E1^E4 protein and the viral capsid.

P-12.11 INTERACTION OF HPV16 L2 WITH THE DYNEIN LIGHT CHAIN TCTEX-1

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Background: Papillomaviruses exploit the cellular microtubule network to deliver their genome into the nucleus during infection. Microtubule plus-ends point towards the plasma membrane, where they can interact with the actin cortex whereas most microtubule minus-ends are stabilized at the microtubule-organizing centre (MTOC) near the nucleus. Minus-end directed transport of cargo is performed by the motor protein complex dynein. We have shown earlier that the minor capsid protein L2 interacts with dynein required for subsequent intracytoplasmic transport towards the nucleus and productive infection.

Objectives: Since the dynein motor complex consists of two heavy chains (HC), two intermediate chains (IC), two light intermediate chains (LIC), and up to six light chains (LC) we wanted to decipher which dynein component mediates the interaction with L2.

Methods: Protein interaction assays like yeast two-hybrid, co-immunofluorescence, and co-immunoprecipitation were performed.

Results: Using yeast two-hybrid the dynein light chain DYNLT1 (Tctex-1) was identified as a specific cellular interaction partner of L2. The interaction was further analyzed by immunofluorescence and immunoprecipitation studies in mammalian cells.

Conclusions: After disassembly of the papillomavirus capsid during infection, L2 performs transport of the viral genome towards the nucleus through interaction with the microtubule motor complex dynein. Our data suggest that the dynein light chain member DYNLT1 (Tctex-1) constitutes the adaptor mediating association of L2 with the dynein complex. This interaction is probably essential for productive infection.

P-12.12

COMPARATIVE STUDY ON DNA DELIVERY BY DIFFERENT PAPILLOMAVIRUS L1/L2 VLPS

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Background: Virus-like particles (VLPs) made from papillomavirus L1 and L2 capsid proteins are promising vectors for high efficiency deliver of DNA in vitro and in vivo. Many studies have chosen HPV16 L1/L2 VLPs as the delivery vehicle. But no studies have been done to compare the delivery efficiency of different types of L1/L2 VLPs.

Objectives: In the current study, we compared delivery efficiency of L1/L2 VLPs from HPV16, HPV18, HPV58 and cottontail rabbit papillomavirus (CRPV).

Methods:L1/L2 VLPs from HPV16, HPV18, HPV58 and CRPV were produced in 293TT cells. A GFP DNA-expressing reported plasmid were attached externally to the VLPs and incubated with rabbit or 293TT cells. The expression levels of GFP were determined by flow cytometry analysis.

Results: CRPV and HPV58 L1/L2 VLPs showed significantly higher levels of plasmid delivery in both rabbit and human cell cultures when compared to HPV16 and HPV18 L1/L2 VLPs. A VLPs/DNA ratio of 2:1 showed the highest delivery efficiency. These data suggested that VLPs formed from different HPV types showed different delivery efficiencies. Conclusions: We concluded that choosing higher delivery VLPs could improve the DNA take up in cells.

P-12.13

THE HPV18 E1^E4 PROTEIN IN THE PRODUCTION OF INFECTIOUS VIRUS.

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The papillomavirus E1^E4 protein is highly expressed in the upper layers of differentiated host tissues constituting approximately 90% of the viral protein. Numerous functions have been attributed to E1^E4, including assistance in virion release from cornified squames allowing for infection of a new host, the collapse of the keratin cytoskeleton, induction of G2/M cell cycle arrest, and interaction with DEAD-box proteins to name a few. Expression of the E1^E4 protein occurs in tissue layers concomitant with viral genome amplification, late gene expression and virion morphogenesis. E1^E4 is believed to have multiple roles in the HPV life cycle, such as a role in the regulation of viral genome amplification, late gene expression, and virus maturation. A potential role for regulating viral genome amplification has been demonstrated by creating E1^E4 null mutations in the Cottontail Rabbit Papillomavirus (CRPV), HPV16, HPV18, and HPV31, where loss of E1^E4 resulted in the lack of genome amplification. Contrary to these studies, a similar mutation in HPV11 did not have any effect on viral genome amplification upon host tissue differentiation. To date no one has tested directly the role of E1^E4 in the production of infectious viral particles. Using a HPV18 E1^E4 null virus we investigated whether or not this mutant was still able to produce infectious virus. In organotypic culture this mutant was unable to amplify its genome upon host tissue differentiation. Putative viral stocks were prepared and infectivity assayed using quantitative real-time PCR (QTPCR). Results demonstrated that the HPV18 E1^E4 null mutant was able to produce infectious virus although less efficiently than wild-type. We are in the process of measuring the integrity of this mutant virus and analyzing other HPV18 E1^E4 mutants.

P-12.14 CAPSID SURFACE CHARGE AS A POTENTIAL DETERMINANT OF HPV TROPISM

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Papillomaviruses can roughly be divided into two tropism groups, those infecting the skin, including the genus beta PVs, and those infecting the mucosa, predominantly genus alpha PVs. The L1 capsid protein determines the phylogenetic separation between beta types and alpha types and the L1 protein is most probably responsible for the first interaction with the cell surface. Virus entry is a known determinant for tissue tropism and to study if interactions of the viral capsid with the cell surface could affect HPV tropism, the net surface charge of the HPV L1 capsid proteins was analyzed and HPV-16 (alpha) and HPV-5 (beta) with a mucosal and cutaneous tropism respectively were used to study heparin inhibition of uptake. The negatively charged L1 proteins were all found among HPVs with cutaneous tropism from the beta- and gamma-PV genus, while all alpha HPVs were positively charged at pH 7.4. The linear sequence of the HPV-5 L1 capsid protein had a predicted isoelectric point (pI) of 6.59 and a charge of -2.74 at pH 7.4, while HPV-16 had a pI of 7.95 with a charge of +2.98, suggesting no interaction between HPV-5 and the highly negative charged heparin. Furthermore, 3D-modelling indicated that HPV-5 L1 exposed more negatively charged amino acids than HPV-16. Uptake of HPV-5 (beta) and HPV-16 (alpha) was studied in vitro by using a pseudovirus (PsV) assay. Uptake of HPV-5 PsV was not inhibited by heparin in C33A cells and only minor inhibition was detected in HaCaT cells. HPV-16 PsV uptake was significantly more inhibited by heparin in both cells and completely blocked in C33A cells.

SESSION 13

HUMORAL IMMUNITY, BASIC SCIENCES

PROGRAMME

Oral presentations

THE 25TH INTERNATIONAL PAPILLOMAVIRUS CONFERENCE MAY 8-14 2009, MALMÖ, SWEDEN

TIME	NUMBER	TITLE	ROOM
2009-05-12			
SESSION 13: HUMORAL IMMUNITY			SCANIA
16.00-16.30	O-13.00	L2 MINOR CAPSID PROTEIN-BIOLOGY AND VACCINES R Roden	
16.30-16.40	O-13.01	SEROLOGIC RESPONSE TO EXPERIMENTAL TRANSMISSION OF MFPV-3 IN FEMALE MACAQUES RD Burk, Z Chen, Y Studentsov, C Wood	
16.40-16.50	O-13.02	MULTIPLEX HUMAN PAPILLOMAVIRUS SEROLOGY BASED ON HEPARIN COATING AND VLPS H Faust, P Knekt, J Dillner	
16.50-17.00	O-13.03	SEROEPIDEMIOLOGY OF HUMAN PAPILLOMAVIRUS 11, 16 AND 18 IN MEN B Lu, R Viscidi, Y Wu, E Lazcano - Ponce, L Villa, D Smith, M Papenfuss, M Abrahamsen, A Giuliano	
17.00-17.10	O-13.04	DETERMINANTS OF SEROCONVERSION AMONG HPV-16/18 DNA POSITIVE YOUNG WOMEN C Porras, C Bennett, M Safaeian, A Hildesheim, A Rodriguez, P Gonzalez, S Wacholder, D Solomon, Lj Van Doorn, C Bougelet, W Quint, R Herrero, M Schiffman, for the CVT Group	
17.10-17.20	O-13.05	HPV SEROLOGY IN WOMEN FOLLOWED-UP FOR 36 MONTHS POST PARTUM S Syrjänen, T Waterboer, M Sarkola, Km Michael, M Rintala, K Syrjänen, S Grenman, M Pawlita	
17.20-17.30	O-13.06	IGA ANTIBODIES AND VIRAL LOAD IN HPV INFECTED WOMEN. A L Combita Rojas, M Molano, P Coursaget, A Touzé, N Muñoz, Df Duarte	

L2 MINOR CAPSID PROTEIN-BIOLOGY AND VACCINES

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While L1 is sufficient to form capsids, the minor capsid antigen L2 facilitates their assembly and plays an important role in viral genome encapsidation. In addition, L2 is necessary for papillomavirus infection, although L1 mediates the initial interaction with the cell surface. How L2 contributes to the process of infection is an area of active investigation, and L2 may act by binding to critical cellular entry factors to facilitate exit from the endosomes and delivery of the viral genome to appropriate domains within the nucleus. Studies in animal papillomavirus models have indicated that L2 is also a protective antigen. These pioneering studies demonstrated that immunization of cows or rabbits with L2 polypeptides produced in bacteria protected from experimental papillomavirus challenge at both mucosal and cutaneous sites respectively. L2-induced immunity appears to be broad and mediated by cross-neutralizing antibodies.

Why should second generation approaches based on L2 be considered since two preventive vaccines comprising recombinant HPV L1 virus-like particles (VLPs) have been licensed? Firstly, the current cost of the licensed vaccines precludes sustained global delivery to those most in need. Secondly, they target only two of the ~15 known oncogenic HPV types, although ~70% of cervical cancer cases are attributed to these two types and there is evidence for some degree of cross-protection against other closely related types. A possible approach to broader immunity at lower cost is to consider vaccination against L2. L2 vaccines can potentially be produced inexpensively as a single antigen in bacteria and they also have the promise of conferring much broader cross-type protective immunity than observed with L1 VLP immunization. However, L2 vaccine development lags behind L1 VLP vaccines and several technical hurdles remain, most notably the lower immunogenicity of linear L2 candidate vaccines in comparison with L1 virus-like particles.

O-13.01

SEROLOGIC RESPONSE TO EXPERIMENTAL TRANSMISSION OF MFPV-3 IN FEMALE MACAQUES

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INTRODUCTION: Female macaques are the only nonhuman species in which naturally occurring PV-associated cervical neoplasia has been described. Survey of a group of cynomolgus macaques (Macaca fascicularis) revealed the presence of a highly prevalent PV (MfPV-3) associated with CIN3. MfPV-3 (RhPV-d) is phylogenetically related to the alpha9/11 (HPV16) HPVs. We previously reported the experimental transmission of oncogenic MfPV-3 in a group of experimentally challenged animals. The sequential time course of antibody response and switching, to cervical PV infection has not been characterized.

METHODS: To characterize the IgM, IgG and IgA humoral response to experimental MfPV-3 infection, virus-like particles (VLPs) using the L1 ORF of MfPV-3 were generated. MfPV-3 VLPs were structurally similar to VLPs from oncogenic human PVs by electron microscopy. Natural MfPV-3 seroprevalence in a subset of animals was found to be 35.1% (53/131). A group of sero-/MfPV-3 DNA- female cynomolgus macaques were experimentally challenged with infectious MfPV-3 material. Sera was obtained from 1 - 4 wks over the course of experimental transmission for over one year, allowing unprecedented accuracy in establishing time of events and humoral response.

RESULTS: Experimental inoculations resulted in 5/10 new infections in sero-/DNA- animals. Four animals showed development of IgG to MfPV-3 VLPs. One animal showed a sequential IgM, IgG and IgA response over the course of 2 years. One animal did not mount a humoral response. Cervical cytological abnormalities were detected after experimental infection.

CONCLUSION: Female cynomolgus macaques are a robust model of cervical PV infection, humoral response and pathogenesis.

MULTIPLEX HUMAN PAPILLOMAVIRUS SEROLOGY BASED ON HEPARIN COATING AND VLPS

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Background: Multiplexing of antigens attached to fluorescent beads enables high throughput serology. HPV serology requires conformationally intact VLPs. Heparin binds intact VLPs.

Objectives: To establish a high quality, multiplexed method for HPV serology using native VLPs produced in mammalian cells. To assess the relative risk of future cervical cancer in relation to serology for multiple HPV types.

Methods: We produced VLPs based on transfection of the mammalian cell line 293TT with codon-modified HPV capsid genes L1 and L2 (Buck, C.B. et al, J.Vir 78,751-7,2004) and selected for native VLPs using heparin-coated fluorescent beads. VLPs from 8 sexually transmitted HPV types (HPV6, 11, 16, 18, 31, 45, 52, 58) and 2 cutaneous HPV types (HPV5 and 38) were produced. Following validation using positive and negative control serum panels, our mammalian VLP multiplex method was used to determine the HPV-serology-associated relative risk for cervical cancer using a prospective study of 18814 women followed for 23 years (Lehtinen M. et al, BMJ 312, 537-9, 1996). 71 women who developed cervical cancer and 143 matched controls were studied.

Results and Conclusions: The multiplexing method gave similar results as the previous VLP-based ELISA method. HPV16 seropositivity had the highest risk for cervical cancer (OR=7.7, CI95%=2.6-23). HPV31 was also associated with increased risk ((OR=4.1, CI95%=1.6-10.8). The other sexually transmitted HPV types had non-significant tendencies to associate with cervical cancer risk, whereas the cutaneous types had no tendency to associate with cervical cancer. In summary, multiplexed HPV serology using mammalian-derived VLPs selected for native conformation finds the expected HPV-type-specific risks for cervical cancer.

O-13.03

SEROEPIDEMIOLOGY OF HUMAN PAPILLOMAVIRUS 11, 16 AND 18 IN MEN

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Background: Information on vaccine-type HPV seroprevalence is essential for understanding the role of host immunity in the natural history of HPV infection as well as for vaccine dissemination. Evaluation of seroprevalence of vaccine-type HPV and its determinants has been scarce, especially in men.

Objectives: The goal of this study was to examine the baseline serum antibody IgG to HPV-11, -16 and -18 virus-like particles (VLPs) in 1477 men aged 18-70 years residing in the United States, Brazil and Mexico, and to investigate factors associated with seropositivity to individual HPV.

Methods: Serum antibodies to HPV-11, -16 and -18 were measured by the use of VLP-based enzyme-linked immunosorbent assay. Unconditional logistic regression was performed to assess factors associated with seropositivity.

Results: The HPV-11, -16 and -18 seroprevalence was 22.7%, 6.1% and 3.2%, respectively. Peak age-specific seroprevalence was observed among those aged 46-55 years for HPV-11 (30.9%), and 36-45 years for HPV-16 (10.2%) and HPV-18 (5.0%). Brazilian and Mexican participants were more likely to be seropositive for HPV-11 (OR 2.6, 95% CI: 1.7-3.8 and OR 2.5, 95% CI: 1.7-3.7, respectively) than the U.S. participants after the adjustment for age, lifetime sexual partners and condom use. Age, circumcision status and sexual orientation were significantly associated with HPV-16 seropositivity in men, with a significant interaction detected between circumcision status and sexual orientation. Uncircumcised men who had sex with men only or both men and women were 3-4 times more likely to be HPV-16 seropositive as compared to uncircumcised heterosexual men. Sexual orientation was the only significant determinant for HPV-18 seroprevalence independent of age.

Conclusion: Our results suggested that in addition to age, the key determinants of seroprevalence in this cohort of men are different aspects of sexual behavior.

DETERMINANTS OF SEROCONVERSION AMONG HPV-16/18 DNA POSITIVE YOUNG WOMEN

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Background: Not all women infected with HPV-16/18 develop detectable levels of HPV-16/18 antibodies, those who seroconvert develop low antibody levels, and seroconversion typically occurs several months post-infection. Little is known about the predictors of serological response to HPV-16/18 among women infected with HPV-16/18.

Objectives: To investigate determinants of seropositivity among HPV-16/18 DNA positive women 18-25 years.

Methods: Data and specimens collected at enrollment (pre-vaccination) phase of the 7,466 women NCI-sponsored Costa Rica HPV Vaccine Trial (CVT) were analyzed. Serum specimens obtained from women were tested for antibodies against HPV-16/18 L1 VLPs by ELISA. Cervical specimens collected from sexually active women were tested for HPV DNA using the HC2 test, SPF10PCR/DEIA/LiPA25 system version 1, and type-specific PCR primers for HPV-16/18. Analyses were restricted to the 455 (HPV-16) and 172 (HPV-18) women positive for HPV-16/18 DNA. ORs and 95% CIs were computed using multivariate logistic models.

Results: Among HPV-16 DNA positive and HPV-18 DNA positive women, seropositivity was 63.7% and 57.0%, respectively. Among HPV-16 DNA positive women, antibody detection was associated with increasing time since initiation of sexual activity with the most recent partner, a proxy for time since likely exposure to HPV (OR=1.9 for 13+months versus <4 months; 95% CI: 1.1-3.2). HPV-16 antibody detection was also associated with high viral load detected by HC2 (OR=1.7=; 95% CI: 1.1-2.7 for >28 rlu/co versus <28), current hormonal contraceptive use (OR=1.9; 95% CI: 1.1-3.3 versus never users) and current use of a condom (OR= 0.5; 95% CI:0.3-0.9 versus never users). Less consistent patterns were observed for HPV-18.

Conclusions: Among women positive for HPV-16 DNA, markers of time since exposure and viral burden were associated with seroconversion. Patterns were less consistent for HPV-18. These findings add to our insight on mechanisms involved in antibody production following HPV infection.

O-13.05

HPV SEROLOGY IN WOMEN FOLLOWED-UP FOR 36 MONTHS POST PARTUM

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Background: Most serological studies on women with genital HPV infections have been cross-sectional in design which precludes the analysis of seroconversion or antibody decay over time.

Objectives: The aim of the present study is to describe HPV seroprevalence, seroconversion and antibody decay among 290 women followed-up for three years after delivery.

Methods: L1 antibodies for HPV types 6, 11, 16, 18 and 45 were determined by multiplex serology from sera sampled at base line, and at 12-, 24- and 36 months. Time-dependent generalized estimating equation was used to explore predictors of seroconversion to either LR- or HR-HPV types in univariate and multivariate mode.

Results: At baseline, seroprevalence of HPV 6, 11, 16, 18 and 45 was 53.3%, 21.5%, 34.9%, 21.5% and 9.0%, respectively. Seropositivity to low-risk HPV (LR-HPV) was significantly associated with age at onset of sexual activity (p=0.001), number of sexual partners until age 20 (p=0.018), life-time number of sexual partners (p=0.0001), history of genital warts (p=0.0001) and being seropositive to HR-HPV (p=0.0001). The same covariates predicted also seropositivity to high-risk HPV (HR-HPV). During follow-up, 26.7%, 13.9%, 17.0%, 16.8% and 6.6% of the women seroconverted to L1 antigen of HPV 6, 11, 16, 18 and 45 between 18.2 to 23.8 months. Independent predictors of seroconversion to LR-HPV were unemployment (p=0.019) and absence of anal sex practice (p=0.031), and to HR-HPV, absence of smoking history and life-time number of sexual partners. Decay of HPV 6, 11, 16, 18, and 45 antibodies was observed in 2.3%, 4.0%, 5.3%, 4.5% and 1.5% of the women, respectively, times of decay varying from 27.2 to 35.8 months.

Conclusions: These data implicate that i) substantial proportion of young women are seropositive to both LR- and HR-HPV types, ii) they frequently undergo seroconversion within 18-24 months, predicted by common covariates, whereas iii) antibody decay is rare.

IGA ANTIBODIES AND VIRAL LOAD IN HPV INFECTED WOMEN.

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Background: In a previous study in women with normal cytology from Bogotá it was observed an increase of HPV16 DNA prevalence in older women. This second peak could be due to HPV re-infection, re-activation of a latent infection or presence of new infection. It has been postulated that an increase in IgA with increasing age could be due to persistent HPV infection. However, there are not conclusive data of IgA role and its association with the HPV infection.

Objectives: In the present study we analyzed the systemic immunological IgA response in 1508 women with normal cytology from the general population of Bogotà, Colombia, in order to evaluated the relationship between IgA antibodies prevalence and host or viral cofactors.

Methods: IgA antibodies were detected by ELISA using HPV16, 18, 31 and 58 VLPs. HPV DNA was analysed by GP5+/GP6+PCR-EIA and typing was done using a reverse line blot assay. Viral load was determined by EIA

Results: A significant association between IgA seroconversion with increasing age was observed for types 16, 31 and 58. However, detection of HPV16 IgA was only significantly associated with high viral load. A multivariate assessment adjusted by age, social status, smoking, number of regular partners, oral contraceptives use, specific HPV infection and viral load shown an increase in IgA seropositivity in women \geq 35 years old. In addition, IgA detection was significantly associated with HPV16 DNA and high viral load.

Conclusions: our data demonstrate an increase in HPV 16 IgA detection with increasing age. This is significantly associated with HPV16 DNA detection and high viral load among women aged 35 or more, suggesting that the second peak of infection is probably due to re-infection or new HPV infection.

POSTER ABSTRACTS SESSION 13

POSTER SESSION I MONDAY 10.00: ODD NUMBERS

POSTER SESSION II

TUESDAY 10.00: EVEN NUMBERS

P-13.07 COMPARISON OF MULTIPLEX ASSAYS TO ASSESS NATURALLY ACQUIRED HPV ANTIBODIES.

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Background and objectives: Several multiplex immunoassays (MIA) are available to assess the prevalence of HPV antibodies in human sera. At present, in HPV vaccine trials different assays are used to monitor the vaccine effects. This will hamper the comparison of immunological responses induced by the two available vaccines. Comparison of techniques with quality serum panels will help to standardize HPV serology. The assignment of an international reference for HPV type 16 is a first step towards standardization. In March 2009, HPV vaccination will be implemented in the Netherlands in the national immunization program. The Dutch Public Health Institute will perform serological surveillance in the prevaccination era using a serumbank of a cross-sectional sample of the Dutch population collected in 2006/2007 (Pienter-2) and in the postvaccination era.

Methods: The presence of HPV specific antibodies will be determined in a subset of 600 sera of women between 11-26 years of age from Pienter-2. These samples will be analyzed with different HPV antibody assays. The first MIA is developed by Opalka et al (Merck USA). It is an inhibition assay with specific monoclonal antibodies for HPV type 6, 11, 16 and 18 using virus like particles bound to microspheres. A second available MIA is based upon direct recognition of serum antibodies against GST-fused HPV-L1 proteins developed by Waterboer et al (DKFZ, Germany).

Results and discussion: The results from this subset of sera obtained with both MIAs will be presented. This study will contribute to the standardization of serological assays determining HPV antibodies. Furthermore, it will contribute to the discussion if these assays are able to measure antibodies derived after natural infection adequately. For future research, it will be important to study the quality of the antibody responses in more detail.

P-13.08

DETECTION OF HPV58 NEUTRALIZING ANTIBODIES IN AN ENDEMIC HPV AREA.

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Background: After HPV 16, HPV 58 is the second most prevalent viral type in women with normal cytology in Colombia and Latin America and it is also detected in the majority of co-infections with HPV 16. The detection of anti-HPV 58 antibodies by a specific test should confirm the high rate of HPV 58 DNA detection observed. Moreover, as this viral type is not included in currently available HPV vaccines, it would be important to determine whether the immune response to HPV 16 thus generated can also induce cross reactivity to HPV 58.

Objectives: The aim of this study was to develop a highly sensitive neutralization assay for human papillomavirus type 58 and to investigate HPV58 antibodies both after natural infection and after immunization.

Methods: HPV58 antibodies were investigated in a selected population of 320 Colombian women. All sera were tested for anti-HPV 16, 18, 31 and 58 antibodies by ELISA and also for the presence of HPV-58 neutralizing antibodies using HPV58 pseudovirions. Recombinant HPV58 pseudovirions were generated by intracellular encapsidation of a luciferase reporter gene in virus-like particles obtained with codon-optimized L1 and L2 protein coding sequences.

Results: Detection of HPV58 neutralizing antibodies was correlated with the number of sexual partners, and an increased proportion of positive women was observed with advancing age. However, poor correlation was found between ELISA and the neutralization tests in the detection of anti-HPV58 antibodies. Preliminary findings in HPV vaccine recipients also indicated that immunization with the quadrivalent vaccine induced low levels of neutralizing antibodies to HPV58 in some subjects.

Conclusions: The results obtained confirmed the high proportion of HPV58 infections in Colombia. Evaluation of neutralizing antibodies can be of both clinical and epidemiological importance and a means of monitoring vaccinated subjects.

A PLANT PRODUCED PAPILLOMAVIRUS L2 VACCINE PROTECTS DOGS AGAINST COPV

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Background: The minor capsid protein (L2) of human papillomavirus (HPV) contains epitopes that can induce antibodies with cross-neutralizing activity. The recently-licensed HPV vaccines are expensive, and hence might not be available for use in developing countries where the burden of cervical cancer is highest. Additionally, the L1-based virus-like particle vaccines do not induce strong cross-protective immune responses. An inexpensive L2-based monovalent vaccine could reduce the global incidence of HPV-linked cancers.

Objectives: The present study aimed to determine whether a COPV L2 vaccine could induce protective immune responses in the canine oral papillomavirus (COPV) model.

Methods: Two overlapping domains, COPV L261-170 and COPV L25-260, were fused to the streptavidin (SA) protein. The vaccines were manufactured in Nicotiana benthamiana using a tobacco mosaic virus (TMV)-based gene expression system, purified and used as immunogens in beagle dogs. Animals were vaccinated three times, at two week intervals. Study endpoints included serology; analysis of COPV neutralizing titers in a pseudovirus-based neutralization assay; and development of oral papillomas after challenge with a high titer stock of infectious COPV.

Results: All vaccinated animals produced antibodies to L2 and the SA carrier protein. The COPV L25-260 vaccine induced good levels of neutralizing antibodies and protected all 5 vaccinated animals against challenge with COPV, while the COPV L261-170 vaccine induced protective immunity in 4 out of 7 vaccinated animals, with the remaining 3 partially protected. The degree of protection against challenge in this cohort was correlated with L2-reactive antibody titers. Two L1 VLP-vaccinated animals were protected from challenge, and two mock-vaccinated animals developed large oral warts.

Conclusions: These data provide strong proof of concept that a plant-produced L2 vaccine can protect against mucosal papillomavirus challenge in a relevant animal model.

P-13.10

MULTITYPE L2 PROTEIN VACCINATION WITH L1 CAPSOMERS OR VLPS

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BACKGROUND: A low cost HPV vaccine that yields cross-protective antibodies and broad protection against more than a dozen oncogenic HPV types is urgently needed. Immunization with L1 capsomers or VLPs generates high and longlived titers of neutralizing antibodies and protection primarily against the papillomavirus type from which the vaccine was derived. Consequently, multivalent L1 VLP vaccines have been developed to expand the breadth of coverage, e.g. Cervarix which contains L1 VLPs that are derived from HPV16 and HPV18 and formulated in alum and monophosphoryl lipid A (MPL) adjuvant. Vaccination with L2 induces lower titer, but more broadly neutralizing and protective antibody responses in animals. To enhance cross-protection we generated multi-type L2 proteins by fusing cross-neutralizing epitopes of divergent HPV types, e.g. 11-88x5 that is a concatamer of L2 residues 11-88 of HPV1, HPV5, HPV6, HPV16 and HPV18. OBJECTIVES: We sought to combine the advantages of each protective antigen by immunization with titrated doses of multi-type L2 11-88x5 in alum+MPL, either alone or with either HPV16 L1 capsomers or Cervarix. METHODS: Mice were vaccinated three times every alternate week and bled two weeks later. Serum antibody titers were determined by ELISA using HPV16 L1 VLP or L2 11-88x5 antigen. RESULTS: The absolute titers and dose response to 11-88x5 were similar in mice vaccinated with 11-88x5 alone or in combination with either L1 capsomeres or VLPs. Importantly, the serum IgG titers specific to HPV16 L1 VLP were not influenced by vaccination in the presence of L2 11-88x5. CONCLUSIONS: We did not find evidence of interference between the L1 and L2 serum antibody response to co-administration of L1 and multi-type L2 vaccines based on ELISA titers, and further analysis of neutralization titers is ongoing.

INDUCTION OF CROSS-NEUTRALIZING ANTIBODIES WITH PSEUDOVIRIONS PACKAGED WITH L2 GENE.

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Background: Current vaccines against HPVs are constituted from L1 protein self-assembled into virus-like particles (VLPs) and they have been shown to protect against natural HPV16 and HPV18 infection and associated lesions. Limited protection against related types (HPV31 and HPV45) has also been reported. Vaccination with L2 protein in animal models has been shown to provide cross-protection against distant papillomavirus types, suggesting that the L2 protein contains cross-neutralizing epitopes. However, vaccination with VLPs composed of L1 and L2 protein or recombinant L2 peptides does not induce high titres of anti-L2 antibodies.

Objectives: In order to develop a vaccine with the potential to protect against all high-risk HPV types, we generated two different chimeric particles based on either the L2 proteins linked outside the HPV16 L1 capsid or a HPV16 L2 peptide (13-88) inserted within the DE loop of the HPV31 L1 protein. HPV58 pseudovirions encoding the HPV16 L2 gene were also produced.

Methods: Levels of cross-neutralizing antibodies against HPV18, HPV31 and HPV58, induced in Balb/c mice after immunization with these three L2 vaccines were compared to the levels of cross-neutralization induced by immunization with L2 protein alone, or with L2 gene or HPV31 L1L2 VLPs or HPV58 pseudovirions encoding GFP.

Results: L2 gene, L2 protein alone and the two chimeric particles were unable to induce cross-neutralizing antibodies. Low levels of cross neutralization were observed in mice immunized with L1L2 VLPs, and the highest levels of cross neutralizing antibodies were observed in mice immunized with pseudovirions encoding the HPV16 L2 gene.

Conclusions: The results obtained confirmed that L2 protein is less immunogenic than L1 VLPs and indicate that high levels of cross-neutralizing antibodies are only produced after immunization with pseudovirions encoding the L2 protein.

P-13.12

GENERATION OF A VACCINE AGAINST CUTANEOUS PAPILLOMAVIRUSES

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Human papillomavirus (HPV)-induced skin warts represent a major burden for immunosuppressed patients, such as organ transplant recipients. We aim to generate an L1-based prophylactic vaccine to prevent infections by the alpha-HPV types 2, 27, and 57, the most frequent causative agents of skin warts in immunocompromised patients.

An improved protocol for the efficient recombinant production of virus-like particles (VLPs) in insect cells has been developed. As determined in preclinical studies, high titers of cross-reactive L1-specific antibodies are triggered upon immunization with HPV 2, 27, and 57 VLPs. However, pseudovirion-based assays demonstrated that the antibodies generated by immunized mice were type-restricted in their neutralizing capacity. These findings are supported by a comprehensive analysis of 94 monoclonal antibodies (MAbs) raised against the same antigens. Even though crossreactive and type-specific MAbs were generated, only some of the typerestricted MAbs are neutralizing. Significant levels of neutralizing antibodies against all three HPV types could be achieved by immunization with a trivalent VLP pool.

Our findings suggest that L1 VLP-based vaccination may constitute a promising strategy to prevent infections by cutaneous HPV types. Clinical studies will be conducted to explore this prospect.

EX-VIVO MONITORING OF MEMORY T-CELLS FOUR YEARS AFTER HPV VACCINATION

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OBJECTIVES: The virus-like-particle (VLP)-based quadrivalent AAHS-adjuvated vaccine Gardasil and bivalent AS04adjuvated vaccine Cervarix provide prophylactic protection against infections with the human papilloma virus types 6/11/16/18 and 16/18 respectively. Serological data form large phase II/III clinical trials showed sustained antibody titers, above those following natural infection. Cellular immune responses, in particular T helper cell responses are important for induction and maintenance of humoral responses. We conducted a non-randomized, cross-sectional study including subjects, immunized with either Gardasil or Cervarix four years prior to enrolment, to assess cellular immune responses to both vaccines. We monitored HPV antigen-specific CD4 and CD8 T cells from whole blood and peripheral blood mononuclear cell (PBMC)-suspension.

METHODS: One ml of blood and one ml of PBMC-suspension were stimulated with different HPV-L1 and HPV-E6/E7 peptide pools for 16 hours or 10 days. PBMC-suspensions were restimulated on day 10 with respective peptide pools. Antigen specific memory CD4 and CD8 T cells were identified by intracellular staining for CD4/CD154/IL-2/IL-4/IFN- γ or CD8/CD137/IL-2/IFN- γ and analysed by flow cytometry. Blood plasma was analysed for the presence of antibodies to HPV- proteins by multiplex HPV serology.

RESULTS and CONCLUSIONS: Four years after vaccination with either Gardasil or Cervarix, vaccine-type specific memory CD4 and CD8 T cells can be detected ex vivo or after restimulation in all vaccinees. T cell frequencies do not differ markedly with both vaccines. HPV vaccines induce long lasting memory T cell responses that may support sustained antibody concentrations and may be important for boosting if necessary.

P-13.14

NANOPATCH SKIN IMMUNE CELL TARGETING FOR IMPROVED CERVICAL CANCER VACCINES.

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Background: Four out of five cervical cancer deaths occur in developing nations where cervical cancer screening programs and treatments are rare or largely unavailable. Also, at \$120 per injection, current HPV vaccines are prohibitively expensive for wide-scale vaccination inaccessible in many countries. Indeed, it has been projected that in countries with a gross income of under \$1000 per capita, this vaccine may have to cost less than \$2 per dose to be economically viable.

One way to tackle this problem is with practical needle-free delivery technologies, achieving protection with a small fraction of the vaccine dose. Here we introduce single-use dry coated micro-nanoprojection arrays ("Nanopatches") as a viable candidate to meet this need. With thousands of tiny projections invisible to the human eye, the Nanopatch targets vaccines to skin immune cells making delivery much more efficient than intramuscular injection. This can produce: an immune response comparable to traditional delivery routes but with significant dose reduction; or achieve a higher response with the same dose depending on the antigen, thus reducing vaccine cost.

Objectives: To demonstrate effective immune response in C57BL/6 mice via Nanopatch inoculation against HPV virus like particles (VLPs) and demonstrate dose reduction as compared with subcutaneous injection.

Methods: We dry-coat existing HPV-VLP vaccines to the Nanopatch projections using novel protocols. Using these Nanopatches, we inoculated mice (n=4) and compared immune responses with control groups of higher-dose control needle and syringe subcutaneous injections. Sera were collected and analyzed for virus neutralizing antibodies using the colorimetric neutralization assay method.

Results: Nanopatches were successfully dry-coated with HPV-VLP, and innoculation with Nanopatches elicits a neutralizing antibody response. Dose reduction results will be presented.

Conclusions: Nanopatches can elicit neutralizing antibody responses against HPV in C57BL/6 mice, and show promise as an alternative delivery platform in prophylactic cervical cancer vaccination in women.

EX VIVO ANALYSIS OF MEMORY CD4 T-CELLS AFTER GARDASIL VACCINATION

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Objectives: The virus-like particle (VLP)-based quadrivalent vaccine Gardasil provides prophylactic protection against infections with the human papillomavirus types 6, 11, 16 and 18. Since September 2006 it is available on the European market and was widely accepted.

Serological data from the large phase II/III clinical trials describe antibody levels above those of natural infections, though antibody concentrations decrease in the first years after vaccination. T helper responses in particular are important for memory, recall, and anamnestic responses. Only very few reports on cellular immune responses to the prophylactic HPV vaccine are available to date. We present data from a cross sectional study on 63 subjects distinguished by their vaccination status as well as the results of a confirmatory longitudinal study following up 10 young women during the course of vaccination. We applied a feasible ex vivo test to monitor HPV antigen-specific CD4 T cells from whole blood.

Methods: One ml of blood was stimulated with different HPV-L1 peptide pools for 16 hours. Antigen specific memory CD4 T cells were identified by intracellular staining for CD4, CD154, IL-2, and IFN- γ and analysed by flow cytometry. Blood plasma was analysed for the presence of antibodies to capsid protein L1 of a broad variety of HPV types by multiplexed human papillomavirus serology based on in situ-purified glutathione S-transferase fusion proteins.

Conclusions: Memory T Helper cell responses can be detected already after the first injection, increase after the second, but not after the third vaccination. Low-risk HPV VLP induced higher specific T cell frequencies than high-risk HPV VLP. Both the cross sectional and the longitudinal study showed consistent results.

P-13.16

AGE-SPECIFIC PREVALENCE OF HUMAN PAPILLOMAVIRUS DNA AND ANTIBODY: SYSTEMATIC REVIEW

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Background: Global data on human papillomavirus (HPV) DNA infection and serum-antibody positivity are essential to understanding exposure to HPV. To date, no review on the simultaneous presence of HPV DNA and serum antibodies has been reported.

Methods: We conducted a systematic review from Medline or cited sources using search terms of human papillomavirus, DNA, and serology or antibody through August 2008. Studies with cross-sectional prevalence data of both HPV DNA and antibody responses in population-based, representative clinical samples, or control patients of a case-control study were included. Studies were required to provide data on age among women.

Results: Of 35 abstracts identified, 15 studies met eligibility criteria. Most included studies were from Europe (40%), North America (26.7%) and Asia (20%). The mean age of study participants ranged from 16.1 to 45 years. All included studies were low-risk populations (e.g. HIV-negative, non-homosexual or non-commercial sex workers). HPV infection was ascertained by PCR techniques in all but 3 studies (80%). Serological antibodies were detected by ELISA in most studies (80%), followed by detection with the GST Capture Assay or competitive Luminex Assay. Most serological data were based on immunoglobin type IgG (73.3%), although roughly one quarter of studies (26.7%) presented data on IgG in combination with IgA or IgM. For HPV 16 and 18, age-stratified DNA prevalence peaked around 20 and generally declined with age, while the peak in 16/18 seroprevalence was consistently later at 30 to 45 years of age. The overall range of positivity of DNA and/or antibody was 6.7-28.2% for HPV 16, and 9.3-21.7% for HPV 18.

Discussion: Prevalence of HPV DNA and serum antibodies varied by age, HPV type, and geographical region. Further data are needed on the cumulative exposure of type-specific HPV infection over a woman's lifetime.

MULTI-VALENT VLPS VACCINES CONTAINING HPV 58 INDUCE LONG-TERM HUMORAL IMMUNITY

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Background: Both Human Papillomavirus (HPV) type 16/18 and 16/18/6/11 VLP-based vaccines have been licensed for public use. However, these two vaccines do not quite match the HPV type distribution in China, Southeast Asia and Latin America, where HPV 58 is more prevalent. Thus it is important to develop vaccines which cover HPV type 58. Methods: Mice were immunized intramuscularly with pentavalent (HPV type 16/18/58/6/11 VLPs), trivalent (HPV type 16/18/58 VLPs), bivalent (HPV type 6/11 VLPs), and five types of monovalent vaccines (HPV type 16, 18, 58, 6, 11 VLPs)

individually). The HPV specific antibodies were detected by ELISA and pseudovirus neutralization assay. Results: The ELISA titers of VLP-specific serum IgG induced by multi-valent vaccines were as high as those induced by corresponding monovalent vaccines, and could persist for at least one year. The neutralizing antibody-inducing ability of HPV 18 VLPs was not influenced when co-administrated with other VLPs and all the sera from pentavalent, trivalent and type 18 monovalent groups showed neutralization of HPV 18 pseudoviruses even at a dilution of 1:10000. However, the neutralizing antibodies induced by HPV 16 and HPV 58 VLPs were interfered significantly. For HPV 16, the percentage of mice sera that showed neutralization at a dilution of 1:10000 was 75%, 50% and 25% in monovalent, trivalent and pentavalent groups respectively, and all increased to 100% at a dilution of 1:2000. For HPV 58, the percentage was 100%, 25% and 0% in monovalent, trivalent and pentavalent groups at a dilution of 1:10000, and increased to 100%, 100% and 75% at a dilution of 1:2000 respectively.

Conclusions: The neutralizing antibody-inducing ability of HPV 58 VLPs is the weaker, and the ability could be further weakened when mixed with other types of HPV VLPs. The results are of great value for development of multi-valent VLP vaccines containing HPV 58.

P-13.18

PRODUCTION OF HPV16 L1 VLP WITH A MODIFIED L1 GENE

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Background: Virus-like Particle (VLP) consisting of HPV capsid protein L1, has been reported to induce significant humoral immunity and provide protection against virus infections and infection related diseases.

Objective: To enhance HPV 16 L1 protein expression level for production of HPV 16 L1 VLP and study its prophylactic efficacy.

Methods: A modified HPV 16 L1 gene, named HPV 16 L1M C, was generated by combination of codon optimization, optimization of the corresponding mRNA secondary structure, and deletion of 35 amino acids at the carboxy-terminal of L1 proteins. The expression level of the modified L1 gene in baculovirus expression system was analyzed by Western blot, and the purified recombinant L1 proteins were analyzed by transmission electron microscopy. The prophylactic study of the modified HPV 16 L1 VLP vaccine was performed in Balb/c mice by immunization intramuscularly twice with a two-week interval.

Results: The expression level of HPV 16 L1M C in sf-9 cells is about 1.77 fold as high as that of HPV 16 L1 wild type. The L1 proteins coded by the modified L1gene could self-assemble into VLPs with a diameter approximately 50 nm. The titers of sera antibodies from modified HPV 16 L1 VLP immunized mice against HPV 16 L1M C VLP were the same as that against HPV 16 L1 VLP by ELISA analysis, and hemagglutination inhibition analysis showed that the sera form the HPV 16 L1M C VLP vaccinated mice could inhibit hemagglutination of mouse erythrocytes induced by HPV 16 L1 VLP. The titers of the anti-HPV 16 VLP specific antibodies could persistent at a level above 104 for more than 3 months.

Conclusions: The modified HPV 16 L1 gene could enhance the expression level of L1 proteins. The present study may have some implications for producing HPV L1 VLP vaccine with a higher expression level.

OPTIMISATION OF EXPRESSION OF PLANT-PRODUCED PAPILLOMAVIRUS CANDIDATE VACCINES

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Background: The L1 VLP vaccines against HPV that are currently on the market remain unaffordable in developing countries where they are needed most and development of cheaper vaccines are required.

Objectives: In this study we investigated the levels of expression of L1 and L2 proteins in plants with the aim of optimising expression and developing cheaper new-generation broadly protective vaccines.

Method: We expressed the L1, L2 and L1-L2 chimaeric proteins in various plant systems by means of infectious TMVbased vectors, Agrobacterium-mediated transient expression and by transgenic expression plants.

In Agrobacterium-mediated transient expression we investigated expression by targeting the proteins to the chloroplast, endoplasmic reticulum (ER), apoplast, specific protein bodies or cytoplasm. Various codon optimisations of the corresponding genes were studied for optimal expression.

Results and Conclusions: Initial expression of wild type HPV-16 and 11 L1 genes in transgenic plants yielded ~4 μ g/kg and ~10 mg/kg of fresh plant material of protein, respectively. Expression by means of infectious TMV-based vectors yielded L1 levels of 1 mg/kg for CRPV, 20-37 μ g/kg HPV16 L1 and 10 mg/kg of HPV-11. HPV-16 L1 and L2 genes were then codon optimised for expression in plants or mammalian cells. Unexpectedly codon optimisation for expression in mammalian cells increased protein yields by up to thousand fold whereas plant codon optimisation did not improve protein expression. A yield of ~533mg/kg of HPV-16 L1 was obtained when it was targeted to the chloroplast whereas L2 expressed to the same level in all compartments. We then further analysed the GC content of the L1 gene and varied GC levels from 35 – 62%. Initial results indicate that the GC content of the gene has a profound effect on protein expression in plants with optimum expression levels with 50 – 60% GC content.

P-13.20

PROPHYLACTIC AND THERAPEUTIC VACCINE AGAINST MULTIPLE HUMAN PAPILLOMAVIRUS TYPES

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Background: Cervical cancer, the most common cancer affecting women in developing countries, is caused by persistent infection with high-risk types of human papillomavirus (HPV), most notably types 16 and 18, for which two vaccines have recently come on the market. However, there is still a need for a vaccine preventing infection by other types of HPV, especially in the developing world, where HIV and co-infection with multiple types of HPV is prevalent. Secondly, there is a need for a vaccine with therapeutic potential. Thirdly, current vaccines are unaffordable to the developing world.

Objective: The objective of this project is to produce chimeric L1 prophylactic and/or therapeutic vaccine candidates, containing L2 epitopes previously shown to elicit cross-neutralising antibodies, or E7 epitopes shown to be effective in immunotherapy, in insect cells using a baculovirus expression system,

Methods: Chimeric genes were designed by replacing segments of L1 with L2 and/or E7 peptides and synthesized by Geneart. Genes were cloned into insect cell expression vectors and their presence confirmed by PCR. All inserts were sequenced. Sf21 insect cells were transfected with recombinant bacmid or baculovirus shuttle vector DNA. Expression of proteins was confirmed by Western blotting. Proteins were purified by 24% Optiprep gradient ultracentrifugation. Future work includes structural confirmation by transmission electron microscopy, large-scale expression, large-scale purification, mouse immunogenicity studies, tumour regression studies and pseudovirion neutralization assays to assess cross-neutralising abilities of the chimeras.

Conclusion: All eight chimeric proteins were successfully expressed in insect cells. To date, one of the chimeras could be purified by 24% Optiprep gradient ultracentrifugation. This method can be further optimized in order to purify all eight chimeric proteins after large-scale expression for use in further studies. Using the most promising vaccine candidate, a variety of platforms will be assessed to determine the most cost-effective means of vaccine production.

DEVELOPMENT OF NOVEL VIRAL HPV DNA VACCINE; ACHERV-HP

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Since the Human Papillomavirus (HPV) Vaccine (Gardasil*) was launched at market, the market demands its quality upgrade and cost down. We developed the novel DNA vaccine for HPVs using viral particles. Human endogenous retrovirus (HERV) enveloped chimeric Baculovirus, non-permissible to mammal, was used as a nano carrier for HPV-L1 DNA vaccine delivery (AcHERV-HP). For in vivo test, mice were intramuscularly injected with 107 particles of the constructs. Additional two times of boosts were carried out with 2 week intervals. Compared to Gadasil immunized one (25 ul/dose), the AcHERV-HP immunized group showed similarly high levels of humoral immunity not only in IgG/IgA but also in the neutralization activity against the HPV pseudovirions. Combined immune groups (prime with AcHERV-HP and boost with Gadasil) showed a little higher neutralizing activity than others. In cellular immunity, Gadasil-treated groups induced no significant immunity, whereas the AcHERV-HP treated group showed a highly strong IFN-r stimulation. Combined immune group has lower T cell immunity than AcHERV-HP treated group. Compare to Gadasil, our novel AcHERV-HP vaccine has advantages; higher immunogenicity especially in cellular immunity, much lower cost for production, and comparable safety in mammal.

P-13.22 INDUCTION OF IMMUNE RESPONSES AFTER SUBLINGUAL IMMUNIZATION OF HPV16 L1

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Although sublingual route is one of conventional routes for drug administration, the route has been little studied for administration of vaccines. Here, we tested whether the sublingual route can induce the immune responses to HPV16 L1, and if so whether the use of adjuvants can enhance the immune responses. Recombinant maltose binding protein-fused HPV16L1 was expressed from E. coli, and purified by column chromatography. Since there are increasing reports that toll-like receptor (TLR) agonists or nucleotide binding oligomerization domain (NOD) agonists may work as potential adjuvants of subunit vaccines, we compared the adjuvant capability of a TLR agonist and an NOD agonist. Imiquimod, L18-MDP or CTB was formulated with HPV16 L1 protein and administered to mice via sublingual route. Among the adjuvants tested, CTB showed the highest mucosal IgA levels in saliva and vaginal secretions than did other adjuvants tested. The systemic IgG immune responses were most effectively enhanced by sublingual CTB as compared to other adjuvants. Moreover, the levels of antibodies have been sustained for prolonged period after sublingual coadministration of CTB. Taken together, our results suggest that the sublingual route might be one of alternative route for HPV16 L1 vaccine delivery, and that the use of effective sublingual adjuvants needs to be done.

ENHANCEMENT OF THE IMMUNOGENICITY TO HPV VACCINE GARDASIL® BY CIAS

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Background: Human papillomavirus (HPV) is the causative agent of cervical cancer that is the second leading cause of death from cancer in women world-wide. A licensed HPV vaccine Gardasil[®] is a quadrivalent vaccine containing VLPs of the L1 proteins from HPV types 6, 11, 16 and 18 as immunogen and alum as adjuvant. CIA07 is an immunostimulatory agent comprised of CIA02 (bacterial DNA fragments) and CIA05 (modified LPS), and has been shown to have an antitumor activity in a mouse bladder cancer model and an adjuvant activity to viral and bacterial vaccine antigens.

Objectives: In order to determine the possibility of using CIAs combined with alum as an adjuvant for HPV vaccine, we investigated whether CIAs are capable of promoting the immune response to HPV vaccine Gardasil[®].

Methods: Balb/c mice were immunized intramuscularly twice with a 3-week interval with the vaccine alone or in combination with CIA02, CIA05 or CIA07, and the immune responses were assessed 1 and 4 weeks after the second immunization.

Results: The serum anti-HPV16 L1 IgG antibody titer was significantly higher in mice given the vaccine combined with CIA05 or CIA07 as compared with the animals given the vaccine alone. HPV pseudovirus-neutralization assays showed increased neutralization antibody titers in both groups. Coadministration of CIA05 or CIA07 with the vaccine led to a marked increase in serum IgG2a antibody titer and the number of IFN- γ + cells in splenocytes, indicating that both CIA05 and CIA07 effectively elicit Th1-type cellular immune responses as well as Th2-type antibody responses.

Conclusion: These data indicate that CIA05 and CIA07, when combined with alum, are capable of promoting the immune response to the HPV vaccine and suggest the potential as an adjuvant for the development of a potent prophylactic vaccine against HPV infection.

P-13.25

A MULTIPLEXED HPV VLP-SPECIFIC TOTAL IGG SEROLOGY ASSAY

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M Esser, Merck & Co., Inc., North Wales, USA

Background:Vaccination with a human papillomavirus (HPV) 6/11/16/18 virus-like particle (VLP) vaccine has been shown to elicit a strong neutralizing antibody response, immune memory and to provide protection through 5 years. A competitive Luminex immunoassay has been effectively used to measure vaccine-induced neutralizing antibodies.

Objectives: To more fully understand the complete antibody response to HPV virions following natural infection or vaccination with a HPV 6/11/16/18 VLP vaccine, we developed a multiplexed assay to measure total human IgG antibodies in serum to VLPs 6, 11, 16, 18 and to cross-reactive types 31, 33, 45, 52, and 58.

Methods:Antibody concentration was determined in a direct binding format, where the IgG1-4 specific, phycoerythrin (PE)-labeled monoclonal antibody (HP6043), detects human serum IgG antibodies bound to microsphere-coupled VLPs (VLP-MS). Three methods were used to evaluate serostatus cutoffs.

Results:VLP adsorption experiments showed that the assay is >99% specific for VLP specific antibodies. Ruggedness was measured as < 1.3-fold different across analyst, VLP-MS lot and HP6043 lot. The total precision across HPV types and assay conditions tested was less than 19% RSD. The overall linearity was < 7% per 10-fold dilution. Seropositivity of individuals at 48 months post-vaccination with a quadrivalent HPV vaccine ranged from 70% to 100% depending on the method used to determine the serostatus cutoff.

Conclusion:The HPV-9 total IgG assay is a sensitive, specific, rugged, precise, and dilutable assay that is fit for its intended purpose of measuring the total anti-HPV serological IgG response following vaccination or natural infection. Using 3 different methods of setting the serostatus cutoff produced broad variation in the levels of seropositivity at 48 months following vaccination. Since no serological correlate of protection has been established for HPV, disease endpoints remain the most important measure of the long-term duration of vaccine efficacy.

P-13.26 MATHEMATICAL MODELLING OF THE IMMUNE RESPONSES INDUCED BY HPV VACCINATION

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Background: Patients suffering from recurrent respiratory papillomatosis must be operated several times per year to continue breathing. To date, no therapeutic approach has succeeded in reducing the risk of relapse.

We hypothesize that, by immunizing these patients with an HPV-VLP vaccine, it would be possible to empower their immune system to block the viral life cycle once reactivation occurs.

However, defining the optimal protocol is not straightforward: i) L1 alone or with other proteins (e.g. E2)?, ii) With or without adjuvant?, iii) Combined with other therapeutic agents (e.g. artemisinin)?, iv) What immunization schedule?, v) Intramuscular (systemic) administration?.

We propose a systems biology approach (i.e. in-silico simulations) to predict the way different prophylactic/therapeutic stimuli (would) elicit different immune responses.

Objective: To develop, implement and validate a mathematical model to describe the dynamics of the cell-level interactions underneath the systemic immune responses induced by the immunization with an HPV-VLP vaccine.

Methods: The model consists of 14 delay differential equations, which describe the behaviour over time of 14 variables representing the fate of 3 cellular (APCs, helper T and B cells) and 2 molecular (HPV-VLPs, anti-HPV antibodies) entities. For being solved, the model needs to be fed with 31 parameters. Since for most of these parameters the literature does not provide sufficient data, we devised and implemented a parameter estimation procedure based on a genetic algorithm (optimization tool borrowed from the field of artificial intelligence).

Results: Our model successfully converged towards the set of clinical data (seroconversion dynamics following HPV immunization) that we used as a target throughout the optimization process. The in-silico predicted behaviour for most of the cellular entities seems plausible according to several experts' opinion.

Conclusion: Our model resulted to be an adequate means for simulating the systemic immune reactions following immunization with an HPV-VLP vaccine.

SESSION 14

VIRAL GENE EXPRESSION

PROGRAMME

Oral presentations

THE 25TH INTERNATIONAL PAPILLOMAVIRUS CONFERENCE MAY 8-14 2009, MALMÖ, SWEDEN

TIME	NUMBER	TITLE	ROOM
2009-05-12			
SESSI	K1-3		
08.30-09.05	O-14.00	PAPILLOMAVIRUS GENE EXPRESSION: PUZZLES AND CHALLENGES Z-M Zheng	
09.05-09.16	O-14.01	ESTABLISHMENT OF TRANSCRIPTION FROM INCOMING VIRAL GENOME REQUIRES L2 PROTEIN M. Sapp, L. Hilbig, M Bienkowska - Haba	
09.16-09.27	O-14.02	ASF/SF2 AND SRP30C REGULATE HPV-16 EARLY AND LATE MRNA SPLICING M Somberg, X Li, S Schwartz	
09.27-09.38	O-14.03	P300 NOT CBP ENHANCES HPV TRANSCRIPTION BY DISTINCT AP-1 COMPLEXES W-M Wang, A Lee, S-y Wu, C-M Chiang	
09.38-09.49	O-14.04	FUNCTIONAL MAPPING OF THE HUMAN PAPILLOMAVIRUS TYPE 16 E1 CISTRON TH Haugen, MJ LACE, JR Anson, LP Turek	
09.49-10.00	O-14.05	A REPORTER SYSTEM TO STUDY HVP SPLICING J.I Gonzalez - Montoya, M Guido, A Garcia - Carrancá	

O-14.00 PAPILLOMAVIRUS GENE EXPRESSION: PUZZLES AND CHALLENGES

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Papillomaviral gene expression in infected cells depends on cell differentiation and is tightly regulated at the transcriptional and post-transcriptional levels. A noteworthy feature of all papillomavirus transcripts is that they are transcribed as a bicistronic or polycistronic form containing two or more open reading frames (ORFs) and are polyadenylated at either an early or late poly (A) site. Despite that remarkable progress has been made in understanding how this complex viral gene expression is regulated at the level of transcription and particularly post-transcription, we know very little, in most cases, which transcript is used for translation of what protein (s) and how a particular protein is translated from a native bicistronic or polycistronic mRNA. In high-risk HPVs, several important ORFs, including E6, E1, and L2, bear an intron in their coding regions; splicing of the individual intron disrupts the ORF and abolish production of the full-length protein. How do these introns escape recognition while other introns in the same pre-mRNA are removed by cellular splicing machinery? More strikingly, how are these mRNAs with a retained intron efficiently exported from the nucleus to the cytoplasm before their nuclear degradation since an intron-bearing RNA is in general not exportable? To address these questions will be certainly challenging and should be a prospective research priority in coming years. In addition, cellular microRNA-papillomavirus interaction in regulation of gene expression is becoming a new research direction and may shed some light on understanding HPV gene expression and life cycle in a cell differentiation manner.

O-14.01

ESTABLISHMENT OF TRANSCRIPTION FROM INCOMING VIRAL GENOME REQUIRES L2 PROTEIN

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The minor capsid protein, L2, serves essential functions during infection. It mediates egress of the viral genome from endosomes as well as retrograde intracytoplasmic transport along microtubules. It also accompanies the viral genome to the nucleus, where both accumulate at PML nuclear bodies (PML NB). PML protein is essential for efficient establishment of transcription from incoming viral genome (Day et al., PNAS 101:14152-57, 2004). Here we present evidence that L2 protein is required to establish transcription as well. We have identified L2 point mutants that allow efficient delivery of viral genome to PML NB. Delivery of DNA to PML NB was determined by confocal microscopy, and nuclear delivery was confirmed by quantitative PCR. However, these mutants fail to initiate viral transcription. Both, unspliced and spliced transcripts were strongly reduced following infection with mutant virions compared to wild-type (wt) virus, suggesting a block at the transcriptional level. The defect is not due to covalent modifications of encapsidated DNA, like methylation or degradation, during virus assembly or intracellular transport. Similarly, infection in the presence of histone deacetylase inhibitors did not rescue mutant virus. Also, histone acetyl transferase inhibitors present during virus assembly did not affect infectivity of wt and mutant particles. These data suggest that histone acetylation is not the underlying reason for transcriptional repression. Taken together, our data indicate that L2 protein may recruit transcriptional activators. To our knowledge, this is the first report of a viral capsid protein contributing to establishment of transcription from incoming viral genome.

O-14.02

ASF/SF2 AND SRP30C REGULATE HPV-16 EARLY AND LATE MRNA SPLICING

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We speculate that inhibition of HPV-16 late gene expression is a requirement for establishment of persistence and development of cancer. Late gene expression is regulated at the level of RNA processing, i.e. polyadenylation, splicing and RNA stability. We identified an area that is unusually rich in potential ASF/SF2 binding sites in the HPV-16 genome. This region is located between SA3358 and SD3632 and overlaps with the splicing enhancer at SA3358. This enhancer that was previously identified by us, promotes the usage of SA3358 and the production of the early E4 mRNA. By deleting the potential ASF/SF2 binding sites, splicing is redirected from early splice site SA3358 to late splice site SA5639, at the same time bypassing the early polyA signal. Deletion of the potential ASF/SF2 sites also results in a dramatic decrease in early polyadenylation, suggesting that ASF/SF2 cooperates with the early polyA signal to produce early mRNAs. Furthermore, deletion of some, but not all potential ASF/SF2 sites, activates SD3632, indicating that interactions between ASF/SF2 and polyadenylation factors suppress SD3632, that is used exclusively by late L1 mRNAs. Experimental data that strengthening our hypothesis include: 1) Over-expression of a mutant ASF/SF2 protein that lacks the RS-domain induces splicing directly into the late region. These results suggest that it acts as a trans-dominant mutant and inhibits wt ASF/SF2, thereby preventing early polyadenylation and relieving SD3632 suppression. In contrast, SRp55 with deleted RS-domain has no effect on HPV-16 late gene expression, whereas SRp30c does. 2) Overexpression of adenovirus E4orf4 that regulates adenovirus late gene expression by dephosphorylating ASF/SF2, also induces HPV-16 late gene expression. In contrast, an AdE4orf4 mutant that does not bind ASF/SF2 fails to induce HPV-16 late gene expression.

O-14.03 P300 NOT CBP ENHANCES HPV TRANSCRIPTION BY DISTINCT AP-1 COMPLEXES

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Activator protein-1 (AP-1) regulates diverse gene responses triggered by environmental cues and virus-induced stress. Although many signaling events leading to AP-1 activation have been well studied, the fundamental features underlying DNA binding site selection and factor recruitment of selective AP-1 family members to their target genes remain to be defined. Using recombinant full-length AP-1 dimeric complexes formed between c-Jun and the Fos family members (c-Fos, FosB, Fra-1, Fra-2) for DNA binding and transcription analysis, we found that each of these AP-1 complex exhibits differential activity for distinct non-consensus AP-1 sites present in HPV-11 and each is capable of activating transcription from HPV chromatin in a p300- and acetyl-CoA-dependent manner. Interestingly, recruitment of p300, but not its close relative CBP, correlates with HPV transcription activity and AP-1 occupancy to both promoter-proximal and promoter-distal AP-1 sites in endogenous HPV-18 genomes in HeLa cells with or without exogenous E2 expression. This comparative study uncovers common and unique properties of different human AP-1 complexes and a selective requirement of p300 acetyltransferase activity for AP-1-dependent HPV chromatin transcription.

O-14.04

FUNCTIONAL MAPPING OF THE HUMAN PAPILLOMAVIRUS TYPE 16 E1 CISTRON

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Replication of the double-stranded, circular human papillomavirus (HPV) genomes requires the viral DNA replicase E1. Here, we report an initial characterization of the E1 cistron of HPV type 16 (HPV-16), the most common oncogenic mucosal HPV type found in cervical and some head and neck cancers. The first step in HPV DNA replication is an initial burst of plasmid viral DNA amplification. Complementation assays between HPV-16 genomes carrying mutations in the early genes confirmed that the expression of E1 was necessary for initial HPV-16 plasmid synthesis. The major early HPV-16 promoter, P97, was dispensable for E1 production in the initial amplification because cis mutations inactivating P97 did not affect the trans complementation of E1– mutants. In contrast, E1 expression was abolished by cis mutations in the splice donor site at nucleotide (nt) 226, the splice acceptor site at nt 409, or a TATAA box at nt 7890. The mapping of 5' mRNA ends using rapid amplification of cDNA ends defined a promoter with a transcription start site at HPV-16 nt 14, P14. P14-initiated mRNA levels were low and required intact TATAA (7890). E1 expression required the HPV-16 keratinocyte-dependent enhancer, since cis mutations in its AP-2 and TEF-1 motifs abolished the ability of the mutant genomes to complement E1– genomes, and it was further modulated by origin-proximal and -distal binding sites for the viral E2 gene products. We conclude that P14-initiated E1 expression is critical for and limiting in the initial amplification of the HPV-16 genome.

O-14.05

A REPORTER SYSTEM TO STUDY HVP SPLICING

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Background. DNA tumor viruses use splicing as a strategy to generate complex messages from compacted information from their genomes. Among Human Papillomavirus (HPV), type 16 exhibits the most complex patterns, with 20 different transcripts identified as a result of alternative splicing. E6 and E7 messangers are transcribed from the same promoter (p97) as a bicistronic mRNA, and alternative splicing produces E6*I, E6*II and E6E7 mRNAs. Objective. Create a reporter system to study HPV splicing in vivo. Methods. GFP and lacZ genes were modified to introduce restriction sites where different introns (amplified by PCR and containing intron-exon junctions, branch point and polipyrimidine tract sequences) were cloned. Plasmids were transfected into different cell lines to assay for intron splicing by reporter activity, cDNA sequencing, and western blot analysis. Results. We first generated a plasmid with a restriction site introduced 100 nt from GFP start codon where the E6*I intron was cloned. Constructs were transiently transfected into SiHa, CasKi, C33A and 293T cell lines. Although initially none of them showed fluorescence under the microscope, RT-PCR and western blot analysis showed existence of both processed GFP mRNA and protein in all cell lines. cDNA sequencing showed the presence of GFP mRNAs containing no intron sequences and only exon junctions. We have now generated a new construction where a restriction site was introduce into a different location and where HPV introns are being introduced to study HPV intron splicing. Conclusions. Our preliminary results show that early introns from HPV 16 are spliced in vivo, although they contain only processing signals within the intron sequence and not exonic signals. HPV positive and negative cell lines showed no differences in splicing activity, meaning that HPV intronic splicing sequences are not type specific, and that they are recognized by the basal spliceosome machinery.

POSTER ABSTRACTS SESSION 14

POSTER SESSION III TUESDAY 20.00: ODD NUMBERS

POSTER SESSION IV

TUESDAY 21.00: EVEN NUMBERS

BPV-1 E5 LOAD AND TRANSCRIPTION IN PBMCS OF SARCOID-AFFECTED HORSES

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Background: In horses and other equids, bovine papillomaviruses types 1 and 2 (BPV-1, BPV-2) induce locally aggressive skin lesions termed sarcoids, which represent the most common neoplasm in ungulates. In addition, BPV may contribute to other malignancies affecting the equid integument, including dermatitis and hoof canker. Recently, we have shown that BPV DNA not only resides in cutaneous cells but also in peripheral mononuclear blood cells (PBMCs) of BPV-1/-2-infected horses.

Objectives: To further investigate this issue, we determined viral DNA loads and transcription of the major transforming gene E5 in PBMCs of BPV-1-infected equines by

Methods: PBMC DNA was obtained by Ficoll gradient centrifugation and subsequent DNA isolation from blood of 9 BPV-1-infected individuals (4 sarcoid patients, 2 hoof canker patients, 3 cases of dermatitis) and 11 uninfected control horses. E5 copy numbers/cell were determined by quantitative PCR (qPCR) and given after normalisation to the amount of input DNA measured by equine Interferon β qPCR.

E5 transcription was assessed by RT-qPCR from DNase-digested PBMC mRNA of 10 horses and 1 donkey with confirmed cutaneous BPV-1-infection (7 sarcoid patients, 4 hoof canker patients) and 2 healthy individuals.

Results: E5 qPCR revealed 1 to 3 E5 copies/PBMC for 3 individuals severely affected by sarcoids (n = 1) or hoof canker (n = 2), and <0.06 E5 copies/PBMC for 6 horses with mild to moderate sarcoid affection (n = 3) or dermatitis (n = 3). E5 mRNA was detected in PBMCs of 6/7 sarcoid-bearing equids and 4/4 hoof canker patients. All control horses scored negative for E5 DNA or transcripts throughout the study.

Conclusions: To our knowledge, this is the first report on E5 major oncogene transcription in equine PBMCs. This finding strongly suggests that infected blood cells are actively involved in papillomavirus-induced pathologic processes including the spread of disease within affected individuals.

P-14.07

INTERACTION OF P53 FAMILY WITH CUTANEOUS HPVE6

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HPV20 has often been demonstrated in benign and malignant cutaneous lesions. Extensive UV irradiation and p53 mutations (hot-spot mutations) are key factors in the pathogenesis of non-melanoma skin cancer, but the role of cutaneous papillomaviruses remains unclear.

We have demonstrated the wtp53-mediated degradation of HPV20E6 through caspase-3. Both deltaNp63alpha and mutant p53R248W convey a protective effect on HPV20E6 under these conditions.

We extended our investigation to included 10 additional cutaneous HPV types belonging to different genera and species. The conserved caspase-3 recognition site in HPV20E6 is located close to the N-terminal, whereas the putative caspase-3 recognition site in the 10 other HPV types were scattered throughout the E6. Our results demonstrate a wtp53-mediated stimulation or degradation of cutaneous HPV E6 depending on HPV type. Co-expression of deltaNp63alpha or mutant p53R248W with HPV E6 exert a differential effect depending on HPV type. A role for these interactions in the pathogenesis of cutaneous lesions will be discussed.

MODULATION OF TGF-BETARII AND HPV-16 E5 EXPRESSION IN PRENEOPLASTIC LESIONS

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Expression of the E5 product of high-risk human papillomavirus type 16 (HPV-16) appears to be involved in cervical cancer progression, although the molecular mechanisms of its oncogenic role remain to be established. Altered expression of transforming growth factor beta (TGF beta) and its receptor serine/threosine kinase TGF-betaRII are also involved in the pathogenesis of cervical carcinomas. To analyze the possible relationship between HPV-16 integration, E5 transcription and TGF-betaRII modulation in the progression of cervical neoplasia, we selected 17 cytological samples of Low-Grade Squamous Intraepithelial Lesions (L-SIL) and 10 of High-Grade Squamous Intraepithelial Lesions (H-SIL), all E6/E7 positive for HPV-16. Total mRNA was extracted using the RNeasy Miniprotocol (Qiagen) and HR-HPV E6/E7 mRNA were detected by PreTect HPV-Proofer kit (NorChip). Quantitative Real Time PCR was then performed for the detection of HPV-16 E5 and of TGF-betaRII mRNA. The results showed that the transcript levels of TGF-betaRII, compared to control samples, were down-regulated in all HSILs, while in LSILs we observed a clear decrease of the receptor expression only in relation to high levels of E5 mRNA, suggesting that E5 expression could play a transforming role in cervical lesions through down-modulation of TGF-betaRII expression and deregulation of TGFbeta signaling.

P-14.09 DETECTION OF HPV E6 AND E7 ONCOPROTEINS IN BLADDER CANCERS

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HPV may have a great role in progression of transitional cell carcinoma (TCC) by inactivation of the tumor suppressors or other unknown mechanisms. Previous studies showed presence of HPV correlated with malignancy of urothelium transformation, but some reported contradictory results. In this regards, more sensitive detection of HPV in bladder cancers is required to demonstrate the association of HPV and cancer progression in TCC. Detection for expression and localization of HPV oncoproteins in different stages of bladder cancer will help understanding the mechanism. The objectives of this study are to 1). detect HPV proteins in TCC by immunohistochemistry (IHC) using our novel antibodies to HPV E6, E7 and L1 proteins; 2). compare HPV E6, E7 oncoprotein expression with HPV DNA and HPV mRNA transcript in TCC specimen. The preliminary results demonstrate expression of HPV E6, E7, and L1 proteins present in TCC specimen compared to normal urocystic tissue or malignant melanoma as negative control used in the HPV IHC assays. For the HPV DNA positive TCC samples, about 30% show expression of HPV E6 and E7 oncoproteins by IHC. More clinical samples are required to analyze the correlation of HPV DNA and HPV protein expression.

DNA METHYLATION PATTERNS OF HPV16 GENOMIC REGIONS IN CERVICAL CANCER

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Background. Deregulation of gene expression is the most distinguishing feature of cancer. Aberrant epigenetic modifications play a central role in neoplastic development and progression.DNA methylation is used for the epigenetic modulation of chromatin structure and regulation of gene expression in vertebrates. DNA methylation in promoter regions is associated with transcriptional silencing of the corresponding gene. High-risk human papillomavirus are the most common etiological agents associated with human cancers, including cervical, anogenital and some oral cancers. Progression of persistent infections to cancer is caused mainly by HPV type 16, with deregulated expression of E6 and E7oncogenes. Objective. Study methylation patterns of the LCR (enhancer & promoter), L2-L1 and E7 regions of HPV-16 and correlate them with regulatory mechanisms influencing the productive life cycle and transcriptional program, in precursor lesions and invasive cervical cancer. Methods. We used cervical samples (normal precursor and cancer lesions) and epithelial cell lines (SiHa, & Caski). We modified DNA with sodium bisulfite, followed by PCR amplification with specific primers for modified HPV-16 DNA. Amplicons were cloned in TOPO vectors and between four and ten positive clones of each analyzed region were sequenced. Results. The HPV-16 genome contains 112 potencial sites for 5' methylation at CpG residues, 78 of them are within the early region, 16 are on the LCR and the others are in the late region. Methylation patterns obtained so far with 10 different clinical samples show an hypermethylation profile within the L2-L1 region and an heterogeneous pattern within the E7 gene and the LCR region, with some frequency of site-specific methylation at the positions 7683, 43 and 745; however it is necessary to complete the analysis with a larger number of cervical samples to try to establish a possible relationship between methylation patterns and transcriptional regulation of HPV-16.

P-14.11 EXPRESSION AND PURIFICATION OF HUMAN PAPILLOMAVIRUS TYPE 58 L1

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Human papillomavirus (HPV) type 16 and 18 cause about 70% of cervical cancer in the Western world. However, the HPV type distribution is quite different in Asia from Western world. In particular, HPV type 58 is common in cervical cancers as well as in the general population throughout south Asia, China, Japan, Korea and Mexico. HPV type 58 is more prevalent than HPV type 18 in women with cervical cancer in China and is founded in 28.5 % of women with HPV positive in Mexico. However, there is no report providing method for expression and purification of HPV 58 L1. Therefore, we developed the method for expression and purification of HPV 58 L1. The HPV 58 L1 gene was cloned into the yeast expression vector YEGa-HIR525, and transformed into Saccharomyces cerevisiae. The purification method of HPV 16 L1 and HPV 18 L1 using ammonium sulfate precipitation, size-exclusion chromatography and cation-exchange chromatography was well established by many studies and showed efficient purification. However, the purification of HPV 58 L1 was unsuccessful when the previous purification method for HPV 16 L1 and HPV 18 L1 was applied. The HPV 58 L1 was not efficiently separated by the cation-exchange chromatography. Therefore, the HPV 58 L1 was serially purified by ammonium sulfate precipitation, heparin chromatography, cation-exchange chromatography and hydrophobic interaction chromatography. Over 90% of contaminants were removed by the heparin chromatography, and some parts of the contaminants were removed by the cation-exchange chromatography. Finally, the HPV 58 L1 was successfully separated by the hydrophobic interaction chromatography. It was confirmed that the purity of HPV 58 L1 obtained by the method was over 95%. This result indicates that the purification method for HPV 58 L1 could be useful for immunogenicity study of HPV type 58.

HPV TRANSCRIPTION, REPLICATION AND HOST CELL INTERACTIONS IN TROPHOBLASTIC MODELS.

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Background: Hallmarks of HPV infection include a restricted tropism for human epithelial cells and a viral life cycle tightly linked to the differentiation program of the host cells. This has hampered the study of the HPV vegetative life cycle. Previous studies reported that the tissue and differentiation dependence seemed to be dictated by viral transcription rather than viral DNA replication.

Objectives: 1. To compare HPV transcription in cervical and trophoblastic cells and to study in those models the regulation of the LCR activity by various hormones and the viral early proteins. 2. To study the impact of the early viral proteins, especially E5, E6 and E7, on cell adhesiveness, migration and invasiveness.Methods: To study transcription, we analyzed the activation of a reporter gene under the control of the HPV-16 LCR. To study replication, we measured, in RT-PCR after DpnI/MboI digestions, the amount of replicated DNA. Cellular properties were studied using various biological assays. Results: The LCR activity was similar in both cell types and could be regulated by various hormones. To analyze the effect of all early proteins expression on the LCR activity and on viral replication in both cell types, the reporter plasmid was cotransfected with a plasmid allowing the expression of the entire early coding region under the control of its own HPV-16 LCR. Viral early proteins activated viral transcription and replication. Using various plasmids harboring point mutation in E1 or E2 ORF, we were able to observe that neither E1 nor E2 did play a role in the increased viral transcription. Early proteins could also modify the adhesion, the migration and the invasion of trophoblastic cells. Conclusions: We will discuss about the interest of this model to identify new cell host (trophoblast) /pathogen (HPV) interactions.

P-14.13

CYTOPLASMIC POLYADENYLATION: POSSIBLE REGULATORY MECHANISM OF HPV-16 EARLY MRNAS

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The regulation of mRNA is an important mode of controlling gene expression. Lengthening and shortening of the poly(A) tail can modulate stability and translational activity of mRNAs in the cytoplasm. This has been shown to be important in Drosophila and C. elegans embryogenesis (1), Xenopus oogenesis (2) and neuronal activity in Xenopus oocytes (3).

Cytoplasmic polyadenylation elements (CPEs) in the target mRNA 3'UTR has been shown to be essential for this posttranscriptional mechanism, as well as CPE binding proteins (CPEBs) (4). The early 3'end of the Human Papillomavirus type 16 (HPV-16) contains five CPE like sequences.

HPV-16 can induce tumor formation through the expression of the E6 and E7 oncogenes. All transcripts encoding these oncoproteins are polyadenylated at the poly(A) signal within the early 3' untranslated region.

In this study, we are investigating the role of the CPE like sequences in the HPV-16 early 3' end in somatic cells and the impact of the human CPEB1 and the human cytoplasmic poly(A) polymerase hGLD-2 specifically on the regulation of HPV-16 early mRNAs.

1)Kuersten S. and Goodwin E. B. (2003). The power of the 3'UTR: translational control and development. Nature Review Genetics 4, 626-637.

2)Mendez R. and Richter J. D. (2001). Translational control by CPEB: a means to the end. Nature Reviews Molecular Cell Biology 2, 521-529.

3)Wu L., Wells D., Tay J., Mendis D., Abott M. A., Barnitt A., Quinlan E., Heynen A., Fallon J. R. and Richter J. D. (1998). CPEB- mediated cytoplasmic polyadenylation and the regulation of experience-dependent translation of α-CaMKII mRNA at synapses. Neuron 21, 1129-1139.

4)Hake L. E., Mendez R. and Richter J. D. (1998). Specificity of RNA binding by CPEB: requirement for RNA recognition motifs and a novel zinc finger. Molecular Cell Biology 18, 685-693.

DETECTION OF HPV E6/E7 ONCOPROTEINS IN CERVICAL CANCER.

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BACKGROUND: The oncological mechanism associated with HPV is one of the most stable of all known. Two events very prevalents are dysfunction of the regulatory proteins p53 and pRB for the viral proteins, E6 and E7 respectively. Excellent indicators of the dysfunction of the p53 and the pRB are the expression of E6 for p53 and the expression of E7 and overexpression of p16INK4a for pRB. OBJETIVES:1)To determine in 234 cases of invasive cervical cancer the frequency of cases with expression of HPV E6, E7 and L1 proteins.2)To Determine in the same cases the frecuency of them with overexpression of the protein p16INK4a3)To compare the results obtained of the objective 1 and 2 with HPV genotype to establish the sensitivity and specificity of the antibodies used for genotypes 16 and 18. METHODS: A) Tissue array preparation: Tissue slides were sectioned from paraffin-embedded blocks. Each tissue microarray contains 22 cervical squamous cell carcinomas or adenocarcinomas and its corresponding controls (their normal epithelial counterparts, vaginal or cervical mucosa of at least 15 mm away from the gross tumor border). 234 cases were arrayed onto total of 11 slides. B)PCR method: HPV DNA typing was identified by PCR SPF10-LiPA 25,C)Immunohistochemical method: Recombinant proteins of full length HPV type 16 E6, E7, L1 and HPV type 18 E6,E7 were produced and purified to immunize rabbits and mice for polyclonal antibody production and monoclonal antibody development by Neodiagnostic Labs Inc. Using such antibodies, and the clone E6H4TM to p16INK4a human protein, IHC protocols were developed to stain tissue microarray. PRELIMINARY RESULTS: Results of IHC staining were scored by certified pathologist to give intensity of score 0-3 or percentage of tumor cells stained. Data will be analyzed to obtain the assay sensitivity and specificity compared to PCR and IHC staining by p16INK4a.

P-14.15

ESTABLISHMENT OF C33A CELL LINES STABLY EXPRESSING HPV16 VARIANT ONCOGENES

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OBJECTIVE: The incidence of the aspartic acid to glutamic acid at E6 amino acid 25 (D25E) and asparagine to serine at E7 amino acid 29 (N29S) for HPV16 is predominant variant in our country. The aim of this study is to establish in vitro cell line with stable and effective E6/E7 variant gene expression, so as to investigate the role of variant E6/E7 gene in the signaling pathway involved in human cervical carcinogenesis.

METHODS: E6 D25E and E7 N29S genes were amplified from HPV16 variant infected patient sample and inserted into pCR2.1-TOPO vector. A positive clone was subcloned into pLenti6.3/V5-TOPO lentiviral vector, and then verified by sequencing. The recombinant lentivirus was harvested from 293FT cells cotransfected with the recombined plasmid and lentiviral packing materials. C33A cells were infected with the recombinant lentivirus and the cells with stable maintenance E6 or E7 gene were screened by blasticidin selection. The single clone was obtained by seeding the cells into 96-well plates with one cell per well. E6 or E7 expression in the cells was determined by reverse transcription-polymerase chain reaction (RT-PCR).

RESULTS: By using lentiviral gene transfer technology, the E6 D25E or E7 N29S gene was integrated into the chromosome of C33A cells. We selected 16 clones of C33A cells infected with the E6 or E7 variant DNA from 40 clones and the one clone exhibited continuously expressing E6 D25E or E7 N29S, respectively. Finally, a cell line stably expressing the E6 D25E or E7 N29S fusion protein was established. The fusion protein was confirmed to be expressed correctly by Western-blot.

CONCLUSION: The lentiviral E6 D25E or E7 N29S expression vector capable of stable variant oncogenes expression in C33A cells has been successfully constructed, which provides a basis for further study of the relationship between human cervical carcinoma and E6/E7 variant oncogenes.

EXPRESSION OF HPV-16 IN TROPHOBLASTIC AND CERVICAL CELLS

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HR-HPV can infect the lower layer of cervical epithelia and can cause cervical cancer. HPV could also infect the trophoblast, an epithelial cell from the placenta through which implantation and nutrient exchanges occur and could be involved in some spontaneous abortions, although this should be further confirmed by additional experiments.

This study compared the regulation of the HPV-16 LCR expression by steroid hormones and early viral proteins in trophoblastic and cervical cell lines.

The cells were transiently transfected with a plasmid expressing the luciferase gene under the control of the entire HPV-16 LCR. Fibroblasts were used as a negative control. Dexamethasone, mimicking pregnancy steroid hormones, induced HPV-16 expression in both cell types. One plasmid, expressing all the early viral proteins under the control of the entire LCR, increased HPV-16 LCR transcriptional activity in transfected cells. Using various constructs harbouring mutated early regions, we observed that this induction is probably not due to E1 or E2, but to the other early viral proteins. In both cell types, early viral proteins were also shown to be able to induce viral replication of an HPV origin (Ori) containing plasmid. As expected, absence of E1 expression abrogated replication of the viral genome. To conclude, HPV was strictly epitheliotropic with no significant differences between the cell lines. Furthermore, transcriptional control in trophoblastic and cervical cells can be induced by glucocorticoids and viral early proteins. This latter effect was not due to E1 or E2. On the other hand, replication of the viral genome was seriously impaired when E1 was not expressed.

P-14.17 DNA METHYLATION PATTERNS OF HPV16 GENOMIC REGIONS IN CERVICAL CANCER

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Background. Deregulation of gene expression is the most distinguishing feature of cancer. Aberrant epigenetic modifications play a central role in neoplastic development and progression.DNA methylation is used for the epigenetic modulation of chromatin structure and regulation of gene expression in vertebrates. DNA methylation in promoter regions is associated with transcriptional silencing of the corresponding gene. High-risk human papillomavirus are the most common etiological agents associated with human cancers, including cervical, anogenital and some oral cancers. Progression of persistent infections to cancer is caused mainly by HPV type 16, with deregulated expression of E6 and E7oncogenes. Objective. Study methylation patterns of the LCR (enhancer & promoter), L2-L1 and E7 regions of HPV-16 and correlate them with regulatory mechanisms influencing the productive life cycle and transcriptional program, in precursor lesions and invasive cervical cancer. Methods. We used cervical samples (normal precursor and cancer lesions) and epithelial cell lines (SiHa, & Caski). We modified DNA with sodium bisulfite, followed by PCR amplification with specific primers for modified HPV-16 DNA. Amplicons were cloned in TOPO vectors and between four and ten positive clones of each analyzed region were sequenced. Results. The HPV-16 genome contains 112 potencial sites for 5' methylation at CpG residues, 78 of them are within the early region, 16 are on the LCR and the others are in the late region. Methylation patterns obtained so far with 10 different clinical samples show an hypermethylation profile within the L2-L1 region and an heterogeneous pattern within the E7 gene and the LCR region, with some frequency of site-specific methylation at the positions 7683, 43 and 745; however it is necessary to complete the analysis with a larger number of cervical samples to try to establish a possible relationship between methylation patterns and transcriptional regulation of HPV-16.

SIMILAR CHROMOSOME ABERRATIONS IN BPV NEOPLASIC CELLS AND LYMPHOCYTES.

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Bovine Papillomavirus is a dermotropic oncogenic DNA virus, species and tissue-specific. Previously we reported the presence of BPV genome in non-epithelial cells (including peripheral blood and gametes), without evidences of virus expression. We described chromosomal abnormalities in lymphocytes of infected animals. Now, we compared the frequency of chromosome abnormalities in peripheral lymphocytes and cultured cells obtained from warts (benign lesions), esophagus and bladder neoplasic cells, collected from BPV infected animals with normal skin cells. The detection of papillomavirus genome sequences in blood, warts and through culture passages were confirmed by PCR and sequencing. Increased frequency of chromosome abnormalities was verified in the affected animals compared to control, not infected skin cells (p > 0.05). The observed chromosome aberrations, cromatid breaks, acentric fragments, gaps, rearrangements involving centromeric and telomeric associations were similar when we analyzed the types of BPV infected cells. The presence of the same aberrations in blood and neoplasic cells in similar frequencies and structure provides relevant evidence of virus expression in non epithelial tissues, emphasizing the lymphocyte, that can be a latent viral site but, also, a possible transmission agent.

P-14.19

CHARACTERIZATION OF HPV16 E6 MRNA EXPRESSION IN ADVANCED CERVICAL NEOPLASIA

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INTRODUCTION: Neoplastic transformation induced by HPV requires expression of the virally-encoded oncogenes, E6 and E7. To determine whether E6 mRNA expression correlates with cervical grade, we have analyzed the level of expression of the E6 mRNA for HPV16 in a panel of formalin-fixed and paraffin-embedded (FFPE) cervical tissues using real time RT-PCR.

METHODS: DNA and RNA were extracted from 86 cervical biopsies using a modified MasterPure protocol from Epicentre. The tissues consisted of 25 normal, 34 CIN III, and 27 squamous cervical cancer (SCC) samples. HPV DNA genotyping was performed using real time PCR with primers/probes specific to genomic regions of HPV 16, 18, 31, 33, and 45. Following reverse transcription, HPV 16 E6 mRNA was analyzed with different and non-overlapping primers/probes using real time RT-PCR.

RESULTS: HPV genotyping results indicated that 41/86 specimens were HPV 16 DNA positive, 24 were positive for other high risk HPV types, and 21 were HPV DNA negative. Real time RT-PCR analysis demonstrated that all of the SCC samples that were HPV 16 DNA positive also expressed HPV 16 E6 mRNA. Ninety six percent of CIN III samples expressed HPV16 E6 mRNA with HPV 16 positive DNA. One CIN III sample was DNA positive but did not contain detectable E6 mRNA. Finally, 92 percent of normal samples did not express any detectable E6 mRNA. Two normal samples were positive by our criteria for HPV 16 E6 mRNA. Samples in which HPV16 E6 mRNA was not detected were either negative for HPV or were of another genotype.

CONCLUSION: In this study we have demonstrated that the majority of advanced cervical neoplasia samples that are HPV 16 DNA positive also express E6 mRNA.

CUTANEOUS HPV INFECTIONS AND SKIN CANCER

SESSION 15

PROGRAMME

ROOM

THE 25TH INTERNATIONAL PAPILLOMAVIRUS CONFERENCE MAY 8-14 2009, MALMÖ, SWEDEN

TIME NUMBER TITLE

2009-05-12

SESSION 15: CUTANEOUS HPV INFECTIONS AND SKIN CANCER			
11.00-11.11	O-15.01	PROSPECTIVE STUDY OF HPV SEROPOSITIVITY AND NON-MELANOMA SKIN CANCER K Andersson, Km Michael, T Waterboer, T Luostarinen, R Gislefoss, O Forslund, T Hakulinen, M Pawlita, J Dillner	
11.11-11.22	O-15.02	HPV SEROPREVALENCE IN EPIDERMODYSPLASIA VERRUCIFORMIS PATIENTS, HEALTHY RELATIVES AND CONTROLS Km Michael, T Waterboer, H Pfister, M. Gariglio, S Majewski, M Favre, M Pawlita	
11.22-11.33	O-15.03	INDICATIONS FOR AN ASSOCIATION OF HIGH BETAPV-LOADS WITH SKIN CARCINOGENESIS SJ Weissenborn, R Neale, D Abeni, JN Bouwes Bavinck, MCW Feltkamp, A Green, M De Koning, U Wieland, H Pfister, and EPI-HPV-UV-CA group	
11.33-11.44	O-15.04	CLASSIFICATION OF CUTANEOUS HPV-TYPES WITH RESPECT TO THEIR IMMORTALIZATION CAPACITY RDM Steenbergen, D Claassen - Kramer, CJLM Meijer, L Struijk, PZ van der Meijden, MCW Feltkamp, PJ Snijders	
11.44-11.55	O-15.05	HPV20E6 ONCOPROTEIN MODIFIES EPITHELIAL DIFFERENTIATION AND INDUCES LIPID ACCUMULATION E Herráez - Hernández, R Nobre, L Langbein, S Kaden, H-J Gröne, Em de Villiers	
11.55-12.06	O-15.06	MASTOMYS COUCHA: A MODEL FOR PV-INDUCED PATHOGENESIS OF THE SKIN K Schaefer, T Waterboer, M Mueller, J Nafz, I Nindl, F Roesl	
12.06-12.17	O-15.07	IDENTIFICATION OF B-CELL EPITOPES ON VLPS OF CUTANEOUS SPECIES ALPHA-HPVS T Senger, Mr Becker, L Schädlich, T Waterboer, Cb Buck, L Gissmann	
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PROSPECTIVE STUDY OF HPV SEROPOSITIVITY AND NON-MELANOMA SKIN CANCER

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Objective: Longitudinal studies assessing whether virus exposure is able to predict risk for future cancer development are an essential part of causality inference. We performed a large biobank-based serological study where donors were followed up for future skin cancer incidence for up to 30 years and the risk of skin cancer in relation to antibodies against 35 mucosal and cutaneous HPV types was estimated.

Methods: About 330000 volunteers donated serum samples to the JANUS biobank in Norway from 1972 and onwards. About 524000 donors had samples in the Malmö Microbiology biobank in Sweden, donated from 1969 and onwards. Registry linkages identified 1990 individuals diagnosed with basal cell carcinoma (BCC) and 633 individuals with squamous cell carcinoma (SCC) of the skin during follow-up. For each case, one control without skin cancer was selected, matched for age, sex, county, number of sampling occasions and length of follow-up. When subjects had donated on multiple occasions, all samples were retrieved, resulting in 10 187 serum samples included in the study. Antibodies to the major capsid protein L1 of HPV 1,2,3,4,5,7,8,9,10,13,15,16,17,18,20,23,24,27,32,36,38,41,48,49,50,63,65,75,76,77,92,95,96 ,101 and 103 were determined using a multiplexed fluorescent bead-based assay.

Results: Seropositivity for HPV types 3, 16, 18 and 38 all showed an increased risk for SCC with crude odds ratios (OR) and 95% confidence intervals (CI) of 1.4(1.0-2.1), 1.6(1.1-2.6), 1.7(1.1-2.5) and 1.3(1.0-1.7), respectively. Seropositivity for HPV 15 showed an increased risk for BCC with an OR of 1.2 (95% CI: 1.0-1.4).

Conclusion: In spite of many cutaneous HPV types being tested, only 2 cutaneous HPV types had marginally significant associations with SCC (HPV3 and HPV 38).

The major oncogenic genital HPV types 16 and 18 have a significantly increased risk for squamous cell carcinoma also of non-genital skin, potentially widening the scope of malignancies that are preventable by HPV16/18 vaccination.

O-15.02

HPV SEROPREVALENCE IN EPIDERMODYSPLASIA VERRUCIFORMIS PATIENTS, HEALTHY RELATIVES AND CONTROLS

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Background: Epidermodysplasia verruciformis (EV) is a rare recessive genodermatosis characterized by high susceptibility to infections with HPV of genus beta. Knowledge about seroreactivity against HPV in these patients and also their first-degree relatives is scarce.

Objectives: To compare HPV seroprevalence between 35 EV patients and 22 first-degree relatives as well as 70 and 44 ageand sex-matched, non-related healthy controls, respectively.

Methods: Antibodies to the major capsid protein L1 of 38 HPV types (10 alpha, 16 beta, 9 gamma, 2 mu, 1 nu PV) were detected simultaneously using multiplex serology.

Results: For all 38 HPV types, EV patients showed higher seroprevalences than their relatives with statistically significant odds ratios (OR) for 15 of 16 investigated beta (OR range 4.2 - 24.9) and 3 of 9 gamma PV (OR range 4.2 - 13.0), but not for alpha, mu or nu PV. In comparison to non-related controls, antibodies in EV patients were significantly more prevalent for 7 of 10 alpha (OR range 5.7 - 20.4), for all 16 beta (OR range 7.1 - 30.8), 5 of 9 gamma (OR range 2.8 - 9.8) and 1 of 2 mu PV (OR 3.5). Significant differences between relatives and controls were seen for three beta PV (OR range 12.6 - 14.9).

Conclusions: The considerably elevated HPV seroprevalence in EV patients especially for beta PV reflects the high viral load found in these individuals. Whether the observed differences between relatives and healthy controls depend on heterozygocity for EV-associated alleles needs further investigation.

INDICATIONS FOR AN ASSOCIATION OF HIGH BETAPV-LOADS WITH SKIN CARCINOGENESIS

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and EPI-HPV-UV-CA group

Background: Epidemiological studies provide some evidence that the presence of betapapillomavirus (betaPV) DNA is associated with cutaneous squamous-cell carcinoma.

Aim: To investigate the role of DNA load of eight betaPV types in case-control studies of 439 Italian and 473 Australian individuals.

Methods: Eyebrow hairs were tested for the presence of the 25 fully sequenced betaPV types and loads of HPV5, 8, 15, 20, 23, 24, 36 and 38 were determined by qPCR.

Results: The overall beta-PV prevalence was 95% in Italians and 91% in Australians. The median number of betaPV types detected was 5.6 and 4.9, respectively. The highest loads were found for HPV5, 8 and 20. The median loads of the eight tested types were similar in cases and controls. Interestingly, the top decile loads of HPV-types 5, 8, 15, 24 and 38 were higher in cases compared to controls both in Italy and Australia (2.5, 3.0, 3.5, 3.1 and 1.2-fold in Italy, and 3.2, 1.7, 1.3, 1.1 and 1.8-fold in Australia, respectively). After adjustment for age and sex odds ratios in the range of 1.2 to 2.6 were found for top tertile viral loads in cases of both centers. We assigned participants into cumulative load groups, by ranking them according to tertile for each individual betaPV type and then summing the scores assigned to each tertile. In Australia there was a significant dose-response association between this measure and SCC, but this was not observed for Italy. Conclusion: Infections with multiple betaPV are extremely common in cases and controls. Our data suggest that high

Conclusion: Infections with multiple betaPV are extremely common in cases and controls. Our data suggest that high betaPV loads, particularly for HPV5 and 38, are associated with cutaneous squamous-cell carcinoma.

O-15.04

CLASSIFICATION OF CUTANEOUS HPV-TYPES WITH RESPECT TO THEIR IMMORTALIZATION CAPACITY

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Background: Although betapapillomaviruses(PVs)infections have been associated with cutaneous squamous cell carcinoma, their role in malignant transformation remains controversial. One feature involves the ability to inhibit UVB-induced apoptosis, as demonstrated for HPV5, HPV8 and HPV20. A second, potentially unrelated, characteristic involves the induction of immortality and telomerase activation.

Objective: To demarcate putatively low-risk and high-risk beta-PV types by their transforming capacities in primary keratinocytes. Methods: Primary human keratinocytes from three donors were transduced with E6/E7 of selected betaPVs, i.e. HPV5, HPV8, HPV10, HPV15, HPV20, HPV24 and HPV38, as well as HPV16, serving as positive control. All transductants were analysed for growth characteristics, telomerase activation and differentiation. Moreover, we determined whether transformation could be facilitated by UV-B exposure or ectopic hTERT expression.

Results: HPV5, HPV8, HPV38 and HPV16 E6/E7 expression invariably resulted in an extended lifespan in all donors. This was followed by immortality in 1/3 HPV5, 2/3 HPV38 and all HPV16 containing transductants. An extended lifespan, without evidence of immortalization, was observed in a subset of HPV15 (1/3), HPV20 (1/3) and HPV24 (2/3) transductants. Elevated hTERT mRNA expression and telomerase activation was limited to cells that acquired an immortal phenotype, with highest levels in HPV16E6/E7 expressing cells. No additive effect was found upon UV-B exposure (600-1200 J/m2) orectopic hTERT expression. Raft cultures of HPV16 and HPV38 transduced keratinocytes revealed a loss of differentiation, which became more severe with passaging. In HPV8 E6/E7 expressing cells differentiation was disturbed, though not lost. HPV5, HPV10, HPV15 and HPV20 transduced keratinocytes closely resembled parental cells.

Conclusions: Under growth conditions supporting immortalization of keratinocytes by HPV16, only HPV38 revealed immortalization in more than half of donors. Immortalization by other betaPVs was either infrequent (HPV5) or absent, suggesting differential immortalization properties of the betaPVs studied.

HPV20E6 ONCOPROTEIN MODIFIES EPITHELIAL DIFFERENTIATION AND INDUCES LIPID ACCUMULATION

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The molecular mechanisms involved in the pathogenesis of papillomavirus associated cutaneous lesions have not been elucidated. Epidemiological studies point to the involvement of a large number of HPV types in such lesions and limited published data indicate a varying range of cellular proteins interacting with their oncogenes. We have previously demonstrated that the chronic exposure to UV-irradiation of transgenic HPV20E6/E7 mice resulted in the formation of papillomas and malignant lesions of the skin. The present study describes disorganized and erratic differentiation of retrovirally–transduced HPV20E6-expressing keratinocytes in organotypic raft cultures. Cellular proteins p53, p21CIP1 and Np63 were modified in these cultures and expressed throughout all epidermal layers, whereas proliferation was not markedly affected as measured by BrdU incorporation and expression of PCNA and keratin 16. Expression of the stratification marker keratin 14 was extended into most of the suprabasal layers and keratin 10 expression was delayed. HPV20E6 induced a marked decrease in the expression of involucrin and loricrin. Ultrastructural analyses of these HPV20E6 expressing cultures indicated an accumulation of lipid droplets which was also visualized by Oil Red O staining. The epithelial barrier and tight junctions were perturbed. Disruption of the skin barrier is related to a number of cutaneous diseases, mostly connected with underlying genetic and immune disorders. Future studies will provide insight into whether infections with cutaneous HPV types participate in the pathogenesis of these diseases.

O-15.06

MASTOMYS COUCHA: A MODEL FOR PV-INDUCED PATHOGENESIS OF THE SKIN

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The rodent Mastomys coucha is latently infected with Mastomys natalensis papilloma virus (MnPV). These animals are unique in spontaneously developing multiple benign skin tumors such as papillomas and keratoacanthomas, for which MnPV is the etiological agent. Previous studies demonstrated that MnPV persistence and viral load correlates with the development of skin tumors, thus viral DNA and the virion itself can be found in these lesions. We recently discovered a novel virus, Mastomys coucha papilloma virus 2 (McPV2), which is the first described animal papillomavirus inducing anogenital lesions in its natural host. McPV2 has a similar tropism as MnPV but is apparently less abundant in our colony.

To investigate immunological events during infection, a variety of animals was tested for the presence of both virus types and humoral responses against viral proteins. Here, a strong correlation between high viral copy numbers and L1-specific antibodies predominantly in older, tumor-bearing animals was detected. Interestingly, in contrast to the early proteins E6/E7, we found extensive antibody titers against E2 also in tumor-free animals, which were even higher than the serum responses against the L1 protein. Follow-up studies revealed that E2 seropositivity marks the latent infection state and precedes tumor formation.

Mastomys couch represents an excellent model to study molecular and immunological aspects of virally induced pathogenesis of the skin. Furthermore, these animals will serve as an in vivo system to investigate prophylactic and therapeutic approaches against papillomavirus caused epithelial tumors. In the meantime, we produced virus-like particles and currently test the efficiency of a prophylactic vaccine in the prevention of MnPV infections and the associated skin papillomas.

IDENTIFICATION OF B-CELL EPITOPES ON VLPS OF CUTANEOUS SPECIES ALPHA-HPVS

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Native virions and virus-like particles (VLPs) of the mucosal HPV types, such as types 16 and 18, have been characterized in detail structurally and immunologically. However, little attention has been paid to particle structure and immunogenicity of cutaneous HPV types that may cause skin warts.

We have generated 94 monoclonal antibodies (MAbs) against VLPs of HPV types 2, 27, and 57 (Alphapapillomavirus species 4). They were screened for reactivity to L1 proteins from 18 different PV types, binding to linear and conformational epitopes, and neutralization of HPV 2, 27, 57, and 16 pseudovirions.

Of 58, 28, and 8 MAbs generated against HPV types 2, 27, and 57 respectively, 21, 1, and 3 exhibit type-specific reactivities to linear and conformational epitopes. The remaining 59 MAbs are cross-reactive to both linear and conformational epitopes. One MAb raised against HPV 27 VLPs neutralizes HPV 27 pseudovirion infection and three MAbs generated against HPV 57 VLPs neutralize HPV 57. Interestingly, all four neutralizing MAbs recognize a type-specific linear surface epitope that was mapped to the variable BC loop region.

This is the first analysis of B-cell epitopes on VLPs of cutaneous alpha PVs. It demonstrates that VLPs of the closely related HPV types 2, 27, and 57 contain numerous type-specific linear and conformational epitopes. Our findings provide valuable information for the development of a prophylactic vaccine against these HPV types.

O-15.08

MERKEL-CELL-POLYOMAVIRUS IS PREVALENT IN SKIN AND MUCOSA INDEPENDENT OF HPV-STATUS

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Background: Merkel cell polyomavirus (MCV) is a novel polyomavirus recently found in Merkel cell carcinoma (MCC). Objectives: We performed a retrospective study to determine the prevalence of MCV-DNA in MCC and in normal and lesional HPV-positive and HPV-negative skin and mucosa samples of immunocompetent and immunosuppressed patients.

Methods: Using nested and single-round PCR, 793 samples from different body sites of 230 individuals were analyzed for the presence of MCV-DNA.

Results: We confirmed the high prevalence of MCV in MCC (88%) and MCC-metastases (100%). However, MCV-DNA was also found in non-MCC skin-tumours (16%) and normal perilesional skin (24%) of immunocompetent patients, in anogenital and oral samples (31%) and eyebrow-hairs (50%) of HIV-positive men, in the skin and mucosa of a patient with an immunosuppression-syndrome, and in skin-swabs of healthy controls (1st swabs: adults 71%, children 40%). Concerning HPV-DNA-status, there were no significant differences between MCV-positive and MCV-negative samples. In HIV-positive men, MCV was more frequent in normal (37%), than in benign (29%), dysplastic (28%) or invasive (14%) samples. Persistent MCV-infection could be demonstrated in 5 of 11 healthy families, with the youngest MCV-positive child being less than 1 month old. MCV-DNA was not found in cerebrospinal fluid of HIV-positive men or in urine of renal transplant recipients.

Conclusions: MCV is widespread in the normal population and in immunosuppressed individuals without MCC. In contrast to high-risk HPV-DNA-positivity, MCV-DNA-positivity was not associated with the presence of mucosal premalignant or malignant lesions of HIV-positive men. Therefore, MCV probably does not play a role in the development of HPV-induced anogenital dysplasia. Similar MCV-prevalence in non-MCC skin-tumours and normal perilesional skin and lower viral loads than in MCC suggest that MCV is not involved in the development of non-MCC skin tumours.

POSTER ABSTRACTS SESSION 15

POSTER SESSION III TUESDAY 20.00: ODD NUMBERS

POSTER SESSION IV

TUESDAY 21.00: EVEN NUMBERS

ANTIBODY RESPONSES TO 26 SKIN HPV IN EUROPE AND AUSTRALIA

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Background: Ultraviolet (UV) radiation is the main risk factor for cutaneous squamous cell carcinoma (SCC). Infections with skin HPV types have also been linked to the development of SCC but little is known about the natural history of these infections.

Objective: To understand factors associated with antibodies to skin HPV, particularly exposure to UV light.

Methods: Using Multiplex HPV serology, a recently developed high-throughput method that allows the analysis of up to 1500 sera per day for antibodies to up to 100 different antigens simultaneously, we analyzed sera from 807 immunocompetent controls of a multi-center SCC case-control study conducted in three countries (The Netherlands, Italy, and Australia) with different sunlight intensity for antibodies to 26 skin HPV types from five phylogenetic genera.

Results: Overall HPV seroprevalence was similar across countries (50-57% for beta types, 40-48% for gamma types), and the five and two most frequent beta (HPV 8, 15, 17, 38, and 49) and gamma (HPV 4 and 65) types, respectively, were the same in all countries. Highest seroprevalences for 24 out of 26 skin HPV types were observed in Italy (14 types) and Australia (10 types). Seroprevalences among men were generally higher than among women, and male sex was significantly associated with both beta and gamma antibodies in Australia. The only statistically significant measure of sun sensitivity or UV exposure associated with skin HPV seroprevalence was found for higher levels of sun exposure on weekend days in Australia and beta HPV antibodies.

Conclusions: We show that type spectra and overall HPV seroprevalence are similar in different countries, and our data do not support a strong role of UV exposure in the development of HPV antibodies.

P-15.11

A LONGITUDINAL STUDY OF BETAPAPILLOMAVIRUS INFECTIONS IN AN AUSTRALIAN COMMUNITY

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Although cutaneous betapapillomaviruses (beta-PV) are a possible cause of actinic keratoses (AK) and cutaneous SCC, little is known about whether beta-PV persists over time in the skin. We assessed the extent to which betaPV infections persisted over 8 years in a subtropical Australian community and whether beta-PV persistence increased the risk of AK. In 1996 and 2003 eyebrow hairs were collected from 171 unselected participants of the community-based Nambour Skin Cancer Study. AKs were scored during skin examinations at baseline and again in 2007 for 138 of the participants. Details of skin type and sun exposure were collected by standard questionnaire. Viral DNA was isolated from eyebrow hairs and analysed with a detection-genotyping system able to identify 25 betaPV-types.

Participants' mean age at baseline was 50 years and 49% were males. In 1996 a total of 413 beta-PV infections were found in 73% of participants; in 2003 this rose to 490 infections in 85% of participants. Of the total beta-PVs, 211 (30%) were found to persist in the same individuals 1996-2003. Age was significantly associated with betaPV persistence: those over 60 were over twice as likely as those under 40 to have type-specific betaPV DNA present on both occasions. Skin type, occupational sun exposure and sunburn history were not associated with betaPV persistence. Presence of betaPV DNA at baseline was resulted with a doubling of odds of having AKs on the face in 2006 while persistence of betaPV DNA at follow-up in 2003 slightly strengthened the betaPV-AK association.

We showed that around one third of betaPV viruses persist in community members over an 8-year period, especially in people over 60. While facial AKs were associated with betaPV detection at baseline, the association seemed stronger with persistent virus, suggesting that long-term betaPV persistence enhances the risk of AK independent of age.

STAPHYLOCOCCUS AUREUS AND SQUAMOUS CELL CARCINOMA OF THE SKIN

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Background: Squamous cell carcinoma of the skin (SCC) is a tumour with greatly increased incidence among immunosuppressed patients and therefore an infectious cause of SCC has long been sought.

Objectives: To investigate whether Staphylococcus (S.) aureus is more common in SCC than in other skin lesions or in healthy skin.

Methods: We performed a hospital-based case-control study of S. aureus and biopsies of SCC (n=82), basal cell carcinoma (BCC) (n=142), actinic keratosis (AK) (n=57) and seborrhoeic keratosis (SK) n=72) in comparison with biopsies from healthy skin of these 353 immunocompetent patients with a S. aureus-specific PCR, targeting the nuc gene.

Results: Presence of S. aureus DNA was strongly associated with SCC (29.3% positive specimens, adjusted odds ratio (OR): 6.23, 95% Confidence Interval (CI): (3.10-12.53)) compared to healthy skin (5.7% positive specimens). There was also a tendency for association of S. aureus with AK, but no association was found for BCC or SK. Analysis using cotton swab samples taken on top of the lesions and from healthy skin gave similar results (adjusted OR for SCC compared to healthy skin: 2.67, 95% CI: 1.47-4.83).

Conclusion: There is a strong association between SCC and presence of S. aureus. The study design used can not determine whether the association implies that presence of S. aureus might influence carcinogenesis or whether it may imply that SCC has an increased susceptibility to S. aureus colonization.

P-15.13 SIMULTANEOUS CONDYLOMA ACUMINATUM, BOWEN'S DISEASE AND PAGET'S DISEASE

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An 83-year-old Japanese man with multiple vertucous papules clustering on a plaque located on the frontal aspect of the scrotum. Histologically, there were three distinct epithelial changing compatible with condyloma acuminatum, Bowen's disease, and extramammary Paget's disease (EMPD). By in situ hybridization, the zone of condyloma acuminatum was positive for HPV 6 and well demarcated from HPV 31-positive Bowen's disease. EMPD was negative for targeted HPV 6/11/16/18/31/33 probes. Immunohistochemically, Paget's cells expressing cytokeratin 7 were distributed as scattered single cells or clusters mainly in the lower part of the HPV 6/31-positive epithelium. To the best of our knowledge, this is the first reported case of the occurrence of condyloma acuminatum, Bowen's disease, and EMPD within the same lesion.

PUTATIVELY NEW HPV IN WARTS OF IMMUNOSUPPRESSED ORGAN-TRANSPLANT-RECIPIENTS

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Background Up to 90% of immunosuppressed organ transplant recipients (OTR) develop persistent, atypical cutaneous warts. Wart-associated HPV types are considered to induce this disease, however approximately 50% were not infected with these types. Objective To investigate cutaneous warts of OTR for the presence of unidentified HPV types. Methods We analyzed 69 cutaneous warts of 40 immunosuppressed OTR by the rolling circle amplification technique (RCA) to detect putatively new HPV types. Genomic fragments of approximately 450 bp were subsequently generated by PV specific PCR with degenerated primers and were sequenced. Moreover, full length HPV genomes of a subset were cloned and sequenced. Phylogenetic analyses of these new types were performed using Maximum Likelihood and Bayesian approaches. Type-specific PCR were established to examine the prevalence of 12 new HPV types. Results Twelve putatively new HPV types were identified. Two belong to the alpha-PV, six nested in the beta-PV (three on extraordinarily long branches), and four were closely allied to the gamma-PV. Conclusions The number of warts with so far unidentified HPV types was remarkably high. This may indicate that new HPV types are associated with atyptical warts in immunosuppressed OTR.

P-15.15

PREVALENCE AND PREDICTIVE FACTORS OF BETAPAPILLOMAVIRUS INFECTION

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Background: In epidemiological studies betapapillomavirus (betaPV) infection has been found to be associated with squamous-cell carcinoma.

Aim: To investigate the frequency and possible risk factors of betaPV infection in a population of individuals who never have had cutaneous squamous cell carcinomas.

Methods: Using a standard study protocol, information about age, sex, UV irradiation, skin type, actinic keratoses, and smoking was obtained from 845 immunocompetent and 560 immunosuppressed participants. Eyebrow hairs were collected and betaPV detection and genotyping of the 25 established betaPV types was performed with the PM-PCR RHA method.

Results: The overall frequency of beta-PV positive participants was 89% in the immunocompetent and 90% in the immunosuppressed population. HPV23 was the most prevalent type followed by HPV36. The median number of infecting betaPV types was 5.0 in both study groups. The immunosuppressed participants were 9 years younger than the immunocompetent participants. After adjustment for age and sex, betaPV (co)infection was significantly associated with immunosuppression with an odds ratio of 1.6 (95% CI 1.1;2.5) for betaPV infection and 1.5 (95% CI 1.1;1.9) for betaPV coinfection. Furthermore increasing age in the immunocompetent participants and duration of immunosuppression in the immunosuppressed patients were associated with betaPV (co)infection. In both groups sex, smoking, skin phototype, painful sunburns and sun-exposure were not consistently associated with betaPV (co)infection.

Conclusion: BetaPV infections are extremely common in both the immunocompetent and immunosuppressed participants, whereas type distribution of betaPV was similar in both groups. Increasing age and (duration of) immunosuppression were identified as risk factors for betaPV (co)infections.

HPV109, 112 AND SW-1 ISOLATED FROM CUTANEOUS AND MUCOSAL LESIONS

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Background: There exists a multitude of putative HPV types, where only short PCR amplimer sequences are known. Cloning and sequencing of full-length genomes is required as a basis for further studies of these viruses.

Objectives: To clone and sequence the complete genomes of three HPV types present in a skin cancer, in an "HPV-negative" condyloma and in a CIN I lesion.

Methods and results: HPV109 was selected for cloning as it was present in a squamous cell carcinoma (SCC) of the skin at a high copy number (10 copies per cell). HPV109 is a new species within the genus Gamma as it is only 64% similar to the most closely related type (HPV4). HPV112 was selected for cloning as it was present in a condyloma acuminate that was "HPV-negative" by general primer PCR. Also HPV112 is a new species within the genus Gamma, as it is only 64% similar to the most closely related type (HPV65). The third HPV-type was selected for cloning after the sequence had been found in four low grade cervical lesions. A fragment of this virus had previously been sequenced from a psoriatic lesion on the skin and denoted SW-1. We cloned SW1 from a CINI lesion. It is 84% similar to HPV84 and belongs to the genus Alpha-3.

Conclusions: HPV109 and 112 represent two new species in the HPV-genus Gamma. SW-1 is a new Alpha-3 virus found in low grade cervical lesions.

P-15.17

HIGH THROUGHPUT SEQUENCING OF PAPILLOMAVIRUSES IN POSSIBLY HPV-ASSOCIATED LESIONS

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Background: Both squamous cell carcinomas of the skin (SCC) and healthy skin commonly contain multiple infections with many cutaneous HPV types. Reliable identification of known and unknown HPVs in SCC has required laborious cloning and sequencing.

Objectives: To sequence all HPVs present in SCCs and other possibly HPV-associated lesions using high throughput sequencing (HTS) methods that do not require cloning.

Methods and results: The viral DNA fractions from specimen pools of possibly HPV-associated lesions (SCCs, actinic keratosis (AKs), keratoacanthomas and "HPV-negative" condyloma acuminate) were subjected to HTS using GS FLX technology. Overall, 58223 contigs were obtained.

After preamplification with HPV general primer PCR(FAP), HTS identified 53 known HPV types or putative HPV types and eleven putative new HPV types in a pool of biopsies from 37 SCCs and 36 AKs. In a pool of 91 keratoachantomas, 51 HPV types or putative HPV types and nine putative new HPV types were found.

The SCCs and AKs had previously been tested by 3 different labs using cloning and sequencing after PCR. Although multiple types had been identified in the many clones that were picked, HTS identified many additional HPV types in the same samples.

HTS of viral genomes without preamplification provided sequence of 2 putatively new HPV types, one from a pool of 27 condylomas "negative for HPV" with general primer PCR and one in a pool of 40 SCCs of the skin that had also been HPV-negative by PCR. In addition, sequences of 3 known HPV types were obtained: two in a pool of 93 AK and one in the pool of "HPV-negative" condylomas.

Conclusions: The HTS technology identifies the sequences of many HPV types (both known and previously unknown) in several putatively HPV-associated lesions, also in tumors where HPV was not possible to demonstrate using general primer PCR.

BETA-PAPILLOMAVIRUS TYPE DISTRIBUTION AND PAPILLOMATOSIS IN CUTANEOUS SQUAMOUS CELL CARCINOMA

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Background: Beta-papillomavirus (beta-HPV) infection has been associated with some cutaneous squamous cell carcinomas (SCC), although the characteristics of beta-HPV-associated SCC are ill-defined. Objective: A detailed case-series was conducted to investigate beta-HPV type distribution in cutaneous SCC by the presence of papillomatosis. Methods: Patients (n=131) ages 18-80 with histologically-confirmed cutaneous SCC were recruited from a dermatology clinic. A 3mm punch was obtained from each tumor (n=149) and snap frozen. DNA from 25 beta-HPV types (5, 8, 9, 12, 14, 15, 17, 19, 20, 21-25, 36, 37, 38, 47, 49, 75, 76, 80, 92, 93, 96) was measured using multiplex PCR with HPV type-specific primers and array primer extension for typing. A dermatopathologist reviewed 133 SCC tissues for the presence of a papillomatous growth pattern ("papillomatosis"). Results: Of 149 tumor tissues tested for HPV, 92 (62%) were positive for at least one beta-HPV type, including 53 (36%) positive for 1-2 types and 39 (26%) positive for 3+ types. The most common HPV types observed were HPV23 (24%), HPV38 (17%), and HPV80, HPV17 and HPV5 (13% each). Among 89 males, HPV23 was most common (24%), followed by HPV 38 (21%) and HPV5 (15%). Among 42 females, HPV23 (23%) was followed by HPV15 (20%) and HPV8 (16%). Among 59 tumors exhibiting papillomatosis, HPV23 (27%) was most common, followed by HPV15, HPV17 and HPV80 (15% each). Among 74 tumors without papillomatosis, HPV38 was most common (20%), followed by HPV23 (19%), HPV5 (12%) and HPV36 (12%). Among 16 patients from whom multiple SCC tumors were obtained, 11 had beta-HPV in multiple tumors, with the greatest intra-individual type-specific concordance observed for HPV15 (80%) and HPV80 (75%). Conclusions: In this large case-series, beta-HPV type distributions in SCC differed by gender and papillomatosis in tumor tissues.

P-15.20

CUTANEOUS HPV DETECTION IN RENAL TRANSPLANT PATIENTS WITH SKIN CANCER.

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BACKGROUND Human papillomavirus (HPV) may be involved in non-melanoma skin carcinogenesis in conjunction with sun exposure and immunosuppression. HPV in frequently detected in normal skin is not uncommon. Its carcinogenic role needs to be proven.

OBJECTIVES To determine the presence of different skin HPV types (beta and gamma) among renal transplant (RT) patients with skin cancer in Spain.

METHODS 42 RT patients of Caucasian origin that developed skin cancer (Squamous cell carcinoma, SCC, or Basal cell carcinoma, BCC) were included. These patients contribute with 186 cutaneous scrapes systematically collected in specific skin areas based on sun exposure and 172 biopsies from lesions (83 cancerous and 89 non cancerous). A beta and gamma cutaneous consensus PCR (72bp) and Reverse line blotting system was used for detection and typing of 24 cutaneous HPV types. Samples were tested at the Catalan Institute of Oncology HPV lab (Barcelona) and at the Department of Pathology from the Vrije Universiteit Medical Centre (Amsterdam).

RESULTS HPV was detected in 75,6% of the biopsies and 76.3% of the scrapes. The median number of HPV types identified were 4 in scrapes and 4 in biopsies. In scrapes, the types most often found were HPV15 (53,5%), HPV20 (52,8%), and HPV23 (51,4%). In SCC/ BCC biopsies the most frequent type identified was HPV15 (52,3%), followed by HPV23 (33,8%) and HPV20 (27,7%).

CONCLUSIONS HPV 15 and 23 were found most frequently in both scrapes and biopsies. Further analysis by site and by type of sample and by lesion will be presented.

ANALYSIS OF MUCOSAL AND CUTANEOUS HPVS IN OESOPHAGEAL CANCER

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Background: Epidemiologic evidence points to a connection between viral infections by the human papillomavirus (HPV) and a subgroup of squamous cell carcinomas of the oropharynx. Still controversial is the association of HPV infection with oesophageal neoplasia.

Objectives: To investigate the presence of mucosal as well as cutaneous HPVs in squamous cell carcinoma and adenocarcinoma of the oesophagus.

Study design: HPV DNA has been searched by PCR and characterized by nucleotide sequence analysis in paraffin embedded biopsies from Italian patients with oesophageal squamous cell carcinoma (n = 36), sarcomatoid cell carcinoma (n = 1), adenocarcinoma (n = 20) and esophagitis lesions (n = 27).

Results: A broad spectrum of HPVs, primarily cutaneous types was demonstrated in 27.8% (10/36) of squamous cell carcinomas with a significantly higher frequency in well (G1) and moderately (G2) differentiated grades (47.3%, 9/19) compared to poorly (G3) differentiated (5.9%, 1/17) squamous cell carcinoma (p = 0.008), and in 10% (2/20) of adenocarcinomas and in 29.6% (8/27) of esophagitis. HPV types detected included mucosal types HPV 6 and 16, cutaneous types HPV 8, 15, 20 and 25; and the putative new HPV types X14, X15, DL473, PPHL1FR and CJ198.

Conclusions: The higher detection frequencies of cutaneous HPV types in esophagitis lesions and in well and moderately differentiated squamous cell carcinoma, and the lower frequency in poorly differentiated squamous cell carcinoma and in adenocarcinoma, lead to the hypothesis that HPV infection may have a possible association with the subset of differentiated oesophageal squamous cell carcinomas.

P-15.22

P16INK4A POSITIVITY IN NON-MELANOMA SKIN CANCER OF RENAL TRANSPLANT RECIPIENTS

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Background: Immunosuppression is a predisposing factor for non-melanoma skin cancer (NMSC), in particular in renal transplant recipients. Mucosal HR-HPV are suggested to play a role in the carcinogenesis of these tumours, in particular in Bowen's disease.

Objective: Since p16INK4a is used as a biomarker for transforming HR-HPV infection in cervical lesions, we asked whether p16INK4a can be used to identify HR-HPV related NMSC. Therefore we investigated p16INK4a expression and mucosal HR-HPV infection in a series of renal transplant patients (RTX) with multiple NMSC and tumours of immunocompetent controls.

Methods: 88 NMSC of 37 RTX recipients and 48 NMSC of immunocompetent controls, including basal cell carcinomas (BCC), Bowen's diseases (BD) and squamous cell carcinomas (SCC) were immunohistochemically stained for p16INK4a expression. Multiplex PCR based typing of mucosal HR-HPV was performed from the same paraffin embedded specimens. Results: Overall a large diversity of p16INK4a expression patterns was observed in NMSC, including classical diffuse staining as observed in cervical dysplasia but also strong inhomogeneous clusters and broad scattered p16INK4a expression in single cells. A strong p16INK4a expression was observed in 63% (55/88) of NMSC from RTX recipients and 56% (27/48) of NMSC from immunocompetent individuals. HR-HPV could only be detected in a subset of the tumours in immunosuppressed and immunocompetent individuals, but with a significant correlation to p16INK4a expression (p<0.005).

Conclusions: Since mucosal HR-HPV types could only be detected in a small subset of p16INK4a positive lesions, a transforming role of other HPV classes and other reasons than HPV that might cause p16INK4a up-regulation in NMSC should be considered. Especially the different p16INK4a expression patterns should be evaluated carefully in correlation to other molecular markers in future studies. However, these data further corroborate the notion that carcinogenic mucosa types can infect the skin and cause dysplatic lesions in immunocompromised individuals.

TYPE-SPECIFIC PERSISTENCE OF ASYMPTOMATIC CUTANEOUS HPV INFECTIONS IN HEALTHY SKIN

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Background: Cutaneous human papillomavirus (HPV) types are commonly found in normal skin, and some of them have been suspected to play a role in the development of non-melanoma skin cancer (NMSC).

Objectives: To examine if certain HPV-types persist over time and if HPV-types are shared within families.

Methods: Swab samples from foreheads were collected for three longitudinal studies from one family with a newborn baby. Two families were also followed up 6 years later from a previous study, and four healthy females were sampled weekly for four months. Specimens were tested for HPV-DNA by PCR using the broad range HPV-type primer pair FAP59/64. The positive samples were HPV-type determined by cloning and sequencing and obtained sequences were compared in GenBank.

Results: Five specific HPV-types were isolated from the family with a newborn, with HPV-5 and FA67 being found at various time points and prevalence rates in all four members of the family. From the six-year follow-up study, only one family member did not have a HPV-type that was detected six years earlier. Six of the family members were found to have at least one of the HPV-types identified in the family six years earlier. Many of the HPV-types identified were shared within the families studied. Among the four healthy individuals, 11%, 65% and 56% were HPV-DNA positive with one individual HPV-negative.

Conclusions: Specific cutaneous HPV-types persist over long periods of time in healthy skin in most individuals investigated and certain HPV are shared between family members.

P-15.24

PROSPECTIVE AND RETROSPECTIVE STUDIES OF HPV AFTER ORGAN TRANSPLANT

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Renal and cardiac organ transplant recipients (OTR) experience a high incidence of squamous cell skin cancer (SCSC), which is thought to develop in part due to infection with cutaneous HPVs. We are enrolling OTR in two complementary studies to investigate the putative role of HPV in SCSC after transplant. Within a retrospective cohort of OTR in the Seattle area, a nested case-control study will assess 1) if there are high-risk cutaneous HPV types that are associated with an increased risk of SCSC; 2) whether pre-transplant HPV antibodies are associated with future SCSC risk; and 3) if patient characteristics (including history of sun exposure, skin type, eye color, medication use, and other exposures) gathered at in-person interview and integrated with measures of HPV types together determine the association between HPV, risk factors, and SCSC in OTR. The second study is designed to explore the natural history of beta HPV infection in OTR with longitudinal collection of blood and eyebrow samples over a 2-year period. Samples will be collected pre-transplant and at 1 and 2 years after transplant. In this longitudinal study we will assess 1) whether the number of HPV DNA types, antibody response, or viral persistence increases with time since transplant and 2) the effect of changes in levels of cell-mediated immunity as measured by a CD4 assay on HPV infection status (as measured by various markers of HPV) over time. Our studies may contribute to a more complete understanding of the role of HPV in SCSC and the role immunosuppression plays in HPV activation. We plan to present preliminary data from this ongoing study.

EPIDERMODYSPLASIA VERRUCIFORMIS TREATED USING Q-SWITCH RUBY LASER

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BACKGROUND: Epidermodysplasia verruciformis (EV) is an autosomal recessive disease characterized by the lifelong eruption of disseminated tinea versicolor-like lesions. Although various treatments have been applied to treat EV, cure of EV is still unsuccessful. Pigmented lesions including pre-malignant/malignant skin lesions develop in patients with EV after a long period of HPV infection. OBJECTIVE: To evaluate the clinical effects of Q-switch ruby laser therapy on pigmented skin lesions. METHODS: Widespread pigmented lesions of a patient with EV were treated with Q-switch ruby laser. RESULTS: Following laser therapy, lesions healed completely within two weeks with slight scarring. Twelve months after treatment, a few lesions had recurred. Marked clinical improvement has been demonstrated. CONCLUSION: Although permanent cure of EV cannot be achieved, Q-switch ruby laser therapy is useful in better control of HPV-induced pigmented lesions.

SESSION 16

HPV AMONG THE HIV-INFECTED

PROGRAMME

THE 25TH INTERNATIONAL PAPILLOMAVIRUS CONFERENCE MAY 8-14 2009, MALMÖ, SWEDEN

SWEDEN			
TIME	NUMBER	TITLE	ROOM
2009-05-12			
SESSION 16: HPV AMONG THE HIV INFECTED			
14.00-14.12	O-16.01	SCREEN-AND-TREAT USING HPV TESTING IS HIGHLY EFFECTIVE AMONG HIV-INFECTED WOMEN L Kuhn, L Denny, M DeSousa, T Wright	
14.12-14.24	O-16.02	SAFETY AND IMMUNOGENICITY OF GARDASIL® IN HIV-INFECTED CHILDREN AB Moscicki, A Weinberg, L-Y Song, E Handelsman, J Patterson, A Saah, D Radley, JS Read, C Sattler, M Levin	
14.24-14.36	O-16.03	HLA ASSOCIATIONS WITH HPV/SIL DECREASE WITH IMMUNOSUPPRESSION IN HIV-POSITIVE WOMEN H Strickler, M Martin, X Xue, Rd Burk, A Hildesheim, H Minkoff, L. S Massad, K Anastos, A Kovacs, M Fazzari, G D'Souza, T Harris, M Young, V Bonagura, J Palefsky, M Carrington	
14.36-14.48	O-16.04	HIGH EXPRESSION PROTEINS IN HIV/HPV CO-INFECTED WOMEN A Nicol, G Nuovo, B Grinsztejn, A Pires, S Souza, A Tristão, F Russomano, L Velasque, K C Pessanha, J R Lapa e Silva, C Pirmez	
14.48-15.00	O-16.05	HIV AFFECTS HPV CONCORDANCE AND VIRAL LOAD IN COUPLES Z Z.A Mbulawa, D Coetzee, D Marais, I Gustavsson, M Hoffman, U Gyllensten, Al Williamson	1
15.00-15.12	O-16.06	RISK OF HPV-ASSOCIATED CANCERS AMONG PERSONS WITH AIDS A Chaturvedi, Mm Madeleine, R Biggar, E Engels	
15.12-15.24	O-16.07	ARE EXTIRPATIVE PROCEDURES FOR CIN EFFECTIVE IN HIV-POSITIVE WOMEN? LI Reimers, D Daniel, S Sotardi, L G Chiu, A Van Arsdale, D L Wieland, J M Leider, D J Gerry, D Gupta, G L Goldberg, H Strickler, Mh Einstein	

Oral presentations

SCREEN-AND-TREAT USING HPV TESTING IS HIGHLY EFFECTIVE AMONG HIV-INFECTED WOMEN

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Background: Cervical cancer prevention should address the special needs of HIV-infected women.

Objective: To examine efficacy of screen-and-treat among HIV-infected women.

Methods: 6553 unscreened women 35-65 years in Cape Town, South Africa were randomized to: (1) cryotherapy if HPV positive, (2) cryotherapy if visual inspection with acetic acid (VIA) positive, or (3) delayed treatment (controls). HPV testing used Hybrid Capture II (Digene). At baseline 956 women (14.6%) were HIV-positive. All women underwent colposcopy/biopsy 6 months after randomization. Two-thirds were followed by design at 12, 24 and 36 months when colposcopy/biopsy was repeated. The endpoint was adjudicated histological diagnosis of cervical intraepithelial neoplasia grade 2/3 (CIN2+).

Results: At baseline 45.9% of HIV-infected were HPV-positive vs. 17.2% of uninfected women (p<0.0001). In controls, 14.9% of HIV-infected women had CIN2+ by 36 months vs. 4.6% of uninfected women (RR=3.27 95% CI: 2.21–4.83). Sensitivity of HPV testing was similar in HIV-infected (94%) and uninfected women (87%); but specificity was worse among HIV-infected. In intent-to-treat analyses in the screen-and-treat HPV arm, CIN2+ by 36 months occurred among 3.05% of HIV-infected vs. 1.43% of uninfected women. In comparison among controls, CIN2+ by 36 months occurred among 14.9% of HIV-infected and 4.6% of uninfected women. Screen-and-treat using HPV testing led to an 80% reduction in CIN2+ among HIV-infected women (RR=0.20 95% CI: 0.06 – 0.69) similar to reduction observed among uninfected women (RR=0.31 95% CI: 0.20 – 0.50).

Conclusions: HIV-infected women are at high risk of being co-infected with HPV and a third of HIV-HPV co-infected women have biopsy-confirmed CIN2+ within 36 months. Screen-and-treat using HPV DNA testing is an effective means of reducing cervical cancer precursor lesions and should be considered as part of HIV treatment programs.

O-16.02

SAFETY AND IMMUNOGENICITY OF GARDASIL® IN HIV-INFECTED CHILDREN

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Objective: The quadrivalent HPV vaccine (qHPV) was studied in perinatally HIV-infected by investigating the vaccine's safety and immunogenicity.

Methods: This randomized, double-blinded, placebo-controlled study stratified HIV-infected 7-12 year old boys and girls receiving optimal antiretroviral therapy into 3 groups according to their CD4% status. Vaccine was administrated at wks 0, 8 & 24 and blood for immunogenicity and CMI was drawn at Wk 28.

Results: A total of 126 children were enrolled (90 vaccine, 36 placebo recipients), 55 male. Median (95% CI) baseline CD4% and log10 plasma HIV RNA concentration (VL) were 34% (32; 39) and 2.6 copies/ml (2.4, 2.9), respectively, and were similar according to vaccine vs. placebo receipt. There were no treatment-related grade \geq 3 adverse events. Comparing baseline and week 28 data, there were no significant changes in either CD4 % or plasma VL vaccination. All vaccine recipients were seropositive for HPV 6, 11 and 16 at 28 wks; and 97% for HPV 18 [geometric mean titer (GMT) of 549.4, 1416.6, 5230.6 and 916.4mMu/ml, respectively]. Among placebo recipients, less than 6% were seropositive at 28 wks. Among 60 vaccinees, 36 had IFNg-ELISPOT responses to HPV 16, and 31 responded to HPV 31. One of 21 placebo recipients had a positive response to HPV 16 and 31. The magnitude of the response was highest in the least immunocompromised vaccinees.

Conclusions: QHPV was generally safe in HIV-infected children and nearly 100% of those initially seronegative seroconverted. Antibody titers against HPV 6 and 18 were lower compared to results previously reported for healthy children. Some children were seropositive at baseline or became positive during the trial, underscoring the importance of immunizing children before sexual activity. A majority of HIV-infected children developed cell-mediated immunity, which may confer cross protection against HPV vaccine-related types not included in the vaccine.

HLA ASSOCIATIONS WITH HPV/SIL DECREASE WITH IMMUNOSUPPRESSION IN HIV-POSITIVE WOMEN

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Human leukocyte antigens (HLA) are highly polymorphic molecules that present antigens to T-cells. However, the diversity of HLA raises issues of multiple comparisons, and the exact HLA alleles associated with cervical HPV/neoplasia remain uncertain following conflicting reports. We focused on a narrow list of HLA alleles with good (if inconsistent) prior evidence of association with HPV/neoplasia. Second, we studied both HIV-seronegative and HIV-seropositive women to determine whether there was a biologic gradient between the strength of these associations and immune status. Women with low CD4+, we reasoned, were unlikely to mount strong immune responses regardless of their HLA. Third, we assessed the consistency of the findings across several stages of cervical tumorigenesis from infection to HSIL.

High definition HLA class I/II genotyping was conducted in a large, long-term prospective cohort of HIV-positive/negative women, called the WIHS. Women were followed semiannually with cytology and HPV PCR. All cases of HSIL, HPV-16/18 infection, and incident SIL were genotyped along with a representative subcohort of 300 HIV-negative, 500 HIV-positive women. Several strong, a priori predicted, associations between HLA allelotypes and HPV/SIL were observed. In particular, DRB1*1501 was associated with HPV16 (OR=10.7;95% CI:2.55-45), HPV18 (OR=7.65;95% CI:1.82-32), and HPV 16/18-positive HSIL (OR=22.7;95% CI: 1.99-259), among HIV-negative but not HIV-positive women (Pinteraction<0.05). Similar results were observed for DRB1*0301, and the predicted inverse associations between DRB1*13 and HPV16/18 were found. Among HIV-positives these associations showed weakening with worsening host immune status. The data provide confirmation of several reported HLA associations with HPV/SIL. The most striking finding, though,

The data provide confirmation of several reported HLA associations with HPV/SIL. The most striking finding, though, was that these HPV/SIL associations relate to HLA class II (relevant to T-helper) and not class I (relevant to CTLs). This has gone largely unappreciated, and may point to a fundamental aspect of immunity in the female genital tract.

O-16.04

HIGH EXPRESSION PROTEINS IN HIV/HPV CO-INFECTED WOMEN

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Background: The oncoproteins of human papillomavirus (HPVs) directly effect cell-cycle control. We hypothesize that regulatory and cell cycle protein expression might be additionally modified in the cervix of HIV/HPV co-infected women. Methods: We analyzed the expression of Rb, p27, VEGF and Elf-1 transcriptor factor by immunohistochemistry in 163 paraffin-embeded cervical samples using Tissue Micro-Array (TMA) and correlated this to HIV-1 and HPV infection. Results: HIV/HPV co-infection was associated with a significant increase in expression (p<0.001) of VEGF and p27 in both low and high grade CIN when compared to the cervices of women infected by HPV alone. Decreased Rb expression was evident with increased CIN grade in the cervices of women infected with HPV alone (p=0.003 average of cells/mm2 in CIN I: 17.9, CIN II/III: 4.8, and tumor 3.9). Rb expression increased 3-fold for both low and high grade CIN with HPV/HIV-1 co-infection compared to HPV infection alone but did not reach statistical significance. There was a significant increase in Elf-1 expression in HPV+/HIV- women with CIN II/III and tumor (average of cells/mm2 in CIN I: 63.8; CIN II/III: 115.7 and tumor: 112.0, p=0.005), in comparison to controls.

Conclusions: Co-infection of HPV and HIV leads to significant increase in the VEGF and p27 expression when compared to HPV+/HIV-negative infection that could facilitate viral persistence and invasive tumor development.

HIV AFFECTS HPV CONCORDANCE AND VIRAL LOAD IN COUPLES

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Background: This study examined the impact of HIV seropositivity (HIV+) on the prevalence, concordance and viral load of genital HPV infection in heterosexually active couples. Methods: Participants were 478 heterosexually active black couples; 206 were HIV seronegative (HIV-), 99 were HIV+ and 173 were HIV discordant (one partner was HIV+). Highrisk (HR) HPV viral loads in cervical and penile cells were determined by the hpVIR real-time polymerase chain reaction. Results: HPV concordance was found in 100 couples (21%) and 80% (80/100) of these shared 1 to 6 identical HPV type(s). Type-specific concordance of one HPV type was observed in 58 (73%) couples, 2 HPV types in 16 (20%) couples and 3-6 HPV types in 6 (8%) couples. HIV+ and discordant couples were significantly more likely to share \geq 2 HPV types compared to HIV- couples (38% 11/29; 6% 1/18 P=0.01 and 32% 10/31; 6% 1/18 P=0.04 respectively). Women sharing HPV types with male partners were found to have a significantly higher HR-HPV viral load per cell compared to women that were not sharing HPV types (median 6 per cell compared with 0.4 per cell, P<0.0001). Men sharing HPV with female partners had a higher viral load compared to men not sharing HPV with female partners but the difference was not significant. Conclusion: HIV+ and HIV discordant couples shared more types than HIV- couples indicating that HIV co-infection in

Conclusion: HIV+ and HIV discordant couples shared more types than HIV- couples indicating that HIV co-infection in one partner has a significant impact on HPV genital infection and transmission. Men and women sharing HPV types with their partner were found to have a higher HPV viral load compared to those who were not sharing HPV types. A high HPV viral load in women was associated with the presence of HPV in male partners suggesting that high HPV viral load may play a role in HPV transmission between partners.

O-16.06

RISK OF HPV-ASSOCIATED CANCERS AMONG PERSONS WITH AIDS

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Background: Although risk of HPV-associated cancers (anus, cervix, oropharynx, penis, vagina, and vulva) is increased among persons with AIDS, the relationship with immunosuppression is unclear. Furthermore, incidence trends for these cancers over time, particularly since the availability of highly active antiretroviral therapy (HAART) in 1996, are not well described.

Methods: Data on 499,230 individuals with AIDS were linked with cancer registries in 15 U.S. regions (1980-2004). We evaluated incidence of in situ and invasive HPV-associated cancers during 5 years of follow-up following AIDS diagnosis. Risk among persons with AIDS relative to the general population was measured using standardized incidence ratios (SIRs). For individuals diagnosed with AIDS during the HAART era (1996-2004), we evaluated the relationship of HPV-associated cancer incidence with CD4 count measured at AIDS onset. Incidence of HPV-associated cancers was compared across three periods (1980-1989, 1990-1995, and 1996-2004).

Results: Compared to the general population, risk of all HPV-associated cancers (n=699 in situ and 602 invasive) was significantly increased among persons with AIDS (SIRs for in situ cancers: anus (male)=68.6, anus (female)=33.0, cervix=8.9, penis=19.7, and vagina/vulva=27.2. SIRs for invasive cancers: anus (male)=34.6, anus (female)=14.5, cervix=5.6, oropharynx=1.6, penis=5.3, vagina/vulva=5.8). During the HAART era, low CD4 counts were associated with significantly increased risks of invasive anal cancer among men (RR per 100 cells/mm3 decline in CD4=1.34; 95% CI=1.08-1.66), invasive cervical cancer (RR=1.32; 95% CI=1.00-1.75), and in situ vagina/vulva cancers (RR=1.52; 95% CI=1.06-2.18). Among men, incidence of both in situ and invasive anal cancer was significantly higher during 1996-2004 than 1990-1995 (61% and 104% increases, respectively). Incidence of other HPV-associated cancers was stable over time.

Conclusions: HPV-associated cancer risk is elevated among persons with AIDS, and risk rises with increasing immunosuppression. The increasing anal cancer incidence during the HAART era suggests that prolonged survival may manifest in rising morbidity from certain HPV-associated cancers.

ARE EXTIRPATIVE PROCEDURES FOR CIN EFFECTIVE IN HIV-POSITIVE WOMEN?

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Background: In well-studied cohorts, women with poorly controlled HIV have been shown to be at increased risk of cervical intraepithelial neoplasia (CIN) than immunocompetent women.

Objectives: To determine predictors of treatment failure after surgical extirpative procedures for CIN in women infected with HIV in a population-based cohort.

Methods: All patients infected with HIV who had an extirpative procedure for CIN between 1999-2005 in two large hospital systems in the same urban and ethnically diverse US city were included in this study. Data was abstracted from charts and large comprehensive databases. Patients with adequate follow-up within 6 months after procedure were classified for treatment failure if they had a result of CIN 2+ at initial follow-up.

Results: Data was available for 164 HIV+ women of which 99 had adequate follow-up. Treatment failure occurred in 70/164 (43%) of all women, among those defined as having adequate follow-up failure occurred in only 35/99 (35%). Out of those pts with CD4 \leq 200 at time of procedure, 16/30 (53%) had treatment failure. Women who frequently (3+) missed appointments were significantly more likely to have treatment failure than those who rarely or never missed appointments (p=0.003). When controlling for missed appointments, pts with CD4 \leq 200 were 18 times more likely to fail (p=0.01). Among women who rarely or never missed an appointment those noncompliant or not on HAART were 13 times more likely to fail than those on HAART (p=0.009).

Conclusions: In this population-based cohort of HIV+ women treatment failure was extremely high, especially those with poorly controlled HIV infection. Risk factors or behaviors that are unique in pts who are non-compliant post-LEEP need further study. In women noncompliant with HAART, maximizing HIV clinical status is likely to be more beneficial than an extirpative procedure to decrease risk of recurrent CIN 2+.

POSTER ABSTRACTS SESSION 16

POSTER SESSION III TUESDAY 20.00: ODD NUMBERS

POSTER SESSION IV

TUESDAY 21.00: EVEN NUMBERS

HYBRID CAPTURE II: A VALUABLE TOOL IN ANAL DYSPLASIA SCREENING

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Background: Anal cancer and HSIL in MSM. ASCUS is the most common abnormal cytology, nonspecific and rarely predicts HSIL.

Objectives: To determine if HCII testing for oncogenic HPV (HPV+) in MSM is useful and if other predictors of HSIL exist.

Methods: A retrospective chart review of MSM with ASCUS cytology, HCII testing and biopsy. In addition all prior and subsequent screening results subjects were analyzed.

Results: 597 MSM enrolled having 1015 ASCUS cytology results with 18.2% having HSIL and 84% HPV+. The sensitivity, specificity, PPV and NPV value were 84%, 53%, 29% and 94%, respectively. The NPV was not significantly different, but the sensitivity was higher and specificity lower (92% and 36% vs. 78% and 59%, respectively) in HIV+ vs. HIV- MSM. Of 390 LSIL cytology results, 36% had HSIL and 90% were HPV+. The sensitivity, specificity, PPV and NPV for MSM with LSIL cytology was 90%, 25%, 40% and 81%, respectively. MSM with prior HSIL or HIV had increased risk of subsequent HSIL (HR=2.2 and HR=1.95, respectively). No difference in subsequent risk of HSIL existed between those that remained HPV- vs. those that cleared oncogenic HPV (HR 1.10[0.47, 2.58]. The HR for subsequent HSIL for HPV- vs. HPV+ MSM was 0.15[0.07, 0.31]. The likelihood of recurrence decreased significantly as a function of disease free time.

Conclusions: HCII testing is useful in MSM with ASCUS, but not LSIL cytology. Referring only those with oncogenic HPV for high-resolution anoscopy reduces the number requiring this by almost half but some HSIL is missed. History of HSIL and HIV are predictors of HSIL while screening intervals might be lengthened absent oncogenic HPV or in those free of HSIL for long periods.

P-16.10 PREDICTORS OF HIGH-GRADE ANAL DYSPLASIA IN HIV-SEROPOSITIVE MEN

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HIV-seropositive men having sex with men (MSM) are at increased risk of high grade anal dysplasia. Objective: We aimed at identifying HPV and other factors associated with detection of high grade anal dysplasia in HIV-seropositive men enrolled in the HIPVIRG cohort study. Methods: HIV-seropositive MSM were invited to participate in a study involving follow-up visits with risk factor interviews, anal HPV testing and anal cytology every 6 months for 3 years. High-resolution anoscopy (HRA) with biopsy was performed yearly unless a previous histology result was abnormal, in which case the HRA was repeated after 6 months. A composite outcome of high grade anal dysplasia as diagnosed by either anal cytology or histology through HRA was the outcome. Results: Of the 247 participants, 78% had completed 5 visits (2 years) at the time of the present analysis. Multivariate period prevalence logistic regression revealed that men whose CD4 counts were lower than 50 cells/mm3 before beginning antiretroviral treatment had a 20-fold increased risk of anal high-grade dysplasia (OR 21.8, 95%CI: 2.0-233) compared to men whose CD4 counts were above 350. A longer time on antiretrovirals was protective against anal dysplasia (OR 0.24, 95%CI: 0.1-0.6 for 2-5 yrs; OR 0.08, 95%CI: 0.03-0.2 for 5 years or more) while a longer time since HIV diagnosis increased risk, the highest risk being for men whose diagnosis was made 5 to 10 years before enrolment (OR 9.9, 95%CI: 2.7-35.5). Other risk factors associated with high grade anal lesions were multiple HPV infection by high risk HPV types and type-specific infection with HPV-16, HPV-18 or HPV-26.

Conclusion: Antiretrovirals protect against development of high grade anal dysplasia, while a lower nadir of CD4 count before beginning antiretrovirals increases the risk of anal dysplasia. Specific types of high risk HPV also increase the risk.

RISK OF HPV IMMEDIATELY AFTER HIV ACQUISITION AMONG ZIMBABWEAN WOMEN

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Background & Objective: A massive depletion of memory CD4+ T cells occurs at mucosal sites during acute HIV infection (HIVa+). HPV-specific T-cell memory in the female genital tract may therefore be rapidly depleted in women with heterosexual HIV acquisition. The objective of this study was to determine whether the density of HPV type-specific infection increases in the first six months following HIV infection.

Methods: 154 HIVa+ seroconverters were matched to 479 HIV-uninfected women in Zimbabwe on follow-up time and age. HPV genotypes were assessed by Roche Linear Array PCR in 3 month intervals starting 6 months prior and ending 6 months after the visit when HIV was first detected by PCR (t-2, t-1, tindex, t+1, t+2). Women with 5 consecutive visits were selected to calculate crude HPV type-specific densities (HIVa+: 92; HIV- 252). Crude type density was defined as the number of new HPV types detected per incident HPV event by HIV group. Differences in type-density were tested using Wilcoxon rank-sum statistics.

Results: Crude type density was similar in the pre-HIV acquisition period (t-2 to t-1) among women who would later become HIVa+ compared to HIV-uninfected women during the same calendar period (1.47 and 1.53 types/incident event, respectively; p=0.95). However, crude type densities in the three consecutive, 3-month intervals post-HIV acquisition were higher (average 1.96 types/incident event) compared to women remaining HIV-negative over the same time interval (average 1.44 types/incident event; p<0.001).

Conclusions: Acutely HIV-infected women had, on average, a 36% higher HPV type density per incident event, despite having similar HPV type densities to HIV-uninfected women in the pre-HIV acquisition period. Crude analysis suggests a change in the natural history of HPV concomitant with HIV-acquisition; multivariate analyses are ongoing to confirm these crude results.

P-16.12

HPV TYPE-SPECIFIC CERVICAL CANCER RISKS ACCORDING TO HIV STATUS

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Background: There is limited data on HPV type-specific risks of cervical cancer in populations with a high prevalence of HIV. Prior reports have indicated that HPV-16 might be under-represented as a cause of cervical cancer among HIV-positive women.

Objective: To investigate HPV type-specific risks of cervical cancer in a population with a high prevalence of HIV.

Methods: A hospital-based case-control study was conducted at the Central Hospital in Maputo, Mozambique, from 2002 to 2007. In total, 221 women with confirmed cervical cancer at the Department of Gynaecology and 203 control women enrolled from an outpatient clinic at the Department of Oto-Rhino-Laryngology with adequate cervical samples (β -globin positive), where included in the analyses. HPV DNA testing was performed by GP5+/6+-PCR followed by typing with LUMINEX. Serum samples where taken for HIV testing and socio-demographic factors where recorded.

Results: Twenty-four different HPV types were detected in the samples. Twenty-two percent of cases and 40% of controls were HIV-positive. After adjustment for age, education, smoking, parity, oral contraceptives, pap smear history and HIV, HPV-16 [Odds Ratio (OR): 18.66 (95%CI: 8.0-43.7)], HPV-18 [OR: 3.61 (95%CI: 1.5-8.7)], HPV-35 [OR: 3.82 (95%CI: 1.1-13.6)] and HPV-45 [OR: 3.05 (95%CI: 1.2-7.8)] were associated with cervical cancer. HPV-16, -18, -35 and -45 attributed to 48.4% (95%CI: 41.0-54.9), 16.2% (95%CI: 9.6-22.3), 6.6% (95%CI: 2.7-10.4) and 13.9% (95%CI: 7.4-19.9) of cervical cancers respectively. There was no evidence of effect modification by HIV on HPV type-specific risks for types associated with cervical cancer in the primary analysis (p-value for interaction: 0.42, 0.87, 0.21, and 0.73 for HPV-16, -18, -35 and -45 respectively), or for other HPV types. HPV-16 was present in 53% and 45% of cervical cancers from HIV-negative and HIV-positive women respectively.

Conclusions: Unlike previously suggested, we found no evidence that HPV type-specific cervical cancer risks are altered by HIV infection.

P-16.13 DETECTION OF HPV IN NON-CERVICAL CANCERS AMONG HIV-POSITIVE WOMEN

DETECTION OF HPV IN NON-CERVICAL CANCERS AMONG HIV-POSITIVE W

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Several novel HPV-cancer relationships have been hypothesized based on occasional reports of viral traces in tumor specimens, including in lung/rectal/breast, and skin cancers. If HPV plays a role in a subset of these tumors, HPV should be particularly prevalent in tumors among HIV-positive patients (patients highly susceptible to HPV). Indeed, rectal, skin, and lung cancer rates were elevated in several studies of HIV-positives patients. While other non-cervical anogenital tumors have clear associations with HPV, little is known regarding the HPV types present among HIV-positive women.

Available paraffin-embedded tumor specimens from the AIDS Cancer Specimen Repository (ACSR), a tumor bank for specimens from HIV-positive patients in the US, were tested for alpha-papillomaviruses using GP5+/6+ PCR. Sectioning involved procedures to minimize risk of viral DNA contamination. The first section was reviewed to confirm diagnosis and presence of tumor cells.

Pos/Tested	%Pos	HPV16	other onc
40/43	93%	n=23	n=7
1/9	11%	n=1	
4/21	19%	n=4	
6/18	33%	n=6	
9/11	82%	n=6	n=1
9/58	16%	n=1	n=3
6/6	100%	n=1	n=1
7/7	100%	n=1	n=3
	40/43 1/9 4/21 6/18 9/11 9/58 6/6	40/43 93% 1/9 11% 4/21 19% 6/18 33% 9/11 82% 9/58 16% 6/6 100%	$\begin{array}{ccccccc} 40/43 & 93\% & n=23 \\ 1/9 & 11\% & n=1 \\ 4/21 & 19\% & n=4 \\ 6/18 & 33\% & n=6 \\ 9/11 & 82\% & n=6 \\ 9/58 & 16\% & n=1 \\ 6/6 & 100\% & n=1 \end{array}$

These preliminary data have important implications to prevention/treatment of these tumors. To further assess causality we will test additional specimens, and assess whether: other HPV types are present (using a broad spectrum PCR), HPV tumor proteins E6/E7 are expressed, HPV DNA is integrated into the host genome.

P-16.14

ANOGENITAL CYTOLOGIC ABNORMALITIES AMONG HIV INFECTED WOMEN -PRELIMINARY DATA

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Background: Cervical human papillomavirus (HPV) infections and related diseases are more prevalent and persistent in HIV-infected compared with uninfected women. Less is known about anal HPV burden in women.

Methods: This is an ongoing cross sectional study of HIV-infected women. Each participant had cervical and anal specimens obtained for cytopathology (Pap tests) and HPV typing with PCR-based RUO Roche Linear Array detecting 37 types. Any abnormal cervical or anal cytology was followed with a colposcopy or anoscopy, respectively, and appropriate biopsies obtained.

Results: Cervical and anal cytology results were available for 88 women. Mean age was 43 years, white 50%, African American 34%, Hispanic 14%, other 10%. The median CD4 count was 564 cells/mm3. Seventy two women (82%) were on combination antiretroviral therapy, 56% had an undetectable HIV viral load. The prevalence of HPV in the anus and cervix was 81% and 68%, respectively. The prevalence of an abnormal cytology in anus and/or cervix was 44%; 26% in the anus, and 28% in the cervix. Ten percent had an abnormal cytology at both anatomical areas, 16% women had an abnormal anal cytology only, and 18% had an abnormal cervical cytology only. Seven of 23 women with an abnormal anal cytology have undergone anoscopy with biopsy, 1 had anal intraepithelial neoplasia, 6 had a normal histology. Nine women of 29 women with an abnormal cervical Pap smear had a colposcopy with biopsy and 2 had cervical intraepithelial neoplasia I. Conclusions: Preliminary results in this cohort of HIV-infected women shows that there is a high prevalence of HPV infection and abnormal cytology in both cervix and anus. Among women with abnormal cytology, the majority had it either in the anus or cervix but not both. Among the few women with biopsy results, only 2 had low-grade neoplasia in anus/cervix.

INTERFERON-ALFA THERAPY ON ANAL HPV-INFECTION, HPV16-INTEGRATION AND PATHOLOGY IN HIV-MEN

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Several studies have suggested that Th1-type cytokines are associated with clearance of cervical HPV infection. However, in HIV-infected population the effect of therapies containing interferon (IFN)-alfa has not been well assessed. The aim of this study was to evaluate the effect of IFN-alfa treatments on the prevalence of HPV infection, HPV16 integration status and HPV-related pathology in anal samples of HIV-infected men. Cross sectional study included 269 HIV-positive consecutive men from the CARH-MEN (CAn Ruti HIV+ MEN) cohort who attended the HIV clinical Unit (Germans Trias i Pujol Hospital) between 2005-2006. Data about IFN-alfa treatment history was obtained from the data base. These patients received IFN therapy (IFN-alfa alone or pegylated interferon with ribavirin) because Hepatitis C co-infection or Kaposi's sarcoma during the period 1999-2005. Type specific HPV infection was determined using the f-HPV typing™ kit (Molgentix, Spain), and HPV16 integration was done by multiplex Real-time PCR. The association between IFN-alfa treatment and type specific HPV-infection, HPV16 integration event and the cytological lesion grade were analyzed by the χ^2 test, Fisher's exact test and multinomial logistic regression model. Prevalence odds ratios and the corresponding 95% confidence intervals (CI) were calculated. Overall prevalence of anal HPV-infection was 78% (209/269). The prevalence of anal HPV-infection in IFN-alfa treated patients (9%, 25/269) was lower than the non-treated group [60% (14/25) vs 80% (194/244), OR=0.39; 95%CI:0.16-0.91, p=0.040]. IFN-alfa was associated with a protective effect against the A9-specie related types [36% (9/25) vs 59% (144/244), OR=0.39; 95%CI:0.17-0.92, p=0.034]. Likewise, the IFN group presented a lower risk for multiple anal HPV infection [28% (7/25) vs 59% (145/244), OR=0.29; 95%CI:0.10-0.86, p=0.031] and a lower frequency of abnormal anal cytology (OR=0.19; 95%CI:0.03-0.9). In contrast, IFN-alfa treatment did not seem to protect against HPV-16 integration. Treatments including IFN-alfa seem to confer some benefit for HPV infection and anal pathology.

P-16.16

HUMAN PAPILLOMAVIRUS TYPES-16 AND-18 INTEGRATION IN HUMAN IMMUNODEFICIENCY VIRUS-INFECTED WOMEN.

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HIV-positive women are at high risk (HR) for acquiring HPV infection and cervical lesions, but little is known whether HIV infection favours HPV integration. Our aim was to assess the prevalence and risk factors of HPV16 and HPV18 integration in cervical samples of HIV infected women (n=251) attending in the HIV Clinical Unit (Germans Trias i Pujol Hospital, Spain) during 1999-2003. The median age of patients (43 years, range:28-73), years since HIV diagnosis (15, range:4-24), baseline CD4 (448 cells/mL, range:2-1780) and nadir CD4 (189 cells/mL, range:2-1884) counts, plasma HIV-1 load (500 copies/mL, range:<50-2.106) and time on Highly Active Antiretroviral Therapy (HAART) (10 years, range:0-15) were included in the analyses. DNA was amplified using F-HPV typing[™] kit (Molgentix, Spain). Multiplex Realtime PCR for simultaneous amplification of E2/E6 regions was used to detect integrated forms. Binary and multivariate logistic regression models were applied to calculate prevalence odds ratios and the confidence intervals (95%CI). Overall prevalence of HPV infection was 53% (133/251) being the HPV16 (27%), HPV33 (15%), HPV52 (8%), HPV58 (7%) and HPV39 (7%) the most prevalent types. Integrated forms were detected in 32% (22/68) of HPV16 positive patients and in the 20% (2/10) of HPV18 positive. The integration of HPV16 was associated with a higher risk of LSIL (OR=6.6, 95%CI:1.4-30) and HSIL diagnosis (OR=15, 95%CI:3-76). The two patients with HPV18 integrated forms presented LSIL and HSIL cytology, respectively. The CD4 nadir counts <250 cells/mL were a risk factor for HPV16 integration (OR=3.5, 95%CI:1-12), whereas longer time on HAART was found as a protective factor for HPV16 integration (OR=0.8, 95%CI:0.6-0.9). In summary, the time on HAART seems to have a protective effect on HPV integration; however, avoiding the drop of CD4 cell counts seems to be the most critical factor for HR-HPV integration and therefore for the development of carcinogenic-related lesions.

P-16.17 INCREASED CERVICAL DYSPLASIA IN HPV AND EBV CO-SHEDDING HIV+ WOMEN

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Background: Previous studies from the New Orleans HIV+ cohort have found an increase (2 fold) of cervical dysplasia in women shedding both high-risk HPV and EBV in cervical fluids. A pilot study of 308 women from the WIHS has indicated a similar relationship but was underpowered. This study is an interim analysis of cervical samples from an early visit from 300 additional HIV+ women.

Methods: EBV was detected using a highly sensitive PCR assays targeting the repeat unit. For those samples who were positive for EBV, the EBV viral load was measured by real time PCR targeting a single copy gene and normalized to the number of cells present using a real time assay for RNAse P. The HPV DNA results and Pap smear results were obtained from the WIHS database.

Results: Ninety-six percent (289/300) of the obtained samples had detectible DNA. Of these, EBV was detected in 29.4% Combined with the previously tested 305 women, EBV was detected in 38.5% of the women. An abnormal Pap smear was found in 74% of the co-shedding women as compared to 53% of those shedding only HPV (p=.008, OR 2.54, CI 1.2 – 5.4). Similarly, dysplasia was seen in 60% of the co-shedding women as compared to 40% of those shedding only HPV (p=.04, OR 2.22, CI 0.94-5.25).

Discussion: The association of EBV and HPV in the development of cervical dyaplasia in HIV+ women is further supported by these studies. The plan is to test an early visit from all HIV+ WIHS participants (n=3,000) to see if these associations hold and to perform a detailed demographic risk factor analysis for the factors associated with co-shedding of HPV and EBV,

P-16.19

ANAL HIGH-GRADE SQUAMOUS INTRAEPITHELIAL LESIONS (HSIL) IN HIV-INFECTED WOMEN

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Background: HIV-infected women have high rates of HSIL of the cervix and may also have high rates of HSIL of the anus. Risk factors for HSIL of the anus in HIV-infected women have not been identified.

Methods: One hundred HIV-positive adult women were enrolled in this prospective, observational study. Subjects had cervical and anal cytology and HPV Hybrid Capture 2 (HC2) testing (Digene) at two visits over a 6-12 month interval with follow-up colposcopy and high resolution anoscopy (HRA) if indicated. Differences in proportions for risk factors between subjects with and without anal HSIL were assessed using Fisher's Exact test in SAS, version 9.1.

Results: Of the 75 subjects who completed both visits and HRA if indicated, 10 had anal HSIL detected and the remaining 65 women were not diagnosed with anal HSIL. Anal HPV was detected in 70% of subjects with HSIL at either visit and in 50% at both visits compared to 17% and 6% of subjects without HSIL (p=0.001). Cervical HPV was detected in 90% of subjects with anal HSIL at either visit and in 40% at both visits compared to 35% and 11% of subjects without HSIL (p=0.002 for either and p=0.035 for both). Thirty percent of HSIL cases had both current and nadir CD4 counts of 200 or less cells /mm3 compared to 5% of women without HSIL (p=0.028). Known HIV infection for greater than 10 years, more than 5 lifetime sexual partners, detectable HIV viral load, and current cigarette smoking were not significant risk factors for HSIL of the anus in this cohort.

Conclusions: Anal and cervical HPV infections and low CD4 counts were associated with increased risk of HSIL of the anus in our cohort of HIV-infected women.

PREVALENCE OF HR-HPV INFECTION IN A MULTICENTER COHORT OF HIV-POSITIVE

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Objectives: To estimate HR-HPV prevalence and associated risk factors in HIV positive subjects.

Methods: CoRIS is a multicenter, open and prospective cohort of HIV positive subjects naïve to antiretrovirals at cohort entry started in 2004 in Spain. Socio-demographic, clinical and immunological variables are updated six-monthly. From June 2007 onwards, an HPV-related module was added to the cohort and subjects are asked about their sexual behaviours with a questionnaire designed to this end. Anal and cervical HPV infection is determined with "Amplicor HPV DNA Test" and genotyped with "Linear Array Genotyping HPV Test" (Roche Diagnostics). Frequencies, means and medians were analyzed with Stata 10.

Results: Of 331 patients, 75% were men who have sex with men (MSM) and 17.5% were women. For MSM, mean age was 35 years (SD: 10.2), mean age of first sexual intercourse was 16.7 years (SD: 3.3) and median number of sexual partners in the last 12 months was 6 (IR: 2-20).

Overall, 54.7% had risk sexual behaviours in the last 12 months, 84.5% in men and 15.5% in women. So far, we have 209 samples for HR-HPV testing. Anal and cervical HR-HPV prevalence was 79.6% (133/167) and 47.6% (20/42) respectively. The percentage of anal HR-HPV multiple infections in men was 65.8%, in women 73.3% and 63% anal and cervical respectively. The most common types were HPV-16 (16.8%) and HPV-18 (11%) in men and HPV-16 (2.1%) and HPV-52 (1.8%) in women.

Conclusions: HR-HPV prevalence is very high in HIV-positive patients; this prevalence is higher in MSM. The prevalence of risky sexual behaviours in MSM is also higher than in women.

P-16.21 ORAL HPV-32 INFECTION IN HIV+ INDIVIDUALS IS ASSOCIATED WITH HAART

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Background: Previous data has demonstrated that HPV-32 is found in most oral warts (65%) seen in HIV+ individuals. Initial studies have also found HPV-32 to be commonly found in the oral cavity of HIV+ individuals without any clear pathology. The risk factors for the presence of oral HPV-32 without any pathology were examined.

Methods: A total of 170 HIV+ individuals were enrolled. Swabs of the buccal mucosa, labia, lingual, sublingual, tonsillar pillar, and gingival were obtained as well as 5 ccs of unstimulated saliva. DNA was isolated using a modified Qiagen extraction kit and HPV 32 DNA detected using a highly sensitive PCR assay. Detailed histories of oral pathology, demographic data, and clinical data including CD4 cell counts, HIV viral load and HAART use were obtained by questionnaire and chart review.

Results: Overall 62% of the individuals have detectible HPV-32 at some site in their oral cavity. Individual sites varied from 15% (sublingual) to 30% labial with tonsillar being approximately 17%. Detailed analysis of the risk factors for HPV-32 shedding is in progress. Initial analysis demonstrated an increase detection of HPV-32 in those on effective HAART (viral load < 400 copies) vs those not on effective HAART (p=.02)

Discussion: HPV-32 is the most commonly found type in oral warts in HIV+ individuals. This virus is also commonly found in HIV+ individuals without oral pathology. It appears that both oral warts and oral HPV-32 infection is increased in HIV+ individuals on HAART. The mechanism of this persistence of HPV-32 despite systemic immune restoration is currently under study

CERVICAL INTRAEPITHELIAL NEOPLASIA IN HIV-INFECTED WOMEN IN PUNE, INDIA

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Objectives: Studies reporting increased prevalence of cervical intraepithelial neoplasia (CIN) in HIV-infected women have relied on detection of cervical lesions by cytology. Unfortunately, cytology has only low to moderate sensitivity and detection rates and thus may not yield precise prevalence estimates. The objective of this study was to evaluate the prevalence and predictors of colposcopic-histopathologically confirmed CIN among HIV-infected women.

Methods: HIV-infected women (n=303) in Pune, India underwent cervical screening and diagnostic colposcopy followed by histopathologic confirmation of suspected CIN lesions to assess the composite colposcopic-histopathological prevalence of CIN. Multivariable analyses using ordinal logistic regression were conducted to determine independent predictors of increasing severity of CIN.

Results: The prevalence of >=CIN1 lesions was 27.7% (95% CI: 22.7, 33.1) and of >=CIN2 lesions was 16.5% (12.2, 21.9) on composite colposcopic histopathological diagnosis. Cervical high-risk HPV-DNA was detected in 41.7% (124/297) of participants. The odds of increasing severity of CIN disease were greater in women currently receiving antiretroviral treatment (ART) [adjusted odds ratios (aOR): 2.16 (95% CI: 1.15, 4.04), p=0.02] as compared to ART-naïve women, and among those with presence of cervical high-risk HPV-DNA [aOR: 1.91 (1.12, 3.26), p=0.02] as compared to cervical high-risk HPV-DNA negative women.

Conclusions: HIV-infected women in Pune, India have high prevalence of HPV-infection and CIN. The surge in funding for HIV/AIDS care programs may offer new windows of opportunity for providing high-value, low-cost preventive clinical interventions like cervical cancer screening to women at elevated risk of this preventable malignancy. Locally appropriate and cost effective screening and treatment services are urgently needed for cervical cancer prevention among HIV-infected women. Increased attention and focus towards most at-risk subpopulations like HIV-infected women may allow optimal utilization of resources.

P-16.23

HPV GENOTYPES AND HPV16 VARIANTS IN HIV- POSITIVE ITALIAN WOMEN

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Background: Human immunodeficiency virus (HIV)-positive women have high rates of cervical squamous intraepithelial lesions (SIL) and concurrent human papillomavirus (HPV) infections with a variety of genotypes whose oncogenic risk is poorly documented.

Methods: Prevalence and persistence of HPV genotypes and HPV16 variants have been analyzed in 112 HIV-positive and 115 HIV-negative Italian women during a 3 year follow up period.

Results: HIV-positive women were more likely than HIV-negative to be infected by HPV at the initial examination (39.3% versus 13.9%, P<0.001) and to have higher period prevalence of HPV infection over a 3-year follow-up (43.8% versus 17.4%, P<0.001), regardless of CD4+ cell counts and anti-retroviral therapy. "High risk" and "probable high risk" HPVs (16, 18, 31, 33, 35, 45, 52, 58, and 66), among the twenty different viral genotypes identified, were predominant in HIV-positive (33.9%) compared to HIV-negative women (13.9%). Among HIV-infected women, with normal cytology as well as with SIL of any grade, the most common genotypes were HPV16 followed by HPV81, 58, 72, 33 and 62. HPV16 isolates from 18 HIV-positive and 8 HIV-negative women were classified into variant lineages based on sequencing analysis of E6, E7 genes and LCR. While HPV16 G350 European variant was prevalent in both HIV-positive (10.7%) and negative women (3.5%), HPV16 African 2 variant was only detected in HIV-positive women (3.6%), suggesting different sexual mixing behaviors. Conclusions: The increased prevalence of uncommon viral genotypes and HPV16 variants in HIV-positive Italian women underscores the need for targeting a wide range of HPV types in cervical screening of high risk women

HPV81 GENETIC DIVERSITY IN MULTIPLE AND SINGLE INFECTIONS

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Background. HPV is the most common sexually transmitted viral infection in the world. HPV81 is the most LR HPV prevalent in Italy. It is frequently found in HIV+ women with multiple infections, including HPV16, 18 and 11 genotypes. Nevertheless, HPV81 is not included in current vaccines.

Objectives. We analysed the genetic variability in L1 region of HPV81 genotype.

Methods. In 5 patients with multiple infections and in 2 patients with single infection, HPV81 L1 region were amplified by specific primers, cloned and biderectionally sequenced. Amino acid sequence were aligned to prototype AJ620209. In HPV-coinfected samples, we confirmed the HPV58 and 61 presence by using type-specific primers.

Results. We identified 27 mutations compared to prototype AJ620209. The Pt422 showed the largest distance (10aa) from the reference. All the sequences differed from prototype in 492-493aa (KS→RT). In samples with single infection we found several mutations (S124N, D192N, Q250L, A478T) moreover none of them is located in immunodominant epitopes of the prototype. A comparison of the BC, DE, FGa, FGb, HI immunoregion with corresponding HPV16 and HPV11 region indicated that HPV81 sequences were highly different. Particularly in BC loop, we found K54I probably associated to distinct protein-protein interaction specificity in their antibody binding. Similarly E355A substitution in HI domain may produce distinct structural feature abrogating binding of HPV16 HI loop type specific monoclonal antibodies (mAbs). Respect to BC of HPV11 loops aa insertions may lead to conformational change.

Conclusions. The present findings seems to indicate that HPV81 mAbs do not cross-react with those specific for HPV16 and 11. Moreover it should be noted that the restricted size of the sample and the depressed immunestatus in HIV+ women could induce in bias. For these reasons further analysis about measurement of neutralising titre antibodies confering HPV immunity are needed.

P-16.25

SEROPREVALENCE OF HPV 16/18 AMONG HIV INFECTED WOMEN IN BRAZIL

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Human immunodeficiency virus (HIV-1) is an important risk factor for human papillomavirus (HPV) infection and for HPV associated lesions in the female genital tract. The HPV types most often associated with cervical squamous intraepithelial lesions and invasive cervical cancer are HPV types 16 and 18; however limited data are available on past exposure to these HPV types in HIV/AIDS infected women in Brazil. Objectives: Our main aim was to investigate the HPV 16 and 18 seroprevalence among a cohort of HIV/AIDS infected women. Methods: 717 serum samples were collected between 2001-2008 from HIV positive women followed at IPEC, Rio de Janeiro, Brazil, including 228 pregnant women and 489 non-pregnant women. Demographic, clinical, HIV and HPV virological and sexual history data were collected on all women. Women with abnormal Pap smear were referred to colposcopy. Serum was tested by virus-like particle (VLPs)-based ELISA for antibodies to HPV types 16 and 18. High and low cut points for seropositivity were determined from reactivity of a low prevalence female population after excluding outliers. Results: The seroprevalence to HPV 16 was 38.5%. At the higher cut point, the seroprevalence was 29.7%. The seroprevalence in HIV positive pregnant women (36.4%) did not differ from that in HIV positive nonpregnant women (41.5%; p-0.22. HPV 18 data is pending. Analyses of associations between seroreactivity and other variables will be presented. Conclusions: The HPV 16 seroprevalence in HIV infected women is higher than that previously reported for HIV negative women in Brazil (15-24%). Knowledge of the burden of past infection with HPV 16 and 18 among HIV infected women from Brazil will allow rationale utilization of HPV vaccine in this population.

CERVICAL HPV INFECTION IN WOMEN ON RENAL DIALYSIS

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Background: Cervical cancer has high prevalence following renal transplantation. However, cervical HPV infection has not been characterized in women on renal dialysis pending renal transplant.

Objectives: Identify the type specific incidence of cervical HPV infection in women on renal dialysis.

Methods: We recruited 17 women on maintenance dialysis who were also on the renal transplant waiting list, and 18 women with normal renal function as controls from the Family Medicine clinic between May 2003 to September 2004. After informed consent, cervical specimens for HPV DNA PCR using MY09/11 were collected.

Results: A total of 35 women were recruited; women on dialysis were average age 42.08 years and controls 46.33 years. There were 58.8% African American women in the study group. Study group women had an average age at sexual debut of <16 years in 35.3% and >4 lifetime sexual partners in 64.7%, compared to sexual debut <16 years in 22.2% and >4 partners in 44.4% control women. There were 12/17 (70.6%) women smokers and smoking initiation occurred at age 13.2 years in study women and 10/18 (55.6%) women smokers with smoking initiation at 18.8 years in control women. Study women positive for any type of HPV at the cervix were 7/17 (41.2%) compared to 4/18 (22.2%) in controls. The incidence of HPV type 16/18 was equal in study and control women and, non 16/18 HPV types is higher in women on renal dialysis.

Conclusions: There is a high incidence of HPV cervical infection in women on renal dialysis pending renal transplant. This suggests the need for a study in a larger number of women to understand HPV infections in women with end stage renal disease on maintenance dialysis pending renal transplant.

P-16.27

HIV-1 PROMOTES RAPID CHANGES IN CERVICAL HPV AND HPV-16 ANTIBODIES

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Background: It is not known how HIV-1 infection impacts on the ability to mount an effective immune response to HPV, which is important in planning vaccine strategies for HIV-1 infected individuals. This longitudinal study investigated changes shortly (8-12 months) after HIV-1 seroconversion on cervical HPV and HPV-16 antibody responses in serum and at the cervix of female sex workers.

Methods: Typing of HPV DNA from cervical cells was by reverse line blot prior to HIV-1 seroconversion and within 1 year and greater than 2 years after HIV-1 seroconversion. Antibody determinations were by HPV-16 virus-like particle-based, enzyme-linked immunosorbent assay.

Results: Shortly after HIV-1 seroconversion a significant increase in multiple (>1) HPV infection (OR 4.0, 95% CI 1.3-11.9) was observed compared with HIV-1 seronegative (HIV-negative) women and changes in HPV type infection in HIVpositive compared with HIV-negative women. HIV-1 seroconversion resulted in a reduced prevalence of serum HPV-16 IgA and cervico-vaginal IgA and IgG but an increased prevalence of serum HPV-16 IgG. All HIV-positive women had been exposed to HPV-16 as all displayed serum HPV-16 IgG. Serum HPV-16 responses were maintained at a high magnitude in the presence of HPV-16 infection irrespective of HIV infection, but decreased in the absence of HPV-16 infection.

Conclusion: HIV-1 seroconversion in sex workers rapidly increased cervical HPV infection and caused a reduced ability to produce cervical HPV-16 antibodies but a continued ability to generate serum IgG antibodies. A sustained high level of serum HPV-16 antibodies was maintained irrespective of HIV infection and only in the presence of HPV-16 infection. HIV seroconversion did not appear to affect the ability to mount HPV-16 IgG serum antibody responses, in fact these were increased. This could be relevant to HIV-positive individual's ability to mount effective responses to HPV vaccines.

SESSION 17

HPV AND HEAD & NECK CANCERS

PROGRAMME

THE 25TH INTERNATIONAL PAPILLOMAVIRUS CONFERENCE MAY 8-14 2009, MALMÖ, SWEDEN

TIME NUMBER TITLE

2009-05-12	20	09.	-05	-12
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ROOM

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16.13-16.24	O-17.02	ORAL HPV PREVALENCE AMONG HEALTHY MEN INTERNATIONALLY AR Kreimer, A Hildesheim, M Abrahamsen, D Smith, LL Villa, E Lazcano, A Giuliano			
16.24-16.35	O-17.03	MESENCHYMAL TRANSFORMATION DURING TUMOR PROGRESSION IN HPV-RELATED OROPHARYNGEAL CANCER EJ Speel, M Stenner, B Yosef, C Huebbers, SF Preuss, HP Dienes, JP Klussmann, M Odenthal			
16.35-16.46	O-17.04	HUMANA PAPILLOMAVIRUS IN TONSILLAR CANCER, AN EPIDEMIC OF VIRAL CARCINOMA T Dalianis, A Näsman, P Attner, L Hammarstedt, J Du, M Eriksson, G Giraud, S Ährlund - Richter, L Marklund, D Lindquist, M Romanitan, T Ramqvist, J Lindholm, P Sparen, W Ye, H Dahlstrand, E Munck - Wikland			
16.46-16.57	O-17.05	HPV INFECTION IS ABSENT FROM ESOPHAGEAL CANCER IN CHINA W Wei, J Koshiol, W Chen, Ar Kreimer, J Wang, P Gravitt, J Ren, C C Abnet, F Kamangar, M Gillison, M J Roth, P Zhao, Pe Castle, G Wang, P R Taylor, Z Dong, S M Dawsey, Y Qiao	1		
16.57-17.08	O-17.06	ASSOCIATION BETWEEN CHROMOSOME STABILITY, HPV AND PROGNOSIS IN TONSILLAR CARCINOMA JJ Mooren, SMH Claessen, A Haesevoets, Ahn Hopman, Fcs Ramaekers, JP Klussmann, JJ Manni, B Kremer, EJM Speel			
17.08-17.19	O-17.07	ADENOID-CYSTIC CARCINOMAS OF SALIVARY GLANDS ARE ASSOCIATED WITH HIGH-RISK HPV AE Albers, C Chen, M Hummel, E Glastetter, U Keilholz, AM Kaufmann			
17.19-17.30	O-17.08	NATURAL HISTORY OF ORAL AND ANAL HPV INFECTION. G D' Souza, E Sugar, M Gillison			

O-17.01 HPV16 E6 INDUCED PTPN13 LOSS ENHANCES ONCOGENE RELATED MAPK SIGNALING

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Human papillomaviruses (HPV's) are a causative factor in over 90% of cervical and 25 % of head and neck squamous cell carcinomas (HNSCC's). The C-terminus of the high risk HPV 16 E6 oncoprotein physically associates with and degrades a non receptor protein tyrosine phosphatase (PTPN13), and PTPN13 loss synergizes with H-RasV12 or ErbB2 for invasive growth in vivo. Oral keratinocytes that have lost PTPN13 and express H-RasV12 or ErbB2 show enhanced Ras/RAF/MEK/Erk signaling. In co-transfection studies, wild type PTPN13 inhibited Ras/RAF/MEK/Erk signaling in HEK 293 cells that over-express ErbB2, EGFR, or H-RasV12, while an enzymatically inactive PTPN13 did not. Twenty percent of HPV negative HNSCC's had PTPN13 phosphatase mutations that did not inhibit Ras/RAF/MEK/Erk signaling. Inhibition of Ras/RAF/MEK/Erk signaling using the MEK inhibitor U0126 blocked anchorage independent growth in cells lacking PTPN13. These findings show PTPN13 phosphatase activity plays a physiologically significant role in regulating MAP kinase signaling.

O-17.02 ORAL HPV PREVALENCE AMONG HEALTHY MEN INTERNATIONALLY

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Background: HPV16 causes of a subset of oropharynx cancers. We aimed to describe the epidemiology of oral HPV infection among healthy adult men.

Methods: Baseline oral rinse/gargle specimens and questionnaire data were collected from 1,200 healthy men aged 18 to 70 (median 32 years), evenly distributed across the United States, Mexico, and Brazil. DNA was extracted using the Qiagen MDx Automated DNA Purification Protocol; Roche Linear Array was used to detect 37 HPV types, of which, HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66 were considered carcinogenic. At the time of this writing, HPV genotyping results were available from 416 men (US, n=145; Mexico, n=271).

Results: 91% of 416 oral specimens were β -globin positive (n=377). Of these, any HPV infection was detected in 7.2% of oral specimens (n=27); HPV prevalence was similar between countries (5.9% in US versus 7.7% in Mexico, p=0.5). Carcinogenic HPV was detected in 1.1% (n=4); of these, HPV16 was detected in 3 (0.8%) men and HPV39 in 1(0.3%) man. Overall HPV prevalence increased with increasing age (4% for men <25 years; 6% for 25 to <35; 10% for 35 to <45; 9% for 45 to <55, 13% for 55 to <65, and 14% for >=65 years; p for trend 0.052).

Future directions: Initial results from our study suggest that oral HPV infection is present in a subset of healthy men and does not vary between the US and Mexico. In contrast to what is known for genital HPV infections, we noted a positive association between HPV prevalence and age. For the meeting, we will present baseline HPV results from 1,200 men, multivariate analysis of behavioral factors associated with oral HPV infection, and type-specific concordance between genital and oral HPV infection.

O-17.03 MESENCHYMAL TRANSFORMATION DURING TUMOR PROGRESSION IN HPV-RELATED OROPHARYNGEAL CANCER

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Introduction: There is growing evidence, that high-risk human papillomaviruses (HPV) are an important risk factor for oropharyngeal squamous cell carcinoma (OSCC). No data exists so far concerning cellular alterations in relation to the patients HPV status in metastases, although lymph node metastasis seems to be the most important prognostic indicator. Methods: In a retrospective design, 48 primary tumor samples and their respective lymph node metastases were examined in regard to their HPV status and for the occurrence of p16, vimentin, E-cadherin and beta-catenin. Results: Correlation between positive HPV status and p16 expression was significant for primary tumor (p = 0.009) as well as for metastasis (p = 0.028). A significant correlation could be found between the expression levels of vimentin and the differentiation grade of the primary tumors (p = 0.021). Expression of E-cadherin in metastases was significantly less abundant than in primary tumors (p = 0.001). In both, primary tumors and metastases, three different patterns of beta-catenin immunostaining were found, and a positive HPV status was significantly correlated to an exclusively nuclear localisation of beta-catenin expression in primary tumors (p = 0.036). Discussion: We could approve the role of p16 as a surrogate marker for the HPV status even in the metastases. The up regulation of vimentin as well as the enhanced nuclear expression of beta-catenin in HPV/p16 positive tumors seems to be part of an epithelial-mesenchymal transition especially during tumor progression of HPV positive OSCC with beta-catenin acting as a transcription factor and being a potential candidate as a prognostic indicator.

O-17.04

HUMANA PAPILLOMAVIRUS IN TONSILLAR CANCER, AN EPIDEMIC OF VIRAL CARCINOMA

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Introduction. In the Stockholm area between 1970 and 2002, we have previously reported a three-fold parallel increase in the incidence of tonsillar squamous cell carcinoma (SCC) and the proportion of HPV-positive SCC. The proportion of HPV-positive SCC was 23% in the 1970s, 28% in the 1980s, 57% in the 1990s and 68% in 2000-2002.

Objectives. Here our aim was to examine the incidence of tonsillar SCC and the proportion of human papillomavirus (HPV) positive tonsillar SCC between 2003-2007 in the County of Stockholm in correlation to our previous data from 1970-2002.

Methods. All patients (n=120) diagnosed with tonsillar SCC during 2003-2007 in the County of Stockholm were included and 98 pre-treatment biopsies were available and presence of HPV DNA and HPV-16 E6 and E7 RNA were tested by PCR and RT-PCR. Incidence data were obtained from the Swedish Cancer Registry. Data reported from 1970 to 2002 were also obtained for comparison.

Results. HPV DNA was present in 83/98 (85%) of the tonsillar SCC biopsies from 2003-2007. Of the 77 HPV-16 positive tumors, HPV-16 E6 and E7 RNA were found in 98% of the analyzed cases. The proportion of HPV-positive cancers had significantly increased both from 1970 to 2007 (p < 0.0001) as well from 2000 to 2007 (p < 0.01), with 68% (95% CI, 53-81) 2000-2002; 77% (95% CI, 63-87) 2003-2005; and 93% (95% CI, 82-99) 2006-2007. The incidence rate of HPV-positive tumors almost doubled each decade between 1970-2007, in parallel with a decline of HPV-negative tumors.

Conclusion. Our data suggest that we are dealing with an epidemic of a virus-induced carcinoma, and that soon practically all tonsillar SCC will be HPV positive, as observed in cervical cancer.

O-17.05 HPV INFECTION IS ABSENT FROM ESOPHAGEAL CANCER IN CHINA

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Background: There are numerous reports that human papillomavirus (HPV) causes esophageal squamous cell carcinoma (ESCC). However, previous studies are widely variable in the fraction of cases testing HPV positive. Even within the same geographic area in China, HPV test results of ESCC have been very inconsistent. This variability may come from differences in laboratory detection methods, from contamination during sample collection or laboratory processing, or both. Objective: To determine the prevalence of HPV infection in ESCC and the activity of HPV in ESCC carcinogenesis. Methods: We prospectively collected tumor specimens from ESCC cases presenting for surgery at Yaocun Commune Hospital in Linxian, China. Rigorous sterile procedures were followed during tissue collection. A sandwich technique was used to prepare the samples. Two hundred and eighty-two paraffin embedded tumor sections were tested for HPV DNA using SPF10 LiPA25 (version 1) PCR and reverse line probe hybridization, which can detect 14 carcinogenic and 11 non-carcinogenic HPV types. DNA positive samples were tested for functionality by immunohistochemistry for p16INK4a. Results: Two hundred seventy-nine cases (98.9%) qualified for further evaluation; 3 were excluded because of the pathological diagnosis review is not the esophageal squamous cell carcinoma. Of those, 277 (99.3%) had no detectable HPV DNA-positive case showed positive immunohistochemical staining for p16INK4a .

Conclusions: We found little evidence that HPV infection is involved in ESCC carcinogenesis in this high risk area in China.

O-17.06

ASSOCIATION BETWEEN CHROMOSOME STABILITY, HPV AND PROGNOSIS IN TONSILLAR CARCINOMA

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Background: Tonsillar squamous cell carcinoma (TSCC) is frequently associated with oncogenic human papillomavirus (HPV). Data on chromosomal alterations in these lesions in relation to HPV, however, are scarce.

Objectives: The aim of this study was to assess chromosome 1 and 7 copy number changes in relation to the presence of HPV as well as the prognosis of TSCCs.

Methods: Seventy TSCCs with known clinical outcome and cell cycle protein expression profiles, and of which 36% showed HPV16 integration, were analyzed by double-target fluorescence in situ hybridization (FISH) using chromosome 1- and 7-specific centromere DNA probes. In addition, 6 HPV-positive, tumor-adjacent dysplasias were analyzed by FISH.

Results: Disomy for chromosome 1 and 7 was present in 26 out of 70 TSCCs (37%) and strongly associated with HPV presence (16 out of 26; p=0.001). Aneusomies for both chromosomes were observed in the remaining TSCCs, of which 24 tumors showed balanced and 20 unbalanced copy numbers (19 cases with higher chromosome 7 copy numbers). Aneusomies correlated significantly with tobacco- and alcohol consumption (p=0.001 and p=0.007, respectively) and a higher T-stage (p=0.03). Both HPV-positivity and chromosome disomy were significantly associated with favorable prognosis (p=0.003 and p=0.011, respectively). Five out of 6 HPV-positive, tumor-adjacent dysplasias also showed disomy for chromosomes 1 and 7.

Conclusions: HPV-positive TSCCs and their precursor lesions are genetically more stable than HPV-negative lesions, suggesting that HPV integration preferentially occurs in (near) diploid lesions. The high chromosome 7 copy numbers in HPV-negative tumors may point to oncogene involvement such as EGFR, which is inversely related to HPV presence. Furthermore, HPV-positivity and chromosome disomy are favorable prognosticators in TSCC.

O-17.07 ADENOID-CYSTIC CARCINOMAS OF SALIVARY GLANDS ARE ASSOCIATED WITH HIGH-RISK HPV

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Background: The etiology of adenoid-cystic carcinoma (ACC) is unknown. The only known risk-factor is previous exposure to radiation. Despite surgery and radiationtherapy, long-term cure is rare. Therefore, a better knowledge of the etiology could lead to innovative therapeutic approaches. HPV was classified as human carcinogen due to an epidemiologic association with cervical cancer and its ability to induce malignant transformation of cells. HPV was also identified to cause a subgroup of squamous cell carcinoma of the head and neck. Methods: 68 ACC were analyzed retrospectively. HPV-typing was carried out using HPV PCR and multiplex genotyping. Immunohistochemistry was used for P16- and EGFR-staining. Results: Of 68 ACC 39% were positive for high-risk HPV. Of these were 44% HPV16, 15% HPV18, 2.5% HPV or -59 single and 1.25% HPV45 and -33 double positive. 57% were HPV negative or positive for low-risk HPV11 (4%). The p16-expression was strong in 23.5%, intermediate in 20%, low in 8% and in 36% positive in single cells. EGFR was strong in 27%, in 13 % intermediate, in 14.5% low, in 5.5% positive in single cells and negative in 40%. Conclusion: The association of ACC with high-risk HPV defines a subgroup of tumors that is most likely caused by infection with HPV. As in other HPV caused cancers, we also found in ACC as the predominant HPV-type HPV16 followed by HPV18. All high-risk HPV positive ACC showed at least a partial p16 overexpression. The EGFR-Expression did not correlate with the HPV-association.

O-17.08 NATURAL HISTORY OF ORAL AND ANAL HPV INFECTION.

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A prospective study of oral and anal HPV infection was performed in a cohort of 404 HIV-positive men and women in Baltimore, MD. At biannual visits oral exfoliated cells were collected by Scope oral rinse and anal exfoliated cells with Dacron swab. HPV DNA was detected in Puregene-purified samples using PGMY09/11 primer PCR and Roche linear array. Incidence rates (IR) were calculated as the number of HPV infections detected over the total person-time contributed. Kaplan Meier and Cox proportional hazards models (clustered by person to account for multiple infections within the same individual) were used to evaluate time to clearance of incident, type-specific infection. Infections were considered cleared if undetected for two consecutive visits (or one visit followed by censor). 18% of subjects participated only at baseline, of remaining 330 subjects median follow-up was 20 months.

Overall, 349 oral HPV infections were detected during the study, including 187 prevalent and 162 incident infections. The prevalence of oral HPV infection at enrollment was 28% (cumulative prevalence=40%). Overall, 2,038 anal HPV infections were detected, including 1,785 prevalent and 253 incident infections. The prevalence of anal HPV infection was 81% at (cumulative prevalence=83%). Oral HPV incidence (IR=6.1 per 100 person-months) was significantly lower than anal(IR=44.3 per 100 person-months, IRR= 0.14, 95%CI=0.12-0.15).

Among newly detected infections, median time to clearance was longer for oral (6.5 months, interquartile range [IQR]=4.8-13.1) than anal (5.9 months, IQR=5.4-8.1) HPV infections. Clearance of newly-detected oral HPV infection was significantly less than anal infections in crude (HR=0.61, 95%CI=0.42-0.87) analysis and was borderline significantl in Cox models clustered by person to yield robust standard error (HR=0.61, 95%CI=0.36-1.0).

Conclusions: Incidence of oral HPV is significantly less and time to clearance of oral HPV infections may be longer than anal HPV infections among HIV infected men and women.

POSTER ABSTRACTS SESSION 17

POSTER SESSION III TUESDAY 20.00: ODD NUMBERS

POSTER SESSION IV

TUESDAY 21.00: EVEN NUMBERS

P-17.09

MOLECULAR CHARACTERIZATION OF HPV-ASSOCIATED HEAD AND NECK SQUAMOUS CELL CARCINOMAS

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Background: Specific oncogenic types of human papillomaviruses (HPV), most frequently HPV type 16 and potentially also other less prevalent high-risk HPV types, are causally associated with a subset of head and neck squamous cell carcinomas (HNSCC). In a further HPV16 DNA positive subset, the causal involvement of HPV16 is still unclear.

Objectives: HPV DNA genotyping alone is insufficient to define the role of the virus in HNSCC pathogenesis. For a better understanding, we included molecular and cell biological markers and also analysed their interactions.

Methods: HPV DNA of 208 fresh-frozen oropharyngeal HNSCC was determined by multiplex papillomavirus genotyping. HPV16 DNA positive tumours were analysed by NASBA and hybridization for expression of E6*II and also integration-associated HPV16 mRNA. Expression of proteins p53, p16INK4a, pRb and Cyclin D1 was examined by immunohistochemistry on tissue microarrays constructed from formalin-fixed paraffin-embedded tumour tissues.

Results: HPV16 DNA was detected in 99/208 (47.6%) of the tumours, with 63(30.3%) showing low (HPV+) and 36 (17.3%) high viral load (HPV++). In three tumours we found HPV18, HPV33 and HPV35, respectively. From the results obtained so far, RNA expression occurs mainly in HPV++ tumours (6 of 10), whereas in the HPV+ group E6*II RNA expression is infrequent (2 of 12).

Most HPV++ tumours showed reduced pRb, low Cyclin D1 and p53, and upregulated p16INK4a. In contrast, HPV+ tumours more often showed normal pRb, increased Cyclin D1 and p53, and reduced p16INK4a. This pattern is typical for HPV negative tumours.

Conclusion: The HPV+ tumours appear to behave as intermediate group but being closer to the HPV negative tumours. These data question an active role of HPV16 in the pathogenesis of these tumours.

P-17.10 PROGNOSTIC VALUE OF HPV AND N-STATUS IN TONSILLAR CARCINOMAS

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Background: In contrast to tumors originating from other sites of the head and neck area, the prognostic value of N-status appears to be controversial in tonsillar squamous cell carcinomas (TSCC).

Objective: The aim of this study was to investigate if HPV16, involved in the carcinogenesis of a subset of TSCC, influences the prognostic value of N-status, taking also into consideration patient treatment.

Methods: We retrospectively analysed data of 81 patients with TSCC for survival-rates (OS, DSS, DFS) related to nodal status and genomic HPV-16 integration (as determined by HPV-specific PCR and FISH analysis, as well as p16INK4A immunostaining), and corrected them for other clinical parameters.

Results: 33 of 81 TSCC were associated with HPV16 (41%). Their primary tumors were significantly smaller (p=0.04) and were more often treated with surgery followed by radiotherapy compared to HPV-negative TSCC. The percentage of cases of cervical metastasis did not differ in both groups. HPV presence and not nodal involvement was correlated with a favorable prognosis (p=0.02). However, within different therapy groups, both parameters did not significantly influence prognosis.

Conclusions: HPV 16, and not N-status, is an indicator for a favorable prognosis in TSCC. However, HPV and N-status seem not to influence prognosis within each treatment modality of TSCC patients. Because HPV-positive TSCC appear to metastasize to cervical lymph nodes at less advanced T-stages, and the frequency of HPV-positivity in TSCC is relatively high, our data suggest that HPV presence significantly reduces the prognostic value of N-status.

P-17.11 HUMAN PAPILLOMAVIRUS INFECTION IN TONSILLAR CANCER IN THE CZECH REPUBLIC

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BACKGROUND: The incidence of tonsillar cancer (TC) is raising almost all around the world despite an opposite trend in its well known etiological factors such as tobacco smoking and alcohol consumption. Hence it is necessary to explore additional risk factors. An important role of high risk human papillomavirus (HR HPV) in the etiology of head and neck cancer has been demonstrated recently. OBJECTIVE: In this study we investigated further the role of HPV in the etiology of TC. MATERIAL AND METHODS: Hundred and eight patients with the diagnosis of primary TC and 185 healthy controls were enrolled. The presence of HPV in the tumour tissue and cytological material, prevalence of HPV-specific antibodies, expression of viral E6 oncogene and a clinical follow up has been assessed. HPV DNA detection and typing has been done by means of polymerase chain reaction and reverse line blot assay. Sera were tested using ELISA with HPV 16 E6/E7 specific antigens. Demographical data were collected from all patients and controls. RESULTS: The prevalence of HR HPV DNA and of HPV-specific antibodies was significantly higher in patients with TC than in controls. HR HPV DNA and HPV16 E6/E7 specific antibodies were found in oral lavages and in sera of 68,1% and 85,5% of patients with the HR HPV DNA positive tumors. Patients with HPV positive tumors differ from those with HPV negative tumors in smoking and alcohol habits but not in the number of sexual partners. CONCLUSION: The results of this study provide an epidemiological evidence for HR HPV types being a strong risk factor for TC of patients from the Czech Republic. The combination of the serology and the detection of HPV DNA in oral rinses have shown a sensitive and specific approach for selection of patients at risk of HPV-associated tonsillar tumors.

P-17.12 TOBACCO, ALCOHOL, AND HPV RISK OF HEAD AND NECK CANCER

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Background: Tobacco, alcohol, and human papillomavirus (HPV) have been identified as major risk factors for head and neck cancer (HNC) but it is unclear whether there are two distinct HNC risk groups, one associated with HPV and the other with tobacco/alcohol use.

Methods: Between 2000 and 2004, 201 cases of HNC and 324 age-gender frequency matched controls were recruited in Iowa to assess anti-HPV VLP (virus like particles) antibodies 16, 18, 31, and 33 using an ELISA assay. Adjusted odds ratios (OR) and 95% confidence intervals (CI) were calculated using multivariate logistic regression analyses.

Results: Seronegative and seropositive/heavy tobacco users had an increased adjusted risk of HNC (VLP-seronegative OR= 2.6, 1.4-5.0; VLP-seropositive OR=2.3, 1.1-4.8), as did seronegative and seropositive/heavy alcohol users (VLP-seronegative OR=4.3, 2.1-9.1; VLP-seropositive OR: 3.9, 1.6-9.4). Among those with oropharyngeal cancer, increased risk was found among heavy tobacco use with greater risk among the seronegative cases (VLP-seronegative OR=11.1, 2.3-54.6; VLP-seropositive OR=6.4, 2.0-20.5) and seronegative/heavy alcohol cases (VLP-seronegative OR=9.5, 2.3-38.6; VLP-seropositive OR=5.0, 1.4-17.6). In contrast, regardless of serology status, an increased risk of cancer of the oral cavity was associated with heavy alcohol use (VLP-seronegative OR=3.7, 1.6-8.3; VLP-seropositive OR=3.8, 1.4-10.1). The risk was less elevated among heavy tobacco users (VLP-seronegative OR=2.0, 1.01-4.1; VLP-seropositive OR=1.5, 0.7-3.4).

Conclusion: We did not find a distinction in HNC risk groups based on HPV serology and on tobacco/alcohol use, suggesting that differences in risk across studies are due to exposure levels among the population examined and to differences associated by tumor site.

P-17.13 HPV IN SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK

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Background: Human Papillomavirus (HPV) contributes to the pathogenesis of head and neck squamous cell carcinoma (SCC). Previous studies have demonstrated a higher prevalence of HPV16 in oropharyngeal SCCs than oral SCCs or laryngeal SCCs.

Methods: Individuals with pathologically confirmed SCC of the head and neck participating in a multicenter study of epidemiologic risk factors through the University of Michigan Head and Neck SPORE program were eligible for inclusion. Paraffin-embedded tissue was examined by a pathologist, and normal, dysplasia, and invasive cancer areas were circled for microdissection. HPV testing was performed using the GP5+/GP6+ reverse line blot method. Adequacy of DNA was assessed by beta-globin gene PCR. Results: Microdissected DNA derived from 222 samples in 203 patients were tested. The prevalence of HPV-positive cancers was 19.2% (39/203). HPV16 was identified in 38 and HPV18 in 1 case. The mean age of HPV positive cases was similar to the mean age of HPV negative cases (58.6 vs. 58.4 p=0.87). Subjects with HPV+ tumors tended to be male (OR= 2.05, 95% C.I., 0.74 – 5.61). The majority of HPV+ cancers were found in the pharynx and there were no HPV+ cases in the larynx. HPV+ cancers were more likely to be stage 3 (OR= 5.46, 95% C.I., 1.09 – 27.4) or stage 4 (OR= 4.67, 95% C.I., 1.06 – 20.6) than stage 1 or 2. Cancers arising in current smokers were less likely to be HPV+ than in non-smokers (OR= 0.32, 95% C.I., 0.15 – 0.69). Conclusion: HPV16-associated squamous cell carcinomas represent an etiologically distinct subset of cancers of the head and neck. Consistent with prior studies, cancers of the oropharynx are more likely to have detectable HPV than other tumor sites. In addition, HPV positive tumors also appear to differ by other epidemiologic features, including gender and possibly smoking.

P-17.14

SHORT-TERM NATURAL HISTORY OF ORAL HPV INFECTION IN HIV+ COHORT

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Oral HPV (O-HPV) is etiologically responsible for a subset of oropharyngeal squamous cell cancers, yet the natural history of O-HPV is poorly understood. To investigate the short term natural history of O-HPV, we compared a two-week sampling period with a six-month sampling period (as established in the cervical HPV literature). Methods: A cohort of HIV+ patients from the Johns Hopkins HIV clinic was prospectively followed over six consecutive months with bimonthly visits(1-14). At each visit, subjects provided an oral rinse sample (ORS). HPV serostatus, CD4 count and HIV viral load were determined at visits 1 and 14. O-HPV DNA was purified from ORS using Puregene (Gentra Systems), genomic DNA was detected by PGMY09/11 L1 consensus primer and type specified by reverse line blot hybridization (Roche Molecular Systems). Results: 92 of 112 subjects completed the study. Average point prevalence of individuals with O-HPV infection for two-week (45%, 95% CI: 42-48) and six-month (47%, 95% CI: 40-54) sampling periods were similar. However, cumulative prevalence of individuals with O-HPV infection was significantly higher with a two-week (82%, 95%CI 73-89%) than a six-month (53%, 95%CI 43-63) sampling period (p< 0.0001). Similar patterns were seen when analysis was performed by infections. Average point prevalence and cumulative prevalence of HPV 16 were 6% (95%CI 4 - 7) and 21% (95%CI 14-31), respectively. When infection patterns were considered, 57 new infections were detected at visit 14 using six-month sampling, while 251 transient infections were observed in two-week sampling. Infections which were persistent or absent in six-month sampling period, held similar patterns in two-week sampling, although a dynamic infection cycle was revealed by the numerous newly detected infections in two-week sampling. Infections that tended to persist were those with higher viral load. Conclusion: Six month sampling period appears to be adequate for studying the natural history of O-HPV.

P-17.15 HUMAN PAPILLOMAVIRUS IN TONGUE BASE – THE PAST 10 YEARS

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Background. In 2004, we reported that 40% of tongue base squamous cell carcinoma (SCC) was positive by PCR for human papillomavirus (HPV) DNA, of 25 pre-treatment samples collected during the period of 1970-2002. However, in that report we did not study the proportion of HPV in the tongue base SCC over time, as we have done for tonsillar SCC. In fact, in the Stockholm area between 1970 and 2002, we have previously observed a three-fold parallel increase in the incidence of tonsillar SCC cancer and the proportion of HPV-positive tonsillar SCC, more specifically from 23% in the 1970s to 68% in 2000-2002. Moreover, this tendency has continued as reported elsewhere.

Objectives. The aim of this project was to examine in the Stockholm area, if there has been a similar increase as that observed in tonsillar SCC between 1998-2007 in the HPV proportion in tongue base SCC.

Methods. All patients (n=117) diagnosed with tongue base SCC between 1998-2007 in the Stockholm area were included. Presence of HPV DNA and HPV-16 E6 and E7 RNA and RT-PCR was analyzed by PCR. Incidence data were obtained from the Swedish Cancer Registry.

Results. So far, we have found that 80% of the samples from 2007 were HPV positive and that all HPV positive samples contained HPV-16 DNA. Moreover, there has also been an increase in the incidence of tongue base SCC in the Stockholm area from 1970 to the present day.

Conclusion. Our data so far, indicate that there is an increase in the proportion of HPV positive cases, and the total incidence of tongue base SCC the past 10 years. Further details of the data will be presented.

P-17.16

HIGH PREVALENCE OF MEDIUM AND LOW-RISK HPV IN OESOPHAGEAL CANCER

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Human papillomavirus (HPV) is increasingly recognized as an important etiological factor of oesophageal cancer. However, its prevalence is highly variable between different geographic areas, and it is also dependent on the different diagnostic tests such as in situ hybridization, southern blot or PCR. The objective of this study was to determine the prevalence of HPV in a selected population of esophageal cancer in Catalonia, Spain. Consecutive patients diagnosed with oesophageal cancer were selected between February 2007-September 2008. Paired endoscopic biopsy samples were obtained from normal mucosa and tumour and collected in RNA later. Extracted DNA was amplified using F-HPV typing[™] kit PCR (Molgentix, Spain) with a set of 24 fluorescently labelled primers recognizing HPV-types 6, 11, 16, 18, 26, 31, 33, 35, 39, 42, 43, 44, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73 and 82. Twenty patients (mean age: 62 ±11, 16 M/ 4 F) diagnosed with 11 squamous cell carcinoma and 9 with oesophageal adenocarcinoma participated in the study. HPV was detected in 6 out of 11 patients (54.5%) with squamous cell carcinoma and in 1 of 9 patients (11.1%) with adenocarcinoma of esophagus. Medium risk HPV types (53, 70, 73) were detected in 5 out of 7 patients in both tumour and the surrounding normal mucosa. Multi-infection with several medium risk HPV types was found in 4/7 (57%). Low risk HPV-42 was also present in 5/7 (71%) of cancer samples, three in combination with medium risk VPH, one isolated, and the remaining sample had medium risk HPV-73 in the surrounding normal mucosa. In our series of oesophageal squamous cell carcinoma we found a high prevalence of medium and low risk HPV. The presence of several medium risk HPV was common in both tumour and normal oesophageal mucosa samples from these patients.

P-17.17 ROLE OF HPV16 IN SQUAMOUS CELL CARCINOMAS OF THE LARYNX

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Background: The mucosal high-risk human papillomavirus HPV16 is a necessary cause for the majority of squamous cell carcinomas (SCC) of the uterine cervix and also of a subset of oropharyngeal SCC. HPV16 has also been found in 15-35% of laryngeal SCC.

Objectives: To clarify the role of HPV16 in laryngeal SCC.

Methods: Formalin-fixed paraffin-embedded laryngeal tumour tissues of 94 patients were analyzed. HPV DNA was determined by multiplex genotyping (MPG). Expression of cellular proteins p53, pRb, p16INK4a and CyclinD1 as surrogate markers for active HPV involvement was evaluated on tissue microarrays by immunohistochemistry. HPV16 DNA positive tumours were analysed by NASBA and hybridisation for expression of E6*II and also integration-associated HPV16 mRNA.

Results: Of 33 laryngeal SCC analysed so far, 8 (24%) were HPV16 DNA positive. However protein expression patterns characteristic for HPV involvement (downregulated p53 and pRb, upregulated p16INK4a and diffuse cytoplasmic CyclinD1) were not revealed in these tumours. Results of ongoing further HPV DNA and RNA analysis will be reported. Conclusions: The preliminary results obtained so far indicate that active involvement of HPV16 in laryngeal tumours appears to be rare. Possibly, HPV16 might act only transiently in larynx carcinomas or is detected as uninvolved bystander.

P-17.18 ORAL SEXUAL BEHAVIORS ASSOCIATED WITH PREVALENT ORAL HPV INFECTION

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Oral HPV infection is a cause of oropharyngeal cancer. We investigated whether sexual behaviors that elevated odds of oropharyngeal cancer in a case-control study similarly elevated odds of oral HPV infection among controls and an additional population of college-aged men. HPV infection was detected among 4.8% of 332 outpatient-clinic controls and 2.9% of 210 college-age men. Odds of infection independently increased with lifetime number of oral (p-trend=0.007) or vaginal-sexual partners (p-trend=0.003) among controls. In college-age men, odds of oral HPV infection increased with recent number of oral-sexual (p-trend=0.046) or open-mouthed kissing partners (p-trend=0.023), but not with vaginal-sexual partners. Oral sex and open-mouthed kissing are associated with oral HPV infection.

P-17.19

HPV IS ASSOCIATED WITH OROPHARYNGEAL CARCINOMAS AND PROGNOSIS IN NON-SMOKERS

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Background: Increasing evidence shows that oncogenic human papillomavirus (HPV) type 16 is etiologically involved in the development of a subgroup of head and neck carcinomas, particularly oropharyngeal squamous cell carcinoma (OSCC). A small part of head and neck carcinoma patients are non-smokers, and data on the HPV frequency and prognosis in tumors of this patient group are scarce.

Objective: Because tobacco smoking may strongly influence the survival of patients with head and neck carcinomas, we decided to examine the prognostic value of HPV positivity in tumors of non-smoking patients only.

Methods: A retrospective study was performed on 23 OSCC and 21 carcinomas from other locations of non-smokers. HPV-presence was identified by HPV-specific PCR and FISH analysis, and immunostaining for p16INK4a expression. Results were correlated with clinical patient data.

Results: In non-smokers HPV16 as well as p16INK4a overexpression was only detected in OSCC (65% vs 0%; p<0.0001). In this patient group HPV-positivity was correlated with a significantly favorable prognosis (p = 0.0495).

Conclusios: In comparison with the literature on OSCC a higher percentage of oncogenic HPV is detected in non-smoking patients (25-35% vs 65%, respectively). In addition, HPV-positivity is associated with a significantly favorable prognosis independent of the confounder tobacco smoking.

P-17.20 PRESENCE OF HPV IN ORAL SQUAMOUS CELL CARCINOMA

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C **Zagalo**, *Instituto Portugues Oncologi Lisboa Francisco Gentil, EPE - Head And Neck Department, Lisbon, Portugal* Most head and neck cancers are associated with tobacco and alcohol abuse, however about 20% of cases aren't associated

Most head and neck cancers are associated with tobacco and alcohol abuse, however about 20% of cases aren't associated with these risk factors.

High proportions of these cancers are occurring in patients who never smoked.

Up to 60% of oropharyngeal cancers have been associated with oncogenic HPV types, while other head and neck cancers, including oral cavity tumours, have lower HPV prevalence (about 20%).

Similar to all other HPV related cancers HPV-16 is the most common type identified in oral cavity cancers followed by HPV-18.

Klozar described that HPV positive patients with oral SCC have higher survival rates than HPV negative patients and this factor should be considered in treatment decisions.

AIM: Study of HPV in oral squamous cell carcinoma as a predictive value of therapeutic outcome.

METHODS: 38 samples from 17 patients, 5 females and 12 males (60.1y), with oral SCC confirmed histologically, were analysed for HPV presence. Smears from the same patient from the healthy part of the mouth were used as controls. HPV presence was evaluated by PCR by GP5+/GP6+. HPV positive samples were typified using MYs/microarrays (PapilloCheck). Negative, positive and internal control were MRC5, Caski and B-globin, respectively,

RESULTS: All control samples were negative for HPV. However, 11,8% (2/17) of the SCC samples were positive for HPV. Positive samples were both from male patients with tongue lesions. 50 % of the positive samples (1/2) were positive for HPV-16 or HPV-33.

CONCLUSIONS: The rate of HPV detection in SCC biopsies in this study is lower (11,8% vs 23.5%) than that found in literature, probably due to the small number of samples. We also can't conclude anything about type distribution because we have a very small number of positive HPV samples. Detection of HPV in SCC can be useful in terms of prognostic and therapeutic approach.

P-17.21 HPV AND IMMUNE RESPONSES IN HEAD AND NECK CANCERS

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Background and objectives: The incidence of head and neck squamous cell carcinomas (HNSCC) is increasing gradually, despite a total decrease in the number of smokers in Western Europe and the USA. This is thought to be due to an increase in the numbers of HPV-associated HNSCCs (~50% of HNSCC cases), particularly tonsillar and base-of-tongue cancers. Several studies have outlined the improved prognosis found in HPV+ HNSCC, and this demonstrates the emerging importance of this subset of HNSCC.

We have commenced an analysis of HPV infection in the aetiology of HNSCC and the associated local and systemic immune responses, which will be linked to treatment outcomes and prognosis.

Study design and methods: A prospective study recruiting patients primarily from the catchment area for the Hospital of Bielefeld, Münster University, Germany, which currently treats approximately 200 cases per year. We will screen for HPV in all cases, define the subtypes involved, and quantify HPV loads in terms of DNA and mRNA by quantitative real-time PCR. The immune responses in the tumour environment will be defined via the cytokine milieu by qPCR and cytokine microarray. Frequencies of infiltrating lymphocytes will be ascertained, together with phenotype. Analyses of PBMC will be undertaken in regards to specific anti-HPV T-cell responses (ELISpots and Intracellular cytokine staining), NK and NKT-cell responses which have been shown to be linked to HNSCC survival outcomes. In this manner, we will analyse the immune and virological parameters in association with prognosis and treatment outcomes by comparing HPV+ and HPV- HNSCC.

Results and discussion: Ongoing recruitment and data analyses. We expect to see distinct immune profiles in both local and systemic compartments for the two groups under analyses. In this way, we hope to define immune parameters which may be augmented to improve treatment and survival outcomes.

SESSION 18

TRANSFORMATION AND CARCINOGENESIS

PROGRAMME

THE 25TH INTERNATIONAL PAPILLOMAVIRUS CONFERENCE MAY 8-14 2009, MALMÖ, SWEDEN

TIME NUMBER TITLE

2009-05-13

SESSION 18: TRANSFORMATION AND CARCINOGENESIS

SCANIA

ROOM

08.30-09.05	O-18.00	ROLES OF VIRAL AND HOST FACTORS IN CERVICAL CANCER P Lambert
09.05-09.16	O-18.01	MODULATION OF P63 IN CERVICAL CARCINOMA AND REGULATION BY E6 F Thierry, Y Ben Khalifa, M M Tan, D S Toh, S Teissier, M Daynac
09.16-09.27	O-18.02	CUTANEOUS HPV38 INDUCES STABILIZATION OF DELTANP73ALPHA BY A NOVEL MECHANISM r accardi
09.27-09.38	O-18.03	ONCOGENE DISRUPTION OF CELL-CELL SENSING UNDERLIES PHENOTYPIC CHANGE DURING NEOPLASIA J Doorbar, Erin Isaacson, Ken Raj, Q Wang, D Jackson
09.38-09.49	O-18.04	INDUCTION OF GENOMIC INSTABILITY BY THE HPV16 E7 ONCOPROTEIN I Hoffmann, R Bahtz, M Arnold, A Krause, O Cizmecioglu, F Settele
09.49-10.00	O-18.05	IDENTIFICATION AND FUNCTIONAL INVESTIGATION OF GENES INVOLVED IN CERVICAL CARCINOGENESIS M Liesenfeld, B. Rudolph, D Steinbach, L Jansen, S. Mosig, H. Funke, I Runnebaum, M Dürst, C Backsch

O-18.00 ROLES OF VIRAL AND HOST FACTORS IN CERVICAL CANCER

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High risk Human Papillomaviruses (HPVs), such as HPV16, are critical etiological agents in cervical cancer, other anogenital cancers and a subset of head and neck cancers. To understand the role of high risk HPVs in cervical cancer in vivo, multiple laboratories have generated HPV-transgenic mice. Using these mouse models the individual roles of the three HPV16 oncogenes, E5, E6 and E7, in cervical cancer have been elucidated. In addition, those cellular targets of these oncogenes that are important in mediating the oncogenic properties of these three oncogenes in cervical cancer in the mouse are currently being identified and characterized. These ongoing studies, which will be described, clearly illustrate the multifunctional nature of E6 and E7 in cervical cancer.

HPVs are not sufficient to cause cervical cancer. Host factors and potentially other environmental factors (e.g. tobacco smoke) likely contribute to the progressive neoplastic disease that culminates in cervical cancer. One factor that is quite apparent from the mouse model studies is the female hormone, estrogen. The cervical epithelium is a highly estrogen responsive tissue. Therefore it is not surprising that estrogen plays a critical role in cervical carcinogenesis. Our recent studies confirm that this role of estrogen is highly dependent upon its nuclear receptor, estrogen receptor (ER) alpha. This knowledge and the fact that estrogen is required not only for the onset of cervical cancers but also their maintenance, led us to test the hypothesis that drugs that can interfere with ER alpha function are of therapeutic value. Studies addressing this hypothesis will be described.

O-18.01 MODULATION OF P63 IN CERVICAL CARCINOMA AND REGULATION BY E6

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In cervical carcinoma cells, the E6 and E7 proteins from oncogenic human papillomaviruses are continuously expressed and induce cell transformation mainly through their interaction with the p53 and pRB pathways, respectively. A critical event on the path to malignant conversion is the loss of the E2 repression of E6 and E7 transcription due to the integration of the viral DNA into the host genome. Using microarrays, we have shown that E6/E7 repression through reintroduction of a functional E2 in the HeLa cervical carcinoma cell line, associated with HPV18, activates a cluster of p53 target genes as well as a novel subset of p63 target genes. These genes belong to the cell-matrix and cell-cell adhesion pathways as previously described (Caroll et al. Nat Cell Biol., 2006). We used an approach that relies on small interfering RNA to demonstrate that the p63 as well as the p53 target genes were activated through silencing of the E6/E6AP pathway in cervical carcinoma cells. We then wanted to determine whether the new pathway discovered was indeed modulated through p63 and not through p53. In addition, and since p63 is expressed as multiple isoforms, it was necessary to define the roles of these different isoforms in cervical carcinoma and their relative regulation by E6. Here we demonstrate that the new set of genes modulated in cervical carcinoma cells are direct p63 targets through gene silencing with siRNA or direct transcriptional modulation by transfected expression vectors of the different p63 isoforms. These targets were confirmed by ChIP experiments in cells overexpressing the Np63 isoform. Modulation of the expression of the p63 isoforms was studied in HeLa and Caski cells and a model will be presented of the putative regulation of p63 and its target genes in cervical carcinoma.

O-18.02 CUTANEOUS HPV38 INDUCES STABILIZATION OF DELTANP73ALPHA BY A NOVEL MECHANISM

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Over the last few years molecular and epidemiological studies have focused on beta cutaneuos HPV types, with the aim to shed light on their potential role as cofactors in skin carcinogenesis. We have previously shown that HPV38 E6 and E7 display transforming activities in vitro and in vivo models. Recently, we have described a new mechanism by which HPV 38 can counteract p53 pro-apoptotic activity, accumulating deltaNp73alpha, a dominant negative inhibitor of both, p53 and p73. This truncated form of p73, is very often found up-regulated in human tumours. In HPV38 E6/E7 keratinocytes and in the skin of HPV38 transgenic mice, deltaNp73 is accumulated and this event is mediated by a specific form of p53, which has high affinity for the promoter of deltaNp73. Here we show that HPV38 induces accumulation of deltaNp73 not only by activating its transcription, but also regulating its protein stability. We observed that IkappaB-kinase-beta (IKKbeta, the key kinase of NF-kappaB signalling, is strongly activated in HPV38 E6/E7-immortalized keratinocytes. In addition, IKKbeta physically interacts with deltaNp73alpha, leading to its phosphorylation and stabilization. Inhibition of IKKbeta by a specific inhibitor (Bay11) led to destabilization of deltaNp73alpha. We have also observed that degradation of deltaNp73 is controlled by the calpain pathway. In fact, inhibitors of calpains, e.g. PD 150606 or E-64, restore the normal levels of deltaNp73 in cells cultered in presence of Bay11. Interestingly, IKKbeta-mediated deltaNp73alpha stabilization was also detected in breast and head and neck cancer cell lines, suggesting that this event is not exclusively restricted to HPV-mediated transformation. In summary, our data highlight a new mechanism of deltaNp73alpha activation and further confirm that characterization of virus-mediated transformation is a valid approach to identify novel biological events implicated in human carcinogenesis.

O-18.03

ONCOGENE DISRUPTION OF CELL-CELL SENSING UNDERLIES PHENOTYPIC CHANGE DURING NEOPLASIA

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High-risk HPV infection can lead to cervical neoplasia of varying grade, and is characterized by changes in viral gene expression and a failure of the virus to complete its productive cycle. The molecular events that underlie these changes are not well characterized, but are thought to involve the deregulation of E6/E7 gene expression in the absence of genome integration. Here we have prepared over 20 HPV16 episome-containing cell lines in the NIKS isogenic background, and have propagated these lines in organotypic raft-cell culture to allow epithelial cell differentiation. To our surprise these lines were heterogeneous in their ability to complete the full productive life-cycle, with some lines differentiating to produce CIN1-like phenotypes that express both E4 and L1, and some producing CIN2/3 like phenotypes in which surrogate markers of E6/E7 expression extended close to the epithelial surface. Although episomal copy number varied between around 50 to 500 copies/cell in the different clonal lines, copy number did not appear to be related to neoplastic phenotype. More important were differences in the levels of E6 and E7 and the concomitant effects on their cellular targets. Cell lines exhibiting CIN2/3-like phenotypes generally had high E6 and low p53/hDLG, and high E7 and low pRb when compared to cell lines that had a CIN1-like phenotype. A strong correlation was noticed in the ability of the different lines to respond to cell-cell contact following growth in monolayer culture. Clonal lines exhibiting a CIN2/3like phenotype continued to proliferate at confluence, with a steady reduction in cell size as space became restricted. By contrast, those exhibiting a CIN1-like phenotype were responsive to cell-contact signals and growth arrested. Our molecular analysis suggests that these phenotypic changes are mediated through changes in beta-catenin activity driven by differences oncogene expression.

O-18.04 INDUCTION OF GENOMIC INSTABILITY BY THE HPV16 E7 ONCOPROTEIN

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Centrosomal aberrations is a hallmark of many human tumors. Duplication of the centrosome is a process that must be completed with high fidelity exactly once per cell cycle to ensure proper formation of a bipolar mitotic spindle. Recent work has expanded our understanding of the functional complexity and importance of this organelle. The centrosomal localisation of proteins has shown that numerous cellular processes can be perturbed by centrosomal dysfunction and may lead to aneuploidy.

The E6 and E7 oncoproteins of high risk HPV-papillomaviruses cooperate to induce mitotic defects and genomic instability by uncoupling centrosome duplication from the cell division cycle while low-risk HPV E6 and E7 proteins do not induce such abnormalities. The mechanisms by which HPV E6 and E7 subvert centrosome homeostasis are strikingly different. Whereas the E7 oncoprotein rapidly drives centrosome duplication errors in cells that appear phenotypically normal, expression of the HPV E6 oncoprotein results in an accumulation of supernumerary centrosomes in multinucleated cells. The major aim of the project is to establish HPV-associated carcinogenesis as a model system to study the development of chromosomal instability. We are studying the effect of high risk E7 expression on cellular proteins involved in centriole duplication. We found that the protein levels of two cellular protein kinases, namely the polo-like kinases Plk2 and Plk4 are modulated in response to HPV16 E7 expression suggesting that these kinases are direct targets of HPV16 E7. Both kinases are overexpressed in cervical cancer cell lines. Furthermore, we could demonstrate that overexpression of both Plk2 and Plk4 leads to the formation of extra centrosomes which can lead to chromosomal instability (CIN) in cervical cancer cells.

O-18.05

IDENTIFICATION AND FUNCTIONAL INVESTIGATION OF GENES INVOLVED IN CERVICAL CARCINOGENESIS

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Background: Persistent infection with a high risk human papillomavirus (HR-HPV) type is a prerequisite for the development of cervical cancer (CxCa). This process is accompanied by numerous genetic alterations which may result in modified gene expression thereby affecting cell homeostasis.

Objective: Our aim is to identify genes which are consistently down-regulated in cervical carcinogenesis and which are likely to contribute to malignant transformation.

Methods: Micro-array analyses of RNA from microdissected cervical precancers (CIN2/3) and CxCa were performed to screen for putative tumour suppressor genes (TSG). Validation of the candidate genes was done by quantitative reverse transcription PCR (qRT-PCR) in normal cervical tissues, CIN2/3 and CxCa and in various cell lines. The most promising genes were ectopically expressed in the above tested cell lines and in primary fibroblasts via transient transfection or lentiviral transduction to analyze their influence on colony formation, cell proliferation and cellular senescence

Results and conclusions: Sixteen putative candidate TSG were identified and validated. Functional tests were performed for SORBS2, HOPX and other genes. Transient reconstitution of SORBS2 expression resulted in a significant reduction of cell proliferation and colony formation in CaSki, HPKII and HaCaT cells. Similiar results were obtained for HOPX in CaSki and HPKII cells. Strong promoter methylation of SORBS2 was observed in the majority of CxCa. The lentiviral experiments and senescence tests are still ongoing.

Our first results thus far suggest that two of the genes identified, SORBS2 and HOPX, have characteristic tumour suppressor gene properties and may contribute to the transformation process. Consistent down-regulation of a subset of genes may proof to be a predictor for progression.

POSTER ABSTRACTS SESSION 18

POSTER SESSION III TUESDAY 20.00: ODD NUMBERS

POSTER SESSION IV

TUESDAY 21.00: EVEN NUMBERS

BRCA1-ASSOCIATED PROTEIN 1 (BAP1) ASSOCIATES WITH HUMAN PAPILLOMAVIRUS PROTEIN E7

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Background: High-risk human papillomavirus (HPV) E6 and E7 oncoproteins function by targeting the p53 and pRb tumor suppressors for degradation. The HPV E6 and E7 oncoproteins affect double strand DNA break repair and the breast cancer susceptibility gene 1 (BRCA1) has been reported to associate with HPV oncoproteins. BRCA1-associated protein 1 (BAP1) is a ubiquitin carboxy hydrolase and a candidate tumor suppressor. It associates with the RING finger domain of BRCA1 and may deubiquitinate either BRCA1 or BRCA1 associated proteins.

Objectives: BAP1 was identified as a candidate HPV16 E7 associated cellular protein by tandem affinity purification The goal of this study is (1) to confirm and biochemically characterize the HPV16 E7/BAP1 complex and (2) to investigate the biological consequence(s) of the HPV16 E7/BAP1 association.

Methods: GST pull-down and co-immunoprecipitation experiments have been used to investigate the BAP1/HPV16 E7 association.

Results: The association of HPV16 E7 with BAP1 was confirmed by GST pull-down and co-immunoprecipitation experiments.

Conclusions: We provide biochemical evidence that BAP1 associates with HPV16 E7 and are in the process of mapping the HPV16 E7 and BAP1 sequences that are necessary. We are using growth suppression and other assays to determine the biological relevance of this association. Given that HPV16 E7 expressing cells have defects in double strand DNA break repair it is possible that binding of E7 to BAP1 may subvert some aspect of BRCA1 associated DNA repair and tumor suppressor activity, thereby providing a promiscuous environment for tumorigenesis.

P-18.07

HPV16 URR CANCER ALTERATIONS INCREASE TRANSCRIPTION, ORI FUNCTION AND IMMORTALIZATION

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HPV DNAs isolated from cervical and head and neck carcinomas frequently contain nucleotide sequence alterations in the viral upstream regulatory region (URR). Our study has addressed the role such sequence changes may play in the efficiency of establishing HPV persistence and altered keratinocyte growth. Genomic mapping of integrated HPV 16 genomes from 32 cervical cancers revealed that the viral E6 and E7 oncogenes as well as the L1/URR regions were intact in all. The viral URRs sequences were found to harbor distinct sets of nucleotide substitutions. A subset of the altered URRs increased the potential of HPV 16 to establish persistent, cell growth-altering viral genome replication in the cell. This aggressive phenotype in culture was not solely due to increased viral early gene transcription but also to greatly augmented initial amplification of the viral genome. As revealed in an ori-dependent HPV 16 genome amplification assay, the altered motifs that led to increased viral transcription also greatly augmented HPV 16 ori function. The nucleotide sequence changes correlate with those previously described in the distinct, geographical North American type 1 (NA1) and Asian-American (AA) variants that are associated with more aggressive disease in epidemiologic studies and encompass, but are not limited to, alterations in previously characterized sites for the negative regulatory protein YY1. Our results thus provide evidence that nucleotide alterations in HPV regulatory sequences could serve as potential prognostic markers of HPV-associated carcinogenesis.

P53 RECOVERY AND APOPTOSIS INDUCTION VIA INHIBITION OF E6-PDZ INTERACTION

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Background: Human papillomavirus (HPV) is the cause of > 99% of cervical cancer (CxCa). Oncogenic E6 protein binds to PDZ domain 1 of MAGI1 via its C-terminal PDZ binding motif. E6-MAGI1 interaction results in MAGI1 degradation and subsequent activation of JNK. Activation of AP1 transcription factor via active JNK stimulates expression of HPV-E6/E7. Consequently, E6 interaction with MAGI1-PDZ1 results in elevated levels of E6. This positive feedback mechanism is hypothesized to function in maintenance of transformation in HPV positive cells.

Objective: To identify small molecules able to disrupt the E6-MAGI1 interaction and to determine their effect on HPV-mediated carcinogenesis.

Methods: The published crystal structure of MAGI1 bound to the C-terminal peptide of HPV18-E6, was used for in silico screening to identify small molecules that inhibit E6-MAGI-1PDZ1 interaction. 650,000 molecules were virtually screened. Molecules predicted to be active were tested in biochemical assays for their ability to disrupt interaction between MAGI1 and E6. Candidate inhibitors were then screened on CaSki (HPV16) and HeLa (HPV18) cells for their effect on E6/E7 levels, p53 levels, and induction of apoptosis via TUNEL assay.

Results: Virtual screening of chemical libraries resulted in identification of 184 small molecules with predicted PDZ binding activity. Three molecules showed inhibition of E6-MAGI1-PDZ1 interaction in a biochemical assay. One molecule reduced HPV-E6/E7 mRNA and protein expression levels and substantially restored the tumor suppressor p53. It also selectively induced apoptosis in HPV positive CxCa cell lines.

Conclusion: A small molecule that targets the E6-MAGI1-PDZ1 interaction results in decreased E6 and increased p53 levels, resulting in apoptosis of CxCa cell lines. Preclinical animal studies are underway.

P-18.09

HUMAN PAPILLOMAVIRUS18E6 PREVENTS STABILIZATION OF P53 BY INHIBITION OF PHOSPHORYLATION

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BACKGROUND: High risk Human Papillomavirus (HPV) abrogates p53 function and its transactivity. Moreover, overexpression of wild-type p53 in HPV positive cells doesnot induce cell cycle arrest or apoptosis. Functional activation of p53 depends on activation of kinases one of them being Cyclin dependent kinase 5 (Cdk5). Though, Cdk5 was discovered in HeLa cells, its function is well-known in neuronal cells. OBJECTIVE: To investigate why overexpression of p53 does not induce cell cycle arrest/apoptosis in HPV positive HeLa cells. METHODS: HeLa-Tet-ON system was exploited for the development of p53 and GFP indicible isogenic models by tranfecting Tet-ON and pTREp53 plasmid sequentially. To evaluate cell-cycle or apoptosis FACS analysis was performed with propidium Iodide (PI) or Annexin V FITC/TUNNEL assay respectively. Immunoblot and RT-PCR was preformed to detect protein or mRNA expression levels. To inhibit protein phosphatase 2A (PP2A) okadaic acid was used. Cell proliferation assay was performed by MTT assay. Specific SiRNA was used to knock down p53 protein. p53 was immunoprecipitated (IPed) with its specific antibody and was immunoblotted with Cdk5 specific antibody. E6 immunoprecipitation was performed with specific E6 antibody and supernatant was post IPed with p53 antibody and blotted with p53 and phospho p53. RESULTS AND DISSCUSSION: Non genotoxic overexpression of p53 does not lead to cell cycle arrest or apoptosis in HPV positive HeLa cells. However inhibition of PP2A results in cell cycle arrest and apoptosis through stabilization of p53 protein by phosphorylation at Ser20 and Ser46 residues. The stabilization of p53 and apoptosis was reversed by inhibition of an upstream kinase Cdk5 which directly interacts with p53. Moreover E6 associated p53 is dephosphoralted as compared to free p53. We propose that Cdk5 phosphorylates only free p53 and not E6 bound p53. Phosphorylation of p53 at Ser20 and Ser46 increases Bax leading to apoptosis.

THE P16INK4A PATHWAY IN CERVICAL CANCER

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Background: Unravelling effects of High Risk HPV oncoproteins has led to the discovery of dysregulated proteins and potential biomarkers. One of these is p16INK4A (p16). Although p16 protein expression is used diagnostically, little is known about its transcriptional regulation and cell cycle involvement.

Objectives: Using the HaCaT, p16 null, cell line we hope to determine the cause and effects of p16 up regulation in Cervical Cancer (CC).

Methods: The HaCaT cell line has been reported as p16 null due to promoter hypermethylation. We have discovered that HaCaT cells are expressing p16 mRNA and the p16 promoter is unmethylated. However western blots have indicated a lack of detectable protein expression. We hypothesize p16 mRNA is being degraded before it reaches the polysome.

Results: Examination of mRNA from polysome extracts is underway. Results show the presence of a small amount of p16 message at the polysome. This is possibly due to interference by small RNAs such as natural anti-sense transcripts (NAT) and microRNAs (miRNA). A large anti-sense non-coding RNA, which overlaps p15, may play a role in regulation of the INK4A/ARF locus. Analysis of total RNA and polysomal RNA for the presence of this NAT has been performed. Using 2 TaqMan[®] assays we have shown the presence of NAT in HaCaT and HeLa cells with both transcripts more highly expressed in HeLa cells. Thus supporting our hypothesis that NATs may play role in the p16 pathway in CC.

Conclusions: Future studies will involve examination of expression of the INK4A/ARF locus and polysomal extracts from other CC cell lines. miRNA profiling of the HaCaT cell line versus other CC cells and normal cervix is underway. This will determine if NATs or miRNAs are a regulatory system for control of the INK4A/ARF locus and also if HPV effects this mechanism.

P-18.11 THE MOLECULAR ROLE OF TOBACCO SMOKING IN CERVICAL CARCINOGENESIS.

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We study the mechanisms behind the synergy between HPV infection and tobacco smoking in cervical carcinogenesis, and, more general, the role of acetylcholine signaling in cervical biology and neoplasia. We have analyzed the expression of mRNAs encoding nicotinic acetylcholine receptors (nAChR) in CaSki, SiHa and HeLa cell lines, which are derived from two squamous and one adenocarcinoma of the cervix, respectively. We detected with reverse transcription-polymerase chain reaction mRNAs for ten of the 16 nAChR subunits, namely strong signals for alpha-5, alpha-7, alpha-9, beta-1, and epsilon, and weak signals for alpha-4, beta-2, beta-4, gamma, and delta. We confirmed the translation of alpha-5 and beta-1, corresponding to the two strongest RNA signals, in SiHa and HeLa cells by Western blotting, and the localization of these proteins to the plasma membrane by immunofluorescence. The beta-1 subunit was detected membrane associated in normal and neoplastic squamous epithelia of the cervix in situ, but appeared to be absent from the underlying mesenchyme and even from adjacent columnar epithelia. These observations suggest that normal and neoplastic cervical squamous epithelial cells express several combinations of the pentameric nAChRs. We also measured that the proliferation of SiHa and HeLa cells is stimulated by nicotine, indicating that cholinergic signaling under normal physiological conditions and stimulated by nicotine in tobacco users affects epithelial homeostasis and neoplastic progression at the cervix in a way similar to the known effects on epithelia of the mouth, the airways and the lung. Ongoing experiments address the interplay between nAChRs, electrophysiology and protein kinases and a potential synergy between nicotinic and muscarinic acetylcholine receptors. Since tobacco smoking is established as risk factor in cervical carcinogenesis, and since nicotine and its derivatives become concentrated in cervical mucus, nAChR dependent signaling is apparently an important molecular cofactor of human papillomavirus dependent cervical carcinogenesis.

CHARACTERIZATION OF GLOBAL GENE EXPRESSION ALTERATIONS INDUCED BY HPV16 E7.

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Background: Viral infections promote the release of regulatory cytokines such as interleukin1 (IL-1), transforming growth factor- (TGF-) and tumor necrosis factor- (TNF). TNF is one of the main mediators of skin and mucosa inflammation and has a potent antiproliferative effect on normal keratinocytes. We and others have previously shown that acute expression of HPV16 or HPV18 E7 oncogene is sufficient to overcome TNF cytostatic effect in monolayer or organotypic cell cultures. Furthermore, we observed that pRb degradation capacity is essential to mediate TNF-resistance. In order to get insights into the molecular mechanisms underlying E7-induced TNF resistance we have analyzed the effects of this cytokine on global gene expression of normal and HPV16 E7-expressing primary human keratinocytes (PHKs).

Methods: Primary human keratinocytes (PHKs) were transduced with recombinant retroviruses containing HPV16 E7 wild type or E26G mutant oncogene expressed from the retroviral 5'-LTR promoter and used to seed organotypic cultures. Total RNA from TNF treated (2 nM TNF for 72 hours) and control cultures were analyzed by cDNA microarray hybridization using glass arrays with 4600 elements with known function (GEO accession: GPL1930). Differential expression of several genes was validated by Real Time-PCR and/or western blot.

Results: We identified a group of 44 genes including TCN1, DEK, HMGB2, INHBA, PCNA, CDKN1B, CCNA2, FUS, MCM2, MCM5 and MMP9, which expression pattern differ between TNF-sensitive and TNF-resistant cells. Moreover, expression of some of these genes, i.e. PCNA, CDKN1B, is down-regulated by the cytokine only in TNF-sensitive cells. Functionally grouping of these genes according to the Gene Ontology database showed that differentially expressed genes are mainly involved in the regulation of different phases of the cell cycle.

Conclusions: Our results suggest that E7 expression overcomes TNF cytostatic effect by up-regulating a set of genes involved in cell proliferation.

P-18.13 PDZ DOMAIN TARGETING BY HIGH-RISK PV TYPES.

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A common feature of all high risk mucosal HPV E6 oncoproteins is the ability to target cellular PDZ domain containing substrates for proteasome mediated degradation. Many of these proteins are involved in processes regulating cell polarity and proliferation control. In the case of HPV-16 and HPV-18 E6, two of the preferred targets are hScrib and hDlg, which together act as key regulators of apico-basal polarity control. Most intriguingly, we have found that Rhesus Paillomavirus E6 lacks PDZ binding potential, and instead the PDZ binding domain is found on the E7 protein. In addition, this domain confers upon E7 the ability to target Par3, a cellular protein involved in the same pathway of polarity control as that regulated by hDlg/hScrib. This demonstrates that PDZ domain targeting is a highly conserved feature amongst cancercausing mucosal PV types. We are currently analysing the potential common roles for the E6AP ubiquitin ligase in these activities of the PV oncoproteins.

PDZ MOTIF OF HPV16E6 IS NOT REQUIRED FOR HFK IMMORTALIZATION

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The high risk human papillomaviruses (e.g. HPV-16 and HPV-18) have been identified as causative agents of nearly all cervical cancers and are etiologically linked to additional human cancers including those of anal, oral, and epidermal origin. The main transforming genes of the high risk HPVs are E6 and E7, which are required and sufficient for the efficient immortalization of genital keratinocytes and mediate the degradation of the cellular p53 and Rb proteins respectively. Our previous analysis of E6 mutants indicated that the degradation of p53 was not required for cell immortalization. However, E6 has other functions such as the targeting of PDZ proteins (e.g. hScrib, hDIg, MAGI, MUPPI, PATJ, PTPN3, PTPN13) and increasing cell telomerase activity via the induction of hTERT. Interestingly, the E6 PDZ (PSD-95/disc-large/Zo-1) motif has been shown to be required for the immortalization and transformation of human tonsil cells. In this study we examined whether E6 PDZ mutants (containing either a deletion or mutation of the PDZ domain) could immortalize human primary genital keratinocytes in cooperation with E7. We confirmed that both E6 mutants (E6 del and E6m) retained the ability to degrade p53 and induce telomerase in human keratinocytes. In addition, despite their lack of a PDZ motif, these E6 mutants immortalized keratinocytes in cooperation with E7. Taken together with our previous studies, we conclude that the ability to degrade p53 or to target PDZ proteins.

P-18.15

TGF-BETA CONTROL OF GENE EXPRESSION DURING PROGRESSION OF HPV16-TRANSFORMED CELLS

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An important step in the development of malignant disease, including cervical cancer, involves a loss of sensitivity to the growth inhibitory effects of TGF-beta. Furthermore, TGF-beta can induce epithelial to mesenchymal transition (EMT), which is associated with invasiveness and involves modulation of expression of focal adhesion and cell-matrix interaction genes. In our in vitro model system for HPV16-mediated multi-step carcinogenesis, normal human keratinocytes (HKc), transformed by HPV16 DNA (HKc/HPV16) progressively acquire differentiation resistance (HKc/DR). We have previously shown that HKc/DR cells are completely resistant to the anti-proliferative effects of TGF-beta, due to a partial loss of TGFbeta receptor type I. However, Smad-responsive reporters can be activated by TGF-beta in HKc/DR, although at a reduced rate. To investigate the functional significance of this residual TGF-beta signaling uncoupled with proliferation control in HKc/DR, we explored the effects of TGF-beta treatment on gene expression profiles of HKc/HPV16 and HKc/DR, using Agilent 44k human microarrays. As expected, we found that TGF-beta treatment altered the expression of genes belonging to the cell cycle and MAP kinase pathways in HKc/HPV16, but not in HKc/DR. However, TGF-beta modulation of the expression of genes involved in focal adhesion was comparable between HKc/HPV16 and HKc/DR in both extent of change and specific gene targets. In addition, a number of genes also associated with EMT and cell-matrix interactions were modulated de novo by TGF-beta in HKc/DR. These findings indicate that the signaling pathways through which TGF-beta elicits growth inhibitory responses are separate and independent from those involved in the modulation of focal adhesion and possibly EMT, and support the concept of a dual role of TGF-beta as an inhibitor of carcinogenesis at early stages, and as a tumor promoter at late stages of HPV-mediated transformation and progression. (Supported by grant #P20 RR-016461, from NCRR, NIH.)

INSERTIONAL MUTAGENESIS OF C-MYC ONCOGENE BY HPV16 INTEGRATION

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Persistent infection with high-risk HPV is the causal risk factor for cervical cancer. Correlated with tumor progression, the circular HPV DNA usually becomes integrated into the host genome with diverse target sites. Viral-cellular hybrid mRNAs triggering expression of the HPV oncogenes E6 and E7 are transcribed from the integrated genome. Apart from enabling the constant expression of E6/E7, HPV integration itself can also induce genetic alterations of neighboring cellular genes (insertional mutagenesis). Here we present the results for HPV16 integration at chromosomal region 8q24.21 and insertional mutagenesis of the c-myc oncogene in the cervical carcinoma cell line MRI-H186. HPV16 DNA co-localizes with c-myc on all chromosomes 8, as shown by fluorescence in situ hybridization (FISH) assays. The integrated HPV16 DNA is located less than 2 kb upstream of c-myc. Consequently, a hybrid transcript starting with the HPV16 E6/E7 exon (or E6*/E7) followed by c-myc exons 2 and 3 is produced, in addition to the genuine c-myc mRNA. The c-myc oncogene was found to be overexpressed on both mRNA and protein levels. From a second variant of integrated HPV16, a prominent hybrid mRNA encoding a novel Myc-E2 fusion protein is transcribed. The (potentially oncogenic) activity of this mutant protein is under investigation. As determined by flow cytometry, MRI-H186 cells show an unusual DNA profile with two distinct cell subpopulations. Single-cell cloning was performed to separate and further characterize the two subpopulations. In summary, HPV16 integration analysis in the cell line MRI-H186 has revealed several striking integration-induced alterations, and the data strengthen the assumption that insertional mutagenesis, in this case activation/mutation of c-myc oncogene, contributes to cervical carcinogenesis.

P-18.17 UPREGULATION OF LIPOCALIN-2 IN HPV-POSITIVE KERATINOCYTES AND CUTANEOUS SCC

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To gain insight into cellular genes, which get deregulated due to the expression of HPV8-E7, cDNA array analysis was performed on human primary adult keratinocytes (PAK), which had been infected with recombinant retroviruses coding for HPV8-E7. The gene coding for lipocalin-2, known to be overexpressed in many human cancers, appeared markedly induced at RNA level. This finding led us to speculate that lipocalin-2 might be involved in cutaneous HPV-induced tumourigenesis. We therefore studied its expression in HPV positive and negative cells and tissues.

Western blots of total cell extracts from HPV8-E7 positive PAK confirmed the increased expression of lipocalin-2. Higher levels of Lipocalin-2 were also observed in PAKs expressing E7 of HPV1, 4, 5, 15, 20 or 38, but not of HPV16. ELISA found the same relative levels when measuring secreted Iipocalin-2 in cell culture supernatants of these cells. In organotypic skin cultures we found lipocalin-2 overexpressed in the upper-most layers of HPV5, 8, 15, 16, 20 or 38 but not of HPV1 or 4 E7-expressing keratinocytes. Immunohistochemical staining of HPV positive and negative human skin squamous cell carcinomas (SCC) revealed lipocalin-2 expression mostly in differentiated, filaggrin positive areas of 15/17 HPV-positive and 3/9 HPV-negative SCCs.

In conclusion, lipocalin-2 expression appears to be related to keratinocyte differentiation in infected skin and correlates with HPV positivity of cutaneous SCC.

DISTINCT GENE EXPRESSION PATTERNS IN CERVICAL CANCER PROGRESSION

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While most infections with oncogenic human papillomaviruses (HPVs) are transient, a fraction persists and may progress to cervical cancer(CC) by pathways that remain poorly understood. Pathologists subjectively grade non-invasive histological manifestations of HPV, termed cervical intraepithelial neoplasia(CIN), from the mildest morphologic changes(CIN1) to the most proximate precursors of CC(CIN3).

To aid management of this progression to prevent CC, identification of biomarkers for improving this classification to better reflect risk for CC is desirable.

Methods: We performed genome-wide expression analysis of 128 histologically assessed, microdissected frozen cervical tissues using the Affymetrix U133 Plus2.0 GeneChip in the molecular epidemiology-directed Study to Understand Cervical Cancer Early Endpoints and Determinants(SUCCEED).

Our initial analyses show the feasibility of performing mRNA expression studies from microdissected cervical lesions and reveal different patterns of gene expression by pathologic diagnosis. Expression levels of genes associated with DNA replication/repair and cell proliferation were significantly upregulated in CIN1 and CIN2 lesions compared to normal and thereafter sustained in CIN3 and CC. Of note, several of these genes were previously found to be upregulated by HPV infection. Among several pathways altered in CIN3 relative to earlier disease stages, genes in the IL-8 signaling pathway, which triggers chemotaxis and angiogenesis, were upregulated in CIN3 and maintained thereafter in CC. By far the greatest number of differences in gene expression were found between CIN3 and cancer, including overexpression of genes in the actin cytoskeleton signaling, fibrosis, tight junction, leukocyte extravasation, and vascular endothelial growth factor pathways in CC.

Our data demonstrate that gene expression patterns differ for histological lesion grades, with the biggest contrast between CIN3 and CC. Further assessment of gene expression by disease category and most importantly, by HPV genotype may permit an improved understanding of CC progression mechanisms and allow the identification of markers for improved risk stratification.

P-18.19

NONAMPLIFICATION OF THE CHROMOSOME 3Q26 REGION PREDICTS LSIL REGRESSION

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Background: The chromosome 3q26 region is amplified in patients with integrated HPV infections and cervical cancer. The frequency of the 3q gain is related to the neoplastic severity of the lesion. The majority of women with LSIL cytology will spontaneously clear without persistence or progression to CIN2+.

Objective: The objective of this study was to determine the negative predictive value of the 3q26 amplification test for regression of LSIL after one year.

Methods: Archival specimens of 36 women, aged 17-67 years, with index LSIL cytology, followed for at least one year (range 13-85 months) were obtained from several pathology laboratories. 31 women had subsequent NILM cytology reports and 5 women had subsequent HSIL cytology reports. Biopsy was available for 19 women. The index cytology specimens of the 36 women were hybridized with a probe for the chromosome 3q26 region and a control centromeric chromosome 7 probe, using standard FISH methods. 3q gain was defined as a positive test result.

Results: Of the 36 cases, 26 showed a negative FISH test result for a sensitivity of 100% (95% CI: 48, 100), specificity of 84% (95% CI of 66, 95) and a negative predictive value of 100% (95% CI: 87, 100). Of the 19 cases with histology follow up at least one year after the index LSIL, 4 women had progressed to CIN2+, all FISH positive; and 14 of 15 women with normal histology were FISH negative for a sensitivity of 100% (95% CI: 40, 100), specificity of 93% (95% CI: 68, 100) and a negative predictive value of 100% (95% CI: 40, 100), specificity of 93% (95% CI: 68, 100) and a negative predictive value of 100% (95% CI: 77, 100).

Conclusions: This study of archival cases indicates that 3q26 amplification can discriminate which women with LSIL cytology can be safely followed without immediate colposcopy. Large scale prospective studies will be needed to confirm these results.

HPV108E7 (LACKING THE E6 GENE) INDUCES DYSPLASIA IN RAFT CULTURES

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The genome organization of novel HPV108 isolated from a low-grade cervical lesion, deviates from other human papillomaviruses in lacking an E6 gene. The 3 related human papillomavirus types HPV103, HPV108 and HPV101 were isolated from cervico-vaginal cells taken from normal genital mucosa, low-grade and high-grade cervical intraepithelial neoplasia, respectively (Chenet al., 2007, this report). Their unusual genome organization (lacking E6 gene), against the background of considerable phylogenetic distance from the other HPV types usually associated with lesions of the genital tract, prompted us to investigate whether HPV108E7 per se is sufficient to induce the above mentioned clinical lesions. Expression of HPV108E7 in organotypic keratinocyte cultures increases proliferation and apoptosis, focal nuclear polymorphism and polychromasia as being characteristic of squamous epithelial dysplasia. This is associated with irregular intra- and extracellular lipid accumulation and loss of the epithelial barrier. These alterations are linked to HPV108E7 binding to pRb and inducing its decrease, an increase in PCNA expression and BrdU incorporation, as well as increased p53 and p21CIP1 protein levels. A delay in keratin 10 expression, increased expression of keratins 14 and 16 and loss of corneal proteins involucrin and loricrin are also noted. These modifications are suggestive of infection by a high-risk papillomavirus

P-18.21

TRANSFORMING PROPERTIES OF HPV38 E6 AND E7 IN TRANSGENIC MICE

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Several lines of evidence support the hypothesis that cutaneous beta-HPVs play a role in the development of nonmelanoma skin cancer (NMSC), although very few copies of the viral genome are usually found in late stages of the transformation process. Previous studies from our group showed that E6 and E7 from HPV38 display transforming activities in vitro and in vivo models. To further elucidate the role of beta-HPV types in carcinogenesis we have developed a novel animal model. Three transgenic (tg) mouse lines carrying the HPV38 E6 and E7 genes under the control of the K14 promoter have been generated, to drive viral protein synthesis to the natural site of HPV infection, the basal undifferentiated layer of epithelia.

Quantifications of viral mRNA, histological analysis and cell cycle marker analysis have been performed in different tissues of the three generated mouse lines. Viral mRNA was detected in all analyzed epithelia (dorsal skin, ear skin, tongue and esophagus), but not in the liver, confirming that the protein synthesis was tissue specific. Cell cycle protein analysis showed varying levels of expression in the analyzed epithelia. Ki67 and cyclinA were expressed stronger in keratinocytes from tg lines compared to the wild type. These observations indicate that HPV38 E6/E7 proteins lead to an enhanced cell proliferation in the epithelia. Furthermore, in tg lines expressing high levels of viral proteins, about 50% of 12 months old mice developed dysplasia, and a pronounced inflammation in the ear skin.

UVB irradiation resulted in a high accumulation of the cell cycle regulator p21 in exposed keratinocytes of wild type mice, while in tg mice this event was strongly attenuated.

These data demonstrate that the proteins E6 and E7 of HPV38, when expressed in keratinocytes, modify the normal cell cycle and influence the cellular response to external stresses.

SPLICED HTERT RNA TYPES AND CERVICAL CANCER PROGRESSION.

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Telomerase is a specialized reverse transcriptase that synthesized repeat DNA sequences at the ends of chromosomes. The absence of telomerase activity in most normal human cells results in progressive shortening of telomeres with each of cell division. In contrast to most human somatic cells immortalized and tumor cells contain detectable telomerase activity and consequently maintain their telomere length and proliferation capacity. It was published earlier that in cervical cancer cells harbouring high risk HPV E6 gene the increase of telomerase activity was observed. The main component of telomerase complex contains protein with catalytic activity encoded by so called hTERT RNA. This RNA persists in tumor cells in few spliced forms from which four forms are more abundant: full sized, alfa-splice, beta-slice and alfa + beta splice forms. We analyzed in the same samples presence of HPV genes, level of telomerase activity detected by TRAP-assay, and pattern of spliced hTERT RNA on different stages of cervical tumor progression (in cervical intraepithelial neoplasia – CIN I, II, III after colposcopy and cervical carcinomas) using specific primers to each form of spliced RNA. All tumor samples contain telomerase activity but in CIN of different stages its activity greatly varied. Full size genomic hTERT RNA was detected in all carcinomas as well as in CIN I, II, III samples. The quantity of samples with detected alfa-splice form hTERT RNA decreased on later stages of tumor progression. There are no stage-dependent variations in beta-slice and alfa + beta-splice forms hTERT RNA.

P-18.23

HPV AND TELOMERASE ACTIVITY IN WOMEN FROM A FOLLOW-UP STUDY

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Background: A few studies in cervical cancer have showed an increase in telomerase activity with grade of lesion. However until now there are no follow-up studies measuring the telomerase activity along the time. We report results of the telomerase activity and HPV infection in a nested case-control study of women from the Bogotá cohort.

Methods: Telomerase activity was analyzed in 22 women that during follow-up developed HGSIL and in 84 women that had normal cytology, using a TeloTAGGG Telomerase PCR ELISA kit. The HPV infection was previously tested using a GP5+/GP6+PCR-EIA and typing using a RLB assay. We chose HPV positive and negative controls.

Results: Telomerase activity was detected in 72.7% of the cases and in 27.3% of the controls (p= 0.000). HPV infections were detected in 86.3% of the cases and in 27.3% of the controls (p=0.000). 68.1% of the cases had telomerase activity and HPV infection at the same time, 18.1% had only HPV infection, 4.5% had only telomerase activity and 9.0% were negative for both. 9.5% of the controls had telomerase activity and HPV infection at the same time, 17.8% only had telomerase activity, 17.8% only HPV infection and 54.7% were negative for both. All cases with telomerase activity had infections with high risk types principally with the alpha 9 specie (86.6%). The multivariate analysis showed a significant increased risk of HGSIL in women with telomerase activity (OR = 4.6 CI 1.14-18.74), HR HPV infection (OR = 16.05 CI 3.64–70.7) and more than 2 parities (OR=1.61 CI 0.95–2.75). When the analysis included only women with HPV infection, the telomerase activity increased the risk of HGSIL (OR = 15.35 CI 1.79–131.33).

Conclusions: This is the first epidemiological study that describes the telomerase activity as an important and independent risk factor of HGSIL.

HORIZONTAL TRANSFER OF VIRAL ONCOGENES: AN ALTERNATIVE PATHWAY OF CARCINOGENESIS

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In 1999, Holmgren et al. revealed that horizontal transfer of genes between two eukaryotic cells was possible. Authors showed that apoptotic bodies obtained from EBV-infected lymphoid cells incubated with fibroblasts or endothelial cells resulted in transfer of viral DNA sequences in recipient's cells, leading to their transformation. Moreover, it was observed that in non-Hodgkin's lymphomas due to EBV resurgence, a high rate of intra-tumoral apoptosis was of bad prognosis. Futhermore, a relationship between apoptotic index and tumor progression was found in many cancers including cervical cancer.

In this context, we hypothesized that horizontal transfer of HPV DNA could constitute an alternative mechanism of carcinogenesis associated with papillomaviruses (HPV).

Apoptotic bodies derived from HeLa and CaSki cells, were obtained after cell exposure to UV irradiation / staurosporine, and cocultured with human primary fibroblasts or NIH/3T3 from 4 up to 48 hours. Incorporation of apoptotic bodies by fibroblasts was monitored by confocal microscopy after specific and non-specific staining. Clonogenic assays in softagar have been optimized to ensure fibroblast transformation. Transformation efficiency was evaluated by counting growing colonies on soft-agar, after crystal violet staining. Presence of E6 HPV16/18 DNA in transformed fibroblasts was determined by real-time PCR.

Carcinoma cell exposure to 20 mJ/cm² of UVB irradiation followed by 300 nM staurosporine for 48h permitted to obtain a suspension of apoptotic bodies devoid of living cells. Microscopic analyses highlighted that apoptotic bodies were adhering to fibroblasts from 4h of coculture, and located near the nucleus after 48h. Clonogenic assays confirmed that fibroblasts cocultured with apoptotic bodies were efficiently transformed. Real-time PCR analysis showed that transfer of HPV sequences in fibroblasts was effective.

Our data support the assumption that fibroblast transformation may be due to horizontal transfer of viral oncogenes.

P-18.25

EFFECT OF HPV16 INFECTION ON IGF1 SPLICING IN CERVICAL CANCER

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Background. Human papillomaviruses infect squamous epithelium and its life cycle is determined by epithelial differentiation. The insulin-like growth factor 1 (IGF1) is involved in proliferation, apoptosis and differentiation of many cell types and the IGF1 gene activity is regulated by both endocrine and paracrine/autocrine mechanisms. The gene comprises of 6 exons. Exons 1 and 2 encode two alternative signal peptides, whereas exons 5 and 6 undergo alternative splicing, giving rise to E peptide type A, B, or C. The expression of six possible variants depends on tissue type and developmental stage.

Aberrant splicing may occur either as a result of mutations in DNA sequence in exon/intron junctions, or via changes in spliceosome components, particularly in virus infected cells.

Objective. The aim of the study was analysis of IGF1 isoforms and major splicing factors mRNA expression levels in HPV positive (CIN(I-III), cervical cancer) and non-tumor HPV negative epithelial cells. Additionally, in HPV positive cells expression of viral HPV16 proteins E2,E6 and E7 was determined.

Methods. The research material was comprised of DNA and RNA isolated from tumor and non-tumor epithelial cells. HPV16 DNA samples were identified using PCR methods. Real-time PCR technique was applied for IGF1 isoforms, viral proteins and cellular splicing factors mRNA quantification.

Results. The real-time PCR indicated upregulation of IGF1B and downregulation of IGF1A mRNA isoforms in HPV positive cells. Both splicing factors and viral protein expression levels were positively correlated.

Conclusion. Due to correlation between IGF1 mRNA isoforms profile and HPV16 proteins as well as splicing factors mRNA expression levels, it is highly plausible that viral proteins are involved in IGF1 splicing course. We also suggest that the autocrine effects of IGF1 on cervical cell proliferation, and their interplay with other cellular and viral factors, may play an important role in cervical cancer development.

EFFECTS OF HPV 16 E5 ONCOGENE ON CELLULAR GENE EXPRESSION

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Background. Cellular signaling pathways are crucially altered in carcinogenesis. Tumor viruses have mechanisms to modulate signaling pathways for reprogramming host cells and supporting their own life cycle, or for controlling host defense responses.

Objectives and Methods. We studied broad-scale effects of the human papillomavirus (HPV) type 16 E5 oncogene in epithelial cells in a genome-wide cDNA microarray screen. The results were validated by qPCR and further confirmed in western blotting, live cellimaging and immunohistochemistry.

Results. We found the expression of altogether 179 genes significantly altered due to the E5 protein in a stable HaCaT human skin keratinocyte cell line transfected with the E5 gene, as compared to control cells. These included genes involved in cell motility, cell adhesion, cell proliferation, mitogenic signaling, differentiation, and antigen presentation. For example, the expression of matrix metalloproteinases, laminin and lamin A/C was downregulated, and the expression of protein kinase C-delta and p55 regulatory subunit of phosphoinositide-3-kinase proteins were found to be upregulated. Using livecell imaging we established that cell motility was indeed increased in E5 expressing cells. Increased motility was shown to be mostly due to enhanced cell movement, not increased cell proliferation. We have also performed immunohistochemical stainings in a set of HPV-associated cervical lesions in order to study whether some alterations caused by the E5 gene can be seen at tissue level.

Conclusions. The E5 protein seems to affect cellular pathways involved in cell adhesion, cell motility and mitogenic signaling. These alterations may contribute to the inhibition of apoptosis and the establishment of persistent infection and malignant changes in the epithelium. We are currently analyzing the effects of the E5 protein on the transcriptional profile of cellular genes as a function of time.

P-18.27

SYNONYMOUS CODON CHANGES IN CRPV ONCOGENES ALTER THE VIRUS PHENOTYPE

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BACKGROUND: Papillomaviruses use rare codons with respect to the host. This has been well documented and is the basis for the now commonly used technique of "codon optimization" to allow improved protein expression in in vitro systems.

OBJECTIVES: The success of the in vitro work led us to become interested in codon manipulation as a tool to improve immunogenicity of the cottontail rabbit papillomavirus in vivo.

METHODS: We used site directed mutagenesis to create CRPV genomes with codon "optimizations" in the E6 and E7 oncogenes. We tested these in our rabbit model system for infectivity and monitored the infected sites for papilloma outgrowth. We examined the E6 and E7 messages in papilloma tissue via Q RT PCR.

RESULTS: We observed increased immunogenicity in animals infected with codon-modified genomes. Furthermore, we noted a greatly increased propensity to malignancy in papillomas initiated with these genomes. We found that ratios of E6 to E7 transcripts were altered relative to the ratios from wild type infections.

CONCLUSIONS: Synonymous codon changes in the context of the CRPV genome may result in increased immunogenicity, decreased time to cancer, and altered growth rates. These findings suggest that synonymous changes in the papillomavirus genome may not be silent and should not be ignored when attempting to correlate nucleotide changes with disease progression.

BENZO[A]PYRENE ALTERS HPV SYNTHESIS AND CELL CYCLE: MODULATING DUAL CARCINOGENESIS?

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Background: Epidemiological studies suggest that HPV infected women who smoke face an increased risk for developing cervical cancer. We have reported that exposure of HPV positive organotypic cultures to Benzo[a]pyrene (BaP), a major carcinogen in cigarette smoke, resulted in enhanced viral titers. Increased viral titers could increase viral persistence which could be important for progression. Additionally, BaP is known to deregulate multiple cellular proliferation and signaling pathways in lung cancer. Therefore, enhanced virion synthesis may result from carcinogen/host-cell interaction or a direct effect of BaP on viral functions.

Objectives: We investigated whether BaP treatment altered cell cycle controls in cervical cells which maintain episomal copies of HPV31b, with primary keratinocytes as controls.

Methods: Organotypic cultures derived from HPV31b positive and primary keratinocytes were grown in the presence and absence of BaP and tested for infectious virion synthesis and cell cycle analysis.

Results: BaP upregulation of virion synthesis was correlated with increased accumulation of hyperphosphorylated pRb, p16INK4 and p27KIP1 proteins, whereas p21WAF1 and p53 levels remained unchanged. Concomitantly, CDK1 kinase activity was increased, CDK2 and CDK6 kinase activities were decreased, whereas CDK4 kinase activity remained unchanged. In contrast, BaP treatment of organotypic cultures derived from primary human keratinocytes resulted in aberrant activation of CDK1, CDK4 and CDK6 kinases which was correlated with pRb inactivation. Aberrant inactivation of pRb and upregulated CDK1 kinase activity was differentiation dependent since HPV positive monolayer cultures treated with BaP became growth arrested in S and G2/M phases, concomitant with a decline in all CDK kinase activities.

Conclusions: BaP modulation of dysregulated cell cycle progression in HPV positive lesions could result in increased host tissue carcinogenesis which may favor persistent HPV infections, and permissiveness for cancer progression. However, BaP regulation of carcinogenesis in primary tissues may increase host susceptibility to future HPV infections.

P-18.29

INFLUENCE OF HPV-58 NUCLEOTIDE VARIABILITY ON VIRAL TRANSCRIPTIONAL ACTIVITY

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HPV-58 is the second most frequent type detected among high-grade cervical lesions at Distrito Federal, Central Brazil. Our aim was to compare E6/E7 promoter activity of HPV-58 molecular variants detected in this geographical area. For this purpose, complete LCR sequences from HPV-58 prototype and three more variants were amplified, cloned upstream of the luciferase reporter gene and transiently transfected in C33 cells. For comparison, plasmids containing HPV-18 or -16 prototype LCR sequence genomes were also analyzed. pCMV-B-Gal plasmid was used as an internal control for transfection efficiency. HPV-58 prototype and HPV-58 variants Bsb-329 and Bsb-327 showed promoter activity similar to HPV-16 but about six times lower than HPV-18. Interestingly, HPV-58 variant Bsb-295 presented a high promoter activity, even comparable to HPV-18. This HPV-58 variant is the only isolate in which we detected a substitution in nucleotide position 7788. Mutants were constructed by site-direct mutagenesis in order to analyze the impact of this nucleotide alteration on viral promoter activity. The introduction of the 7788 mutation in the HPV-58 prototype isolate led to a transcription activity similar to that observed for HPV-58 variant Bsb-295 and HPV-18 prototype. On the other hand, the promoter activity of the Bsb-295 isolate was considerably reduced when this nucleotide alteration was reversed. Taken together, these data indicate that this single nucleotide variation is capable of enhancing viral promoter activity and could lead to a higher expression of E6 and E7 viral oncogenes in vivo. Nevertheless, additional studies are necessary to further identify cellular transcriptional factors involved in this regulation.

INVOLVEMENT OF HPV16 E6/E7 ON THE INVASIVENESS OF CANCER CELLS.

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Background. If association between high-risk HPV (HR-HPV) and carcinogenesis is well described, the role of these oncogenic viruses has been poorly explored in tumor invasion process. Invasive tumor cells are characterized by the loss of cell-cell adhesion and by high degradative properties. Indeed, the reorganization of E-cadherin/b-catenin and occludin-ZO-1 complexes of intercellular junctions and the expression of proteases including matrix metalloproteinases (MMP) during tumor cell migration and invasion have been largely documented.

Objectives. In this study, we examined the impact of HPV16-E6/E7 oncoproteins on MMP and adhesion molecules expression, these latter being particularly involved in tumor invasion process.

Methods. We transiently transfected expression vectors encoding HPV16-E6, -E7 and siRNA against E6, E7 in different cell lines (CaSki, 16HBE, BZR). Affymetrix microarrays were performed on these transfected cells. Expression of MMP and adhesion molecules was explored using RT-PCR, western blotting and immunochemistry analyses. Invasive capacity of the different transfectants was evaluated with a modified Boyden chamber assay.

Results. Using RNA interference strategy to inhibit E6 and E7 expression in CaSki cancer cells, we observed that the E7 siRNA transfectants displayed a decrease of their invasiveness, a reduced occludin and an increase of MT1-MMP expression. Furthermore, 16HBE and BZR cells treated with expression vectors encoding HPV16-E6, -E7 did not show any change neither in MMP expression nor in invasive capacity.

Conclusions. Our results suggest that E6/E7 viral oncoproteins regulate the acquisition of an invasive phenotype by tumor cells. The involved pathways are under investigation. This study will allow to better understand the implication of HR-HPV as a factor promoting tumor invasion.

P-18.31 ANIMAL PAPILLOMA VIRUSES (BPV- 1/2) & THEIR TUMOURS.

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Papilloma viruses are small non-enveloped, icosahedral DNA viruses that replicate in the nucleus of squamous epithelial cells. In the present study, histopathological, ultrastructural and molecular diagnostic investigations were carried out for cutaneous wart (CW) and urinary bladder tumours(UBT) of cows and hamsters. A total of 14 and 8 cases CW and UBT respectively were studied for animal papilloma viruses from various herds located northen part of the country. Representative CW & UBT samples were taken for PCR detection by targeting conserved L1 major capsid protein gene. BPV-2 of genomic size 165 bp mostly found in CW with a few exception of BPV-1 of 300 bp size. In UBT all the tested cases of transitional cell carcinoma case could detect 165 bp long BPV-2 genome without any evidence of BPV-1. UBT extract containing BPV-2 genome could successfully be transmitted to laboratory model hamster on 10 months of fern (Paquilinum and O.contiguum) feeding and induced cutaneous fibromatous growth and fibroma. It was assumed that papilloma virus caused exo& edo-phytic papilloma, fibropapilloma, fibroma, transitional cell carcinoma, transitional cell adenocarcinoma, cystitis cystica glandularis, haemangioma, leiomyosarcoma and intestinal fibroma (experimental)in cows . AgNOR count of the tumour was around 2.5 in CW and much higher in cases of UBT. Bizarre urothelium, interdigitation of lateral cell membrane, plamalemmal scalloping, secondary lysosomes etc. characteristic for transitional cell carcinoma were observed in TEM. Molecular marker like PCNA by immunohistochemistry for preneoplastic lesions induced in fern-fed BPV-2 infected animals is under laboratory investigation which could further elucidate oncogenic potential of papilloma virus infection in animal experimentation.

CHARACTERIZATION OF MONOCLONAL ANTIBODIES TO PDZ-BINDING DOMAINS OF HRHPV-E6 PROTEINS

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Background: High-risk human papillomaviruses (hrHPV) induce dysplasia and carcinomas of the female lower genital tract by expressing the viral oncoproteins E6 and E7. All hrHPV E6 oncoproteins contain a conventional PDZ binding motif (X-S/T-X-V/L) located at the extreme carboxy-terminus. Eighty-seven percent of hrHPV types contain either an E-T-Q-V or E-T-Q-L sequence.

Objective: Our aim was to generate specific monoclonal antibodies (MAbs) against these sequences for use as diagnostic or therapeutic immunoreagents.

Materials and methods: A chimeric peptide consisting of a T-cell epitope fused with the HPV oncogenic PDZ consensus binding motif was synthesized for intrasplenic immunizations of female Balb/c mice. Splenocytes containing B-lymphocytes obtained from immunized mice were fused with P3X63-Ag-653/Bcl-2 mouse myeloma cells to generate antibody secreting hybridoma cell cultures. Enzyme linked immunosorbent assay (ELISA), Immunohistochemistry (IHC), and Western blotting were applied to screen hybridoma cultures for antibodies secreting specific immunoreactivity against hrHPV PDZ binding domains. Specific hybridoma colonies were identified and cloned via limiting dilution to generate MAbs.

Results: Monoclonal antibodies specific for binding to hrHPV16-E6 (E-T-Q-V) or HPV18-E6 (E-T-Q-L) were produced. Western blot analysis confirmed the specificity of the MAbs to hrHPV genotypes. Sandwich ELISA and PDZ capture immunoassays demonstrated antibody binding to the hrHPV types 16 and 18 and not to low-risk HPV types 6b and 11. Hybridoma clones 1A9.1 and 6D9.3 partially blocked binding of hrHPV E6 to the MAGI-1 PDZ protein. Immunohistochemical analysis of the HPV oncopeptide antibodies in SiHa (HPV16) and HeLa (HPV18) control cell lines demonstrated predominantly cytoplasmic staining.

Conclusion: Novel monoclonal antibodies with specificity for sensitive detection of oncogenic hrHPV types were successfully generated and characterized by immunological analysis. Our data indicate that these antibodies can be used in prospective studies to test the value of E6 protein as a biomarker for carcinogenesis or as potential biotherapeutic agents for cervical cancer.

P-18.33

HIGH-RISK HPV INFECTION AND MUC1 MUCIN IN CERVICAL INTRAEPITHELIAL LESIONS

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BACKGROUND. Cell surface MUC1 mucin is involved in cell adhesion and regulatory intracellular signaling in cancer cells. However, in normal epithelial cell, its main function is shielding against pathogens, and it plays a role in antiviral protection. OBJECTIVE. The study aimed at investigating the MUC1 expression in cervical intraepithelial lesions with regard to HPV status of patients. METHODS. Eight women with benign cervical lesions, 19 women with CINI-III, 47 patients after radical trachelectomy or photodynamic therapy, and 6 healthy volunteers were evaluated. The median age was 33.5 years. The median follow-up for patients after treatment was 12 (1-72) months. Clinical status was confirmed by cytological examination and/or biopsy. MUC1 was detected using ICO25 monoclonal antibody in cervicovaginal smears, and HPV status was established using PCR. RESULTS. Intensive ICO25 membrane staining of high-grade squamous lesion (HSIL) type cells was revealed in 18 of 19 patients with CIN (95%), in 4 of 49 patients having more than 6 months after treatment (8%), but not in women having benign lesions nor in those having no pathology. HPV types 16 and 18 were detected in 23 women. Twenty one of them (88%) had ICO25-positive HSIL type cells or stained immature squamous metaplasia. Only 2 of 12 (17%) women having HPV types other than 16 or 18, and only 9 of 48 (19%) women having no HPV had the same positive cells types. CONCLUSION. High membrane MUC1 expression in squamous metaplasia epithelial cells is related to HPV16 and HPV18 types infection. While MUC1 up-regulation could be an adaptive reaction to infection, it can be hypothesized that MUC1 membrane localization disturbance follows HPV integration in host-cell genome. In turn, MUC1 depolarization leads to crucial alterations in intracellular processes resulting in a malignant phenotype formation. Therefore, MUC1 could be a mediator of precancerous events in cervical epithelium.

RARE HIGH-RISK HPV68 IN CERVICAL PRECURSOR LESIONS AND CARCINOMAS

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Persistent infection with high-risk human papillomavirus (hr-HPV) types is the essential basis for cervical carcinoma development, frequently followed by integration of the viral DNA into the host genome. HPV68, a rare HPV type, is classified either as high-risk or as probably high-risk, and is one of a few HPV types divided into subtypes. Subtype HPV68b was identified as integrated DNA in the cervical carcinoma cell line ME180. Subsequent work provided clear evidence that the cloned and sequenced fragment carries a large cloning-induced deletion in one of the HPV68b copies (accession number M73258). In a mutant line, ME180R, which is resistant against growth inhibition by tumor-necrosis-factor alpha (TNFalpha), substantial deletions of the integrated HPV68b DNA were also detected. In this study, we have determined the complete structures of integrated HPV68b in both ME180 and ME180R. ME180 cells contain two truncated HPV68b copies, integrated in an unusual head-to-head arrangement in chromosome 18q21. The E1 and E2 genes are disrupted or deleted in both copies. For ME180R we could demonstrate that the TNFalpha-resistant phenotype is independent of the HPV68b deletions. In addition, we report for the first time the DNA sequence of a complete HPV68b genome isolated from a cervical intraepithelial neoplasia grade 2 (CIN2), which was infected only with HPV68b. A mutant HPV68b genome with a 1.2-kb deletion in the E1 gene was also isolated from the same CIN2. Southern blot data suggest that this mutant genome is probably integrated. The co-existence of the episomal and integrated HPV68b indicates a risk of progression to cancer for this particular lesion. Altogether, the structural features of HPV68b in the cancer cell line and the CIN2 lesion present strong clues for the carcinogenicity of HPV68b.

P-18.35 GENETIC ALTERATIONS AT THE HPV INSERTION SITE IN CERVICAL CARCINOMA

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Objectives: To determine whether integration of HPV DNA could be responsible for structural alteration of the host genome at the insertion site.

Methods: A series of 42 DNAs extracted from cervical cancers (34 cases) and cervical cancer derived cell lines (8 cases) were analysed. HPV integration site were determined using the DIPS technique. Tumour DNA structure was analysed using the SNP GeneChip Human Mapping 500k. The genome status at the integration site was classified as follow: No Alteration when integration site was located in normal region or at more than 100 kb of boundaries of the nearest genomic rearrangement; Amplification when the DNA copy number of the rearrangement was > 4 and its size < 300 kb; Gain when the SNP ratio was between 2.3 and 4 and the size of the fragment in excess longer than 300 kb; Loss when the SNP ratio was lower than 1.14; Gain/Loss when integration site was located between gain and loss.

Results: HPV DNA integration site could be determined in all 42 cases. A single site was found in 34 cases, two sites in 7 cases and 3 sites in one case (51 sites). Comparison between integration and SNP data showed that the genome status at the integration site was altered in 16 cases (31.4%) and showed no alteration in 35 cases (68.6%). Alterations detected corresponded to Amplification in 6 cases, Gain in 5 cases, Loss in 2 cases and Gain/Loss in 3 cases. When these figures were compared to the total number of genomic rearrangements in the series of tumours, a highly significant association was found between genomic rearrangement and integration of HPV DNA (p=1.28 10-11).

Conclusion: Integration of HPV DNA frequently leads to structural alteration of the host genome likely to act in the tumour process.

GROWTH INHIBITION OF CERVICAL CANCER CELLS BY RNA INTERFERENCE

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Background: Integration of high-risk HPV genomes and expression of the viral oncogenes, E6 and E7, are critical steps in the development of Cervical Cancer (CC). E6 and E7 interfere with essential cell cycle pathways including those governed by tumour suppressor proteins, p53 and Rb.

Objectives: The aim of this project was to assess the effect of short interfering RNA (siRNA) targeted towards the E6/E7 oncogenes in CC cells, with an ultimate objective to establish the downstream effects of these silencing events using high density gene expression profiling arrays.

Methods: Two siRNA were designed towards HPV16 E7 region. The HPV16 transformed cell line SiHa was used as a model system. TaqMan[®] PCR specific for E7 and E6, western blotting of E6 and E7 targeted proteins, p53, p21 and Rb, and flow cytometry were applied to examine the effects of the E7 knockdown.

Results: With a concentration of 10nM both the E7 siRNA independently induced in excess of a 70% reduction in RNA levels of E7 and E6. There was also a significant increase in levels of p53, p21 and the hypophosphorylated form of Rb indicating a reduction in E6 and E7 protein levels. The introduction of the E7 siRNA into the SiHa cells resulted in a phenotype changes; cell shrinkage and chromatin condensation. Analysis of the cell cycle showed that there was a substantial decrease in the number of cells entering the G2/M phase of the cell cycle post siRNA treatment.

Conclusions: Our results demonstrate it is possible to silence HPV16 E6 and E7 simultaneously using as little as 10nM of siRNA targeting E7 and this can lead to a reduction in cellular proliferation. This approach may have a potential role for gene-specific therapy in HPV16 associated CC and additionally, will allow identification of a subset of dysregulated genes in CC.

P-18.38

EBV'S LMP-1 PROTEIN INCREASES ONCOGENICITY IN HPV-CONTAINING CERVICAL CANCER CELL-LINES

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Background: Many HIV+ women are infected with high-oncogenic risk HPV-types(HR-HPV). Few progress to cervical dysplasia implying the need for co-factors. Women cervically shedding the oncogenic virus, Epstein-Bar virus (EBV) and HR-HPV are at increased risk for an abnormal Pap smear (76%) or cervical dysplasia (68%) as compared to women only shedding HR-HPV (53%, p<.001, 45%, p=.004, respectively). The role of EBV in HPV-induced cervical carcinogenesis is not known but may involve a direct interaction of their oncoproteins. Initial staining studies have localized EBV in the basal cervical epithelial cells where HPV-E6/E7 is expressed. The goal of this study is to determine if having both HPV-16 E6E7 and EBV's putative onco-protein LMP1 could help in the progression of HPV-related cervical disease.

Methods: Cervical cancer cell lines (SiHa, CaSki, and C-33A) were infected with retroviruses containing LMP1. The polyclonal cell lines were assessed for proliferation (3H-Thymidine assay), apoptosis (TUNEL assay), and ability to invade (BD Tumor-Invasion System). Their ability to cause tumors in nude mice is being assessed. Similar studies using primary ectocervical cells are also underway.

Results: Polyclonal mixtures of SiHa-LMP1 (cells expressing LMP1) proliferate two-fold faster as compared to vector controls. Although apoptosis was seen in vector controls, none was seen in SiHa-LMP1 and CaSki-LMP1 cell lines. A 25% increase in invasiveness of SiHa-LMP1 was observed compared to vector controls while CaSki-LMP1 cells became invasive. No difference in proliferation or invasiveness was detected in C-33A cells.

Discussion: LMP1 is capable of increasing the cancerous phenotype seen in SiHa and CaSki by increasing proliferation or invasion while inhibiting apoptosis. However, the specific changes observed seem to be cell line specific. This likely represents the differences in the genetic backgrounds of the cell lines utilized.

AN IN VITRO MULTI-STEP CARCINOGENESIS MODEL FOR HPV-ASSOCIATED HUMAN CANCERS

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Background: In HPV-associated cancers including cervical cancer, de-regulated expression of two viral oncogenes E6 and E7 in basal cells is considered to be a critical event for disease progression and lines of evidence suggest that additional host genetic alterations are required for cancer development. However, it is difficult to determine which aberration is responsible for each biological step in multi-step carcinogenesis.

Objectives: To clarify the causal relationship between each abnormality and the resultant phenotypic change of the cell, it is important to transduce these abnormalities into natural host cells to examine whether normal cells should acquire tumorigenic phenotype.

Methods: Normal human epithelial cells (cervical keratinocytes, esophageal keratinocytes, tongue keratinocytes and bronchial epithelial cells) were transduced with HPV16 E6 and E7 (HCK1T-E6E7) followed by transduction of oncogenes whose contribution to the cancer has been suggested.

Results and Conclusion: The results with the model suggest that only one or two genetic changes occurring after de-regulated expression of high-risk HPV oncogenes might be sufficient for development of cancer. Notably, combined transduction of Myc and Hras to some human keratinocytes expressing E6 and E7 resulted in the creation of highly potent tumor initiating cells. We will present the in vitro carcinogenesis models, and discuss the essential steps for carcinogenesis.

P-18.41

DNA METHYLATION OF CELLULAR GENOME INTEGRATED HPV 16

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BACKGROUND. Epigenetic modifications alter gene expression and in consequence play an important role in carcinogenesis. The methylation of CpG sites in the promoter region of HPV is associated with transcriptional inactivation and HPV gene expression regulation. OBJECTIVE. Determine the CpGs methylation pattern of the integrated HPV 16 long control region (LCR) and part of the L1 gene. METHODS. Cervical cancer and normal cervical cytology samples from women of the State of Guerrero, Mexico that show HPV 16 infection integrated into the cellular genome in E2 gene were selected. In these samples the methylation pattern of 19 CpG sites located in the LCR and part of the L1 gene was determined. HPV detection was done by PCR (MY09/11 o GP5+/6+) and typing by RFLPs or sequencing. The integration of viral genome was detected by PCR using primers for E2, a gene that is eliminated during viral integration. Methylation detection of the CpG sites was performed by DNA modification with sodium bisulfite, amplifying the regions: LCR 3' (promoter), central region (enhancer) y L1-LCR5', TOPO TA cloning and sequencing. RESULTS. The results show that the CpG sites of the LCR 3' of HPV 16 in normal cytology samples show more methylation than cancer samples. The enhancer has minimal methylation in cancer and is not methylated in normal cytology. The L1-LCR 5' region is methylated in cancer cases but not in normal cytology. CONCLUSIONS. The hypomethylation of the LCR promoter of HPV 16 integrated in cervical cancer samples could be important in the over expression of viral oncogenes. The integration of the viral genome did not result in a homogenous methylation pattern in the HPV 16 LCR in cervical cancer and normal cytology. Methylation is of more importance in the HPV 16 promoter than in the enhancer core.

HPV AND URINARY BLADDER CANCER

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BACKGROUND: The problem of whether or not HPV are carcinogenic for human bladder urothelium is not novel. However it is not solved yet.

OBJECTIVES: To analyse causes of discrepant results by research groups and to design investigation plan by means of literature data analysis. To study possible links between HPV and bladder cancer (BC) in Russia.

METHODS: Transitional cell BC, morphologically normal bladder lining from the same patients and normal bladder specimens from non-oncological patients were studied. PCR with several primer sets, restriction fragment length polymorphism analysis of PCR products, RT-PCR, immunohistochemical detection of HPV 16 oncoprotein E7 were performed.

RESULTS: There seem to exist three different sources of data discrepancies: ethno-geographic heterogeneity of BC, technical peculiarities of some studies (small numbers of specimens tested; use of single test for viral detection, mostly PCR or in situ hybridization; lack of evidence of viral genome expression) and contamination (intralaboratory or intrapatient, the latter due to ano-genital HPV in the patient). Thus use of a test complex is expedient including viral genome expression study. Among 120 BC specimens tested by PCR ~40% turned out to harbour HPV DNA. Type 16 HPV was detected in 40 cases. Not less than 25 % of the cases studied were positive for HPV E6/E7 mRNA, most of them were also positive for protein E7 HPV16. CONCLUSION: The results obtained are in agreement with the notion that HPV play some role in bladder urothelium malignization.

P-18.43

EFFECT OF HPV ON MTDNA STRUCTURE AND APOPTOSIS IN CERVIX

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Background: Human papillomaviruses (HPVs), especially types 16 and 18 are the main agent of cervical carcinogenesis. The E6 viral protein of HPV 16 and HPV 18 interacts and promotes degradation of a variety of cellular proteins such as p53, Bak, and Myc, which are involved in proliferation and apoptosis. Most proapoptotic and antiapoptotic proteins regulate apoptosis by controlling the release cytochrome c (cyt.c) or apoptosis inducing factor (AIF) from mitochondria. The Human papillomavirus E6 protein prevents the release of cyt.c and AIF. MtDNA is also known for its high mutation rates which can alter the mitogenic signal of apoptosis.

Objectives: In the present study we examined the alteration in the structure of mtDNA in non-tumor epithelial cell HPV negative and cervical cancer cell HPV positive and the relation of HPV infection with mtDNA mutation and expression of E6 HPV16/18 viral protein.

Methods: The mtDNA was analyzed by PRC/SSCP and sequencing methods. The HPV DNA was identified by PCR methods. Real-Time PCR was used to study the expression level of mitochondrial genes involved in the apoptosis process and viral genes coding E6 and E7 proteins.

Results: We analyzed 20 different nucleotide changes in the D-loop of mtDNA. They were present in 80% of the DNA isolated from HPV positive women with CIN (II-III) and cervical cancer. In these cells we observed an increase in E6 HPV mRNA level and a decrease in the level of proapoptotic proteins.

Conclusion: D-loop region of mtDNA is important in the replication of mtDNA. Therefore, a high frequency of point nucleotide changes in this region can alter mtDNA replication and its function. These results suggest that mtDNA abnormalities might be involved in cervical cancer development and that mtDNA analysis may represent a new molecular tool for cervical cancer prevention in women with HPV persistent infection.

P-18.44 EGFR, PDGFRA AND VEGFR-2 CHARACTERIZATION IN CERVICAL ADENOSQUAMOUS CARCINOMA

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Adenosquamous carcinoma of the uterine cervix (ASC) is an aggressive subtype of cervical cancer. A better understanding of its biological behavior is warranted to define accurate prognosis and therapeutic targets. Currently, the blockage of tyrosine kinase receptors (RTKs) activity is an efficient target for therapeutic purposes for many different cancers. The objective was to investigate EGFR, PDGFRA and VEGFR2 RTKs overexpression and activating gene mutations in a cohort of 30 adenosquamous carcinomas of the cervix, and to assess the potentiality of these RTKs as therapeutic targets. EGFR, PDGFRA and VEGFR2 Immunohistochemistry was performed in all samples, followed by DNA isolation of the tumor cells. Screening for EGFR (exons 18-21) and PDGFRA (exons 12, 14 and 18) mutations was done by PCR - single-strand conformational polymorphism (PCR-SSCP). VEGFR-2 mutation screening was not performed because no mutation is already known. EGFR immunohistochemical positive reactions were observed in 43. No EGFR activating mutations in the hotspot region were identified. A silent base substitution (CAG>CAA) in EGFR exon 20 at codon 787 (Q787Q) was found in 17 cases (56%). All PDGFRA immunohistochemical reaction was positive and consistently observed in stromal components, as fibroblasts, endothelial cells as well as in cytoplasm of malignant cells. The mutation analyses of PDGFRA were negative. We observed several silent mutations, such as a base substitution in exon 12 (CCA>CCG) at codon 567 (P567P) in 9 cases and in exon 18 (GTC>GTT) at codon 824 (V824V) in 4 cases, and the presence of base substitutions in intron 14 (IVS14+3G>A and IVS14+49G>A), in two different cases, and in intron 18 (IVS18-50insA) in 4 cases. VEGFR-2 l positivity was observed in 22 cases (73.3%), and was significantly associated with lack of metastasis (p=0.038). Anti RTK therapy should be considered for more stringent study as a potential option for ASC.

P-18.45

MODULATION OF THE EXPRESSION OF ERBB-FAMILY RECEPTORS DURING HPV16 CARCINOGENESIS

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Background. The EGFR (epidermal growth factor receptor) family consists of four ErbB tyrosine kinase receptors involved in a complex network of signal transduction pathways, playing a key role in regulating cell proliferation, differentiation, motility, invasion, angiogenesis, and survival. Co-expression of these receptors favours omo-hetero-dimerization among them, enhancing tyrosine-kinase activity promoting the phosphorylation of several tyrosine residues which leads to a complex signalling cascade. The third oncogene of HPV, the E5 protein, leads to a reduction in the down-regulation of EGFR and concomitant recycling of the receptor to the cell surface. When co-expressing HPV-16 E5 and ErbB4 in cells, E5 can abrogate ErbB4-induced c-Jun protein expression resulting in increasing cell proliferation. Objectives Beside the E5 all the early proteins of high risk HPV have been implicated in the growth factor signalling regulation during the viral replication or transformation and therefore there is the need to ascertain "in vitro" the relationship of all these viral genes with the ErbB-family receptors. Methods. The expression of viral protein and ErbB family receptors has been analysed in W12 cell line in which all the phases of carcinogenesis take place during passages, including integration and E2 regulation loss. RT-PCR, western-blot and immunoprecipitation have utilised in order to detect viral and cellular expression at early (episomal virus) and late (integrated virus) passages (>50). Results and Conclusions. Human Papillomavirus (HPV) and ErbB family expression are modulated in W12 cell during the carcinogenesis process. The different expression profile will be showed and the possible relationship discussed. The causal relationship of this family receptor network to disease progression and resistance to therapy as well as to the viral transformation could provide a rationale for future targeting this signalling pathway with innovative and promising "target-therapies" particularly in the HPV associated head and neck tumours.

P-18.46 SYSTEMS BIOLOGY OF THE HPV16 E5-MEDIATED EGFR-SIGNALING

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The three oncogenes E5, E6 and E7 of the human papillomavirus type 16 (HPV16) are known to play a major role in cervical carcinogenesis causing deregulation of cell differentiation, apoptosis and proliferation by interacting with different cellular proteins and providing a selective growth advantage.

Our aim is the quantitative and spatio-temporal analysis of signal cascades that are modulated by E5. The small hydrophobic protein E5 is acknowledged to have an effect on ligand-mediated EGFR activation. The protein causes a downregulation of EGFR ubiquitination and leads to an upregulated activation of the receptor.

We performed quantitative analysis of protein expression to establish the relative levels of total and activated EGFR in E5-expressing human keratinocytes and in the corresponding controls. After EGF stimulation in E5-expressing cells we observed an upregulation and long-lasting activation of EGFR, whereas the amount of total EGFR in both E5-expressing and control keratinocytes was similar.

We analyzed further the intracellular trafficking dynamics after EGF stimulation by treatment with Monensin, an inhibitor of receptor recycling. Treatment with Monensin resulted in a decrease of EGFR in control keratinocytes compared to the untreated cells. Additionally, the relative levels of both total and activated EGFR were higher in E5-expressing cells. In control cells the receptors are dephosphorylated and degraded upon internalization, while EGFR remains phosphorylated and activated in E5-expressing cells, therefore inhibiting receptor degradation. Our results suggest that E5-mediated EGFR activation proceeds via different molecular mechanism such as modulation of phosphorylation, but increased receptor recycling is not responsible for the EGFR overactivation after stimulation.

P-18.47

HPV38 E6/E7 ONCOPROTEINS DOWNREGULATE 5 MEMBERS OF THE ANNEXIN FAMILY

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Papillomaviruses of the beta-genus appear to be involved in the development of non-melanoma skin cancer (NMSC). As previously demonstrated the oncoproteins E6 and E7 of HPV38 are able to immortalize primary keratinocytes by inactivation of pRb and alteration of p53 function through upregulation of Δ Np73. To gain deeper insight into the role of 38E6/E7 in the immortalization process we analysed the proteome of E6/E7-expressing and primary keratinocytes by two-dimensional gel electrophoresis. We found 40 differentially expressed proteins, 27 were down regulated and 13 were higher expressed in the HPV38 cell lines. Interestingly, we found five members of the Annexin family being down-regulated in oncogene-expressing cells: Annexin 1, Annexin 2, Annexin 3, Annexin 5 and Annexin 8. These data were confirmed by Western blot and immunofluorescence. Real time PCR analysis showed opposed regulations at the mRNA level. While Annexin 1 and Annexin 3 showed reduced transcription, Annexin 2 and Annexin 5 mRNA levels were upregulated. This was in concordance with data from a cDNA microarray analysis.

Further, we found by yeast two hybrid screening and pull down analyses Annexin 1 to be an interaction partner of betagenus HPV E7 proteins.

To figure out whether lowered expression is HPV38-specific or a characteristic of immortalization we examined by Western blot the Annexin levels in different HPV positive and HPV negative immortalized cell lines. Since C33A and HaCaT cell lines also showed decreased Annexin levels, we hypothesize that lowered expression is involved in immortalization. Thus, HPV38 E6/E7 modulate Annexin expression during the immortalization process.

BPV-2 CAN BE MAINTAINED IN ACTIVE STATUS IN BOVINE BLOODSTREAM.

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Background. Bovine papillomavirus type 2 (BPV-2) infection has been associated with urinary bladder tumors in adult cattle grazing on bracken fern-infested lands and it was detected in the bloodstream. Objectives. In an attempt to better understand the biological role of circulating BPV we investigated the simultaneous presence of BPV-2 in whole blood and urinary bladder tumours of adult cattle. Methods. 78 cattle clinically suffering from a severe chronic enzootic haematuria were analysed for the presence of circulating BPV DNA. Samples were analysed by: PCR and sequencing for viral detection; rolling circle amplification to determine the presence of episomal viruses, RT-PCR for the detection of viral transcripts; and, finally, immunoprecipitation for the presence of E5 oncoprotein. Results. BPV2 DNA was detected in 61 of the analysed animals A number of affected animals were slaughtered and BPV-2 DNA was evidenced in 78% of urinary bladder tumour samples. Simultaneous presence of BPV-2 DNA in neoplastic bladder and blood samples was detected in 37 animals. Specific viral E5 mRNA and E5 oncoprotein were also shown in blood both by RT-PCR and western blot, respectively, indicating an active role of BPV in bloodstream. Conclusions. It is very likely BPV-2 may persist and be maintained in an active status in the blood stream as reservoir of viral infection which in the presence of co-carcinogens may cause the development of urinary bladder tumours. Data on the subset of circulating cells infected by the BPV2 will be presented.

P-18.49

MAL TUMOR SUPPRESSOR ACTIVITY, SILENCING AND METHYLATION DURING CERVICAL CARCINOGENESIS

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Background: hrHPV-induced cervical carcinogenesis is driven by oncogenic alterations in host genes. We previously identified MAL (T-lymphocyte maturation associated protein) as most significantly down-regulated gene in cervical carcinomas.

Objectives: To examine the mechanism underlying MAL silencing, its functional role in cervical carcinogenesis and potential diagnostic relevance of detecting MAL alterations for risk assessment of hrHPV-positive women.

Methods: We analyzed MAL mRNA expression, either or not following methylation inhibition, in primary keratinocytes, hrHPV-immortalized keratinocytes and cervical cancer cells. MAL promoter methylation was examined by quantitative methylation-specific PCR (qMSP) at two regions (M1 and M2). SiHa cervical cancer cells were transfected with MAL expression or control vector and assayed for proliferation, migration and anchorage independent growth. Next, we analyzed MAL promoter methylation and mRNA expression in cervical biopsies and scrapings.

Results: MAL mRNA was undetectable in (nearly) all HPV-immortalized cells and cervical cancer cells compared with primary keratinocytes, but was strongly upregulated upon methylation inhibition. MAL promoter methylation at both M1 and M2 regions was detected in all HPV-immortalized cells and cervical cancer cells. Ectopic expression of MAL in SiHa cells suppressed proliferation, migration and anchorage independent growth. None (0/22) of normal biopsies, 9.1% (6/66) of CIN1 lesions, 53% (34/64) of CIN3 lesions, 90% (85/94) of SCC and 93% (26/28) of AdCa demonstrated MAL promoter methylation at both regions. Moreover, detection of MAL promoter methylation in cervical scrapings was predictive for underlying high-grade lesions. Both in tissue specimens and scrapings MAL promoter methylation was significantly correlated with reduced mRNA expression.

Conclusions: MAL gene silencing by promoter methylation is a highly frequent and biologically essential event in HPV-induced cervical carcinogenesis. Hence, MAL promoter methylation and/or mRNA expression analysis on cervical scrapes may provide a valuable diagnostic tool to improve the detection of high-grade CIN and SCC as well as cervical AdCa.

BOVINE PAPILLOMAVIRUS INFECTS KERATINOCYTES IN HORSES BEFORE SHIFTING TO FIBROBLASTS

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BACKGROUND AND OBJECTIVES: Bovine papillomavirus (BPV) infection in horses leads to proliferation of skin fibroblasts accompanied by pseudoepithelial hyperplasia. Earlier studies using in situ hybridization consistently show BPV DNA in fibroblasts but not in keratinocytes. However, dysplastic epithelial changes and lack of dermal changes in latently infected normal skin urged us to test the hypothesis that BPV infection in horses might start in the epidermis before shifting to the dermis.

METHODS: Thirty occult sarcoids, 5 advanced sarcoids and 5 normal skin samples latently infected with BPV were examined by histology. Five of 12 occult sarcoids with a dysplastic epidermis but minimal dermal alterations, as well as the 5 advanced sarcoids and 5 latently infected normal skin samples were selected and laser-microdissected to separate keratinocytes and fibroblasts. DNA was isolated using the QIAamp DNA Micro Kit and real time PCR using BPV-1 and BPV-2 specific probes was performed.

RESULTS: Eighteen occult and all 5 advanced sarcoids displayed one or more of the epidermal and dermal changes generally known to be present in equine sarcoids (hyperkeratosis, rete peg formation, dermal proliferation). However, 12 occult sarcoids as well as all 5 latently infected normal skin samples showed focal dysplastic epithelial changes and minimal to no dermal alterations. Real time PCR showed BPV DNA in keratinocytes of 2/5 occult sarcoids, 2/5 latently infected normal skin samples but 0/5 typical sarcoids. All but 2 samples (1 occult, 1 latent) had detectable BPV DNA in the fibroblasts.

CONCLUSION: These results suggest that BPV infection in horses starts in keratinocytes, similar to most papillomavirus infections. However, when sarcoids progress to more advanced forms, a shift of BPV occurs to dermal layers and infection disappears from the epidermis. Further research is needed to elucidate the mechanism of this change in cell tropism.

P-18.51

EARLY HPV8 GENES DEREGULATE EXPRESSION OF ONCOGENIC AND ANTI-APOPTOTIC MIRNAS

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Background: HPV8 is involved in non-melanoma-skin-cancer development. Transgenic mice, expressing all early genes of HPV8 (E1/E2/E4/E6/E7 = HPV8-CER) or E2 or E6 separately under the control of the human keratin-14 promoter, spontaneously develop papillomas, dysplasias and in 2% (HPV8-E2) or 6% (HPV8-CER and -E6) squamous cell carcinomas. Furthermore we could demonstrate that UV-irradiation induced papillomas in about two weeks in HPV8-CER, -E2 and -E6 mice.

Objectives: As it is known that miRNAs are deregulated in several types of cancer, we were interested, if HPV8 gene-expression leads to deregulated miRNA expression.

Methods: MiRNA-microarray, UV-irradiation, qPCR, IHC

Results: MiRNA-microarray-analysis of untreated skin from wild type (wt) and HPV8-CER mice revealed 35 deregulated miRNAs, among them miRNA-21 and miRNA-106a, known to have oncogenic and anti-apoptotic effects. To look for a correlation between HPV8 transgene- and miRNA-expression after UVA/B-irradiation, we determined the transcription kinetics in skin of HPV8-CER and wt-mice. During the first fourteen days after UV-irradiation expression of miRNA-21 and miRNA-106a was increased in both HPV8-CER and wt-mice. Along with healing in wt-mice miRNA expression returned to an almost basal level 30 days after UV-irradiation. In contrast, in HPV8-CER mice both transgene- and miRNA-expression of miRNA-21 and miRNA-106a target gene expression (tropomyosin1 and retinoblastoma1, respectively) was confirmed by IHC. UV-irradiated HPV8-E2 and –E6 mice showed a similar miRNA-21 and miRNA-106a expression profile as HPV8-CER mice.

Conclusions: Our data show that expression of miRNAs is altered during wound healing. While alteration of oncogenic and anti-apoptotic miRNAs returns to a normal level in wt-mice it persists in the skin of HPV8 transgenics. This may point to a contribution of deregulated miRNAs to the early steps of beta-HPV associated skin carcinogenesis.

MMP-1 UP-REGULATION AND INVASIVENESS OF EQUINE BPV-1 TRANSFORMED FIBROBLASTS

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Introduction: Equine sarcoids are the most common dermal tumours in equids and Bovine Papillomaviruse type 1 and 2 (BPV-1/2) are widely accepted as causal agents of this type of tumour. Matrix metalloproteinase 1 (MMP-1) expression was found to be significantly up-regulated in BPV-1 expressing equine fibroblasts and in sarcoid tumours. The aim of this study was to determine the effects of high MMP-1 expression on the invasion potential of equine fibroblasts and identify BPV-1 viral genes associated with regulation of MMP-1 expression.

Methods: Collagen zymography was used to assess collagenase activity produced by BPV-1 transformed equine fibroblasts and an in vitro 3D invasion assay was carried out to study the invasive potential of cells. To study the transcriptional regulation mechanism of MMP-1, transient expression of BPV-1 viral genes E2, E5, E6 and E7 were performed followed by assessment of MMP-1 expression in equine fibroblasts. Furthermore, the functionality of the equine MMP-1 promoter was also assayed in transcriptional assays and by mutational analysis.

Results: BPV-1 transformed fibroblasts expressed high levels of MMP-1 and showed higher collagenase activity than control fibroblasts. The MMP-1 expressing fibroblasts were highly invasive in a 3D/matrigel invasion system. BPV-1 E6 and E7 up-regulated MMP-1 transcription, however E2 inhibited its expression. Mutational analysis of an AP-1 binding site within the equine MMP-1 promoter was shown to be crucial for the up-regulation of MMP-1 transcription by BPV-1. Conclusions: These findings demonstrate that BPV-1 expression has a profound effect on the regulation of MMP-1 transcription and high MMP-1 expression and collagenase activity in BPV-1 transformed fibroblasts are associated with their invasiveness. The high invasion ability of equine sarcoid fibroblasts may explain the high recurrence of the tumour. Acknowledgment: This work was supported by the HBLB.MSC is a Fellow of Cancer Research UK.

P-18.53 NO• INDUCES EARLY RNAS AND MUTATION RATES IN HPV+ CELLS

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High-risk human papillomavirus (HPV) infections are necessary but insufficient causes of cervical cancers. Many other risk factors for cervical cancer (e.g., pregnancy, smoking, infections causing inflammation) can lead to high and sustained nitric oxide (NO) concentrations in the cervix, and high NO levels are related to carcinogenesis through DNA damage and mutation. However, the effects of NO exposure in HPV infected cells have not been investigated. In this study, we used the NO donor DETA-NO to model NO exposure to cervical epithelium. In cell culture media 24h exposure of 0.25 to 0.5 mM DETA-NO yielded a pathologically relevant NO concentration. Exposure of HPV infected cells to NO increased HPV early transcript levels 2-4 fold, but did not increase viral DNA replication levels in cells maintaining episomal high-risk HPV genomes. Accompanying increased E6 and E7 mRNA levels were significant decreases in p53 and pRb protein levels, lower apoptotic indices, increased DNA double strand breaks, and higher mutation frequencies when compared to HPV-negative cells. We propose that NO is a molecular co-factor with HPV infection in cervical carcinogenesis, and that modifying local NO cervical concentrations may constitute a strategy whereby HPV-related cancer can be reduced.

TREATMENT AND POST-TREATMENT FOLLOW-UP

SESSION 19

PROGRAMME

ROOM

THE 25TH INTERNATIONAL PAPILLOMAVIRUS CONFERENCE MAY 8-14 2009, MALMÖ, SWEDEN

TIME NUMBER TITLE

2009-05-13

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11.11-11.22	O-19.02	HC2 AS TEST OF CURE IN STAGE IA CERVICAL CANCER M Sideri, S Costa, S Boveri, C Casadio, M Sandri, N Spolti, G Negri	
11.22-11.33	O-19.03	LONG-TERM INCIDENCE/MORTALITY OF CERVICAL OR VAGINAL CANCER AFTER CIN3-TREATMENT B Strander, J Hällgren, P Sparén	
11.33-11.44	O-19.04	MORTALITY AFTER TREATMENT OF CERVICAL INTRAEPITHELIAL NEOPLASIA I Kalliala, T Dyba, P Nieminen, T Hakulinen, A Anttila	
11.44-11.55	O-19.05	POST-TREATMENT HPV TESTING AND LONG-TERM RISK AT RECURRENCE M Kocken, JA Louwers, WA ter Harmsel, J Berkhof, PJ Snijders, RHM Verheijen, T Helmerhorst, CJLM Meijer	
11.55-12.06	O-19.06	HPV TESTING IN THE FOLLOW-UP AFTER CONIZATION A Trope, K Sjøborg, A Eskild, T Eriksen, C Jonassen, U Westerhagen, AK Lie	
12.06-12.17	O-19.07	HPV TEST OF CURE REMAINS HIGHLY PREDICTIVE AT 5YRS FOLLOW-UP H Kitchener, C Moore, R Hadwin, L Nelson, P Walker, A Sargent, J Wood, GB Anthony, M Cruickshank	
12.17-12.28	O-19.08	SURVEILLANCE AFTER TREATMENT FOR CIN: HPV TESTING IS NOT COST-EFFECTIVE J Melnikow, S Kulasingam, M Kuppermann, LJ Helms, C Slee, G Sawaya	

O-19.01 PTIMUM FOLLOW-UP STRATEGIES AFTER CIN TREATMENT; A SCORING SYSTEM(FUSS)

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Introduction: To evaluate the accuracy of various markers alone or in combination to predict treatment failure (TF) following treatment of CIN.

Methods: In a prospective study, women treated by LLETZ were assessed at regular intervals with cytology and colposcopy. At the same assessments, samples were also collected for HPV testing and typing, viral load, p16 and microspectroscopy. Women with cytologic or colposcopic suggestion of residual disease who had repeat excision that confirmed CIN were TF. Those with normal cytology and colposcopy during the first 2 post-operative years or with negative histology at repeat excision were considered as treatment successes. Accuracy parameters for cytology, colposcopy and the new markers were assessed for each test alone or in combination together with histopathological information and age.

Results: 363 women completed 2 years of follow-up. Out of 33 who underwent a repeat LLETZ, twenty-six were histologically confirmed TF. At 6 months the sensitivity was highest in cases of positive HPV testing, followed in decreasing sequence by positive HPV typing, p16, high viral load, cytology and colposcopy. The simpler combination that provided almost 100% negative predictive value at 6 months was that of negative cytology and HPV testing with favourable histopathological variables and age less than 35.

Conclusion: HPV test, cytology and p16 staining give the highest prediction of TF. The combination of HPV testing, cytology, initial histology and age could allow the distinction of low risk cases that could return to routine screening. All the above parameters should be evaluated in a cost analysis and could be integrated in a TF prediction scoring system, allowing tailored post-treatment surveillance.

O-19.02 HC2 AS TEST OF CURE IN STAGE IA CERVICAL CANCER

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BACKGROUND. HPV testing has been included in many international management guidelines as a test of cure after conservative CIN treatment. However, the role of HPV testing after conservative treatment of stage IA invasive cervical cancer has been never investigated. OBJECTIVE: evaluation of Digene HC2 DNA test as a test of cure after conservative treatment of stage IA invasive cervical cancer.

METHODS: 86 patients treated by cold knife cone, LEEP or laserconization for stage IA invasive cervical cancer were followed up by cytology, colposcopy and with the Digene HC2 DNA test. Persistent or recurrent disease was defined as histology confirmed CIN2+; positive pap smear was defined at a threshold of ASC-US or more.

RESULTS: the cone histology in 78 patients was stage IA1 and in 8 patients was stage IA2; median age of the patients was 38 years (mean 38.5; range 28 - 67); mean follow up time was 60.9 months (median 59.4; range 5-179). There were 12 cases of persistent or recurrent disease. Pap smear was positive in 10 out of 12 cases (83%), while HC2 was positive in all the 12 cases (100%). Positive margins were present in half of the 12 persistent or recurrent cases (50%).

CONCLUSIONS: these results suggest that also in patients conservatively treated for stage IA invasive cervical cancer, the Digene HC2 DNA test has a clinical role as a test of cure in adjunct to cytology as it correctly identified recurrent or persistent disease and was stronger predictor of persistence than positive margins on the original cone specimen.

0-19.03

LONG-TERM INCIDENCE/MORTALITY OF CERVICAL OR VAGINAL CANCER AFTER CIN3-TREATMENT

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OBJECTIVE: To study the long term mortality and incidence of invasive cancer of the cervix or vagina after treatment for cervical intraepithelial neoplasia grade 3.

DESIGN: Population based cohort study.

SETTING: Swedish cancer registry, Swedish registry of causes of death.

PARTICIPANTS: All women in Sweden with CIN3 diagnosed and treated during 1958-2004 (n=137,882) contributing 2,661,318 woman years.

MAIN OUTCOME MEASURES: Standardised incidence and mortality ratios with risk of aquiring or die from vaginal or cervical cancer in the Swedish general female population as reference; relative risks in multivariable log-linear regression model, with internal references.

RESULTS: Women with previous cervical intraepithelial neoplasia grade 3 had an increased risk to die due to invasive cervical or vaginal cancer compared with the general female population (SMR 3.02, 95% CI 2.73 – 3.34). The relative risk increase for mortality was 23% higher than that for incidence, suggesting a worsened prognosis compared with cancer cases in the general population. After age 60 women treated for CIN3 had an accelerated increased risk of acquiring invasiv cancer and a similar steep increase in mortality risk was seen after 70, reaching >100/100,000 women at age 78. This acceleration in risk seems to be confined to women who were >40 years of age at treatment of CIN3.

CONCLUSION: Women previously treated for CIN3 are at increased risk of developing and dying from cancer in the cervix or vagina compared to the general population. An observed accelerated increase in risk at older age seems to be confined to women over age 40 at treatment, suggesting a particular need for life long surveillance in this group.

O-19.04

MORTALITY AFTER TREATMENT OF CERVICAL INTRAEPITHELIAL NEOPLASIA

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Background: After treatment of the cervical intraepithelial neoplasia, CIN, cervical cancer incidence remains elevated at least for 20 years. Whether the overall or cervical cancer mortality after treatment of CIN is elevated is unknown. Objective: To determine the long-term survival and cause-specific mortality among women treated of CIN.

Methods: A retrospective cohort study. The study population consisted of 7104 women treated of CIN between 1974-2001 in one hospital, in Helsinki, Finland, and 35437 control women matched individually for age and place of residence at the time of CIN treatment, gathered trough a registry-based sample. The follow-up of mortality was based on nationwide registries and closed either at death, emigration or 31.December 2005. The possible differences in mortality were assessed using stratified Cox proportional hazard model, adjusting for socio-economic status and histopathological grade of CIN. Results: With follow-up time of approximately 630000 woman-years, overall 2781 deaths were observed, 530 among women treated of CIN and 2251 among reference population (HR 1.1, 95% CI 1.0-1.3). Mortality from any cancer (HR 1.4, 95% CI 1.2-1.7), lung cancer (HR 2.7, 95% CI 1.8-4.1) and HPV-related anogenital cancer (cancers of cervix, vulva, vagina & anus) were in excess among CIN patients, even though mortality from cervical cancer was not (HR 1.0, 95% CI 0.3-4.0).

Conclusions: Elevated cervical cancer incidence after treatment of CIN did not predict elevation in cervical cancer mortality. This confirms high effectiveness of CIN treatments: most of the excess mortality observed among CIN-patients was due to increased risk of other cancers, especially lung cancer. These long-term mortality patterns should be considered when planning and evaluating the management of CIN lesions and related cervical or other cancer prevention activity.

O-19.05

POST-TREATMENT HPV TESTING AND LONG-TERM RISK AT RECURRENCE

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Background: In the Netherlands, each year over 5000 women are treated for high-grade premalignant cervical lesions (CIN 2/3). The prevalence of recurrent/residual disease (rCIN 2/3) is approximately 15%, mostly within two years after treatment. Monitoring women for rCIN 2/3 is presently performed by cytology and/or HPV-testing. Follow-up in most studies is predominantly confined to two years. Here we describe the predictive value of cytology and HPV-testing taken two years after treatment on the development of long-term rCIN 2/3.

Methods: Between 1988 and 2004, 445 women treated for CIN 2/3 were tested for hrHPV (GP5+/6+ PCR) and/or cytology 24 months post-treatment. Women who developed rCIN 2/3 before 24 months of follow-up (n=55) were excluded. All other women were tracked for the development of rCIN 2/3 by the use of the Dutch nationwide network and registry of histo- and cytopathology.

Results: The mean follow-up was 61 months (range 0 - 207). Of the 380 retrieved women, 16 (4.2%) developed rCIN 2/3 during follow-up. Most (9) rCIN2/3 developed between two and five years after treatment. A combined cytology and HPV-test at 24 months has the highest sensitivity (91%) and negative predictive value (98%) for the development of rCIN 2/3.

Conclusions: This study indicates that only women who are negative for cytology and HPV-testing do not need follow-up for rCIN 2/3 and should be monitored within the population based cervical screening programme.

O-19.06

HPV TESTING IN THE FOLLOW-UP AFTER CONIZATION

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Objective: The aim of this study was to assess the value of HPV DNA and mRNA testing after conization for high-grade cervical intraepithelial neoplasia (CIN2+) as a measure of treatment success.

Methods: HPV testing was performed before treatment and on the cytology samples collected at six and 12 month followup. A total of 613 women were included in the study. All participants were tested for HPV using the Amplicor HPV test (Roche Molecular Systems, Switzerland) which detects 13 high-risk HPV types in aggregate and targets L1 DNA and the PreTect HPV-Proofer (NorChip AS, Norway) which is type specific and detects 16,18,31,33,45 E6/E7 mRNA. A total of 384/613 (62.6%) tested positive with both tests before treatment. Six month follow-up test-results were available in 313 of these 384 women. Median age was 34 years (range, 19-76).

Results: Baseline histology revealed CIN2 in 45 cases, CIN3/adenocarcinoma in situ in 267 cases and invasive carcinoma in one case. Resection margins were involved in 52/313 (16.6%) of the cone specimens. At the six month follow-up, 32/313 women had abnormal cytology (10.2%), 99/313 were HPV DNA positive (31.6%) and 28/313 (8.9%) were mRNA positive. HR HPV DNA and mRNA persistence at six month was significantly associated with positive resection margins (p=0.001 and 0.005 respectively). Positive cytology post-treatment was significantly associated with HR HPV DNA and mRNA persistence (p<0.000). Follow-up data at twelve months and the prevalence of persistent disease will be presented.

Conclusions: Preliminary results indicate that the majority of HR HPV infections are eliminated after conization. This study will help us to evaluate whether mRNA testing is a more useful indicator of treatment success compared to DNA testing or cytology. Further data will be presented and therein, the sensitivity and specificity of the assays for detection of residual disease.

O-19.07

HPV TEST OF CURE REMAINS HIGHLY PREDICTIVE AT 5YRS FOLLOW-UP

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Background: HPV testing is known to be clinically effective in the follow-up of women treated for CIN. We hypothesised that HPV test of cure (ToC) could be used to identify a large majority who could be returned to routine recall and the minority for whom colposcopy should be performed. We have published 2 year follow-up data on a cohort of treated women (Kitchener et al, BJOG 2008;115:1001-1007).

Methods: A cohort of 917 women who had been treated for CIN (700 [76%] had CIN2+ and 217 [24%] had CIN1) had undergone cytology and HPV testing at 6 and 12 months and cytology alone annually thereafter.

Results: At 6 months the negative predictive value for HPV, cytology and both combined were 99%, 92% and 100% respectively for residual CIN2+. In the absence of disease at 6 months, the relative risk for HR-HPV+ve women developing non negative cytology at 4 and 5 years was 4.5 (95% C.I. 2.05-8.97) and 6.5 (95% C.I. 1.92-22.06) respectively. The negative predictive value of a HR-HPV test for CIN2+ at 6 months post treatment was 99%, and 94% and 96% at 4 and 5 years respectively.

Conclusion: HPV test of cure at 6 months can safely return HPV-ve cytology-ve women to routine recall within the context of a population based national screening program. HPV+ve women should remain in annual follow up for at least 5 years.

O-19.08

SURVEILLANCE AFTER TREATMENT FOR CIN: HPV TESTING IS NOT COST-EFFECTIVE

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Background: Current guidelines recommend use of HPV testing for surveillance after treatment of CIN. We evaluated the relative cost-effectiveness of this strategy compared cytology or colposcopy. Methods: A Markov model of the natural history of HPV infection and cervical cancer modeled outcomes following treatment of CIN 2 or 3. Post-treatment disease rates were estimated from a large cohort study. Costs were estimated from US Medicare reimbursements. Time tradeoff utilities were elicited from 65 English or Spanish-speaking women who had experienced an abnormal Pap test. We evaluated 8 different surveillance strategies to detect subsequent disease in treated women. Model outcomes included CIN 1-3, cervical cancer, incidental hysterectomy, death from cervical cancer, and death from all other causes. Rates of falsepositive test results were estimated for each strategy, based on test specificity. Total lifetime costs, life expectancy, qualityadjusted life expectancy (QALYs) and incremental cost-effectiveness ratios (ICERs) associated with each strategy were tabulated. Results: Conventional cytology at 6 and 12 months, followed by triennial screening was the least costly strategy. ICER's for more intensive strategies were more favorable for women at greater risk of subsequent disease (CIN 3 treated with cryotherapy). Annual cytology follow-up had an ICER of \$70,000-\$80,000 per life-year gained. Strong disutilities for false-positive results and recurrent disease influenced the quality-adjusted model. Colposcopy was the preferred initial screening strategy for women at greater risk of subsequent disease. Colposcopy as initial follow-up for lower-risk women had a favorable ICER of \$430 per QALY. Strategies involving initial HPV testing were dominated in all QALY analyses. Conclusions: Serial cytology offers cost-effective strategies for follow-up after CIN treatment if the goal is maximizing life-expectancy. Initial colposcopy followed by serial cytology is preferred to optimize quality of life. HPV testing was dominated due in part to relatively high false-positive rates.

POSTER ABSTRACTS SESSION 19

POSTER SESSION III TUESDAY 20.00: ODD NUMBERS

POSTER SESSION IV

TUESDAY 21.00: EVEN NUMBERS

HPV DNA DETECTION IN WOMEN UNDERGOING TREATMENT FOR ABNORMAL CYTOLOGY

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Background: With the advent of standardized assays now available for detection of oncogenic HPVs, evaluation of such tests for effective clinical management is possible. In particular, the role of HPV DNA testing is being considered in the follow-up of patients recently treated for pre-cancerous lesions in order to identify women with residual disease and/or recurrence.

Objectives: To evaluate the role of HPV DNA detection in assessment of adequacy of treatment in women undergoing treatment for abnormal Pap.

Methods: A prospective study of 1700 women in Melbourne Australia, with histologically proven dysplasia, undergoing ablative treatment was conducted. Seven hundred and fifty seven patients were evaluated for HPV DNA status at treatment and at intervals of 4-6 months until 24 months post-treatment. HPV DNA detection was performed using Hybrid Capture II, Roche Amplicor and Linear Array (LA). The results of these assays were compared to each other and with the conventional clinical assessment in order to determine the efficacy of these methods.

Results: Detection by Hybrid Capture showed 64% positivity for high-risk HPV types at the treatment visit and 22.7% at 24 months. Amplicor and LA showed 73.3% and 72.6% HR positivity at treatment visit respectively and 29.5% and 27.9% at 24 month visit. HPV 16 and 18 was detected at 35.1% and 8.5% respectively at treatment visit and 6.3% and 1.1% at 24 month visit.

Conclusions: Overall, a significant number of patients still carry HPV DNA after 24 months, however HPV 16 and 18 positivity decrease significantly.

P-19.10

PERINATAL MORTALITY & SEVERE PREGNANCY OUTCOMES AFTER CIN TREATMENT: META-ANALYSIS

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Study Objective: To assess the relative risk (RR) of peri-natal mortality (PM), severe preterm delivery (PD) and low-birth weight (LBW) after treatment for CIN.

Methods: Eligible studies, published between 1960 and 2007, were retrieved through a literature search, if they provided data on severe pregnancy outcomes for women with and without prior treatment for CIN. Considered outcomes were: PM, severe PD (<32/34weeks), extreme PD (<28/30weeks), LBW <2000g, <1500g and <1000g. Excisional and ablative treatment procedures were analysed separately.

Results: A significantly increased risk of PM was observed for women treated by cold knife conisation (CKC) (RR=2.87; 95%CI:1.42-5.81). Moreover, CKC was associated with a significantly higher risk of severe PD (RR=2.78; 95%CI:1.72-4.51), extreme PD (RR=5.33; 95%CI:1.63-17.40) and LBW<2000g (RR=2.86; 95%CI:1.37-5.97). Laser conisation (LC), described in only one study was also followed by a significantly increased chance of LBW<2000g and <1500g. LLETZ was not associated with a significantly increased RR of serious adverse pregnancy outcome. Neither was ablative treatment using cryotherapy (CT) or laser. However ablation by radical diathermy (DT) was followed by a significantly higher frequency of PM, severe and extreme PD and LBW below 2000g or 1500g.

Conclusions: In contrast to CKC, serious adverse outcomes have not been demonstrated after LLETZ. However, even the milder sequelae after LLETZ such as PD may result in perinatal morbidity, increased socio-economic burden and parental anxiety. The risk of these should be balanced against the patients' characteristics and the need of minimum residual rates. Other factors related to the severity and the extent of disease might affect the degree of tissue destruction and may account for differences in pregnancy outcomes.

ROLE OF HPV TEST AFTER CONIZATION IN PREDICTING RESIDUAL DISEASE

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Objectives; To evaluate the role of HPV test which was performed after conization in predicting residual disease (RD) in subsequent hysterectomy specimen.

Methods; A prospective study was undertaken on 115 patients who underwent hysterectomy after conization due to CIN 3 and IA1 cervical cancer. All patients underwent HPV test with Hybrid Capture II (HC II) system just before hysterectomy. The mean time interval from conization to hysterectomy was 37 days (range, 9-95 days). We correlated several factors with RD in a hysterectomy specimen.

Results; In univariate analysis, age, parity, menopausal status, glandular extension, severity of disease (CIN3 vs IA1 cancer) were not predictive for RD in subsequent hysterectomy specimen, but positive RM and positive HPV test were significant factors for predicting RD. In multivariate analysis, positive RM (Odds ratio [OR], 3.09; 95% confidence interval [CI], 1.19-8.03; P = 0.021) and positive HPV test (OR, 11.05; 95% CI, 4.01-30.49; P < 0.001) were also significant. With RM, the sensitivity, specificity and accuracy in predicting RD were 53%, 75%, and 61%, respectively; with HPV test, those were 67%, 85%, and 73 %, respectively (P = 0.080, 0.454, and 0.044, respectively). Of patients with positive RM, 78 % of patients with negative HPV test have no RD, but 63% of patients with positive HPV test had RD. Of patients with negative RM, no patients with negative HPV test had RD, but 48 % of patients with positive HPV test had RD.

Conclusion; In predicting RD, the accuracy of HPV test after conization was significantly higher than that of RM. Especially, combining with RM, the negative predictive value of HPV test in predicting RD was very high. The integration of HPV test in deciding hysterectomy after conization for CIN 3 and microinvasive cervical cancer is recommended.

P-19.13

EFFECT OF BIORESPONSE-DIM ON CERVICAL HPV INFECTION: THE CRISP-1 TRIAL

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Background: The food supplements indole-3-carbinol (I3C) and its dimer diindolylmethane (DIM) have been identified as compounds in cruciferous vegetables that could potentially prevent or halt carcinogenesis. A well tolerated oral treatment for low-grade cytological abnormalities would be extremely useful.

Objectives: To investigate the effect of oral DIM on cervical HPV infection

Methods: A double-blind randomised placebo-controlled trial in women with low-grade cytological abnormalities within Cervical Screening Wales. Participants took 150mg/day DIM from BioResponse DIM or placebo for six months. Cervical samples were taken at entry and at six months and tested for high-risk HPV DNA using the PCR method of Walboomers.

Results: 479 women attended their six month follow-up appointment, 461 (96%) had an adequate HPV test. Of the women who attended their 6 month visit 56.5% (182/322) of DIM women and 56.1% (83/148) of placebo women were infected at entry. Of those positive at baseline, 25.0% (44/176) of the DIM group were negative at six months compared to 40.0% (32/80) of the placebo group: Risk ratio 1.25, 95%CI: 1.03 - 1.52. Of those who were negative at baseline, 7.5% (10/133) on the DIM and 11.1% (7/63) on placebo became HPV positive by six months (Risk ratio (95%CI): 0.68 (0.27, 1.69)). Analysis of HPV status at six months stratified by baseline HPV status gives a combined risk ratio of 1.17 (95%CI; 0.97, 1.44).

Conclusion: Six months of supplementation with DIM does not substantially increase the rate of HPV clearance during the period of supplementation in women with low-grade cytological abnormalities. However we cannot rule out substantial non-compliance in either the control or placebo group because we have not yet analysed hair and urine sample for DIM levels.

HUMAN PAPILLOMAVIRUS PERSISTENCE AFTER TREATMENT FOR CERVICAL DYSPLASIA WITH LEEP

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Background: Because HPV persistence precedes cervical intraepithelial neoplasia (CIN), HPV persistence detection may be preferable to CIN recurrence as a more rapidly occurring endpoint for monitoring of treatment efficacy, both on the individual patient level and when evaluating new treatment modalities.

Objective: Effectiveness of treatment of cervical dysplasia by LEEP (loop electrosurgical excision procedure) conization was evaluated using persistence of HPV as outcome. Also, a long-term follow-up on the ability of HPV testing, as compared to cytology, to predict recurrence of high-grade CIN was performed.

Methods: 178 women in Umeå, Sweden, with abnormal smears who were treated using LEEP conization were scheduled for HPV DNA testing and Pap smears before and 3, 6, 12, 24 and 36 months post treatment.

Results: HPV persistence was found among 25 (19%) of the 129 patients that were HPV-positive before treatment. Three years after treatment 4.5% of patients were still persistently positive. CIN II+ was found among 9 patients. All of these had HPV-persistence, but only 7/9 had abnormal cytology.

Conclusions: The treatment was moderately effective for clearing HPV and preventing CIN II+ recurrence. Only HPV persistence predicted recurrence adequately.

P-19.15

THE CORRELATION BETWEEN HISTOLOGICAL RESULTS IN HIGH-GRADE SQUAMOUS INTRAEPITHELIAL LESIONS

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Objective: The purpose of this study was to examine the correlation between histological results in patients with high-grade squamous intraepithelial lesions (HSIL) who have undergone large loop excision of the transformation zone (LLETZ) with or without colposcopy-directed biopsy.

Study design: This retrospective study included 321 patients who had HSIL on Pap smear and were treated with LLETZ, with or without colposcopy-directed biopsy, at Yongdong Severance Hospital between March 2004 and June 2008. Patients were divided into two groups based on the treatment they received: see-and-treat group and the standard three-step group. Histological findings were compared for the two study groups. In addition, we compared the final histological results of 269 patients with LSIL/ASCUS or normal cytology on Pap smear.

Results: Of the 136 patients on see-and-treat group, 18 (13.2%) had no evidence of cervical intraepithelial neoplasia (CIN), 7 (5.1%) had CIN 1, 14 (10.2%) had CIN 2, 78 (57.3%) had CIN 3, and 19 (13.9%) had squamous cell carcinoma (81.7% agreement). Of the 185 patients who were treated by the standard three-step protocol, 24 (12.9%) had no evidence of CIN, 11 (5.9%) had CIN 1, 14 (7.5%) had CIN 2, 117 (63.2%) had CIN 3, and 19 (10.2%) had squamous cell carcinoma (81.1% agreement). There were no significant differences in the final histological results between the two study groups. In patients with LSIL/ASCUS or normal cytology on Pap smear, the concordance rate between the initial and final diagnosis was significantly different between the two study groups (P < 0.05).

Conclusions: We conclude that, compared to the standard three-step protocol, a see-and-treat protocol may be a viable alternative in women with HSIL on Pap smear and suggestive of CIN 2 or 3 after colposcopic examination.

TREATMENT OF DIFFUSE HIGH-GRADE ANAL INTRAEPITHELIAL NEOPLASIA WITH 5%-FLUOROURACIL CREAM

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Background: High-grade anal intraepithelial neoplasia (HGAIN) is considered the precursor lesion for anal cancer. Treatment of HGAIN may prevent anal cancer, similar to treatment of cervical high-grade lesions to prevent cervical cancer. Treatment options for HGAIN include physical ablation using trichloroacetic acid (TCA) or liquid nitrogen for small lesions, and infrared coagulation (IRC) or surgical fulguration for larger lesions. However, these methods are often not suitable for diffuse disease. Topical 5% 5-fluorouracil (5FUc) is used to treat diffuse female genital HPV-associated disease but has not been assessed for treatment of diffuse HGAIN.

Objectives: To evaluate the tolerability and efficacy of 5FUc for treatment of diffuse HGAIN.

Methods: Twenty-eight patients (25 HIV-seropositive, 3 HIV-seronegative and 25 men, 3 women) at the UCSF Anal Neoplasia Clinic were given topical 5FUc for diffuse HGAIN (>75% of circumference). Patients applied 5ml per side twice daily in cycles of 5 days on/9 days off. Patients returned for assessment after 4 cycles and were assessed for histologic response or reduction of disease burden.

Results: 27 patients returned for follow-up. Of the 20 patients who completed at least 3 cycles of treatment (mean 6, mode 4), 3 had complete histologic regression of HGAIN, 16 had partial response evidenced by reduction of disease burden to < 25-50% and 1 had no response. Two patients discontinued treatment due to side effects. Four patients were non-adherent, completing < 2 cycles, 1 did not obtain medication, and 1 was lost to follow-up. Side effects included ulceration, bleeding and exacerbation of herpes simplex virus infection. All patients with partial response were subsequently treated successfully with IRC.

Conclusions: Topical 5FUc may successfully treat diffuse HGAIN, allowing for lesion reduction to a size amenable to office ablation. Longer treatment may offer successful primary therapy but needs to be evaluated in clinical trials.

P-19.17

2009 EUROPEAN GUIDELINES (IUSTI/WHO) ON THE MANAGEMENT OF ANOGENITAL WARTS

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The International Union against Sexually Transmitted Infections (IUSTI) produce guidelines on the treatment and management of sexually transmitted infections (STI) and related conditions. Current European guidelines on the diagnosis and management of genital warts were published in 2000 by the European Course on HPV-associated Pathology. These guidelines require updating to incorporate evidence from studies published since 2000, and would benefit from revision in view of the availability of a vaccine which provides protection against human papillomavirus (HPV) types 6 and 11 which cause over 90% of genital warts.

IUSTI/WHO European guidelines are produced according to an established protocol, requiring a systematic review of published articles and relevant clinical guidelines. We will present the search strategy, draft guidelines, and provide an opportunity for consultation and feedback on the guidelines.

4,4'-DIHYDROXYBENZOPHENONE-2,4-DINITROPHENYLHYDRAZONE (A-007) FOR PERIANAL HIGH-GRADE SQUAMOUS INTRAEPITHELIAL LESIONS (HSIL)

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Background: Human papillomavirus infection (HPV) can lead to HSIL and anal cancer. Immunomodulation is successfully used to treat HPV-related low-grade dysplasia (warts). A-007 is an aryl hydrazone with immunomodulating properties when applied as 0.25% gel to anogenital cancer and demonstrated >25% objective responses when applied topically to metastatic breast cancer, melanoma and lymphoma. We conducted a pilot study of A-007 in the treatment of anal/perianal HSIL.

Objectives: The primary objectives of the study were to determine the acceptability, document any toxicity, and monitor local responses when A-007 0.25% gel was applied as treatment of anal/perianal HSIL.

Methods: This open-label, single arm study evaluated the safety and efficacy of A-007 in four subjects with HSIL ineligible for office-based excision. Two grams of 0.25% A-007 gel were applied daily for five days of each 28-day cycle. After two cycles (one course) subjects were biopsied to assess response. Subjects could have received up to two additional courses of therapy.

Results: All subjects were HIV-positive men ranging in age from 45-58 years. Following one course of study drug, two subjects experienced a partial response when their individual 1x1.5cm and 0.4x0.8 lesions regressed by >50% in area. Another subject experienced a partial response when his 2x2cm lesion regressed to 1.5x1.5cm and from HSIL to low-grade squamous intraepithelial lesion. One subject with 2-3cm of circumferential disease had no response to 3 courses of study drug. Hematology and chemistry parameters did not change from baseline. One subject experienced local irritation and another developed malignant melanoma at a distant site. Considering his significant risk factors of heavy sun exposure, tobacco use, and HIV infection, the adverse event was considered possibly related to the study drug. Conclusions:

Topically applied A-007 0.25% gel appears well tolerated. Data from initial subjects suggest clinical benefit in subjects with anal/perianal HSIL.

P-19.20

OBSTETRICAL RISKS DUE TO CONISATION AVOIDABLE BY HPV VACCINATION

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Background and objectives: Recent meta-analytical work has indicated that excisional treatment of cervical intra-epithelial neoplasia (CIN) is associated with adverse obstetrical outcomes. Currently, large loop excision of the transformation zone (LLETZ) is the most frequently used procedure to treat CIN in Belgium as well as in other industrialized countries.

The aim of this study was to estimate the burden of adverse obstetrical outcomes after conisation in Belgian women. Methods: An exhaustive data file of the Intermutualistic Agency together with official population data of the corresponding age group and calendar year (1996-2000) was used to estimate the incidence of conisations in Belgium, by age, as well

as the cumulative incidence up to a given age.

Results: The peak incidence of conisation (including LLETZ) was noted in the age group 30-34 years (2.0/1000 womenyears). In this age group, 34,193 women gave birth, and the cumulative incidence of women with a history of conisation was 2.5%. In the age group 35-39 years, 10,813 women gave birth, with a cumulative incidence of conisations upto the age of 39 of more than 4%.

On average, pregnant women previously treated with LLETZ have a risk of preterm delivery (PD) that is 1.70 (95% CI: 1.24-2.35) times higher than for non-treated pregnant women. Taking into account, the maternal age distribution at delivery (Centre for Operational Research in Public Health, 2006), and the cumulative incidence of conisation over age, we can estimate that 2% (95% CI: 0.7-3.6%) of all PDs in Belgium are attributable to prior LLETZ. However, among treated women, the risk of PD attributable to LLETZ is about 41% (95% CI: 19-57%).

Conclusions: Introduction of prophylactic vaccines for human papillomavirus will result in a considerable decrease in the incidence of CIN requiring treatment, which will subsequently reduce the risk of adverse obstetrical outcomes.

THE D1000 APLICATION IN HPV INFECTION LOW VIRAL LOAD.

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Background: The cervical cancer (cc) is associated among 99.7% of the cases with human papilloma virus of high risk (HPV-HR) and it is the main mortality cause of cancer in women in developing countries. The persistence of infection causes cervical intraepithelial neoplasy (CIN) which as time it advances to CC development. 12% of CIN-1 progresses and 32% remains constantly, 27 % of CIN-2 progresses and 35% persists, 12% of CIN-3 progresses and 56% remains constantly. We have been proposed a new compound (D1000), for the management to this infection which are the causes of damages. D-1000 has effects anti-viral for other viral types among microorganisms such as: coronavirus, herpes virus and SARS virus and anti-carcinogenic properties.

Objective: To evaluate the effect of D-1000 in patients who are only infected with HPV or CIN.

Material and methods: Doses of D1000, between 250-500mg. were applied in patients from 7 to 15 days of compound gel. The viral load was measure with Hybrid Capture-II at the beginning of the study and from 15-21 days post-treatment and the cytology was performed with negative results.

Result: The treatment report result with the D-1000 (thermoreversible gel) in 34 patients. This is the viral load before and after the treatment: 23.14/0.16; 241/0.18; 2306/0.72; 2306.26/0.27; 5.59/0.09; 377.51/0.30, 271.92/0.18; 264.52/0.11; 6.41/0.25; 23.14/0.13; 1.25/0.52; 4.32/0.09; 187.76/0.91, 1336.49/0.12; 1.32/0.35; 78.56/0.16; 2.81/0.74; 1.9/0.16; 31.6/0.16; 254.52/0.36; 12.02/0.17; 1588.14/0.22; 103.54/9.23; 2068.42/94.83; 16.71/0.78; 48.51/0.09; 237.37/0.27; 1.29/0.22; 3.88/0.43; 1788.02/0.21; 310.12/0.16; 209.22/2.26; 798.23/0.11 and 11.43/2.17 URL.

Conclusion: The negative results of this cases lead our spectatives to use D-1000 as a therapeutic of those infections caused by the HPV-HR. We can interfere in the natural history of this disease that without treatment will increase the risk of developing cervical cancer.

SESSION 20

HPV-BASED SCREENING I

PROGRAMME

Oral presentations

THE 25TH INTERNATIONAL PAPILLOMAVIRUS CONFERENCE MAY 8-14 2009, MALMÖ, SWEDEN

TIME	NUMBER	TITLE	ROOM	
2009-05-13				
SESSION 20: HPV-BASED SCREENING I SC				
14.00-14.13	O-20.01	FOLLOW-UP OF THE NTCC RANDOMISED CONTROLLED TRIAL. G Ronco, N Segnan, A Gillio - Tos, R Rizzolo, A Del Mistro, M Zorzi, P Dalla Palma, F Carozzi, C Naldoni, M Confortini, P Pierotti, P Giorgi Rossi, J Cuzick		
14.13-14.24	O-20.02	DURABLE BENEFITS OF HPV-BASED SCREEN-AND-TREAT TO 36 MONTHS T Wright, L Denny, M DeSousa, L Kuhn		
14.24-14.35	O-20.03	HART: LONG-TERM FOLLOW-UP AMONG WOMEN SCREENED BY CYTOLOGY AND HPV TESTING J Cusick, D Mesher, A Szarewski, L Cadman, H Cubie, G Hulman, H Kitchener, D Luesley, U Menon, G Terry, M Desai, C Gie, L Ho, C Pickles, P Sasieni	,	
14.35-14.46	O-20.04	COST-EFFECTIVENESS OF HPV DNA SCREENING IN NON-VACCINATED AND VACCINATED WOMEN J Berkhof, VM Coupé, JA Bogaards, Fj van Kemenade, PJ Snijders, CJIm Meijer		
14.46-14.57	O-20.05	COST-EFFECTIVENESS OF VARIOUS CERVICAL SCREENING MODALITIES IN RURAL CHINA K Canfell, JF Shi, Y Ning, JB Lew, R Legood, FH Zhao, YJ Kang, L Ma, L Simonella, M Smith, YZ Zhang, JF Chen, M Clements, G Clifford, S Francheschi, Y Qiao		
14.57-15.08	O-20.06	ADDITION OF HRHPV TESTING TO CONVENTIONAL CYTOLOGICAL SCREENING: VUSA-SCREEN TRIAL D Rijkaart, J Berkhof, PJ Snijders, CJLM Meijer		
15.08-15.19	O-20.07	AGE-SPECIFIC PERFORMANCE OF HPV DNA TEST IN PRIMARY CERVICAL SCREENING A Anttila, M Leinonen, P Nieminen, L Kotaniemi - Talonen, N Malila, J Tarkkanen, P Laurila		
15.19-15.30	O-20.08	HPV TESTING CAN EXTEND SCREENING INTERVALS; EVIDENCE FROM ARTISTIC TRIAL. C Thomson, H Baysson, M Almonte, M Desai, A Sargent, A Turner, A Bailey, J Peto		

FOLLOW-UP OF THE NTCC RANDOMISED CONTROLLED TRIAL.

G Ronco, CPO, Torino, Italy; *N Segnan*, CPO, Torino, Italy; *A Gillio - Tos*, CPO, Torino, Italy; *R Rizzolo*, CPO, Torino, Italy; *A Del Mistro*, IOV, Padova, Italy; *M Zorzi*, IOV, Padova, Italy; *P Dalla Palma*, S.Croce Hospital, Trento, Italy; *F Carozzi*, ISPO, Firenze, Italy; *C Naldoni*, Regione Emilia-Romagna, Bologna, Italy; *M Confortini*, ISPO, Firenze, Italy; *P Pierotti*, Maggiore Hospital, Bologna, Italy; *P Giorgi Rossi*, ASP Lazio, Rome, Italy; *J Cuzick*, Queen Mary's School off Medicine, London, UK

Background. HPV testing is cross-sectionally more sensitive than cytology in detecting high-grade cervical intraepithelial neoplasia (hgCIN). Two randomised controlled trials (RCT) observed decreased detection of hgCIN after screening by HPV testing than by cytology.

Objectives. Comparing the occurrence of hgCIN after screening with HPV DNA and after cytology as primary tests. Methods. In a RCT women were randomly assigned to a conventional arm (conventional cytology) or to an experimental arm (HPV testing + liquid-based cytology in phase one and alone in phase 2). Women testing negative in each arm were re-invited after 3 years for a new screening round with conventional cytology. We studied the detection of histologically confirmed CIN2+ in the two arms after recruitment.

Results. 22,547 and 24,353 women were enrolled in the conventional during phase one and phase two respectively. Women enrolled in the experimental arm were 22,708 in phase one and 24,361 in phase two. Concerning women recruited during phase one, at the new screening round, there was a reduction of the detection rate of histologically confirmed CIN2+ in the experimental, compared to the conventional, arm (relative DR 0.63; 95% CI 0.32-1.24) and especially of CIN3+ (relative DR 0.24; 95% CI 0.07-0.86). This reduction was stronger among women aged 35-60 than in younger women. We are analysing data from the entire groups (including those testing positive at recruitment) during the two phases.

Conclusion. For phase one our data show that women who had a negative HPV test have a strongly lower risk of subsequent high-grade CIN than women who had a normal cytology, suggesting that the use of prolonged screening intervals for HPV negative women will be safe

O-20.02

DURABLE BENEFITS OF HPV-BASED SCREEN-AND-TREAT TO 36 MONTHS

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Background: Long-term studies to assess the efficacy of screen-and-treat are lacking.

Objective: We followed a subset of women enrolled in a randomized screen-and-treat" trial for 36 months.

Methods: 6553 South African women (ages 35-65 yrs) were screened using HPV (hc2) and visual inspection after acetic acid (VIA) and subsequently randomized to three groups: (1) cryotherapy if HPV positive, (2) cryotherapy if VIA positive, or (3) delayed evaluation (control group). All women were scheduled for colposcopy at 6 months. Women who were HPV or VIA positive at enrollment, and a planned subset of all others, underwent extended follow-up for 36 months with yearly colposcopy (n = 2,712).

Results: After 36 months there was no significance difference in the cumulative prevalence of CIN 2,3/cancer (CIN2+) detected among women randomized to VIA (4.07% [95% CI. 3.06-5.08%]) compared to the control arm (6.05% [95% CI. 4.89-7.21%]). In contrast, the cumulative prevalence of CIN2+ was significantly reduced in women randomized to the HPV (1.63% [95% CI. 0.96-2.31]) compared to those randomized to either control or VIA arms. 31 incident cases of CIN2+ were identified at 24 and 36 months; 17 in the VIA arm, 12 in the control arm, and 2 in the HPV arm. 30 (97%) were HPV positive at the time CIN2+ was diagnosed and 26 (84%) had been HPV positive at the enrollment visit and would have been treated using HPV-based screen-and-treat.

Conclusions: Screen-and-treat utilizing HPV DNA testing produces a significant and durable reduction in the prevalence of CIN2+. This approach not only identifies and treats prevalent cases of CIN 2,3 but it also significantly reduces incident cases of CIN2+ for up to 36 months.

HART: LONG-TERM FOLLOW-UP AMONG WOMEN SCREENED BY CYTOLOGY AND HPV TESTING

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Abstract:Background: Several studies have shown that HPV testing is substantially more sensitive than cytology for primary cervical screening. Results published from the long-term follow-up of the Hammersmith study in 2008 suggested HPV testing offers improved protection from CIN2+ for 6-8 years after a negative test. Here we report the long-term findings of the follow-up of the HART study.

Objectives: This study aimed to investigate long-term follow-up according to HPV and cytology results to determine appropriate management of women.

Methods: At baseline, 10358 eligible women from 5 sites received a cytology and HPV test. All women were followed-up and routine screening and histological outcomes were collected at individual sites. The main outcome of interest was duration of protection against histologically confirmed CIN2+ afforded by a negative HPV test compared to normal cytology.

Results: 5662 women had at least one further smear recorded at least 1 year after entry with a median follow-up of 6.0 years. At baseline 90 women had CIN2 or worse and during the follow-up a further 42 cases of CIN2+ were found. Including any disease found at baseline, the risk of developing CIN2 or worse at 1, 3, 5 and 9 years after a normal cytology was 0.19%, 0.27%, 0.47% and 2.04% respectively whereas it was 0.07%, 0.11%, 0.22% and 1.66% after a negative HPV test.

Conclusions: HPV testing offers improved protection from CIN2+ after a negative test compared to the protection afforded from a normal cytology.

O-20.04

COST-EFFECTIVENESS OF HPV DNA SCREENING IN NON-VACCINATED AND VACCINATED WOMEN

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Background: The HPV DNA test has a higher sensitivity but lower specificity than cytology. Mathematical models have been used to assess the cost-effectiveness of HPV DNA screening strategies.

Objectives: 1. To predict the effect of replacing cytological screening by HPV DNA screening on the cervical cancer incidence. Separate predictions will be given for non-vaccinated women, women vaccinated against HPV16/18, and women vaccinated against HPV16/18/31/33/45 (possible future 5-valent vaccine).

2. To identify cost-effective screening strategies for non-vaccinated and vaccinated women in the Netherlands.

Methods: We developed a simulation model that describes the relation between high-risk HPV, high-grade cervical intraepithelial neoplasia, and cervical cancer. Pre-invasive model parameters were estimated from data collected during the baseline round of a population-based HPV screening trial (POBASCAM) [Bulkmans et al. Lancet 2007;370:1764-72]. We varied model parameters and selected 118 models that were not violated by the observed age-dependent incidences of high-grade CIN, cervical cancer, and death from cervical cancer. The selected models yielded accurate predictions of the POBASCAM results at the second round after 5 years.

Results: The selected models predict that the number of cervical cancer cases does not increase if HPV testing is implemented (with cytology triage) and the screening interval is increased by 1 to 3 years. From a cost-effectiveness perspective, the optimal screening strategy for both non-vaccinated women and for women vaccinated against HPV16/18 is to replace cytology by HPV DNA testing and to extend the screening interval from 5 to 7.5 years. For women vaccinated against HPV16/18/31/33/45, the screening interval can be further extended if the vaccination compliance is high.

Conclusions: Our model supports the implementation of HPV testing with extended screening intervals both for non-vaccinated and vaccinated women.

COST-EFFECTIVENESS OF VARIOUS CERVICAL SCREENING MODALITIES IN RURAL CHINA

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Background: Visual inspection using acetic acid, used as a stand-alone screening modality (VIA-only) or in combination with visual inspection using Lugol's iodine (VIA/VILI) have been proposed as cervical screening modalities in less developed settings. However, problems with test accuracy and standardisation have limited the adoption of the procedure. An alternative new modality, rapid-throughput low cost HPV DNA testing, has recently been shown to have high sensitivity and specificity for screening women in rural China.

Methods: We constructed a model incorporating information on age-specific HPV infection, the natural history of cervical disease, and the accuracy of screening tests and colposcopic diagnosis. The model was parameterised using data on sexual behaviour, test accuracy, and screening and diagnostic practices from large-scale studies conducted in rural Shanxi Province, China. We estimated the incremental cost-effectiveness ratio (ICER) of various screening strategies, including once or twice-lifetime screening, and routine screening at 5-yearly, 10-yearly and IARC-recommended intervals.

Results: For all potential screening strategies, the relative ordering of test technologies in terms of reduction in cervical cancer incidence was VIA-only (least effective); VIA/VILI; HPV@1.0pg/ML threshold and HPV@0.5pg/mL (most effective). For once-lifetime screening strategies, maximum cost-effectiveness was achieved if screening was performed between 35-45 years. Depending on the test technology used, and assuming coverage of 70%, once-lifetime screening at age 35 years would reduce the age-standardised incidence of cervical cancer in the population by 7-10% with an ICER of US\$367-670. IARC-recommended screening would reduce the age-standardised incidence of cervical cancer in the population by 32-41%, with an ICER of US\$367-670.

Conclusions: Once-lifetime screening strategies under feasible coverage assumptions would reduce cancer incidence in rural China by 10% or less. If used as part of a program of regular screening, rapid throughput HPV DNA testing would be an effective and highly cost-effective method of primary screening in rural China.

O-20.06

ADDITION OF HRHPV TESTING TO CONVENTIONAL CYTOLOGICAL SCREENING: VUSA-SCREEN TRIAL

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Objectives: The VUSA-SCREEN trial was designed to investigate whether combined hrHPV testing and cytology can be implemented in the national cervical screening program. Here, we present follow-up results on women with normal cytology at baseline.

Methods: 25,871 women (aged 30-60 years) attending screening in the Netherlands received combined cytological and hrHPV testing. hrHPV-testing was performed using Hybrid Capture 2 (HC2). All women with an hrHPV positive normal smear were invited for retesting after 12 months (instead of 5 years for women with normal cytology in the Netherlands). Each hrHPV positive cytologically normal woman was matched to three randomly chosen hrHPV negative cytologically normal women of the same age who were invited for retesting after 24 months.

The baseline hrHPV test results were blinded for participating women and clinicians. The 3 year follow-up results were obtained from the nation-wide pathology register (PALGA).

Results and conclusion: 1,021 cytologically normal hrHPV+ women were matched to 3,063 hrHPV- women. Followup attendance was similar in hrHPV+ and - women (62% and 60%, resp.). CIN2+ and CIN3+ detection rates were 24 fold (CIN2+; 0.2% vs 5.6%) and 41 fold (CIN3+; 0.1% vs 2.6%) higher in hrHPV+ than HPV- women. Adjusted CIN3+ yield was 10-fold lower in double (HPV and cytology) negative women than in women with normal cytology (0.02% vs 0.19%), indicating that extension of screening interval in case of hrHPV testing seems safe. Moreover, CIN3+ yield was 5-fold lower in HPV- women than those with normal cytology (0.04% vs 0.19%), suggesting that sole HPV testing is an option for primary screening. In conclusion, our data show that HPV testing allows risk stratification of women with normal cytology, permits screening with increased interval length for HPV- women, and is a potentially valuable primary screening tool.

AGE-SPECIFIC PERFORMANCE OF HPV DNA TEST IN PRIMARY CERVICAL SCREENING

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Background: Screening with HPV DNA test has higher sensitivity than cytology. It is uncertain to what extent this represents overdiagnosis, and whether it is dependent on age.

Objectives: To study the age-specific performance of primary HPV DNA test with cytology triage in cervical cancer screening.

Methods: Women aged 25-65 years invited for the routine 5-yearly screening were randomised to primary HPV screening (n=54 207) followed by cytology triage for HPV positives, and to conventional cytology (n=54 218). In HPV arm, an HC 2 assay with the probes for 13 high-risk HPV types was used and samples were classified positive if the rlu ratio was \geq 1.00. In both arms, women with cytology equal to LSIL or worse were referred for colposcopy.

Results: The referral rate for colposcopy was 1.2% overall but compared to the conventional arm, 27% more referrals were made in the HPV arm among women <35 years old. CIN 1 and CIN 2 lesions were detected in excess in the HPV arm but rates of CIN 3+ detected did not differ between arms over ages. Specificity of primary HPV screening with cytology triage was similar to that of cytological screening (99.2% vs. 99.1% for CIN 2+) whereas the specificity of a sole HPV DNA test was clearly inferior. Positive predictive values for primary HPV screening with cytology triage were consistently higher than cytology alone. In both screening arms specificities increased with age whereas highest PPVs were observed among youngest women screened.

Conclusions: Primary HPV screening with cytology triage is more sensitive than conventional cytology. Among older women, it is also more specific and decreases the number of colposcopy referrals and follow-up tests needed. Hence, at least slight increase in the cervical screening programme efficacy can be achieved. However, primary HPV screening of women <35 years is not recommended.

O-20.08

HPV TESTING CAN EXTEND SCREENING INTERVALS; EVIDENCE FROM ARTISTIC TRIAL.

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H Baysson, London School of Hygeine and Tropical Medicine, London, United Kingdom

M Almonte, London School of Hygeine and Tropical Medicine, London, United Kingdom

M Desai, Central Manchester & Manchester Children's Hospitals NHS Trust, Manchester, United Kingdom

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A Turner, Central Manchester & Manchester Children's Hospitals NHS Trust, Manchester, United Kingdom

A Bailey, Central Manchester & Manchester Children's Hospitals NHS Trust, Manchester, United Kingdom

J Peto, London School of Hygeine and Tropical Medicine, London, United Kingdom

Background: Recent data suggests that HPV testing could extend cervical screening intervals to 6 years (Dillner et al, BMJ 2008). Extended follow up of the ARTISTIC study cohort has enabled evaluation of the predictive value of baseline cytology and HPV testing.

Methods: The ARTISTIC study which began in July 01 randomised 24,510 woman aged 20-64, who were screened with liquid based cytology (ThinPrep) and HPV testing (HC2), in a ratio of 3:1 to HPV result revealed (18,386) or concealed (6,124). Women were invited for repeat screening at 3 and 6 years. Management of abnormal cytology was according to national guidelines, but women in the revealed arm with HPV +ve/negative cytology were offered colposcopy if persistent HPV +ve over 12 months. CIN2+ rates are calculated at entry, for the first adequate smear 30-60 months after entry and for the first adequate smear after 60 months.

Results: The cumulative detection rate of CIN 2+ over 60 months+ for women in the revealed arm was 0.4% and 6.2% for cytology -ve/HPV-ve and cytology -ve/HPV +ve women respectively. Amongst all cytology -ve/HPV-ve women in the trial, the cumulative incidence of CIN2+ was 0.39%. In the concealed arm the detection rate of CIN2+ in cytology -ve women (irrespective of HPV status)was 0.58% over 30-60 months of follow-up and reached a cumulative detection rate of 0.99% at 60 months+. For HPV-ve women (irrespective of cytology status) the detection rate was 0.3% at 60 months+.

Conclusion: In this study cohort HPV-ve/cytology-ve women remained at very low risk of developing CIN2+ over 60 months+. HPV -ve women were less likely to develop CIN2+ over a 60 month+ interval than cytology -ve women over a 30-60 months interval. These data suggest that HPV testing could safely extend screening intervals.

SESSION 21

HPV-BASED SCREENING II

PROGRAMME

Oral presentations

THE 25TH INTERNATIONAL PAPILLOMAVIRUS CONFERENCE MAY 8-14 2009, MALMÖ, SWEDEN

TIME	NUMBER	TITLE	ROOM	
2009-05-13				
SESSION 21: HPV-BASED SCREENING II SC				
15.30-15.40	O-21.01	PREVENTION BY OFFERING HRHPV-TESTING ON SELF-SAMPLED CERVICOVAGINAL SPECIMENS TRIAL (PROHTECT) M Gök, DAm Heideman, FJ Van kemenade, L Rozendaal, J Berkhof, PJ Snijders, CJLM Meijer		
15.40-15.50	O-21.02	NATURAL HISTORIES OF HPV DEFINED BY SELF-SAMPLING AB Moscicki, L Widdice, Y Ma, S Farhat, S Miller - Benningfield, J Jonte, J Jay, C Godwin - de Medina, E Hanson, L Clayton, J Simington, S Shiboski		
15.50-16.00	O-21.03	TYPE-SPECIFIC HUMAN PAPILLOMAVIRUS (HPV) STRATIFIED BY ENDOCERVICAL AND VAGINAL SELF-TEST RG Pretorius, Y Qiao, JL Belinson, H Wang, J Smith, J Li, F Taddeo, S Hu, RJ Burchette		
16.00-16.10	O-21.05	TYPING FOR TRIAGING HPV POSITIVE WOMEN AND EXPECTED VACCINATION EFFECT F Carozzi, G Ronco, A Del Mistro, L De Marco, S Girlando, M Zorzi, P Dalla palma, C Naldoni, M Confortini, P Giogi Rossi, J Cuzick, R Rizzolo, N Segnan, A Gillio - Tos		
16.10-16.20	O-21.06	HPV TESTING AND CYTOLOGY IN PRIMARY SCREENING IN RURAL CHINA FH Zhao		
16.20-16.30	O-21.07	TRIAGE OF ASCUS/LSIL: META-ANALYSIS OF HPV TEST POSITIVITY RATE M Arbyn, P Martin - Hirsch, F Buntinx, E Paraskevaidis		

O-21.01

PREVENTION BY OFFERING HRHPV-TESTING ON SELF-SAMPLED CERVICOVAGINAL SPECIMENS TRIAL (PROHTECT)

M Gök, VU University Medical Center, Amsterdam, Netherlands DAm Heideman, VU University Medical Center, Amsterdam, Netherlands FJ Van kemenade, VU University Medical Center, Amsterdam, Netherlands L Rozendaal, VU University Medical Center, Amsterdam, Netherlands J Berkhof, VU University Medical Center, Amsterdam, Netherlands PJ Snijders, VU University Medical Center, Amsterdam, Netherlands CJLM Meijer, VU University Medical Center, Amsterdam, Netherlands

Objective: Women not attending the cervical screening programme are at high risk of cervical cancer. In the PROHTECT trial, we investigate to what extent offering self-sampling of cervico-vaginal specimens for high-risk human papillomavirus (hrHPV) testing to these 'non-responders' increases participation to screening and provides detection of high-grade cervical lesions (CIN2+).

Methods: Women who were invited for the national screening-programme in 2005, but who did not respond, received a device for lavage self-sampling (self-sampling group, n=24,638) or an additional recall for conventional cytology (controlgroup, n=325). The self-sampled specimens were tested for the presence of hrHPV by the digene high-risk HPV hc2 DNA Test(Qiagen). Women with hrHPV-positive self-sample test were advised to visit their general practitioner for conventional cytology and confirmatory hrHPV test. In case of abnormal cytology (>BMD), women were advised to visit the gynaecologist. Women with normal cytology were advised to repeat testing after 1 year.

Results: Participation was higher in the self-sampling group than in the control group (30% vs 6%; p<0.001). Out of 7,384 women in the self-sampling group, 757 women (10%) were hrHPV-positive. So far, 84% participated in the cytological follow-up. 29% of these women had a cytology result of >BMD and were referred for colposcopy-guided biopsy. Histological follow-up data are available for 80%. Five women had carcinoma, 89 CIN2/3, and 52 CIN0/1. On the basis of current findings, the CIN2+ yield among self-sampling responders is 1.3%, which is slightly higher than in screening participants.

Conclusion: Our findings indicate that offering self-sampling of cervico-vaginal specimens for hrHPV testing to nonresponders of the cervical screening programme improves the coverage of the screening programme by almost 10%. Furthermore, our preliminary findings suggest that hrHPV testing on self-sampled cervico-vaginal specimens is a feasible screening tool resulting in a significant yield of high-grade cervical lesions or worse.

O-21.02

NATURAL HISTORIES OF HPV DEFINED BY SELF-SAMPLING

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Objective: The purpose of this study was to determine whether cervical directed clinician collection and self-administered vaginal sampling infer the same natural history of low and high-risk HPV infections.

Methods: 620 women who were participating in the UCSF TEEN HPV study were enrolled in this study. Clinician-collected (CC) cervical and self-collected (SC) vaginal samples were obtained every 4 months at alternate 2-month windows. Data analysis included the nine most common high-risk HPV types (16, 18, 39, 51, 52, 53, 59, 66 and 68) in the cohort and low risk HPV 6. Proportional hazards model and paired Prentice-Wilcoxon test was used to compare curves of time-to-clearance between and within CC and SC samples for each HPV type.

Results: No differences were found for time-to-clearance for any of the 10 types between clinician- and self-collected samples except for HPV 18 (p=0.03) and HPV 66 (p=0.04). Both were found to clear slower by SC means. However, when curves were compared within the same women (both CC and adjacent SC must have been positive for the same type), only HPV 52 was significantly slower to clear by SC. When we examined rate of clearance among the CC samples, the fastest types to clear were HPV 6, 18 and 66. Among the SC samples, HPV 6 and 39 were fastest. CC samples were more often negative when both adjacent self-collected swabs were positive for a specific type than vice-versa.

Conclusions: Our findings suggest that longitudinal studies using SC vaginal swabs would observe similar natural histories of HPV for most types compared to studies using CC specimens. However, the clinical utility of using self-collected means only to detect cervical disease may be limited since it appears that the self-collected samples were more likely to detect HPV types not found in the cervical directed samples.

O-21.03

TYPE-SPECIFIC HUMAN PAPILLOMAVIRUS (HPV) STRATIFIED BY ENDOCERVICAL AND VAGINAL SELF-TEST

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F Taddeo, Merck & Co., Inc., West Point, PA, USA

S Hu, The Cancer Institute/Hospital, Chinese Academy of Medical Sciences, Beijing, China

RJ Burchette, Southern California Permanente Medical Group, Los Robles, CA, USA

Objectives: Determine if decreased sensitivity for \geq CIN 2 of vaginal self-test for HR-HPV by Hybrid Capture 2° (HC-2) is secondary to higher prevalence of HR-HPV or type 16/18 HPV in the endocervix and if decreased specificity of self-test by HC-2 is secondary to higher prevalence of cross-reacting low-risk (LR) HPV in the vagina.

Methods: 2,646 Chinese women age 16-54 years had specimens collected from endocervix and vaginal self-test and cervical cytology. 411 of 420 women with positive endocervical or self-test by HC-2 or cytology of ASC-H or worse had colposcopy and biopsy. Linear Array* tests for specific HPV type were obtained for endocervix and self-test for 410 of 413 women with positive HC-2 in the endocervix or self-test and for a random sample of 75 of 2,233 women with negative HC-2 in endocervix and self-test. HPV prevalences were calculated with and without weighting of random 75 results. Differences in unweighted prevalence were tested with McNemar's. Differences in proportions were tested with Chi-Square.

Results: Unweighted prevalence of endocervical HR-HPV (9.8%) and LR-HPV (2.8%) were lower than in self-test (HR-HPV=10.9%, p=.0002, LR-HPV=5.6%, p<.0001). Prevalence of endocervical LR-HPV without HR-HPV (1.3%) was similar to self-test (1.6%, p=.13). Weighting only accentuated these findings. Proportion of HR-HPV that was HPV 16/18 in endocervix (39.6%) was similar to self-test (37.1%, NS). None of 62 women with HR-HPV in self-test without HR-HPV in endocervix had \geq CIN 2.

Conclusions: Decreased sensitivity for \geq CIN 2 of vaginal self-test by HC-2 is not secondary to higher prevalence of HR-HPV or type 16/18 HPV in endocervix. Decreased specificity for \geq CIN 2 of self-test by HC-2 is likely secondary to HR-HPV present in vagina but not in endocervix and cross-reaction of HC-2 with LR-HPV.

O-21.05

TYPING FOR TRIAGING HPV POSITIVE WOMEN AND EXPECTED VACCINATION EFFECT

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Background. Methods for triaging HPV positive women are warranted. Vaccines for HPV 16 and 18 are available. Objectives. Evaluating HPV genotyping for triaging HPV positive women. Estimating the PPV of cytology and Hybrid Capture2 (HC2) among vaccinated women.

Methods. In the NTCC trial women in the experimental arm were tested by HC2. On stored samples from HC2-positive women we performed PCR by GP5+/GP6+ and typing by reverse line blot hybridisation. We computed sensitivity and specificity for CIN2 or more severe histology among HC2-positive women considering positive only women with infection by some HPV types and estimated the relative sensitivity and referral rate vs. conventional cytology.

Results. We studied 2921 samples. Considering positive only women with infection by HPV 16 or 18(group A) sensitivity was 59.5%(95%CI 52.4-66.4) and specificity 64.9% (63.1-66.7). When also including types 33, 35 and 52 (group B) sensitivity increased to 74.5% (67.9-80.4) and specificity decreased to 58.4% (56.5-60.2). When adding HPV 31 (group C) sensitivity was 85.5% (79.8-90.1) and specificity was 47.9% (46.1-49.9). Positive predictive values(PPV) for groups A, B and C, were 11.1% (9.3-13.1), 11.6% (9.9-13.5) and 10.8% (9.3-12.4) respectively. Among women aged 35-60 relative sensitivity and relative referral rate vs. conventional cytology were 0.96 and 0.83 respectively for group A, 1.18 and 0.99 respectively for group B, 1.33 and 1.21 respectively for group C. By comparison these values were 1.53 and 1.08 respectively with triage by p16INK4A immunostaining. When excluding women with infection from HPV16 or 18, the PPV of HC2 positivity decreased from 6.9% (5.9-7.8) to 4.4% (3.5-5.3) and that of ASCUS+ LBC cytology from 8.8% (7.4-10.1) to 4.9% (3.7-6.0).

Conclusions. Genotyping is less efficient than p16INK4A for triaging HPV positive women. The PPV of both cytology and HPV testing is expected to be strongly reduced among vaccinated women.

O-21.06 HPV TESTING AND CYTOLOGY IN PRIMARY SCREENING IN RURAL CHINA

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Background: The causal relationship between persistent high-risk human papillomavirus (HR-HPV) infection and cervical cancer is widely accepted. HR-HPV DNA testing, alone or in combination with Pap smear testing, may have a role in primary screening. Objectives: To determine the screening performance of Pap and HR-HPV DNA testing for detecting CIN 3 or worse (CIN3+) in an unscreened population of women in rural China. Methods: From 2003 to 2006, 9,057 women were screened by VIA, VILI, Pap, and HR-HPV DNA testing. Colposcopy was done based upon VIA or VILI positivity, Pap result of LSIL or worse (liquid-based cytology), or HR-HPV DNA positivity (digene HPV hc2 DNA Test, Qiagen, Gaithersburg, MD). Results: Co-testing strategies had the overall highest sensitivity for CIN3+ (98.8%, CI: 98.2-100), followed by HR-HPV DNA testing alone (96.3%, CI: 93.6-99.2), Pap alone (80.2%, CI: 74.1-86.2), and reflex testing (75.4%, CI: 68.7-81.9). Reflex testing had the highest specificity (96.7%, CI: 96.5-96.9), followed by Pap alone (93.3%, CI: 93.0–93.6), HR-HPV DNA testing alone (85.5%, CI: 85.0–85.9), and both co-testing strategies (LSIL: 84.8%, CI: 84.3-85.3; HSIL: 84.8%, CI: 84.4-85.3). Of the single-test strategies, HR-HPV DNA testing had a higher sensitivity (96.3% vs. 80.2%) compared to Pap testing. The specificity of the Pap test was higher (93.3% vs. 85.5%) and had a lower percent referred for colposcopy (7.8% vs. 15.8%) than HR-HPV DNA testing. HR-HPV DNA testing with a 10.0 cutoff point (relative light units/cutoff ratio) had a sensitivity estimate comparable to Pap testing. Conclusions: A single-test primary screening strategy with adequate performance would permit less frequent screening and be most appropriate. Of the primary screening strategies investigated in this setting in China, the performance of HR-HPV DNA testing with an increased cutoff-point would best meet these criteria.

O-21.07

TRIAGE OF ASCUS/LSIL: META-ANALYSIS OF HPV TEST POSITIVITY RATE

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Background: Consistent evidence underlines the utility of HPV DNA testing in the management of women with equivocal cervical cytological abnormalities, but not in case of low-grade lesions.

Objectives: We performed a meta-analysis if the Hybrid Capture-II testing is used for these two cytological categories. This test positivity rate reflects the colposcopy referral work load.

Methods: Data were pooled on the HPV test positivity rate in women with atypical squamous cells of undetermined significance (ASCUS/ASC-US) or low grade squamous intraepithelial lesions (LSIL), derived from different cytological classification systems. The meta-analysis was restricted to studies, published between 1991 and 2007, where the high-risk HPV probe of the Hybrid Capture II assay was used. A random-effect model was applied for meta-analytical pooling and the influence of covariates on the HPV positivity rate was analysed by meta-regression. The variation by age was assessed within individual studies since age strata were not defined uniformly.

Results: On average, 43 % (95% CI:40-46%) of women with ASCUS/ASC-US were high-risk HPV positive (range 23-74%). In women with LSIL, the pooled positivity rate was 76% (95% CI:71-81%; range 55%-89%). In spite of considerable interstudy heterogeneity, the difference in HPV positivity between the 2 triage groups was large and highly significant: 32% (95% CI:27-38%). HPV rates dropped tremendously as age and cutoffs of test positivity increased. Other factors (cytological classification system, country, continent, collection method and year of publication) had no statistically significant impact, except in LSIL triage where HPV positivity was significantly lower in European compared to American studies.

Conclusions: Women with LSIL, especially younger women, have high HPV positivity rates suggesting limited utility of reflex HPV triaging these cases.

Research is needed to identify more specific methods to triage women with low-grade squamous cervical lesions.

POSTER ABSTRACTS SESSION 21

POSTER SESSION III TUESDAY 20.00: ODD NUMBERS

POSTER SESSION IV

TUESDAY 21.00: EVEN NUMBERS

P-21.08

REDUCTION IN HPV TESTING UTILITY IN SEQUENTIAL ROUNDS OF SCREENING

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Background: Carcinogenic HPV DNA testing will soon be widely available as a primary screening method to prevent cervical cancer. Like other screening methods, the performance of HPV testing will likely change in sequential rounds of use, which should be anticipated before HPV-based screening is adopted.

Objective: To study the subsequent three-year risk of persistence, CIN2 or CIN3+ associated with carcinogenic HPV infections, comparing those detected prevalently at enrollment versus those newly-detected during active follow-up of the Guanacaste cohort.

Methods: Analysis was done at the infection level; therefore one woman could contribute to one or more infections. The proportion of infections leading within 3 years to persistence, CIN2 or CIN3+ was estimated for infections detected as prevalent (found at enrollment into the cohort) (n=879) or newly-detected (previously negative for that specific HPV type or detected in the first cervical collection among ex-virginal women) (n=592) stratified by four age groups (18-25, 26-33, 34-41 and 42+).

Results: The risks of persistence, CIN2, and CIN3 were 9.1%, 8.8%, and 15.1% among prevalent infections, respectively. The risks were much lower for new infections (6.1%, 1.2%, and 2.4%, respectively). Within prevalent infections the ratio of CIN3+ to CIN2 increased with age group: from 1.1, to 1.3, to 2.5, to 9.1. Persistence of prevalent infections also increased with age. For newly-detected infections, however, the age trend for persistence was weaker, and there was no age trend in the severity of the few cases of CIN3+.

Conclusions: First-time screening of a population using HPV testing detects infections associated with high risk of persistence, CIN2, and CIN3, compared to subsequent screening that detects newly-apparent infections. These new-appearing infections, even at older ages, have a lower positive predictive value, arguing against the use of HPV screening at too-frequent intervals.

P-21.09

ELISA FOR DETECTION OF HIGH-RISK E7 ONCOPROTEIN IN CERVICAL SMEARS

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The main cause for the development of cervical cancer and precancer is a persistent infection by human papillomaviruses (HPVs) of the high-risk group. The high-risk types HPV-16, HPV-18 and HPV-45 together are responsible for ~80% of all cervical carcinomas. The integration of the viral high-risk DNA into the host genome often leads to a dysregulated expression of the viral proteins E6 and E7, which are the major transforming oncoproteins of HPVs. It has been shown by immunohistochemistry that HPV-16, HPV-18 and HPV-45 E7 oncoproteins are expressed continously in biopsies from cervical carcinomas, suggesting that high-risk E7 protein has the potential to be used as a tumor marker for cervical cancer and precancer. Roughly 20-30% of the diagnostic findings using the conventional Papanicolau staining technique (Pap smear), a standard method for cervical cancer screening in clinical diagnosis, are false positive or false negative. To circumvent these problems in detecting abnormal cell types by Pap smear, the aim of this study is to establish a Sandwich-ELISA to detect the E7 oncoproteins of HPV types 16, 18 and 45 in cervical smears and to evaluate its potential as a molecular marker for cervical cancer and precancer. Using our detection system we are able to detect recombinant HPV-16, HPV-18 and HPV-45 E7 protein and also endogenous E7 protein of Caski (HPV-16 positive) and HeLa (HPV-18 positive) cells in the background of HPV-negative cells. We intend to develop this system as a new tool for the detection of cervical cancer and precancer usels.

P-21.10

PROJECTED EFFICACY OF SCREEN AND TREAT PROGRAMS

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Background: In low-resource regions, screen-and-treat programs may use the forthcoming CareHPV test for all women within an optimal age range, with immediate cryotherapy for all HPV+ women. Triage before cryotherapy could identify women not treatable by cryotherapy (i.e., no invasive cancer, large precancers, or polyps or ulcers) and limit unnecessary treatment.

Methods: In a population-based sample of HPV+ Costa Rican women, we considered how a test like CareHPV might perform at enrollment, comparing no triage, visual triage, or viral triage (limiting HPV types to those causing most disease). We categorized as needing treatment those women diagnosed with CIN2+ in 7 years of follow-up or persistent infection throughout. Two lead reviewers determined whether cryotherapy would work by considering all longitudinal clinical information and cervical images. Visual triage performance was estimated from 12 midwives' and 5 gynecologists' independent review of enrollment cervical images.

Results: Among 7132 women with complete follow-up, 314 (4.4%) required treatment (58 with persistent HPV, 256 with CIN2+). Over one-half (n=163) would not have been treated because they were <25 or >45 years (n=206) and/or tested HPV- at enrollment (n=84). Cryotherapy without triage would likely have cured 78 (24.8% of 314) and over-treated 408 (6.0% of 6818) women. A midwife or gynecologist triage would have over-treated fewer women (4.3% and 3.7%) but cured fewer women requiring treatment (19.3% and 14.8%, respectively). Using a viral triage test for 4 types (16, 58, 18, 31) would have cured the same proportion of treatable women as midwife triage (19.7%) while over-treating fewer women (2.5% vs. 4.3%).

Conclusions: A one-time, age-restricted screen-and-treat program targeting persistent infection and CIN2+ using current cryotherapy methods might treat a smaller proportion of the target group than anticipated.

P-21.11

CIN2+ DETECTION: PERFORMANCE OF THE ROCHE PROTOTYPE COBAS®4800 HPV TEST.

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Background. Many studies have demonstrated that high risk (HR) HPV testing is more sensitive than cytology in the detection of CIN2+ lesions. Many methods have been proposed to detect HR HPV in cervical samples, with the Hybrid Capture 2 (HC2) system being the most widely used. The HC2 test provides a negative or positive result, without defining which HRHPV genotypes are present. We have evaluated the prototype, cobas^{*} 4800 HPV test, in terms of sensitivity and specificity for CIN2+ lesions. The prototype cobas^{*} 4800 is a highly automated system that performs sample preparation, real-time HR-HPV amplification and simultaneous detection of 12 HR-HPV genotypes in a single pool, with separate detection of HPV16, HPV18.

Methods. From May till October 2008, aliquots of specimens from 750 consecutive patients for whom a HC2 test was requested were stored for further analysis. HC2 and cobas* 4800 HPV results were available for 584 patients. Cytology and/or histology results were available from all patients. Clinical disease was determined as the highest categorization of either cytology or histology results.

Results. Among the 584 patients, 88 showed a CIN2+ lesion. The sensitivity of HC2 (92%) and the prototype cobas 4800 (88.5%) appeared similar for CIN2+ lesions, 95% CI for difference (-06,0.13). However the specificity of HC2 (70.6%) was different from the prototype cobas 4800 (77.5%) 95% CI for difference (-0.12, -0.01). Calculating the PPV for CIN2+ we found 35.4% and 40.7% for HC2 and cobas[®] 4800 respectively, with identical NPV (98%, 97.5%).

Conclusion. This evaluation of the prototype cobas[®] 4800 HPV test indicates that the system is reliable, easy to use, and shows similar sensitivity, PPV and NPV and slightly better specificity compared to HC2 in the detection of CIN2+ lesions.

P-21.12 FULLY AUTOMATED PERFORMANCE OF A NEXT-GENERATION HYBRID CAPTURE® HPV ASSAY

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A next-generation diagnostic system is under development at QIAGEN. The system includes CE1000, JE2000, and Central Control Unit. JE2000 is a fully automated analytical system for running next generation hybrid capture HPV DNA assay. The JE2000 analytical system analyzes high-risk HPV DNA on extracted DNA from CE1000, or samples directly from proprietary QIAGEN collection medium. JE2000 automates all steps for the next generation Hybrid Capture chemistry. The analytical sensitivity for JE2000 is 1875 copies (95% CI 1615-2290) of HPV 16 plasmid, as compared to 1950 copies (95% CI 1650-2800) of HPV 16 plasmid in the manual assay. Assay specificity was evaluated with 22 HPV LR types in the NextGen[™] assay and compared to the current HC2[®] HPV DNA Test. All 22 HPV LR types were tested at a high concentration of 2.0 ng/mL. Results of the analytical testing show that the number of HPV low risk types that cross-hybridize with the NextGen assay is significantly reduced as compared to HC2 assay. Clinical specimens with 30% prevalence rates were used to evaluate the performance between JE2000 and manual assays. The total, positive and negative agreements were 96% (95%CI 89-98%), 85% (95%CI 64-95%), and 99% (95%CI 74-98%), respectively. The Kappa value is 0.87 for this study. The assay reproducibility on JE2000 was improved over the manual assay using HPV 16 plasmids. The fully automated assay had consistent performance from plate to plate and day to day. No indication of target carryover was found when samples containing up to 10^9 copies/ml of HPV DNA type 16 were processed on JE2000 instruments.

The data presented show that the fully automated next generation Hybrid Capture HPV DNA assay on JE2000 analytical system significantly improves assay specificity and assay reproducibility without compromising sensitivity of detection of HR virus.

P-21.13

NOVEL SOLUTION FOR HPV-TESTING; NEW POSSIBILITIES FOR CERVICAL CANCER SCREENING

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Background HPV testing in cervical-cancer screening has a beneficial effect in patient management and can increase the success rate of population-based screening programs. Cervico-vaginal self-samples have been proven to be as reliable as physician-taken samples. Introduction of cervico-vaginal self-sampling might increase the participation rate and may therefore potentially reduce cancer incidence. Furthermore, it may be used in post-vaccination surveillance.

Objectives This study assesses HPV detection and genotyping in self-sampled genital smears subsequently applied to solid carriers (FTA Elute Card, Whatman) which allow easy storage, transport, and DNA-elution without further purification. Methods The study group consisted of 98 women, divided in two sample sets. All samples were analysed by the HPV-SPF10 LineBlot-25.

Sample set-1 consisted of 48 women attending the gynaecologist, who obtained a self-sampled cervico-vaginal smear, which was applied to an FTA-card. Their HPV results were compared to a cervical smear (liquid-based) taken by a trained physician.

Sample set-2 consisted of 50 women who obtained a self-sampled cervico-vaginal smear at home, which was applied to an FTA-card and to a liquid-based medium. DNA was obtained from FTA-cards by simple elution as well as extraction.

Results Of all self-obtained samples 57% tested positive for HPV. Overall agreement between self- and physician obtained samples was excellent. Furthermore, overall hr-HPV agreement between FTA-card and liquid-based medium for HPV presence was 98%(kappa-value 0.94). Additionally, overall agreement between the elution and extraction method was 100%.

Conclusions This study shows that HPV detection and genotyping in self-obtained genital samples applied to an FTA-card is reliable and shows a high overall agreement with HPV detection and genotyping in physician-obtained cervical smears. Elution of DNA is easy, fast, cheap and reliable. Furthermore, the FTA-card is a convenient medium for collection and safe transport by ambient temperature. Therefore, this method may be a new possibility for cervical cancer screening.

P-21.14 COMPARISON OF THREE HIGH-RISK-HPV TESTS ON ASC SAMPLES WITH FOLLOW-UP.

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Background: HR-HPV testing might help qualifying the ASC-US diagnosis in determining which women should be referred for colposcopy and which could be returned to the screening programme.

Objectives: To evaluate the performance of CINtec[®] p16INK4a Cytology (p16, mtm laboratories), APTIMA HPV (AHPV, Gen-Probe Incorporated) and Linear Array (LA, Roche Molecular Diagnostics) on samples diagnosed ASC-US and ASC-H.

Methods: Residual ThinPrep[®] specimens (Hologic) were collected from consecutive ASC-US and ASC-H samples from the population based screening programme in the Region of Funen, Denmark, and follow-up results were registered. The p16 staining and the LA tests were conducted in our department and the AHPV tests were performed by Gen-Probe (San Diego). The clinical sensitivity and specificity were calculated with histological CIN2+ as endpoint.

Results: A total of 195 ASC samples including 78 ASC-US (40 %), 99 ASC-H (51 %) and 18 ASC-US in two consecutive cytology specimens (9 %) were tested with the three HR-HPV tests. Follow-up results were available for 182 (93 %) of the women. A follow-up result of CIN2+ was found in 10 % of ASC-US, 31 % of ASC-H and 24 % of ASC-US twice. For p16, AHPV and LA the clinical sensitivity was 92.1 %, 95.1 % and 97.6 % with 3, 2 and 1 false negative diagnoses respectively. The clinical specificity for p16, AHPV and LA was 54.5 %, 34.0 % and 26.2 % with 56, 93 and 104 false positive diagnoses respectively.

Conclusions: In our experience the perfect HR-HPV test for ASC triage does not exist yet, but the results are promising. For clinical use the level of the sensitivity and specificity must be as high as possible in order to avoid false diagnoses. The choice of HR-HPV test is highly dependent on the clinical significance, financial possibilities and local laboratory capacity and expertise.

P-21.15

PERFORMANCE OF VIA, HPV AND PAP TESTING IN PERI-URBAN INDIA

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Objectives: A population based study was conducted in peri-urban villages in Andhra Pradesh, India to compare the performance of Pap testing, visual inspection with acetic acid (VIA) and HPV DNA test for the detection of high grade cervical intraepithelial neoplasia (CIN 2/3) and cancer.

Methods: All women living in Medchal Mandal who were age 25 years and older, had an intact uterus, and were not currently pregnant were invited to participate in a cervical cancer screening study, which included a gynecologist administered Pap smear, VIA, and HPV DNA testing at a local hospital. Women positive by one or more screening tests were asked to return for a colposcopic follow-up exam; twenty percent of screen negative women were randomized to a colposcopic examination. All estimates are corrected for verification bias.

Results: Of the 5,724 eligible women systematically recruited from 35 villages, 2,110 (36.9%) women enrolled; an additional 248 women enrolled through passive recruitment strategies. Of 2,358 women enrolled, 2331 (98.9%) women have completed screening test results. A total of 732 (31.4%) women screened positive by one or more screening test (342 (14.7%) Pap+, 297 (12.7%) VIA+, and 240 (10.3%) HPV+); 65.1% of these women returned for a colposcopic follow-up exam with 19 total cases identified (8 CIN2, 7 CIN3, and 4 invasive cancers). HPV sensitivity was 74.1% and 100%, Pap sensitivity was 58.4% and 82.7%, and VIA sensitivity was 22.2% and 34.6% for CIN2+ and CIN3+, respectively. Specificity for CIN2+ was 90.6%, 86.0%, and 87.4% for HPV, Pap, and VIA, respectively; specificities were similar restricting to CIN3+.

Conclusion: HPV testing was relatively more sensitive and specific compared to Pap and VIA. However, compliance with screening, follow-up and treatment remains a significant barrier to community based screening programs.

P-21.16 HPV MRNA DETECTION IN CYTOLOGY SPECIMENS WITH THE APTIMA® ASSAY

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Background: The APTIMA HPV Assay (AHPV, Gen-Probe Incorporated) is a new, qualitative nucleic acid amplification test designed to detect the E6/E7 mRNA of 14 high-risk HPV types in women undergoing cervical cancer screening. Objectives: Evaluate the ability to detect high-risk HPV mRNA and DNA in disease positive (CIN2+) specimens stored for up to 3 years at room temperature.

Methods: 298 clinical specimens were collected from patients with abnormal cytology and at follow-up visits after LEEP conization or laser vaporization. Conventional and liquid based cytology (LBC), colposcopy, and histology were performed. Samples were subsequently stored in the original liquid based cytology (LBC) vials at room temperature for up to 3 years. Detection of high risk HPV DNA was determined with the Hybrid Capture 2 HPV DNA Test (HC2, Qiagen Incorporated). AHPV results were compared to HC2, conventional cytology and histology results. 122 of the samples were CIN3 or worse (10 cervical carcinomas) in a punch biopsy or cone tissue, 60 CIN2, 36 CIN1 and 80 disease negative.

Results: Sensitivity/specificity for detection of CIN2+ were: AHPV 90,1%/78,4%, HC2 91,2%/62,9%, conventional cytology (ASCUS) 85,2%/73,3%. Sensitivity/specificity for detection of CIN3+ were: AHPV 97.5%/60,2%, HC2 96,7%/48,3%, conventional cytology (ASCUS) 94.3%/59,7%.

Conclusions: These results indicate that the AHPV Assay is able to detect high-risk HPV mRNA in retrospective LBC specimens stored at room temperature for up to three years with strong correlation to disease. The AHPV assay was as sensitive as the HC2 assay, but clearly more specific. The AHPV was not only more sensitive than conventional cytology, but also more specific.

P-21.17

HPV IN PUERTO RICAN WOMEN: CLINICIAN- VERSUS SELF-COLLECTED ANOGENITAL SPECIMENS

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The prevalence of anogenital HPV infection is unknown in Puerto Rico (PR). We aimed to estimate the prevalence of anal and cervicovaginal HPV infection; to describe distribution of HPV types in these areas; and to determine the agreement between self-collected and physician-collected HPV specimens in a clinic-based sample of women in PR. We recruited 100 women aged 18-34 years attending an OBGYN clinic in PR for routine Pap smear. Clinician-collected and patient self-collected anal and cervicovaginal specimens were obtained for HPV-DNA testing. HPV testing was performed using L1 consensus primer PCR with MY09/MY11 primers; PCR products from positive samples were typed by dotblot hybridization using 38 individual type-specific probes. Frequency distributions were used to describe the HPVtypes detected. Agreement between the results for the two sampling methods was determined with the kappa statistic. In clinician-collected cervicovaginal and anal samples, 51.8% and 53.9% of women were HPV+ with the consensus primers, while 38.4% and 33.7% were positive for at least one of the type-specific probes. There was a good agreement between clinician and the patient collected samples for both cervicovaginal (kappa=0.71) and anal (kappa=0.67) samples. The most common HPV types identified in the cervix were 16 (9.1%), 90 or 106 (6.1%), and 53 (6.1%); the most frequent in the anus were 16 (5.6%), 51 (4.5%) and 56 (4.5%). The clinic-based prevalence of anal and cervicovaginal HPV infection in this study was high. We found a large variety of HPV types not included currently on available HPV vaccines. Our findings highlight the need for further research to better estimate the overall and type-specific prevalence of HPV infection in PR. Further, the good agreement between clinician- and self-collected samples supports the feasibility of utilizing the selfsampling method in future population-based studies of HPV infection in PR.

P-21.18

A SCHOOL BASED HPV PREVALENCE STUDY IN SCOTTISH ADOLESCENTS

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Background: HPV immunisation was introduced in Scotland in 2008. To enable us to monitor the impact of the immunisation programme on HPV infection, we conducted a direct survey of adolescents before vaccination started to determine the baseline prevalence in this population.

Objectives: To perform a cross sectional survey of type specific HPV prevalence in school and college settings.

Methods: Population proportional to size sampling was employed to select representative educational administrative areas, and within these, representative schools and school years. Further convenience sampling was undertaken in further education colleges to reach early school leavers. We invited boys and girls aged 11-18 to provide a urine sample for HPV screening and typing. We collected data on and analysed by age, sex and social deprivation. Sampling was weighted towards older age groups to provide sufficient power to enable further analysis of type specific prevalence Nucleic acid was extracted from washed cellular pellets originating from 5 ml of urine specimen. Type specific HPV detection was using short fragment (SPF10) PCR and the INNO-LiPA HPV Genotyping test.

Results and Conclusions: 58/114 schools, 9/15 colleges and 2575 individuals agreed to participate. 2446 samples were suitable for testing. The median age of respondents was 16 years. 53% were female. Preliminary results indicate that the sample was broadly representative by age, sex and social deprivation. At time of submission of abstract, HPV prevalence was 6%. Type specific information will be presented - in addition to association of HPV positivity with deprivation.

This population based survey in adolescents had the advantage of enabling direct testing of both boys and girls in the target age for immunisation by using non-invasive urine tests. The survey enabled the measurement of baseline type-specific prevalence data against which to monitor the impact of the immunisation programme.

P-21.19

PRIMARY HPV SCREENING IS THE BEST STRATEGY FOR DEVELOPING COUNTRIES

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BACKGROUND: VIA is an effective screening tool for cervical cancer in low resource settings, but its low specificity leads to high referral rates. Adjunctive testing may overcome this drawback. The new rapid, affordable HPV test may be another option in the near future.

OBJECTIVES: To assess test performances of VIA by a health worker, human papillomavirus (HPV) testing by Hybrid Capture 2 (HC2) and conventional Pap smear, individually and in simulated combinations, to determine the probable best screening option.

METHODS: 512 women with persistent vaginal discharge, irregular or post coital bleeding or unhealthy cervix provided self-collected vaginal samples for HC2 (HPV-S); physicians/health workers collected cervical samples for Pap and HC2 (HPV-P), health worker performed VIA and physician performed colposcopy and biopsy, if indicated, in this screening order. Statistical Analysis: Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for diagnosis of biopsy-proven CIN2+ as the reference standard were calculated for each of the tests. Simulated parallel and sequential combinations for VIA/Pap, HPV-P/VIA, HPV-S/VIA, HPV-P/Pap and HPV-S/Pap were calculated and compared with individual test performance.

RESULTS: Prevalence of abnormal Pap smears was 5%, VIA positive 51% and HPV positive 16%. Sensitivity and specificity were as follows: VIA 82.5% and 51.3%; HPV-P 90.0% and 90.2%; HPV-S 80% and 87.6%; Pap > ASCUS 77.5% and 87.3% respectively. The best simulated combination with a balance of sensitivity and specificity was of HPV-P followed by Pap (sensitivity 75%, specificity 96.7%) or VIA (sensitivity 75%, specificity 94.5%). HPV-S compared well, with overall diagnostic accuracy of 93.6%. Parallel testing improved sensitivity of all combinations from 90% (HPV-S/Pap) to 97.5% (VIA/Pap and HPV-P/VIA). HPV-S/VIA had a sensitivity of 95%.

CONCLUSION: Primary HPV screening followed by VIA and directed biopsy is a feasible and effective strategy for developing countries. Self-sampling is a comparable method for inaccessible populations.

P-21.20 HIGH THROUGHPUT HPV SCREENING BY ONE-STEP MULTIPLEX REAL-TIME PCR

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Various high-risk HPV detection systems are currently available, however none of the current systems address the need for an automated, robotic system for high throughput screening.

A molecular beacon based one step multiplex real-time PCR (MB-RT PCR) system has been designed for the high throughput detection of 14 high-risk HPVs (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) on the AB7900HT instrument, in 96 or 384 well plate format. Detection is achieved in 3 channels. Recognizing the demand for the identification of HPV vaccine types, HPV 16 and 18 are detected together in one channel. The other high-risk types are detected in group in a second channel. The artificial internal control added before DNA preparation is detected in a third channel.

The clinical performance of this MB-RT-PCR system has been assessed on 139 liquid based cytology specimens. The MB-RT PCR was compared to Full Spectrum HPV Amplification and Detection System (GenoID, IVD-CE mark) results. The MB-RT PCR system identified correctly high-risk HPV DNA positivity/negativity with a detection rate of 97.12%, according to the above mentioned grouping. The analytical sensitivity was ~100 DNA copies/reaction for each detected type.

A further 250 samples from a colposcopy referral population were examined and correlation with cytology, histology, Amplicor HPV Test (Roche) results and Hybrid Capture II (HCII, Qiagen) was assessed. The MB-RT PCR showed 94% sensitivity for CIN2+ samples (Amplicor 94%, HCII 91%), and its specificity was 61% (Amplicor 56%, HCII 66%).

A second version of the system allows the detection of HPV types 6 and 11 in a fourth detection channels, using a corresponding fourth dye for the specific MBs. The evaluation of this version on a screening population is ongoing. We propose the MB-RT PCR as a high throughput HPV based cervical screening tool.

P-21.22

POPULATION-BASED PREVALENCE AND AGE-SPECIFIC DISTRIBUTION OF HPV IN KOREA

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Introduction: A multi-center prospective randomized controlled trial for developing a new cervical cancer screening and prevention program in Korea (HPV DNA test with cervical cytology; KGOG-1015) is ongoing at 8 centers in Korea throughout the country. Women of age between 30 and 66 were accrued for the national cancer screening program. , They were randomized into two groups: Group I is for only Pap smear test every 2 years and Group II is for both Pap smear & HPV DNA test every 4 years. After 4 years, test accuracy and cost effectiveness will be examined with respect to the diagnosis of CIN II or worse and the total expense for the diagnosis. One of the goals of KGOG-1015 is to establish a nationwide HPV epidemiology database in Korea. As an interim report of KGOG-1015, we summarized the prevalence of HPV and Pap test among 2,680 women accrued so far in this study.

Method: HPV test was performed with Hybrid Capture 2 (HC2; Quiagen) (high-risk and low-risk probes). All 2,680 women took the classical Pap smear from Sep. 2007 through Aug. 2008. Those 1,168 women who were randomized into Group II and took additional HPV DNA test.

Result: We analyzed samples from 2,680 women; 96.96% had normal cytology, 2.09% atypical squamous cell undetermined significance (ASCUS), 0.58% low-grade squamous intraepithelial lesion (LSIL) and 0.37% had high-grade squamous intraepithelial lesion (HSIL). The HPV prevalence was 8.06% with decreasing pattern with age and a second peak in older women over 60. (Table 1)

Conclusion: The prevalence of HPV and Pap test over age 30 on a population level in Korea is consistent with reports from other countries.

P-21.23 DEVELOPMENT OF SINGLE-TUBE SEMINESTED PCR ASSAY FOR DETECTING HPV

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Background: There is a growing appreciation of the potential value for routine screening for the presence of HPV not only for cervical specimens but also from oral cavity.

Objectives: The purpose of this study was to develop and clinically evaluate a single-tube seminested PCR assay for the detection of HPV.

Methods: Primers specific for L1 region of the HPV genome namely MY09, MY11 and GP6+ were employed in this study. Several parameters such as primer annealing temperature, the number of PCR cycles and concentration of PCR components were optimized. The assay was evaluated using HPV inserts of type 6, 11, 16, 18, 31, 33, 38 and 51. Clinical evaluation was performed with cervical scrapings from 30 clinically suspected patients and buccal swabs from 30 of head and neck cancer patients and results were compared with that of two-tube nested PCR.

Results: The results were found to be comparable with a total of 60% (36/60) of samples being positive for HPV using the single-tube assay, while 62% (37/60) positivity was found with two-tube PCR assay.

Conclusion: We succeeded in developing a single-tube seminested PCR method for HPV DNA detection which is easier than the conventional nested PCR and can be used for screening and detection of HPV in oral and cervical regions.

P-21.24 POTENTIAL IMPACT OF SELF-COLLECTION IN A LOW INCOME SETTING

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Background: Self collection for HPV as a method for screening for cervical cancer has yet to be widely applied in low income settings, but may be an approach to improve access to screening in at risk populations.

Objective: We modeled the impact of one-time screening interventions of self-collection for HPV (SCHPV), cliniciancollection for HPV (CCHPV), visual inspection with acetic acid (VIA) and cytology (CYT) on identification of lesions for colposcopic evaluation, biopsy and treatment in a low income setting.

Methods: We developed a high level model of one million women aged 35, using Microsoft Excel[™] and assumed a prevalence of 10% of LSIL/HSIL warranting colposcopic evaluation. In this model, women were offered a once in a lifetime screen of one of the following: SCHPV; CCPHV; VIA; CYT. HPV testing was assumed to be conducted with careHPV. Rates of uptake of the screening method and the diagnostic characteristics of each screening method were applied to the model. Uptake rates of the tests were derived from published literature; SCHPV – 70%; CCHPV – 30%; VIA – 30%; CYT – 30%. The main outcome of the model was the number of women in the population identified for colposcopic evaluation.

Results: In a population of 1,000,000 women, 700,000 received screening with SCHPV, while 300,000 received screening with other screening methods. Of the 100,000 women in the model requiring colposcopic evaluation, overall 51,000 were missed with SCHPV; 74800 with CCHPV; 82750 with CYT; 80200 with VIA. The model was highly sensitive to the diagnostic characteristics of the screening method and the rates of uptake of the initial screening method.

Conclusion: Policy makers should continue to examine the benefits and drawbacks of SCHPV as an additional option for screening for cervical cancer in low income settings.

P-21.25 TAMPON SUITABLE FOR DETECTION OF HPV MRNA FROM CERVICAL CELLS

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Background: Self-collection of samples for human papillomavirus (HPV) testing is a feasible method for women declining to participate in screening programs. Furthermore, screening by cytology is often not implemented in low resource settings. Thus, HPV detection on self-collected specimens not requiring the use of speculums or brushes may be used as a primary screening test in developing countries and may also be a valuable tool for increasing coverage of screening programs.

Objectives: To prove the validity of tampon collection for detecting high-risk (HR) HPV E6/E7 mRNA, using the PreTect HPV-Proofer assay in cervical cancer patients.

Methods: A total of 180 samples from patients in Norway and South Africa have been included; 88 patients from South Africa, with newly diagnosed cervical cancer and 92 patients from Norway. The latter comprise both follow-up patients with a previous cytological diagnosis and routinely examined patients. Samples were collected using tampons inserted for one hour, thereafter with a brush.

Extraction of DNA/RNA was performed using the NucliSENS easyMAG (bioMerieux, France). E6/E7 mRNA detection and direct typing of the five HR HPV types HPV 16, 18, 31, 33 and 45 was performed using the NASBA based PreTect HPV-Proofer assay (NorChip AS, Norway). Detection of E6/E7 mRNA from additional HPV types was also done using the NASBA technique.

Results : 180 sets of samples have been analysed. Of the 159 sets of samples with adequate sample quality 153 had concordant results. In 4 (2,5%) cases the tampon sample was HPV negative while the brush sample was positive and in 2 cases (1,25%) the tampon sample was HPV positive while the brush sample was negative.

Conclusions: The data from these studies indicate that tampon collection of cervical samples provides cell material of equal quality for mRNA testing as material collected with traditional brushes for all stages of neoplasia.

P-21.26

IN ASCUS, HC2 IDENTIFIES 33% MORE CIN2-3 THAN REPEAT CYTOLOGY.

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Background. In Sweden around 7% of cervical cancer cases have a history of an atypical smear, mostly ASCUS, not followed by histological assessment. An HPV test has been suggested as alternative to biopsy in all these cases.

Methods. A study was undertaken in which 197 women, with a mean age of 39 years, an age range of 21 – 60 years, and a diagnosis of ASCUS in the primary screening were selected for re-examination with an additional smear collection, which was evaluated with a cytological examination in combination with a high risk HPV test (hc2). Based on the test results in the secondary screening, the women were grouped in four categories namely; a) Cyt+/HPV+, b) Cyt-/HPV+, c) Cyt+/HPV- and d) Cyt-/HPV-. Women within group a-c were admitted for colposcopy and cervical biopsy, whereas women in group d were considered as of very low risk for tumour development and were not further examined.

Results. The groups a-c consisted of 108 women with a mean age of 38 years. In group a) comprising 58 women the prevalence of CIN2-3 histopathology was 41%, in group b) comprising 41 women it was 20% and in group c) comprising 9 women it was 0%. Among the 89 women of group d) one case of CIN3 was detected in the following screening round.

Conclusions. The study shows that adding a high-risk HPV test in the secondary screening improves the identification of women with CIN2-3 histopathological lesions with 33%. The increase was highly statistically significant, p=0.01 The results also demonstrate that adding high-risk HPV testing in the secondary screening increases the sensitivity, compared to only repeat cytology, considerably all age categories and in women 50 years and older both sensitivity and specificity (83%) of the high-risk HPV test was higher in comparison with repeat cytology.

P-21.27 PERFORMANCE OF GENOTYPING AND MRNA TESTING IN DETECTING CIN3+ LESIONS

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Background. Persistent infection with high-risk (HR) HPV is the major cause of cervical cancer and methods to detect the presence of HPV DNA represent a very useful tool in cervical cancer screening. However, these tests are very sensitive but lack specificity, and additional tests should be investigated in this context. The aim of this study was to compare the performance of HPV genotyping and E6/E7 mRNA testing in detecting CIN3+ lesions in hc2 positive women.

Methods. Cervical samples from 166 patients testing positive with hc2 (Digene) and in whom the histological result was available were included in this study. All the samples were tested with the Pretect HPV Proofer (Norchip), by which the expression of mRNA of HPV 16, 18, 31, 33 and 45 is detected, and the Linear Array HPV genotyping test (Roche).

Results. CIN3+ lesions were found in 77 patients; among the 89 remaining women we found 43 CIN2, 22 CIN1 and 24 negative samples. The prevalence of mRNA was greater in higher grade lesions, ranging from 35% in women with negative histology to 100% in carcinoma patients. The sensitivity of Pretect HPV Proofer for CIN3+ lesions was 87.2%, while Linear Array showed a sensitivity of 93.6% when considering only the 5 genotypes included in the Pretect test (P=0.17 for comparison) and of 100% when considering all the HR genotypes (P<0.005). The specificity of mRNA test (40.9%) was significantly better when compared to Linear Array, both considering the 5 common genotypes (17.2%)(P<0.001) and the 13 HR HPV (5.3%)(P<0.001).

Conclusion. The Linear Array HPV genotyping test showed better sensitivity than the Pretect HPV Proofer when including all high risk genotypes, while the mRNA test resulted in a significant improvement of the specificity (from 17.2% to 40.9%) and could be used for triaging hc2 positive women.

P-21.28

HPV PERSISTENCE IN COLLEGE STUDENTS: THE SC WOMEN'S CARE STUDY

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The Carolina Women's Care Study explores the molecular basis for HPV persistence, to identify biomarkers for the identification of women at the highest risk for cervical cancer development. Study participants, recruited as freshmen at the University of South Carolina, are followed throughout their college studies with biannual Pap smears, cervical mucus collections, and questionnaires on lifestyle factors, stress, smoking, diet and physical activity. We monitor: cytology, HPV infection by specific type, viral load, HPV E7 expression and cellular gene expression profiles in RNA from the Pap smear material, and the profile of sixteen cytokines in cervical mucus. In addition, we are exploring genetic polymorphisms (SNPs) in the promoter region of several cytokine genes in DNA isolated from blood collected their first visit. As of November 14, 2008, 445 participants were enrolled (1622 visits) and 270 participants have completed three or more visits. The racial distribution of participants is: 70% European-American (EA), 24% African-American (AA) 3% Latina/Hispanic, and 3% Asian. We observed a trend for higher rates of HR HPV positivity in AA women (28%) vs. EA (23%) and a significant difference in the rate of SIL (10% vs 5.9%, respectively, with a p<0.004). Persistent HPV infection, defined as 3 consecutive Pap tests positive for the same HPV type, was detected in 16 participants. HPV16 accounted for 69% of the persistent infections while the remaining 31% is made up of participants with persistent HPV31, 52, 53, 59, and 66 infections. Current studies focus on evaluating the outcomes of the persistent infections by viral type, and identifying biomarkers that can distinguish persistent from transient infections. (Supported by grant # 1P20MD001770 from the NCMHD, NIH.)

P-21.29

EVALUATING PRIMARY HPV TESTING IN A CANADIAN ORGANIZED SCREENING PROGRAM

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Background: The British Columbia Cervical Cancer Screening Program processes over 600,000 conventional cytology slides per year. This is the first large randomized trial in a North American organized screening program evaluating primary HPV testing followed by cytology triage of HPV positives.

Objectives: Establish the efficacy of high-risk, HPV (HR-HPV) testing, followed by liquid based cytology (LBC) triage of HPV-positives, for primary cervical cancer screening compared to cytology alone within an organized cervical cancer screening program; establish appropriate screening interval for HPV-negative women.

Method: A randomised controlled trial comparing LBC to HPV testing as primary screening for cervical cancer. 33,000 women aged 25 to 65, from the BC CCSP are randomly assigned to one of three study arms:

Control Arm: LBC testing. Negatives, screened again at 2 and 4 years. Colposcopy referral at ASC-US threshold or HPV-positive.

Safety-Check: HR-HPV testing. Exit screen at 2 years with LBC. HPV-positives undergo reflex cytology testing and managed identical to intervention arm.

Intervention: HR-HPV testing. Negatives, exit screen at 4 years. HPV-positives undergo reflex cytology testing. Exit colposcopy referral at ASC-US threshold or HPV-positive.

Outcome measures: Confirmed ≥CIN3 detected at exit screen in control and intervention arms; confirmed >CIN2 in control arm at 2 years, safety arm at exit; clearance of HPV infection in HPV-positives at recruitment.

Results: As of November 21, 2008, results available for 1671 women. Median age in control (n=558), safety (n=561) and intervention arms (n=552) are respectively 45; 45; and 46. Percentage of women who never smoked is 46.6%; 44.6% and 44.9%. Women who screened positive in each arm are 4.8% (control); 6.8% (safety); 7.2% (intervention).

Conclusions: The trial will demonstrate if HPV testing as primary screening in an organised screening program will enhance cervical cancer precursor detection, allow for extension of the screening interval, and be cost-effective.

P-21.30

RELEVANCE OF HPV PRIMARY SCREENING USING GENOTYPING AND MRNA DETECTION.

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Background. It is now widely established that cervical cancer and its immediate precursor CIN2/3 are caused by persistent infection with high-risk HPV (HR-HPV). Since the recognition of this causal relationship, cervical cancer screening algorithms tended to combine cytology and HR-HPV DNA detection to improve clinical sensitivity and specificity of the screening. There are now increasing arguments to consider HR-HPV DNA detection as the initial sole screening test. In this context, the management of transitory HR-HPV infections will be an important challenge and a valuable triage test for HR-HPV positives will be required.

Objectives. In this study, we are investigating the clinical relevance of HPV genotyping and of E6/E7 mRNA detection for the triage of HR-HPV positive women.

Methods. Among the patients enrolled in the Reims HPV Cohort Study (France), we selected all women with a positive Hybrid Capture 2 test and stored prospectively cervical material since June 2006 for subsequent DNA and RNA isolation. DNA extracts are being analyzed with the Inno-Lipa HPV genotyping kit (Innogenetics). Samples will be classified as HPV16/18 positive or other HR-HPV positive. RNA extracts are being analyzed with the Nuclisens Easy-Q HPV kit (bioMérieux), which allows the detection of E6/E7 mRNA of 5 HR-HPV types (HPV16, 18, 31, 33, 45). Samples will be classified as E6/E7 mRNA positive or negative. Follow-up data will be used to establish whether the HR-HPV infection will clear or persist. Available histological data are also being collected and will assess the effective development of CIN2+ lesions. Complete analysis of the data will be provided.

Conclusions. Detection of HR-HPV genotypes and/or E6/E7 mRNA could be relevant biomarkers to improve the specificity of a HR-HPV positive screening test. This would improve the selection of women at risk before cytological diagnosis and colposcopy referral.

P-21.31 FEASIBILITY OF RECRUITING WOMEN TO HPV SELF-SAMPLE IN CHICAGO, ILLINOIS

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Background: Infection with high-risk Human Papillomavirus (HPV) has been linked to nearly all invasive cervical cancer (ICC). Although ICC is preventable with regular screening, many women, especially in rural and low-resource areas, do not receive screening. In Cook County, which includes the city of Chicago, more than 10% of women report never having a Pap smear in their lifetime, which is notably higher than the 6.6% statewide. For women disenfranchised from the medical community and who refuse traditional Pap smear screening, a self-administered cervico-vaginal sample (self-sampling) for HPV may aid in the identification of women at high-risk for ICC. Data on effective recruitment methods for these women are sparse.

Objective: Determine the feasibility of recruiting African American women in Chicago, Illinois, who haven't had a Pap smear in five years, to self-sample for HPV.

Methods: Beginning November 2008, women who were non-pregnant, ages 30-50 years, residents of Austin community in Chicago, with no previous history of hysterectomy or ICC, and who had not had a Pap in 5 years are being recruited. Recruitment occurs through community lectures, advertisements, community testing parties and outreach. All women fitting the eligibility criteria are offered a test until the target of 200 women is reached.

Results: Six women, mean age of 38 years, have been recruited into the study. Four were recruited directly from a community lecture attended by 10 women for a recruitment rate of 40%. Two women were enrolled through community outreach. Descriptive characteristics of all women enrolled, their method of recruitment and an overall assessment of feasibility will be presented.

Conclusions: Effective recruitment methods are essential to the success of any research. This study will provide data on the feasibility of recruiting hard to reach women through community outreach for a study on HPV self-sampling.

P-21.32 SELF SAMPLE HPV TEST - INCREASING SCREENING COVERAGE IN FINLAND?

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Background: Since the 1990's cervical cancer incidence among women younger than 40 years has been increasing in Finland. Reasons for this could be an increase in smoking, and changes in sexual behaviour that are likely to increase the prevalence of HPV infections. Yet, the most significant reason is their low participation rate in the screening programme. A low attendance rate in a particular municipality correlates with high cancer incidence, indicating that by increasing the coverage the increasing incidence rates could be stopped or even decreased.

Objectives: To study 1) whether the self-sampling method increases screening coverage among non-responders as compared to a reminder letter, 2) how the chosen self-sampling device works and its reception and feasibility among women, 3) the prevalence of HPV-infections and precancerous lesions in self-samplers in comparison to first invitation attendees.

Methods: The study is performed within the organised cervical screening programme in Espoo, Finland. Women who didn't respond to a screening invitation during the year 2008 are randomised to self-sampling and reminder letter arms with a sampling ratio of 1:2.6. Women in the self-sampling arm receive a self-sampling device (Panterhei Screener*). They return the self-taken sample by mail to the screening laboratory, where it is tested for hrHPV-DNA using an HC 2 assay. A sample tested positive for hrHPV-DNA will result in a recall for further examinations (Pap-smear or colposcopy). The control group receives a reminder letter according to current guidelines. Reasons for non-attendance and views on the new test will be clarified with an enclosed questionnaire.

Current status of the study: Mailing of self-sampling devices and reminder letters started in November 2008. Further examinations of the participants will be done in spring 2009 and comparative analysis will begin after this. Analysis of the questionnaire data will begin in spring 2009.

P-21.33 HPV GENOTYPES IN ASCUS AND LSIL PRIOR TO PUBLIC VACCINATION

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Background: High-risk (HR-) HPV infection is necessary for development of cervical intraepithelial lesions (CIN). High prevalence of HR-HPV has discarded HPV "reflex" testing in young women with LSIL. Distribution of HPV genotypes in minor abnormalities in all age-groups is not known in Sweden.

Objectives: To identify HPV genotypes in minor cytological abnormalities, to describe the prevalence of multiple infections, and to define an age limit for HPV "reflex" testing in women with ASCUS or LSIL.

Methods: 343 liquid-based cytological samples with ASCUS or LSIL from primary population-based screening were genotyped using Linear Array HPV Genotyping Test (Roche).

Results: HR-HPV was found in 71% of LSIL compared to 49% of ASCUS cases (p<0.001). HR-HPV prevalence was agegroup dependent in LSIL (p=0.01), with decreasing prevalence until 50 years. Stratified in age-groups, only women 20-24 years differed significantly in HR-HPV prevalence between LSIL (85%) and ASCUS (44%, p<0.001). HPV16 was the most common HR-HPV and found in 23% of HPV-positive women. HPV18 was found in 9.9% as number six (p<0.001). Multiple infections were equally common in women with ASCUS and LSIL (66% of HPV-positive women) and followed a quadratic trend dependent on age (p=0.01). Most common high-risk types to co-infect with HPV16 (clade 9) were HPV39 (28%), 45 (38%) and 59 (46%), which all belong to HPV18 clade 7. The frequency of low-risk versus probable HR+HR genotypes also followed a quadratic trend dependent on age.

Conclusion: After 25 years of age HR-HPV prevalence is similar in LSIL and ASCUS motivating a low age-limit for HPV "reflex" testing. Multiple infections are common and age-dependent as well as a shift in low-risk/high-risk HPV dominance. Genotyping in longitudinal studies is needed to elucidate the importance of multiple infections in cancer progression as well as cross-protectoral effects of vaccination.

P-21.36

A POINT OF CARE HUMAN PAPILLOMAVIRUS DNA TEST

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Background: Human Papillomavirus (HPV) is a known causal agent for cervical cancer. Thus, HPV nucleic acids are a primary target for diagnostic tests. Great Basin Scientific has developed a small card assay that serves as a rapid extraction, amplification and detection platform, combining thermophilic helicase-dependent amplification (tHDA) with a sensitive chip-based visual detection platform. Objectives: Here, we describe a novel on-chip amplification/detection that can be used to detect several high risk genotypes of Human Papillomavirus DNA. The current Great Basin format allows automated sample extraction, tHDA, and detection - all within 60 minutes. The reduction in time to diagnosis may permit testing for high risk HPV in the point of care setting. Methods: The sample is extracted by a simple lysis, followed by isothermal amplification, at 65oC. There are fewer than 12 total steps and 5 minutes active time for manual users. The process requires only a simple heat block, while the detection signal is permanent and visually interpretable by the naked eye or CCD imaging. Results & Conclusions: DNA extracted from cells can be successfully amplified via tHDA for HPV genotypes 16 and 18. HPV plasmids for genotypes 16, 18, 31 and 45 are rapidly amplifiable and can be subsequently detected on a silicon chip, by visual means. The methods demonstrated here will be the basis for a fast manual Human Papillomavirus card assay that can offer inexpensive, reliable results in a point of care environment, in a first or third world setting, without the requirement of highly trained personnel or complex instrumentation.

P-21.37 HC2 FOR ASC-US TRIAGE AND RESCUE OF INADEQUATELY SCREENED WOMEN

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The age-adjusted cervical cancer (CC) incidence in Spain is 7,6/100.000 and screening is opportunistic. Cytology covers between 50-70% of the population in Catalonia and around 2% of the cytology results are abnormal squamous cells of undetermined significance (ASC-US). 76% of CC cases have no prior history of Pap smear. A new screening protocol was implemented in 2006 to increase coverage among non participants in screening programs and increase management efficacy of women with ASC-US. At the time of attending any health service in the public system for any reason, women were asked for their screening history.

HPV DNA testing (HR HC2) was offered as an adjuvant to cytology for women aged \geq 40 with an interval since their last pap of 5 years or more HPV testing was also offered within 3 months following a diagnosis of ASC-US.

Preliminary data are presented for 3 of the 7 health regional counties for the interval January to December 2007. Women entering this protocol were followed-up until November 2008.

1372 women had inadequate screening. 5.8% were positive in the HPV DNA test and 1.8% had abnormal cytology. 93.1% were negative in either test. 9 CIN2+ were found following the diagnostic referral protocol. All of them were HPV positive and 5 had a baseline normal cytology. Of the 394 women with ASC-US diagnosis, 35.8% were HPV positive. All 8 CIN2+ cases diagnosed belonged to this group.

HPV testing combined with cytology in inadequately screened women has doubled the CIN2+ detection rate when compared to cytology alone (6.55/1000 vs. 2.91/1000).

The established long term negative predictive value of HPV testing allows women with negative results (93.1% with inadequate screening history and 64.4% of ASC-US cases) to join the routine protocol with a triennial cytology recommended to the general population.

P-21.38

GENERATING EVIDENCE ON NEW SCREENING STRATEGIES FOR CERVICAL CANCER

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Cervical cancer, a highly preventable neoplasia related to human papillomavirus (HPV), affects almost half a million women each year, causing 270,000 deaths annually. Nearly 85 percent of women dying from cervical cancer reside in developing countries, due mostly to lack of infrastructure and trained health workers. These women account for 90 percent of the years of life lost to cervical cancer.

HPV—a sexually transmitted virus—is the underlying cause of cervical cancer. The World Health Organization recognizes HPV-DNA testing as an acceptable tool for primary cervical cancer screening but also notes that appropriate and affordable molecular screening tests for developing countries are needed. PATH partnered with QIAGEN Inc. (Gaithersburg, MD; formerly Digene Corporation) to develop careHPV[™]—a test that is inexpensive, culturally acceptable, safe, accurate, and reliable. This rapid batch test detects 14 oncogenic HPV types and will be ready for public health introduction in early 2009.

In developing countries, several challenges hinder widespread adoption of screening options like careHPV[™] into national cervical cancer prevention strategies. Policymakers need evidence that the new HPV test is affordable and appropriate for their health-system infrastructure, as well as their geographical, cultural, and economic circumstances.

PATH is conducting a five-year demonstration project (2008–2012) to generate evidence on available screening options (Pap smear, VIA, and careHPV[™]) in countries (India, Uganda, and Nicaragua) on three continents.

The main objectives of the project are to:

1. Provide ministries of health with data comparing various screening approaches— Pap smear, VIA, and careHPV[™]—to allow them to plan for long-term, wide-scale adoption of screening and treatment programs.

2. Provide the manufacturer with information needed to respond to public-sector investment risks, constraints, and opportunities in developing countries.

3. Share tools for progress with neighboring countries to strengthen and expand regional readiness for cervical cancer prevention programs.

P-21.39

INTEGRAL STUDY OF SQUAMOUS INTRAEPITHELIAL LESIONS IN MEXICAN WOMEN

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BACKGROUND. Currently invasive cervical carcinoma is still a public health issue in Mexican women. Infection by high risk human papillomma virus (HR-HPV) is a sexually transmitted disease frequent among these women. Squamous intraepithelial lesions (SIL) that precede invasive cervical carcinoma are associated with HR-HPV and early detection of these lesions allow adequate clinical treatment. OBJECTIVE. Determine the frequency of SIL associated with HR-HPV. METHODS. Two-hundred and sixty women from Chilpancingo, Gro., Mexico that attended the Cytopathology Laboratory at the Universidad Autonoma de Guerrero to have a Papanicolaou test performed were included in the study. The sample was exo-endocervical making sure to take material from the squamo-cilindrical transformation zone with a wooden Ayre spatula and a cytobrush. Liquid cytology was used (LiquiPREPTM) with Papanicolau staining and PCR-RFLP's for the detection and typification of HPV. RESULTS. Of the 260 cytologies performed, 103 (39.6%) were diagnosed as negative for SIL or malignancy, 142 (54.6%) were low grade squamous intraepithelial lesions (LSIL) of which, 137 (52.7%) corresponded to koilocytic changes for HPV infection and 5 (1.9%) presented koilocytes, plus mild displasia, finally 15 (5.9%) were inadequate samples. All the cytologies diagnosed to be LSIL were positive for HPV DNA by PCR. Twenty-four HPV types were found, the most frequent was type 16 (17.7%) and 12 cases of multiple infection (4.6%). CONCLUSIONS. Liquid base cytology and molecular study by PCR-RFLP together, allow the detection of LSIL with infection by HR-HPV.

P-21.40

RANDOMIZED HEALTH-CARE POLICY OF HPV-BASED MANAGEMENT OF LOW-GRADE CYTOLOGICAL ABNORMALITIES

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Background: The use of Human Papillomavirus (HPV)-based management of women with borderline (ASCUS) or mildly abnormal (CINI) cervical cytology has been extensively investigated in research studies, but generalizability to real-life screening policies is uncertain.

Methods: The organized population-based screening program in Stockholm, Sweden randomized the implementation of HPV triaging of ASCUS/CINI. All 15 outpatient clinics involved in the screening program in the region were randomized to either continue with the established policy (colposcopy of all women with ASCUS/CINI) or to implement a policy with HPV triaging and colposcopy only of HPV-positive women. Incidence of CINII+ and health care cost consumption was evaluated using registry linkages.

Findings: The trial enrolled the 3319 women that were diagnosed with ASCUS (n=1335) or CINI (n=1984) in Stockholm during 17th March 2003 to 16th January 2006. 64% of women with ASCUS and 77% of women with CINI were HPV-positive. HPV-positivity was age-dependent, with 81% of women below 35 years of age and 44% of women above 45 years of age testing HPV-positive. Registry linkages found the proportion of histopathology-verified CINII+ to be similar for the 2 policies (395/1752 women (22.5%) had CINII+ diagnosed with HPV triaging policy, 318/1567 women (20.3%) had CINII+ with colposcopy policy).

Interpretation: À real-life randomised health care policy evaluation of HPV triaging of women with ASCUS/CINI demonstrated similar detection of CINII+ as colposcopy of all women.

SESSION 22

ACCEPTABILITY, BEHAVIOURIAL AND PSYCHOLOGICAL ASPECTS OF SCREENING AND VACCINATION

PROGRAMME

THE 25TH INTERNATIONAL PAPILLOMAVIRUS CONFERENCE MAY 8-14 2009, MALMÖ, SWEDEN

TIME NUMBER TITLE

ROOM

2009-05-13

SESSION 22: ACCEPTABILITY, BEHAVIOURIAL AND PSYCHOLOGICAL ASPECTS OF K1-3

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08.54-09.05	O-22.03	ASCCP GUIDELINE ADHERENCE ON MANAGEMENT OF DISCORDANT AND ASCUS/HPV - RESULTS M Saraiya, Z Berkowitz, V Benard, L Wideroff, S Kobrin
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O-22.01 COLPOSCOPY AFTER CERVICAL DYSPLASIA: PSYCHOLOGICAL ASPECTS

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Presentation of a prospective investigation of anxiety and its long-term effects in 100 women in Malmö Sweden, referred for colposcopy after an abnormal cervical smear. The study comprises aspects of anxiety, depression, psychosexuality, and quality of life measured at their first visit, at six months and after two years.

Upon referral for colposcopy, women exhibited high state anxiety levels and 52% feared they had cancer. By the time of the six-month follow-up visit, anxiety levels were comparable to the reference group. Women with high initial depression scores had a nine-fold risk of high state anxiety. At the end of two years, those with the highest initial depression scores still scored high on state anxiety and depression. At their first baseline visit, women scored low on all health-related mental subscales of Quality of Life and after two years the levels were still lower than normative data. The physical subscales, however, were comparable to normative data at all three visits. Two years after referral for colposcopy, women still reported experiencing a negative impact on their sexual functioning, i.e., lower "spontaneous interest" in sex and a decrease in "frequency of intercourse".

In conclusion, the most important risk factor for high state anxiety upon referral for colposcopy was a depressive mood. Treatment with LLETZ had no effect on anxiety, depression, sexual functioning, or quality of life. After two years, women with an initially depressed mood still had state anxiety levels and depression scores significantly higher than normal and low levels on the assessment of their mental quality of life.

O-22.02

U.S. PHYSICIANS' RECOMMENDATIONS ON SCREENING INTERVALS AND HPV DNA TESTING

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OBJECTIVE: To assess primary care physicians' (PCPs) in the United States recommendations for extending screening intervals that incorporate adjunct HPV DNA tests compared to Pap testing alone. METHODS: We surveyed a nationally representative sample of practicing PCPs in 2006-2007 (cooperation rate = 73.4%). We limited our main analysis to 950 Pap test providers familiar with HPV DNA testing. We examined Pap test recommendations using 2 clinical vignettes of a 35-year old female with: 1) no new sexual partners and 3 consecutive negative Pap tests or 2) a negative Pap and negative HPV DNA test. Differences in practices were assessed by physician specialty. RESULTS: Approximately one third of PCPs recommended HPV DNA testing as an adjunct test for cervical cancer screening, with little variation noted by specialty. For a 35-year old with three negative Pap tests, 32.4% would conduct the next Pap test in 3 or more years. For a 35-year old with a negative HPV DNA test and a normal Pap test, 19.6% would conduct the next Pap test in 3 or more years. HPV DNA test recommendations were variable, with the majority of responses split between no HPV testing, HPV testing at the same frequency as the Pap test, or unknown HPV testing.

CONCLUSIONS: The majority of PCPs do not recommend extending the screening interval to 3 years with adjunct HPV DNA testing. Implementation of effective interventions that focus on physician familiarity with extended screening intervals, combined with not reimbursing more frequent screening in women with negative HPV DNA and negative Pap tests, or with several consecutive negative Pap tests, will be important for improving efficient and cost-effective screening practices.

O-22.03

ASCCP GUIDELINE ADHERENCE ON MANAGEMENT OF DISCORDANT AND ASCUS/HPV - RESULTS

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BACKGROUND: The 2006 American Society for Colposcopy and Cervical Pathology (ASCCP) guidelines recommend that women screened with both human papillomavirus (HPV) and Pap tests who have discordant results (Pap normal/ HPV positive) should have both tests in 12 months, a change from 2003 guidelines recommending both tests in 6-12 months. Both guidelines recommend women with mildly abnormal results (atypical squamous cells of undetermined significance ASC-US/ HPV negative) should have their next Pap test (no HPV test) in 12 months.OBJECTIVE: To assess adherence to these management guidelines among U.S. primary care physicians (PCPs)

METHODS AND MATERIALS: We surveyed a nationally representative sample of practicing PCPs in 2006-2007 (cooperation rate = 73.4%). We limited analysis to 950 Pap test providers familiar with HPV testing for screening or management. We examined adherence using 2 clinical vignettes of a 35 year old with the following results: 1) normal Pap/HPV positive, or 2) ASC-US Pap/HPV negative.

RESULTS: Among women with discordant results, 11% of PCPs recommended the next Pap test in <1 year, and 80% in one year. Of the 80%, only 59% recommended an HPV test in one year, while 28% did not recommend an HPV test. For ASC-US/HPV negative results, 25% of PCPs recommended the next Pap test in <1 year and 67% in one year. Of the 67%, 69% would conduct the next HPV test in a year.

CONCLUSIONS: When results were discordant, the majority of PCPs were aware of newer guidelines for the next Pap test but less aware of the timing of the next HPV DNA test. When results showed ASC-US/HPV negative, PCPs were aware of timing of the next Pap but seemed unaware that guidelines do not recommend an HPV test. These results indicate further educational efforts are needed to emphasize the recommended guidelines of the newer HPV DNA test.

O-22.04

ATTITUDES TO HPV VACCINATION AMONG PARENTS TO CHILDREN 12-15 YEARS

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Background: To design successful HPV vaccination programs, understanding predictors of individuals' HPV knowledge and willingness to vaccinate is crucial.

Objectives: A population based survey was undertaken in Sweden to investigate correlates of HPV knowledge and attitudes to HPV vaccination among parents to children aged 12-15 years.

Methods: We invited 16,000 parents to girls and 4,000 parents to boys, randomly selected from the Swedish population. Response rates were 70% and 69%, respectively. Multinomial and binomial logistic regression models were applied to investigate correlates of attitudes to HPV vaccination and HPV knowledge, respectively.

Results: 24 % parents of girls and 21.0 % of parents of boys had heard of HPV before participating in the study. Mothers had significantly higher knowledge than fathers (OR = 2.17, 95% CI: 1.98-2.37). The odds ratio for those with more than high school education was four times that of those with less than high school, while being born outside a Nordic country indicated less knowledge of HPV. The willingness to vaccinate was high among parents (76%), and also the willingness to pay (63%) for the vaccine. A strong correlate of willingness to vaccinate their child was knowledge on HPV (OR = 1.42, 95% CI: 1.21-1.66 for willingness to vaccinate and OR = 1.96, 95% CI: 1.75-2.20 for willingness to pay). Parents born outside Europe were less willing to pay for the vaccine, while parents with higher education were less willing to pay compared to those with lower education (OR = 0.61, 95% CI=0.52-0.71). Beliefs about vaccine safety and efficacy were also strong correlates of willingness to vaccinate.

Conclusions: HPV knowledge was poor, while the willingness to vaccinate was high and cost was not considered a major barrier. Information about vaccine safety and efficacy is important and parents need information about HPV and the HPV vaccination.

O-22.05

SOCIO-CULTURAL ISSUES FOR INTRODUCTION OF HPV VACCINE IN LOW-RESOURCE SETTINGS

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Background: Successful planning for HPV vaccine introduction in developing country settings requires an integrated and comprehensive approach that addresses factors influencing acceptance or willingness to support vaccine delivery at multiple levels.

Objectives: To synthesize sociocultural results from diverse populations related to vaccine decision-making; understanding of cervical cancer and its etiology; experiences with previous vaccinations; human papillomavirus (HPV) vaccine concerns; and information needs to foster acceptance; (2) to contextualize findings in light of recent studies; and (3) to discuss implications for communication strategies to facilitate vaccine acceptance. Methods: We performed a descriptive qualitative synthesis of socio-cultural studies in four countries—India, Peru, Uganda and Vietnam—utilizing iterative, theme-based analyses. We conducted 252 focus group discussions and 470 in-depth interviews with children, parents, teachers/administrators, health workers/managers, and community/religious leaders, who were purposively sampled. A knowledge, attitudes, and practices survey was administered to 879 children and 875 parents in Vietnam.

Results: We found that vaccine decision-making was primarily done by parents, with children having some role in assent. Understanding of cervical cancer and HPV was limited; however, the gravity of cancer and some symptoms of cervical cancer were recognized. Vaccination and government-sponsored immunization programs were generally supported by respondents. Sentiments toward cervical cancer vaccines were positive, but concerns about quality of delivery, safe and side effects and impact on fertility were raised. Communities requested comprehensive sensitization to address these concerns.

Conclusion: Socio-cultural studies help elucidate complexities of new vaccine introduction from the perspective of children, parents, and communities. HPV vaccine introduction strategies should address community concerns through effective communication, appropriate delivery, and targeted advocacy to make the program locally relevant.

O-22.06

EARLY UPTAKE OF HPV VACCINATION IN GERMANY

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Background: HPV vaccination using Gardasil[®] was introduced in Germany in December 2006 and costs were covered by most Health insurances from the beginning. In February 2007 a recommendation was given by the National vaccination committee (STIKO) to vaccinate all girls aged 12 to 17 years.

Objectives: We investigated how many women have already been vaccinated or were willing to get vaccinated in the future. The effect of education and socio-economic status was considered.

Methods: A survey was conducted in 55,000 German households in September and October 2007. A set of three questions for adult women and one question for girls aged 9 to 17 years was prepared on the issue of HPV vaccination within the overall questionnaire. All females were asked if they would get vaccinated themselves. Adult women were additionally asked if they would have their daughters or sons vaccinated.

Results: The overall household participation rate was 51%. Data were available for 21,587 women aged above 18 years and for 1,906 girls aged between 9 and 17 years. Only 0.6% of the women above 18 years of age have already been vaccinated. Many plan to get vaccinated (17.1%) or were considering it (42.6%), while 13.8% were not sure and 18.0% were declining to get vaccinated themselves. Most women are considering having their daughters vaccinated (72.0%), while 10.0% are not sure and 4.5% declined it. Half of the women were considering having their sons vaccinated against HPV, but 13.8% were not sure and 18.9% declined this. Already 17.4% of the participating girls 9 to 17 years of age have been vaccinated. Furthermore 61.5% were considering to get vaccinated, while 16.3% were not sure and 4.8% were declining HPV vaccination.

Conclusions: Early interest in HPV vaccination was high in Germany.

O-22.07

RISK PERCEPTIONS AFTER HPV VACCINATION

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Introduction: Concerns about changes in adolescent risk perceptions and sexual disinhibition after HPV vaccination may impact clinician and parental acceptance of vaccination. The aims of this study were to examine adolescent risk perceptions after HPV vaccination.

Methods: Participants were 13-17 year-old girls receiving their first HPV vaccination in a hospital-based practice, who completed a confidential survey immediately after vaccination. The outcome variables were three items assessing: concern about HPV acquisition after vaccination, concern about acquisition of other sexually transmitted infections (STI) after vaccination, and perceived need for condom use after vaccination. Responses were recorded on a visual analog scale from 0 (strongly disagree) to 10 (strongly agree). Spearman correlation coefficients were used to determine whether demographic factors, knowledge about HPV/HPV vaccines, sexual history, and beliefs about HPV/HPV vaccines were associated with risk perceptions.

Results: Of the 60 participants recruited to date, 54% were African-American. Concern for HPV acquisition was lower (mean score 4.2, SD 3.6) than concern for other STI acquisition (mean 5.5, SD 3.0) and perceived need for condoms (mean 8.2, SD 3.0). Knowledge was poor (mean score 3.8/10). Higher concern for HPV acquisition was associated with perceived risk of cervical cancer (r=.28, p=.03) and perceived severity of HPV (r=.27, p=.04). Higher concern for other STI acquisition was associated with belief HPV vaccine would not protect against cervical cancer (r=-.30, p=.02) and would not protect one's sexual partner from HPV (r=-.39, p=.003). Perceived need for condom use was associated with later age of sexual initiation (r=.42, p=.035) and belief that HPV vaccine would not protect one's partner from HPV (r=-.37, p=.004).

Conclusions: Despite poor understanding of HPV and HPV vaccines, adolescent girls vaccinated against HPV tended to appropriately report less concern about HPV acquisition than other STI acquisition, and believe that safer sexual behaviors were still important after vaccination.

O-22.08

CHALLENGES OF DELIVERING SCHOOL-BASED HPV VACCINATION IN MANCHESTER, UK

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BACKGROUND: Programmatic experience of delivering HPV vaccination is limited. Ahead of the introduction of the UK national HPV vaccination programme, we sought to identify potential challenges to successful delivery of Cervarix to adolescent schoolgirls.

OBJECTIVES: To assess factors affecting vaccine uptake and completion of the three dose schedule.

METHODS: Cervarix was offered (free of charge) to 2822 girls aged 12 to 13 years attending 36 schools in the catchment area of two primary care trusts. Anonymised data from the school child health systems was used to calculate uptake. Postal questionnaires were sent to parents who had agreed to be contacted about their views on HPV vaccination. RESULTS:

Uptake for Dose 1 was 70.8% (1998), 69.9% (1972) for Dose 2 and 69% (1948) for Dose 3. Taking into account the questions asked at parent information evenings before vaccination, as well as 651 questionnaires returned by parents after Dose 1, safety was parent's prime concern. Compared to parents who had consented to vaccination, those who had refused were more dissatisfied (47%) than parents who had consented (90%) with the information they received (p<0.001), which also did not influence their views. After consent for vaccination 97.6% of girls received three doses. To achieve this level of course completion required school nurses to provide individually scheduled vaccinations to late consenters and to girls who missed their designated school appointments (25% of girls at Dose 2). As a result of bringing girls back onto the school timetable for Dose 3, 10% were vaccinated earlier or later than the recommended interval.

CONCLUSIONS: As this was a research study and public awareness was low, uptake should be higher in the national HPV vaccination programme. It is feasible, but requires time and careful follow-up of individual girls, to prevent attrition after the first dose.

POSTER ABSTRACTS SESSION 22

POSTER SESSION III TUESDAY 20.00: ODD NUMBERS

POSTER SESSION IV

TUESDAY 21.00: EVEN NUMBERS

ATTITUDES TO HPV VACCINATION AMONG YOUNG ADULTS IN SWEDEN.

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Background: In order to design successful cervical cancer vaccination programs, understanding predictors of individuals' willingness to vaccinate is crucial. We therefore conducted a cross-sectional study to elucidate these factors.

Objectives: To investigate knowledge and attitudes of young adults in Sweden towards HPV, its related diseases and HPV vaccination.

Methods: We invited 16 000 women and 4 000 men aged 18-30 years, randomly selected from the Swedish population. Correlates of willingness to vaccinate (for free or through payment) was assessed in multinomial logistic regression models.

Results: Response rates were 55% among women and 43% among men. Median age was 24 years and 23 years, respectively. Only 20% of women and 13% of men had heard of HPV. Of women, 7% stated they were unwilling to vaccinate, 18% were unsure, 34% were willing to vaccinate for free and 40% were willing to pay for vaccination. Corresponding figures among men were 11%, 20%, 37% and 31%. Willingness to vaccinate or to pay for vaccination increased with consideration of vaccines as efficient or not (OR=2.69, 95% CI=1.83-3.93, and OR=4.07, 95%CI=2.61-6.33), having heard of HPV (OR=1.32, 95% CI=1.11-1.57, and OR=2.09, 95%CI=1.78-2.45), and considering oneself to be at a high or fairly high risk of a STI (OR=1.69, 95% CI=1.31-2.19, and OR=2.27, 95%CI=1.77-2.91). A higher disposable income increased the willingness to pay for vaccination while both willingness to vaccinate and to pay was lowered with higher education. Younger age at vaginal sex debut, anal sex, and having had two or more sex partners the previous year increased the willingness to vaccinate and to pay for vaccination. None of these factors differed between men and women.

Conclusions: Knowledge of HPV was very low. Willingness to vaccinate was high and increased with general confidence in vaccines and factors related to the risk for HPV infection.

P-22.10 KNOWLEDGE OF RUSSIAN GYNAECOLOGISTS ABOUT HPV AND HPV-VACCINATION

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Background. It is known HPV is capable to cause a cervical cancer. Anti-HPV vaccine is registered now in Russian Federation but knowledge of gynaecologists and general population in this area is unknown.

The purpose. To estimate the knowledge of Russian gynaecologists about the ethiology and opportunities of a cervical cancer prevention.

Methods. Within the limits of 1-st International conference "Cervical cancer prevention: looking into the future", which was held in Moscow in March 2008 the survey of 350 gynaecologists from 16 regions of Russia has been undertaken.

Results. The interpretation of questioning have shown, that 98% surveyed doctors know, that HPV is the reason of cervical cancer, less than 70% know that vaccine against HPV was created and registered in Russia. Among them 88% are ready to vaccinate patients under 18 years. Nevertheless 65,9% doctors have no opportunity to apply a vaccine into the practice because of different reasons (high cost, no vaccine in their city, etc.). 24,9% of gynaecologists suppose that less then 10% of their patients know HPV to be a reason of cervical cancer, 14,9% of surveyed doctors consider that 40-60% of their patients are informed about HPV and only 20% gynaecologists think, that 70-100% of their patients know about HPV. The majority of doctors (78%) consider that less than 10% of patients are aware of a vaccine against the cervical cancer.

Conclusions. Survey has shown a high level of awareness among the doctors about the HPV- infection, the opportunities of prevention of a cervical cancer and has revealed the readiness of doctors to vaccinate the patients. However, in opinion of surveyed, lack of patients are informed properly about HPV and vaccine acceptability and enhancement of educational programs among patients is neded.

AWARENESS OF HUMAN PAPILLOMAVIRUS IN DENMARK

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BACKGROUND: Both in men and women HPV causes great morbidity, such as cervical cancer, penile and anal cancer and genital warts. The awareness of HPV infection and its consequences is essential to successful vaccination programs against HPV.

OBJECTIVES: The aim of the present study was to assess awareness of HPV among women and men in the Danish population. Furthermore, we wanted to identify risk factors associated with awareness of HPV infection.

METHODS: The study was based on data from two cross-sectional surveys conducted in 2004 (female survey) and 2006 (male survey). The study includes a random sample of women and men aged 18 - 45 years from the general Danish population. A total of 28,000 women and 33.000 men were invited to participate and respectively 22,199 women (response-rate 81.4%) and 23,080 men (response-rate 71.0%) were included in the study. The participants filled in a self-administered questionnaire with questions concerning awareness of HPV, lifestyle and sexual habits.

RESULTS: We observed a low awareness of HPV in the Danish population. In all, 25% of the women and 10% of the men reported to have heard of HPV. In the multivariate analyses, higher educational level and a self-reported history of genital warts significantly increased the likelihood of having heard of HPV infection in both women and men.

CONCLUSIONS: We found that awareness of HPV in the Danish population in general was limited. The low level of awareness of HPV can be a barrier in the prevention of HPV related diseases. Education is warranted to increase such awareness to ensure success of HPV vaccination. A full risk factor analysis will be presented.

P-22.12

ADHERENCE WITH THE QUADRIVALENT HPV VACCINE AMONG INSURED US FEMALES.

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Background: The US Centers for Disease Control and Prevention (CDC) recommends HPV vaccination for females ages 11-12 and catch up for 13-26 year-olds not previously vaccinated. In the US, the quadrivalent (type 6,11,16,18) HPV vaccine can be administered to females starting at age 9. The vaccine's efficacy was studied among patients receiving all three doses within 12 months. Adherence issues have been raised for other vaccines. Data are lacking regarding adherence with quadrivalent HPV vaccination.

Objectives: To quantify dosage adherence with quadrivalent HPV vaccination among females age 9-26.

Methods: We used an administrative claims database from a large U.S. managed care health plan to determine completion rates among patients who received their first dose between January 1, 2007 and April 30, 2007. Patients with a claim related to pregnancy or cervical cancer were excluded as were individuals not continuously enrolled in the health plan from June 1, 2006 to April 30, 2008. All eligible patients were followed for 12 months from their first dose.

Results: Among 37,211 female patients ages 9-26 who received their first dose, 29,643 (79.7%) received their second dose and 18,833 (50.6%) received their third dose within 12 months of their first dose. Patients ages 9-18 were more adherent than patients ages 19-26: 80.7% vs. 77.7% for second dose completion and 52.7% vs. 46.7% for third dose completion, respectively (p<0.0001). Median time between first and second doses and between the second and third doses was 2.2 months and 4.2 months respectively, and was similar between age groups.

Conclusions: In this population, about one-half of patients received three doses of the quadrivalent HPV vaccine within a 12 month period. Adherence was somewhat lower among 19-26 year olds. In order to maximize prevention of HPV-related diseases, innovative efforts are needed to improve adherence with HPV vaccination across all ages.

HPV KNOWLEDGE AND VACCINE KNOWLEDGE AND ACCEPTABILITY IN PUERTO RICO

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Background: Knowledge and attitudes about HPV and HPV vaccine, and the acceptability of HPV vaccine among Puerto Ricans is unknown.

Objectives: This study assesses Knowledge about HPV and knowledge and acceptability of HPV vaccine in men and women in Puerto Rico.

Methods: Cross-sectional household survey to assess mental health services and rehabilitation services was conducted in a probability sample of men and women aged 15-74 years in PR (n=3,187). 1,476 subjects responded to an HPV module. Data was collected by face to face computerized interview and self-administered computerized questionnaire. Sociodemographic factors associated with knowledge of HPV and HPV vaccine were assessed by multivariate logistic regression models. Percentage of acceptability of HPV vaccine among women and parents of 9-26 year old females is reported.

Results: 37.2% of participants had ever heard about HPV and 33.4% had heard about HPV vaccine. Women were more likely to have heard of HPV (OR=5.3, 95% CI=4.1-7.0) and HPV vaccine (OR=6.8, 95% CI=5.1-9.2); as were those with more than high school education (OR=2.7,95% CI=1.9-3.8 and OR=3.3, 95% CI=2.2-4.8, respectively); whereas, those with lower income were less likely to have heard of HPV (OR=0.69, 95% CI=0.47-0.99) and HPV vaccine (OR=0.52, 95% CI=0.35-0.76). Eighty percent of women responded they would be vaccinated if their physician would recommend it, and 89% of participants with daughters aged 9-26 years responded that they would vaccinate them if recommended by their physicians.

Conclusions: Knowledge of HPV and the HPV vaccine is poor, particularly in men, younger and older age groups, and those with lower educational and income level. Acceptability of the vaccine was high. These findings identify the need for educational interventions to increase knowledge about HPV and HPV vaccine. Future studies are needed to identify other factors associated with HPV knowledge and acceptability of HPV vaccine.

P-22.14

TRANSLATING FORMATIVE RESEARCH FOR HPV VACCINE DELIVERY IN DEVELOPING COUNTRIES

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Background: Formative research is recognized as a powerful tool for new vaccine introduction, as it provides a mechanism to gather critical information on communities and systems during the planning process. PATH conducted formative research in low-resource countries from 2006-2008 to inform the design of HPV vaccine demonstration projects, currently underway in these countries. Objective: To describe the design and implementation of vaccine delivery, communication and advocacy strategies for HPV vaccine demonstration projects in Peru, Uganda, India and Vietnam.

Methods: Consultative meetings with ministries of health and key stakeholders were held to synthesize formative research results. Outcomes included vaccine delivery, communication and advocacy strategies in each country. Vaccine delivery focused on girls 10-14 years old immunized at schools, health centers, routine outreach settings, or combined with the Child Days Plus program. IEC materials and communication strategies included leaflets, posters, radio spots and information booklets in each country. Advocacy strategies focused on meetings with key stakeholders, media involvement, and local champions.

Results: Three doses of vaccine were successfully delivered in schools in Peru and Uganda and through the Child Days Plus program in Uganda, with preliminary estimates of coverage over 80%. By May 2009, two doses of HPV vaccine will delivered in schools and health centers in Vietnam, and preliminary coverage data will be presented. Radio spots and community sensitization were instrumental in facilitating parental acceptance. Strong support for the HPV vaccine demonstration projects by national and local leaders positively influenced community acceptance.

Conclusion: HPV vaccines can be successfully delivered to girls in low-resource settings. Comprehensive understanding of the socio-cultural environment, health systems capacities, and policy milieu was critical to the design of vaccine delivery, communication and advocacy strategies that worked, illustrating the importance of translating research results in a directed way for maximum benefit.

ATTITUDES TO HPV VACCINATION AMONG PARENTS TO CHILDREN IN SWEDEN

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Objectives: A population based survey was undertaken in Sweden in 2007 to investigate correlates of attitudes to HPV vaccination among parents to children aged 12-15 years.

Methods: We invited 16 000 parents to girls and 4 000 parents to boys, randomly selected from the Swedish population. Response rates were 70% and 69%, respectively. Multinomial logistic regression models were applied to investigate correlates of attitudes to HPV vaccination.

Results: The willingness to vaccinate was high among parents (76%), and also the willingness to pay (63%) for the vaccine. The preferred age of vaccination of children was 15-17 years (50%). A strong correlate of willingness to vaccinate their child was knowledge on HPV (OR= 1.42, 95% CI: 1.21-1.66 for willingness to vaccinate and OR= 1.96, 95% CI: 1.75-2.20 for willingness to pay). Parents born outside Europe were less willing to pay for the vaccine, while parents with higher education were less willing to pay compared to those with lower education (OR= 0.61, 95% CI=0.52-0.71). Beliefs about vaccine safety and efficacy were also strong correlates of willingness to vaccinate.

Conclusions: The willingness to vaccinate was high and cost was not considered a major barrier. Information about vaccine safety and efficacy is important and parents need information about HPV and the HPV vaccination.

P-22.16

NURSES OPINIONS: TWO YEARS POST-HPV VACCINE APPROVAL IN CANADA

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Background. Little is known about nurses' level of support for HPV vaccination.

Objectives. 1) To assess nurses' opinions regarding HPV vaccines: safety, efficacy, acceptability, usefulness for a immunization program, and willingness to recommend them; 2) To assess priority rating of HPV vaccines.

Methods. This anonymous mail-based survey was conducted in June-July 2008. 500 randomly selected nurses were invited to participate. Descriptive statistics were generated. Multivariate logistic regression models were used to determine variables associated with the intention to recommend the HPV vaccines. The Basic Priority Rating approach was used for vaccines priority assessment.

Results. Participation rate was 60%. 56% of respondents were directly involved in vaccination; 98% agreed that the vaccines recommended by public health authorities are very useful. The HPV vaccines were perceived as useful to implement in a public program by 92% of nurses; 87% and 93% estimated HPV vaccination is well accepted by the public and vaccinators, respectively; 57% agreed that the HPV vaccines are safe and 67% that they are efficacious; 39% and 28% selected the "Do not know" response for vaccine safety and efficacy, respectively; only 37% of nurses self-estimated the information they received on HPV vaccines as sufficient for their needs. No significant difference was observed between responses given by different categories of nurses. Perceived usefulness of HPV vaccination, perceived vaccine safety, support of HPV vaccination by the public and colleagues as well as the positive attitude towards vaccination in general, were associated with the willingness to recommend the HPV vaccines. Consistently, the HPV vaccines had the 6th priority rating out of seven vaccines.

Conclusion. The majority of nurses perceive HPV vaccines as a useful tool in disease prevention and manifested willingness to recommend them. However, around 1/3 of nurses remain unconvinced about HPV vaccines' safety and effectiveness. Additional educational efforts are needed.

INTELLECTUAL PROPERTY CHALLENGES TO REGIONAL MANUFACTURING OF HPV VACCINES

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Background: Widespread access to HPV prophylactic vaccines in low- and middle-income countries (LMCs) will be determined by vaccine cost. Tiered pricing programs by Merck and GSK may make these vaccines affordable to high-income populations in developing economies. GAVI and other international public health agencies may also finance vaccine purchases for distribution at highly discounted prices in eligible countries. However, large scale vaccination will only be cost-effective in low-income countries at prices of ~ \$1 per dose. Hepatitis B vaccines illustrated that the emergence of competitive developing country manufacturers dramatically reduced vaccine prices. In the last decade, Indian manufacturers have developed the technological capacity to manufacture high-quality recombinant vaccines, emerging as leading global suppliers of low-cost vaccines. Consequently, Indian manufacturing of HPV vaccines for domestic and international low-income markets appears viable provided patents do not bar access to necessary technologies.

Objectives/Methods: The HPV vaccine intellectual property (IP) landscape is complex, with several for-profit and non-profit institutions owning patents in the US and other OECD countries. However the status of IP protection for these technologies in developing countries is unknown. To assess whether Indian manufacturers face IP-related barriers, we interviewed researchers and performed patent searches to determine which HPV vaccine technologies are patented in India. Results: Our analysis yields reasons for optimism and concern. University and NIH- generated enabling technologies for L1-based VLPs are not patented in India. However, several pending patent applications from Merck and GSK create uncertainty. Increased IP transparency can facilitate regional manufacturing by allowing interested companies to foresee IP barriers. Indeed, two Indian companies, in collaboration with US and European researchers, are developing second generation vaccines for which they have access to enabling IP. Additional technology transfer partnerships involving exchange of know-how and biological materials are necessary to accelerate low-cost HPV vaccine manufacturing in LMCs.

P-22.19

DETERMINANTS OF ATTENDANCE AT CERVICAL CANCER SCREENING AND HPV VACCINE

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Background: Public screening programmes have brought about considerable decrease of cervical cancer mortality in highly developed countries. In recent years, incidence rates of cervical cancer are persisting at a moderate level, though. Main reason is the neglect of regular screening attendance. With the introduction of the vaccine against carcinogen humane papillomavirus (HPV), a novel preventive measure is now available which might make a difference in lowering the incidence rates further. Yet for developing optimal policy strategies for applying both screening and the vaccine for a more effective prevention of this type of cancer, we need to know more about the motives and determinants of women's attendance at screening and HPV vaccine.

Objectives: This contribution analyses the determinants of cervical screening and HPV vaccine attendance.

Methods: The study is based on recent representative survey data of 760 women aged 14-65 years in the German federal state of Mecklenburg-Western Pomerania. Women were interviewed to their screening and vaccination behaviour, knowledge and attitudes towards screening and HPV vaccine.

Results: Results of multivariate logistic regression analyses revealed that attendance both at screening and at HPV vaccine was best predicted by attitudinal factors. Positive connotations of cancer prevention measures and utility expectations, but also fear and high subjective risk perception were conducive to attendance at screening and HPV vaccine. Women with a lower socioeconomic status attended at screening more irregularly. In contrast, HPV vaccine was more often utilised by young women with lower level of education and lower social class. Knowledge did not impact prevention behaviour significantly.

Conclusions: Utilization of the existing prevention measures can be enhanced by fostering perceptions of utility and positive connotations of attending at screening regularly and getting vaccinated against HPV. Education about the necessity of prevention should reach women across all social classes and ages.

FACTORS ASSOCIATED WITH UPTAKE OF HPV VACCINE IN BRITISH COLUMBIA

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Background: In September 2008, the Canadian province of British Columbia (BC) launched a publicly funded, school based HPV vaccination program for a cohort of over 20,000 girls born in 1997.

Objectives: To determine the parental factors that predicted acceptance of HPV vaccination for school aged daughters in the province of BC

Methods: In December 2008/January 2009, 2000 randomly selected parents of girls eligible for the HPV vaccine in BC were contacted by phone as part of HPV vaccine program evaluation. Parents were asked if their daughter(s) received the first dose of the HPV vaccine and other vaccines offered in school. Demographics of parents and children were assessed. Participant knowledge of cervical cancer, HPV and HPV vaccine, participant attitudes, subjective norms and perceived behavioural control with respect to the HPV vaccine, including their decision to vaccinate pre-teen daughters, attitudes to vaccines, past history of cervical cancer/cervical dysplasia were assessed.

Results: Descriptive analyses of demographic characteristics will be conducted and HPV vaccine uptake rate in the province will be determined, and compared to rates of other school based vaccine programs. Bivariate analyses will be conducted to compare the responses of parents who had their daughters vaccinated against HPV with those of parents who did not for the province overall. For the entire province, variables that achieve p < 0.05 will be included in a multivariable model to achieve a best-fit model. Backward logistic regression will be performed to calculate adjusted odds ratios (AORs) to identify the factors associated with a parents' decision to have their daughters receive the HPV vaccine.

Conclusions: We will present the factors that were predictive of actual uptake of the HPV vaccine in a provincial school based publicly funded program in Canada. Data from this evaluation will inform vaccine program planning.

P-22.21

PHYSICIANS' ATTITUDES TOWARDS HPV VACCINE'S IMPACT ON CERVICAL CANCER SCREENING

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Background: In 2006, the human papillomavirus (HPV) vaccine was recommended for females ages 11 to 26 years in the U.S. Although current cervical cancer screening recommendations have not changed since vaccine introduction, it is anticipated from cost effectiveness and epidemiological studies that the HPV vaccine will affect screening recommendations for fully vaccinated women, such as delayed initiation and lengthened screening interval.

Objectives: To assess current attitudes on HPV vaccine impact on future cervical cancer screening among primary care physicians in the U.S.

Methods: A nationally representative sample of 1,212 primary care physicians was surveyed in 2006-2007 (cooperation rate: 73.4%). Our study included 1,114 physicians providing Pap testing. The survey included questions about physician and practice characteristics, Pap test screening practices and attitudes on HPV vaccine's impact on cervical cancer screening. Differences in attitudes were assessed by physician specialty and other characteristics using chi-square statistics.

Results: Overall, 39% and 42% of physicians do not believe that HPV vaccine will impact cervical cancer screening initiation and frequency, respectively. Approximately 19% of physicians were unsure of the vaccine's impact on initiation and frequency. Internists were more likely to agree that vaccination would impact screening initiation and frequency than family medicine doctors and obstetric/gynecologists (p<0.05 for both initiation and frequency). Significant associations were found with the following covariates: provider age, provider race, percent of uninsured patients, patient ethnicity, physician attitudes towards new screening technologies and adherence to current guidelines on pap screening frequency. Conclusions: Currently, the majority of physicians either do not believe or are unsure that HPV vaccine will lead to changes in cervical cancer screening initiation or frequency. Our findings provide a baseline for directing educational resources if and when these changes occur in order to maximize the vaccine's benefit.

HPV VACCINE UPTAKE AFTER INITIATION OF ELECTRONIC PROVIDER PROMPTING SYSTEM

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Background. In the USA, the quadrivalent HPV vaccine was introduced in June 2006 for females ages 9-26. Objective. To examine HPV vaccine uptake in practices using an electronic medical record (EMR) with provider vaccine prompts at appointments with eligible women.

Methods. Five community-based Family Medicine practices with a common EMR adopted the quadrivalent HPV vaccine. Algorithms for the EMR were developed to prompt providers at all appointments of women eligible to receive HPV vaccine and at appropriate intervals for the subsequent doses. Providers were required to respond to prompts as: done, ordered, patient declined, patient not eligible, discussed, or not addressed. EMRs of women age 9-26 years with appointments in the 18 months since the algorithms were implemented were reviewed to determine whether the vaccine was given, interval between doses, and patient demographic characteristics.

Results. During the 18 months, 4705 women eligible for HPV vaccine were seen. At all encounters, providers received the HPV vaccine prompt. 1670 (36%) received the first HPV vaccine dose, and this was significantly more common (p<0.001) among African Americans (41%) than Asians (25%), Hispanics (30%), and Caucasians (35%). Mean age at first dose was 19.5 years with no significant difference between those getting and not getting the vaccine. Mean interval between the first and second doses was 2.7 months (range: 11 months). Mean interval between the second and third doses was 4.3 months (range: 16 months).

Conclusions. These Family Medicine practices using an EMR requiring provider response to HPV vaccine prompts had high HPV vaccine uptake by eligible patients. Future studies should compare vaccination rates between practices that do and do not have access to this type of vaccine-reminder system to determine if this intervention impacts vaccine utilization among eligible patients.

P-22.23

ASSOCIATION BETWEEN HPV KNOWLEDGE AND HIGH-RISK BEHAVIORS IN PUERTO RICO.

JC Reyes, University of Puerto Rico, San Juan, Puerto Rico

Objectives: Information of HPV knowledge among high-risk populations is limited. This study aimed to determine the association of high-risk behaviors and HPV knowledge in Puerto Rico (PR). Methods: A cross-sectional household survey was conducted in 2008 among a probability sample of persons 15 to 74 years old living in PR (n=3,187). Questions regarding HPV knowledge were administered to a sub-group of study participants (n=1,476). A face to face computerized interview and a self-administered computerized questionnaire assessed information on high-risk behaviors. Logistic regression models were used to assess the age- and sex-adjusted associations of history of STD, health care coverage, drug use (cocaine, heroine, marihuana, PCP, amphetamines) alcohol abuse/dependence (DSM-IV) and current tobacco use with the following two outcomes (1) knowledge of HPV and (2) knowledge of HPV vaccine. Results: Overall, 37.2% of the study sample had ever heard about HPV and 33.4% had heard about the HPV vaccine. Multivariate logistic regression models showed that after adjusting for sex and age, smokers were less likely to know about HPV (OR=0.57, 95% CI=0.45, 0.72). Meanwhile, smokers (OR=0.60, 95% CI=0.47-0.78) and persons with alcohol abuse/dependence (OR=0.39, 95% CI=0.18-0.85) were less likely to have heard about the HPV vaccine. No significant association was observed between drug use, history of STD or health care coverage and the studied outcomes. Conclusions: Knowledge of HPV and the HPV vaccine was poor in this population. In addition, smokers and persons with alcohol abuse/dependence seem to have an even lower knowledge about HPV. This is of special relevance as these populations are potentially at increased risk of HPV infections and HPV related malignancies. Preventive interventions should be developed aimed at integrating HPV screening and education in primary health care settings, particularly in those settings which attend these populations.

HPV IMMUNISATION: WILL IT WIDEN EXISTING CERVICAL CANCER INEQUALITIES?

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Background: Many countries have recently commenced immunisation programmes, routinely vaccinating adolescent girls against cervical cancer, a disease already characterised by pronounced patterns of health inequality. Risk factors for cervical cancer, lower rates of cervical screening and immunisation all have a shared propensity to cluster among socially excluded population subgroups, thus it is important to ensure that the immunisation programme does not widen existing health inequalities.

Methods: A review of incidence, mortality and survival data, and trends in uptake of screening and immunisation by deprivation was undertaken for Scotland. A simple model estimated the impact of differential immunisation uptake on future levels of cancer. Focus groups with providers of services to 'hard to reach' young people were held to identify ways to improve equitable access to cervical cancer prevention.

Results: Screening has reduced the incidence of cervical cancer across the socio-economic spectrum in Scotland. However, the inequality gap remains with those at most socio-economic disadvantage twice as likely to be diagnosed than the most well off. Simple modelling illustrates that this gap could widen further with the introduction of HPV immunisation unless equitable uptake is achieved.

Conclusions: Proactive approaches to ensuring equal access to immunisation will be needed to ensure that those who have most to gain from this intervention have access. These approaches should be informed by the experience of those already providing services to populations poorly served by public health interventions. Careful monitoring of vaccine uptake and the impact of immunisation on disease outcomes should measure progress in addressing inequalities in disease.

P-22.26

ACCEPTABILITY OF HPV VACCINE AMONG PARENTS OF ADOLESCENTS IN COLOMBIA

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Background: The recent licensed vaccine to prevent HPV and cervical cancer offers a new opportunity for cancer control. Given the sexual transmission and the recommended age for vaccination, parental consent is a key aspect in this vaccine. Objectives: We investigated knowledge and acceptability of the vaccine among parents of adolescents in four cultural differentiated regions of Colombia.

Methods: We performed a qualitative study with parents, who were selected from a sample of schools. In each region we carried out 4 focus groups according to gender and type of school (private/official). A brief presentation of general aspects of HPV and vaccine was given during the focus group. All groups were tape-recorded for further transcription and analysis. A content analysis was performed with the following steps: reading, coding, structural analysis and critical appraisal. We developed a qualitative, conceptual and relational matrix, comparing results among regions, type of school and gender.

Results: 185 parents participated in 16 focus groups. Most parents had never heard about HPV. Vaccine acceptability did not show important regional variations being in general good, and higher among parents without knowledge and from lower socioeconomic conditions. Concerns on adverse effects and long term protection were related with lower acceptability. Parents would prefer higher ages for vaccinating their children; in two regions parents expressed that HPV vaccination could foster earlier and non-protected sexual relations; costs were perceived as an important barrier although some would do an effort. The importance of the clear orientation from the government was mentioned in all regions.

Conclusions: HPV knowledge is not related with vaccine acceptability; countries decided to implement HPV vaccination need to develop important communication efforts with parents of adolescents in terms perception of risk, ideal age of vaccination, and aspects not yet established as period of immunity.

SYSTEMATIC REVIEW: CERVICAL CANCER AND SCREENING AWARENESS IN SUB-SAHARAN AFRICA

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Background: Cervical cancer (CC) is the most frequent female cancer in sub-Saharan Africa (SSA). Organized screening programmes are almost non existent and coverage of opportunistic screening is very low. Estimation of current level of awareness may be useful for planning the introduction of prevention strategies in Africa and identifying major barriers. Methods: Literature published between 1998-2008 was retrieved from PubMed. Twenty-six studies from nine countries reporting on the proportion of women with knowledge of CC and screening were included. Additional information on barriers to cytological screening (Pap) and willingness to be screened in the future was extracted. Most surveys were conducted in urban areas, including women attending hospitals, family planning clinics and rarely a random sample of the general population (women). In 8 studies the target population was female health workers (FHWs).

Results: Knowledge of CC, knowledge of Pap and uptake of test ranged from 10-93%, 3-87% and 0.2-60%, respectively. Estimates were slightly better for FHW but with a low Pap usage (6-38%). Pap uptake tends to cluster in urban areas and well-off groups.

Among women, barriers to cytological screening were: lack of test knowledge (46-97%), no medical referral (5-40%), perception of being healthy (3-94%), access to the test (5-85%), costs (2-73%), and fear or embarrassment of the procedure (3-88%). 42-100% reported their willingness to be screened in the future.

Conclusions: Lack of access to health services and lack of knowledge of Pap are the main reported barriers to get screened although large variability between studies was observed. CC preventive strategies need to be implemented in SSA and health education of medical workers and health promotion to the general population are necessary. A Monograph on novel opportunities for CC prevention in Africa will be prepared by the WHO/ICO HPV Information Centre (hpvcentre@ iconcologia.net)

P-22.28

WILL SCHOOL NURSES VACCINATE UNDER-16S AGAINST HPV WITHOUT PARENTAL CONSENT?

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BACKGROUND: Under UK law policies exist to allow children below 16 years to consent to routine immunisation if they fully understand what is involved, although ideally their parents would be consulted. Anecdotal reports suggest that use of "Gillick Competence"- as it is known - varies in practice.

OBJECTIVES: In 2007/08, as part of a study to investigate the feasibility of offering Cervarix[®] to schoolgirls in two Primary Care Trusts in Greater Manchester, we interviewed school nurses who had delivered the vaccine. We sought their views on vaccinating 12-13 year old girls in the forthcoming national HPV programme without parental consent.

METHODS: Semi structured interviews were conducted with 15 school nurses at the end of the study.

RESULTS: School nurses knew how to assess the competency of under-16s but were unwilling to vaccinate 12-13yrs old girls if parents had refused permission. Most thought it unlikely that such a request would arise. Their views were influenced by the young appearance and age of the school year group rather than an individual child's level of maturity. They acknowledged the child's right to vaccination and strongly supported preventive vaccination but still believed in the parent's right to give consent. Most school nurses were themselves parents and shared other parents' concerns about the vaccine's novelty and unknown long-term side effects. They also saw no urgency for individuals to be vaccinated. If parental consent had been withheld and the child requested vaccination, they were willing to negotiate later with parents. They seemed unaware that parental involvement required the child's consent to avoid breaking confidentiality.

CONCLUSIONS: School nurses were not willing to offer HPV vaccination without parental consent to 12-13 year old children. Current approaches for allowing minors to consent to HPV vaccination in a school based vaccine programme have not been adequately addressed.

HPV VACCINE ACCEPTANCE BY ADOLESCENTS AND PARENTS IN SOUTHERN MEXICO

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BACKGROUND. Prophylactic vaccines against HPV are a new method of cervical cancer (CC) prevention, particularly in countries like Mexico in which CC mortality is high. OBJECTIVE. Examine the grade of acceptance of the vaccine in parents of adolescent daughters and adolescent students. METHODS. Four-hundred parents with adolescent daughters and 445 adolescent students of Chilpancigo, Gro. Mexico were interviewed to obtain information about their HPV and CC knowledge as well as their perception of HPV vaccination. The questionnaires included: socio-demographic characteristics, knowledge about HPV and CC, knowledge and attitude towards HPV prevention and vaccination. The adolescents were also asked about their sexual behavior and knowledge of STDs. After the interview, the participants were informed about the etiology of CC. RESULTS. 80.8% of the parents were women and 19.2% were men, only 24% had higher education. 60.8% of the parents and 63.8% of the adolescents knew what HPV is. Only 30.5% of the parents identified viral infection as the cause of CC. 67% of the adolescents did not know how HPV is transmitted and 76.2% how it is prevented. 73.5% had heard about CC. Before the interview, 53.5% of the parents and 33.5% of the adolescents knew of the existence of the HPV vaccine. We observed acceptance of the HPV vaccine by the parents for their daughters (92.8%) and by the adolescents (94.6%). The parents as well as the adolescents showed more acceptance of the vaccine if it were to be provided free or charge (92.3% y 94.6%). CONCLUSIONS. The acceptance grade of the HPV vaccine in the sample studied was high, however, it is necessary to implement a public policy that includes information about HPV, CC etiology, and viral infection prevention and vaccination campaigns to ensure its impact on the population susceptible to infection and in parents of adolescent daughters.

P-22.30

HPV VACCINE INTRODUCTION IN INDIGENOUS COMMUNITIES REQUIRE DIFFERENT APPROACHES

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INTRODUCTION: In Australia HPV vaccine mass immunisation was targeted to females between ages 9 to 26 years. Its introduction was considered highly sensitive in some cultural groups and raised challenging issues for those who administer, and those who are offered the vaccine. The HPV vaccine is particularly pertinent in Australian Aboriginal communities where there is a higher incidence of cervical cancer incidence and deaths than non-Aboriginal communities. This Australian study examined the attitudes of Aboriginal Health Workers (AHWs) toward mass immunisation with the human papillomavirus (HPV) vaccine in one remote and one urban Aboriginal region. The initial uptake of the third dose was low in some Australian regions.

METHODS: Participants for the Aboriginal cultural group's qualitative focus group discussions were purposively selected according to Aboriginal descendency, gender (female only), and AHW status. As one group cannot speak for another the results are different between communities. Recruitment was through Aboriginal health associations and clinics; and the Aboriginal Reference Group that advised the study. AHWs were educated about HPV vaccine prior to the study and disseminated the knowledge to their communities.

RESULTS: The findings from AHWs demonstrated that the national introduction of sensitive vaccines cannot be generalised to Indigenous populations. Whilst there were similarities in attitudes toward support for the vaccine; adolescent behaviours; concerns about vaccine safety and efficacy there were significant differences in how communities should be educated; age of vaccination; stigma; perceived risk of promiscuity and infrastructure needs.

DISCUSSION: By understanding the perspectives and development needs from two Aboriginal communities for culturally appropriate HPV vaccine information, education and resourcing, it enable insights into what is required for optimum and culturally sensitive delivery of the HPV vaccine. Global mass immunisation strategies need to consider specific issues relevant to Indigenous communities for high uptake.

PSYCHOSOCIAL IMPACT OF HPV-RELATED DISEASES: THE PASQUAL STUDY

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Objective: Although data for health-related quality of life of patients with cervical cancer has been well reported, data for other HPV-related diseases are scarce. Our aim is to assess the psychosocial impact of HPV-related diseases other than cervical cancer in the UK, using specific and generic instruments.

Methods & Results: PasQuaL (Papillomavirus ASsociated QUAlity of Life) is a cross-sectional study with a nested subpopulation longitudinal follow-up. From May 2008 to March 2009, a total of 1,250 subjects aged 18-64 years, including women with HPV-related lower genital tract diseases, men with genital warts, and appropriate controls, are recruited from 16 community and hospital healthcare clinics and asked to complete a series of self-administered instruments: CSFQ (Changes in Sexual Functioning Questionnaire); EQ-5D (European Quality of Life Index); CECA (Cuestionario Especifico en Condilomas Acuminados); HIP (HPV Impact Profile). The HIP allows to assess the psychosocial impact of HPV in women. It comprises 29 items grouped into 9 domains: worries and concerns; emotional impact; sexual impact; self image; partner and social relations; interaction with doctors; health perception; cognition and sleep. Socio-demographic and clinical data are also collected. The methodology of this ongoing study will be presented, together with recent data from previous studies using the HIP.

Conclusion: Our results will allow to demonstrate the extent of any psychosocial impact of HPV-related diseases; to estimate the long-term impact of these diseases; and to help improving our knowledge of this aspect of the burden of disease that can be prevented by HPV vaccination.

P-22.32 LESSONS FROM SUCCESS AND FAILURE OF CERVICAL CANCER PREVENTION

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The cervical cancer screening program was introduced in the late 1950s and enacted as a national program in 1982 in Japan. The age-adjusted mortality rate fell from 21.3% (1960) to 5.3% (1993). The cytological screening for the detection of cervical cancer demonstrated a sensitivity of 94.7%, specificity of 98.9% and a false negative rate of 5.3%. Cervical cancer screening program was successful to reduce the incidence and the mortality until 1995 in Japan. In 1997, poorly informed mass media reports claimed that mass screening for cervical cancer might not be effective. Furthermore, the Japanese national government stopped specific funding for cancer screening in 1998 for financial reasons, local governments should provide funding for cancer screening. Recent coverage of cervical cancer screening is lowest among 22 OECD countries (Japan 23.7%). Because there is not enough reproductive health education in schools, adolescent and young women do not have the knowledge about cervical cancer or cervical cancer screening. Coverage between 20-39 aged women is only 7%. Older women have continued participation but remarkably fewer younger women participate in screening programs which may explain the increase in the incidence and mortality rate among young women. Low screening coverage must be due to insufficient knowledge of cervical cancer screening and less financial support by government. There are many problems facing the prevention of cervical cancer in Japan, such as low screening coverage, education and a political strategy for HPV vaccination. In Japan, it is now urgent to educate people, doctors and the government on how to prevent cervical cancer. Public funding for school-based mandatory vaccination and high coverage of cervical cancer screening are most important for cervical cancer prevention. We should learn a lesson from the success and the failure afterwards in cervical cancer screening, and we are straightening the strategy for cervical cancer prevention.

PERCEPTIONS AND ATTITUDES OF LOCAL HEALTH DECISION-MAKERS REGARDING HPV VACCINE

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Background: In most developing countries although HPV vaccines have been licensed, clear national policy recommendations and decisions to introduce them as part of a vaccination program are still lacking. It is necessary to explore perceptions, knowledge and attitudes of local health decision-makers as they might influence national decisions and outcomes.

Objectives: We investigated perceptions, attitudes and knowledge of HPV vaccine and its implementation among local health-decision makers in four regions of Colombia.

Methods: In a qualitative approach, a total of 14 in-depth interviews were done with local health authorities, including the general health secretary and the professionals responsible for public health, immunization and sexual and reproductive health. All interviews were tape-recorded for further transcription and analysis. A content analysis was performed with the following steps: reading, coding, structural analysis and critical appraisal. We developed a qualitative, conceptual and relational framework comparing results among regions.

Results: Most of the actors had scarce knowledge on the HPV vaccine and most had been approached by the manufacturers; two clear opposite attitudes towards HPV immunization were observed: a supportive and favorable one and a clear skeptic one. Neither of these attitudes was related with the level of knowledge regarding technical and operational criteria for implementation, nor with the knowledge on available scientific evidence. Within actors of the same local team there were different attitudes. Although both, equity and need for governmental guidelines were concerns expressed by all actors, some local authorities have invested financial resources on vaccination initiatives that clearly reflect equity problems.

Conclusions: The licensure of HPV vaccines has raised renewed interest for cervical cancer at all levels. Within a country, local decisions and initiatives need to be strengthened technically and supported with national- based decisions, guidelines and follow-up.

P-22.34

ACCEPTABILITY OF HPV CERVICOVAGINAL AND ANAL SELF-SAMPLING IN PUERTO RICO

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Self-sampling techniques have shown to be reliable for determining HPV infection, although this has not been studied in Puerto Rico (PR). We aimed to determine the acceptability of cervicovaginal and anal self-sampling for HPV testing among women in PR. We recruited 100 women aged 18-34 years undergoing routine Pap-smear in an OBGYN clinic. Interviewer administered and ACASI-computer based interviews were used to collect information. Clinician- and selfcollected cervicovaginal and anal samples for HPV-DNA testing were obtained from women. Fisher's exact test was used to assess the association between anogenital sampling acceptability (clinician vs. self-sampling) and relevant covariates. A four-item acceptability scale (range: 4-16) which measured comfort, pain, privacy and embarrassment was used (Dzuba et al., 2002). Differences in the overall acceptability indices between clinician- and self-collected anal and cervical samples were evaluated using the Wilcoxon signed rank sum test. Although acceptability of both sampling methods was high for both the cervix and the anus, there was a higher acceptability of the clinician collection methods for the cervix (mean±SD) (14.1±1.8 vs. 14.8±1.5, p=0.0009) and the anus (13.7±2.3 vs. 14.3±1.9, p=0.0105), as compared to self-collection. Overall, more women preferred the clinician collection of cervical (67%) and anal (61%) samples. Nonetheless, more women who preferred the cervical clinician collection method did so because they felt the sample would be more appropriately taken (85.6%) as compared to women who preferred the self-sampling technique (6.1%). Meanwhile, more women who preferred cervical self-sampling stated they felt more comfortable (72.7% vs. 20.9%) and felt less embarrassment (27.3% vs. 1.5%)(p<0.0001). Similar results were observed for the preference of anal sampling. In conclusion, it is feasible to perform self-sampling techniques in future population-based studies of anogenital HPV infection in PR, as women feel comfortable with these methods.

CRITICAL ISSUES FOR HPV VACCINATION IN MIXED CULTURE COMMUNITIES.

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INTRODUCTION: This study investigated the complexities of HPV vaccination in multicultural Australia.It examined the influence of culture on the attitudes and intentions of parents from three diverse cultural groups in Australia towards HPV vaccination among preadolescent children; General Practitioners [GPs] (family physicians) and Aboriginal Health Workers [AHWs].It showed that cultural difference and geography does matter and how attitudes, social norms and experiences of both consent givers and health providers impact on vaccine uptake, education resources and infrastructure needs.

METHODS: Participants for the qualitative semi-structured interviews were purposively selected according to ethnicity, gender, age and drawn from two specific categories :(i) Australian parents [male and female] of Anglo, Aboriginal and Chinese descendency (ii) practicing GPs; and AHWs. Recruitment was through hospital clinics; AHW networks; related cultural and medical associations.

RESULTS: Challenges facing uptake of HPV vaccine were varied reflecting Australia's cultural and geographic diversity and its influence on (i) HPV vaccine communication and education (ii) informed consent (iii) vaccine uptake. GPs and AHWs demonstrated a positive attitude but conceded it was difficult to explain to some parents why sexually naïve children will benefit from a vaccine for a sexually transmitted virus. Parents displayed diverse social and cultural values, attitudes and information needs toward the vaccine that influenced HPV education and communication interventions; and intentions to consent.

DISCUSSION: There has been a low uptake of the third dose of the quadrivalent HPV vaccine in some regions of Australia. Culturally sensitive messages and planning will play a key role in HPV vaccine education, delivery and uptake. Successful implementation and uptake of the HPV vaccine in multicultural nations is complex and challenging. It requires a coordinated national effort and understanding of the role of culture and diversity as integral to successful HPV vaccine mass immunisation programs.

P-22.38

POTENTIAL BARRIERS TO HPV VACCINE IMPLEMENTATION IN INDIA

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Background: Establishing effective HPV vaccine delivery programs requires understanding the logistics of current vaccine delivery and unique issues associated with HPV vaccine.

Objective: To identify perceived barriers to HPV vaccine introduction through elicitation of information from vaccine delivery personnel.

Methods: In-depth interviews were conducted among vaccine delivery personnel involved with the Universal Immunization Program in Rangareddy district, Andhra Pradesh (AP), India, including nine government multipurpose health workers and their two supervisors. Three health workers and a supervisor from a local hospital were also interviewed.

Results: Supervisors were better educated about vaccine delivery issues. Most participants (11/15) had not heard about HPV infection, though all knew about cervical cancer and associated symptoms. Participants were less knowledgeable about risk factors for cervical cancer, reporting both accurate (multiple sex partners) and inaccurate (improper hygiene during menstruation) information. Problems with cold chain maintenance and storage were not reported, though some expressed concerns with transportation. Nearly two-thirds of the participants believed HPV vaccination of 9-12 year old children was possible; however most (8/11) expressed a preference for vaccination at 16 years or older. Almost all delivery personnel believed that the community would accept girls-only vaccination. Lack of cervical cancer awareness and the need to protect against a disease with a long latency between infection and disease onset were identified as primary barriers to HPV vaccine introduction. Other barriers included concerns about adverse events and cost.

Conclusions: Messages targeting both delivery personnel and community members are needed to increase awareness of HPV and the benefits of early protection from cervical cancer in order to achieve broad acceptance of HPV vaccines. Logistical issues like age at vaccination, perceived adverse events, cost and transportation may interfere with vaccine introduction.

ACCEPTABILITY OF TECHNOLOGY IN PREVENTION OF CERVICAL CANCER

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OBJECTIVE: To determine the acceptability of reflex HPV-DNA testing, HPV vaccines and to assess knowledge about HPV amongst a subset of Ontario women who participated in the Ontario HPV Pilot implementation study.

METHODS: Subjects were recruited from the intervention arm of the Ontario HPV Pilot, conducted by Cancer Care Ontario to assess reflex HPV-DNA testing in women with recent ASC Pap test results.

Telephone-based semi-structured interviews and questionnaires were conducted with consenting subjects (N=51), all of whom were tested for oncogenic HPV types using reflex HPV-DNA testing. Subjects also completed the Impact of Event Scale and Mental Health Inventory and sociodemographic questionnaire items. Qualitative analyses of transcribed verbatim interview content were undertaken focusing on acceptability of HPV-DNA testing, HPV vaccines and knowledge related to HPV and cervical cancer.

RESULTS: Most of the 51 women interviewed were highly accepting of reflex HPV-DNA testing as well as preventive and therapeutic HPV vaccines. Most women were aware of the role of Pap tests but had limited awareness and knowledge about HPV. While women reported feelings of distress, confusion and anxiety, those with the highest levels of anxiety were simultaneously coping with other significant stressors in daily life.

DISCUSSION: Based on interview and questionnaire results, high levels of acceptance for reflex HPV-DNA testing are likely, if this form of testing is introduced on a widespread basis. Whether subjects received positive results (indicating infection with oncogenic HPV types) or negative results (indicating no detectable HPV infection), subjects appeared to be highly accepting of the procedure, particularly because it is non-invasive and painless, apparently definitive, accurate and because results can be obtained simultaneously with a Pap test result. HPV vaccines are also seen as highly acceptable, even when the presumed effectiveness of these vaccines is defined on a hypothetical basis.

P-22.41

EDUCATION IN SCREENING FOR HUMAN PAPILLOMAVIRUS, THE PSYCHO-SOCIAL IMPACT.

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The Human Papillomavirus association with cervical cáncer should be knowledge of the women. Nevertheless the disinformation of that are object even for the professional health create them malformation of ideas of the disease. Therefore several state of health emotional altering the personal, couple, familiar even professional environment.

Objective. To inform correctly the patients, analizing environment psycho-social and diverse state of alteration that can have with the report screening positive.

Method: Every patients go to colposcopy clinic for first time with citologycal and/or hibrid capture-II report positives, should to take part of information and orientation talk. Subsequently are interviews in the moment operture files.

Results. We did observed that patients presented diverses state emotions, from confusion, doubt, anguish and desperation even depresión and mourning stage when diagnosis of cáncer do hers. Present changes in your sexual behaviour, they feeling rejection, culpability, dirty, punishing. They don't know that attitude take with the couple. This situation sometime is flattering for the medical opinión who proyect your personality aspects in the patients and at the same time to have repercussions on the treatments and manegements.

Conclusion. Nowadays exist few experience in the manegement integral of patients from environment multi-disciplinary because of the fact that many factors do not analizing psycho-social aspects in women with HPV infetion, pre-cancer lesions or in situ cáncer, from the perspective of gender in health, in screening preventive for that is recomendable a educative program of information in the materia for general population.

LAUNCH OF A NATIONAL HPV AWARENESS CAMPAIGN IN CANADA

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The Society of Obstetricians and Gynaecologists of Canada (SOGC) has launched a national HPV awareness campaign across Canada.

Given the unfortunate controversy that has developed around HPV vaccination in Canada unbiased, reliable information about HPV is needed.

Parents want to make the right decision about vaccination but are confronted with confusing messages from their school boards, online blogs and in the mainstream media. As a result, parents are not proceeding with the HPV vaccination for their daughters at school and uptake rates have been alarmingly low. In some provinces in Canada it has been reported that fewer than 50% of parents, and in some areas as low as 28% are accepting to vaccinate their girls.

Despite the high prevalence of HPV in Canada, there are substantial knowledge gaps surrounding this virus in the general public and with educators and public health professionals. As a result, the SOGC has engaged national education and public health partners including the Public Health Agency of Canada to identify knowledge and education gaps around HPV. Consultations were held with members of the general public, including mothers, teens, nurses and teachers and the subsequent resources created by the SOGC were focus tested to ensure that they were understood and met the needs of the target populations.

The presentation will discuss the results of the consultations and provide an overview of the SOGC's comprehensive resource materials in a toolkit for educators and public health professionals across Canada. The toolkit has been designed to addresses these gaps in education and knowledge surrounding HPV, its associated diseases and the vaccine. The educational resources that were created include materials for frontline professionals and counselling tools to reach the general public.

SESSION 23

TRANSFORMATION AND CARCINOGENESIS, II

PROGRAMME

TIME NUMBER TITLE

2009-05-13

SESSIC	ON 23: TRA	INSFORMATION AND CARCINOGENESIS II	K1-3
11.00-11.13	O-23.01	CELLULAR ZINC HOMEOSTASIS AS A NATURAL ANTI-HPV BARRIER M Lazarczyk, P Cassonnet, C Pons, Y Jacob, M Favre, M Lazarczyk, m Lazarczyk	
11.13-11.24	O-23.02	NFX1-123 INCREASES HTERT POST-TRANSCRIPTIONALLY IN HPV 16E6- KERATINOCYTES R Katzenellenbogen, P Vliet, D Galloway	
11.24-11.35	O-23.03	PI3K SIGNALLING DURING HPV MEDIATED TRANSFORMATION IN VITRO Fe Henken, Pj Snijders, Cjim Meijer, Rdm Steenbergen	
11.35-11.46	O-23.04	PHOSPHORYLATION OF HSCRIB REGULATES ITS INTERACTION WITH HPV E6 ONCOPROTEINS. K Nagasaka, L Banks	
11.46-11.57	O-23.05	DOWN-MODULATION OF THE P53-NOTCH1 PATHWAY BY HPV-16 E6 T Yugawa, M Narisawa - Saito, M Fujita, T Kiyono	
11.57-12.08	O-23.06	IDENTIFICATION OF THE NUCLEAR TRANSPORT SIGNALS OF HPV16 E7 ONCOPROTEIN J Moroianu, A Knapp, P McManus, K Bockstall, Z Piccioli	
12.08-12.19	O-23.07	CUTANEOUS HPV38E7 INTERACTS WITH HUMAN EEF1A AND MODULATES ITS FUNCTIONS B Sylla, J. Yue, I. Zanella - Cleon, R. Accardi - Gheit, I. Hussain, M. Becchi, E Oswald, A Alonso, M Tommasino	
12.19-12.30	O-23.08	HPV-16 INFECTION ALTERS CELLULAR MICRORNA EXPRESSION IN SCCHN Al Boster, RL Ferris, SA Khan	

ROOM

O-23.01 CELLULAR ZINC HOMEOSTASIS AS A NATURAL ANTI-HPV BARRIER

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The amazing ubiquity of beta-HPV, among which there are also some widespread oncogenic genotypes, remains in a sharp contrast with unapparent course of the infections by these viruses in the general population. On the other hand, their full oncogenic potential emerges only in Epidermodysplasia Verruciformis (EV) suffering patients, where infections by HPV5 or HPV8 leads to skin cancer development. This led us to form a hypothesis that a natural anti-HPV barrier might exist and that just disruption of this enigmatic barrier confers EV.

We have demonstrated that a central role in the function of these HPV-specific protective mechanisms is played by a protein complex involved in zinc transport (EVER1, EVER2, ZnT-1). This complex was shown to maintain cellular zinc homeostasis in keratinocytes and to serve as a negative regulator of some cellular transcription factors needed for viral genome expression. However, EVER-deficiency caused by a homozygous mutation in either of EVER genes imposes zinc imbalance in keratinocytes and confers an extreme susceptibility to HPV infections. We demonstrate that a proper cellular zinc balance might constitute a limiting factor for papillomaviruses and discuss the presumable mechanism of this restriction. More importantly, deregulation of cellular zinc balance emerges as an important step in the life cycle of not only cutaneous but also genital HPV, though the latter viruses have developed an evolutionary conserved mechanism, by which they can break the natural anti-HPV barrier and impose zinc imbalance. Namely, we have demonstrated that E5 protein, which is encoded by alpha- but not beta-HPV, disrupt the function of EVER/ZnT 1 complex, leading to the metabolic changes in keratinocytes strikingly similar as mutation in EVER genes does.

O-23.02

NFX1-123 INCREASES HTERT POST-TRANSCRIPTIONALLY IN HPV 16E6-KERATINOCYTES

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Background: E6 induces telomerase activity through upregulation of hTERT, the catalytic subunit of telomerase. Full activation of hTERT by 16E6 in keratinocytes (HFKs) requires the endogenous protein NFX1-123. NFX1-123 contains a PAM2 motif, to which cytoplasmic poly(A) binding proteins (PABPCs) bind, and an R3H domain, to which single-stranded nucleic acids putatively can bind. The mechanism by which NFX1-123 affects 16E6 activation of hTERT is unknown.

Objective: To determine whether NFX1-123 affects hTERT at the transcriptional or post-transcriptional level and the domains required for this effect. Methods: HFKs expressing tagged (F-) NFX1-123, with or without 16E6, were stained for immunofluorescence. 293T cells transfected with F-NFX1-123 were collected for immunoblot as well as treated with leptomycin B and stained for immunofluorescence. HFKs expressing 16E6 and either wildtype F-NFX1-123 or mutant F-NFX1-123, with the PAM2 motif or R3H domain deleted, were collected for qPCR and telomeric repeat amplification protocol. Luciferase RNA fused with the 5' untranslated region (5'UTR) of hTERT or beta-actin was transfected into HFKs expressing wildtype F-NFX1-123 or mutant F-NFX1-123, with or without 16E6, and protein was collected.

Results: NFX1-123 was cytoplasmic and unlike PABPCs, did not shuttle between the cytoplasm and nucleus. In 16E6 F-NFX1-123 HFKs, the two to three fold increase in hTERT mRNA and telomerase activity required both the PAM2 motif and R3H domain of F-NFX1-123. Three fold more luciferase was produced in 16E6 F-NFX1-123 HFKs when luciferase RNA was fused with the 5'UTR of hTERT. This post-transcriptional increase in expression required the PAM2 motif and R3H domain of F-NFX1-123 and co-expression of 16E6.

Conclusions: NFX1-123 is a cytoplasmic protein that increases the hTERT mRNA and telomerase activity in 16E6 HFKs post-transcriptionally through its PAM2 motif and R3H domain.

O-23.03

PI3K SIGNALLING DURING HPV MEDIATED TRANSFORMATION IN VITRO

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Background: Several lines of evidence indicate involvement of phosphoinositide-3 kinase (PI3K) signalling in hrHPVinduced cervical carcinogenesis. A gain of 3q26, where the active subunit phosphatidylinositol 3-kinase catalytic alpha (PIK3CA) of PI3K is located, is common in cervical carcinomas. PIK3CA copy number aberrations are correlated to increased mRNA and protein levels during progression to cervical cancer. Moreover, the viral oncoprotein E7 can activate protein kinase B (PKB/AKT), a downstream effector of PI3K, in differentiated keratinocytes, the target cells of a productive HPV infection. There is however limited functional evidence supporting an active role of PI3K signalling in a so-called transforming hrHPV infection, associated with malignant transformation.

We previously demonstrated that hrHPV-mediated transformation in vitro, characterized by successive acquisition of an immortal, anchorage independent and tumorigenic phenotype, is associated with differentiation loss and 3q26 gain. Objective: To assess the role of PI3K signalling in HPV-induced transformation in vitro.

Methods: The activity and functional role of PI3K signalling was tested in consecutive passages of HPV16 and HPV18 transfected keratinocyte cell lines, representing immortal and subsequent anchorage independent phenotypes.

Results: Phosphorylation of downstream effector PKB/AKT doubled with progression to anchorage independence in both HPV16 and 18 containing cell lines, which could be reversed by treatment with the PI3K inhibitor LY294002. Moreover, LY294002 treatment reduced proliferation of both cell lines, without inducing apoptosis, excluding any toxic effects of the inhibitor. Vice versa ectopic expression of PIK3CA in early passage cells increased the levels of PKB/AKT phosphorylation and resulted in an increased proliferation. In late passage cells inhibition of PI3K activity resulted in strongly reduced colony formation in soft agarose and less coherent colony morphology. Additionally, LY294002 treatment strongly reduced cellular migration after wound induction.

Conclusions: Present data provide evidence for activation of PI3K being functionally involved in HPV-induced transformation in vitro.

O-23.04 PHOSPHORYLATION OF HSCRIB REGULATES ITS INTERACTION WITH HPV E6 ONCOPROTEINS.

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The cell polarity regulator, hScrib was previously identified as a substrate of high-risk human papillomavirus (HPV) E6 which targets it for ubiquitin-mediated degradation. Recent studies have suggested that hScrib localization in the cell could be regulated following phosphorylation by MAPK after exposure to osmotic stress. However, there is no information on how phosphorylation may affect hScrib function nor its interaction with E6. In this study, we have combined biochemical and proteomic approaches to analyse the role of hScrib phosphorylation on these aspects. We show that osmotic shock-induced phosphorylation of hScrib increases the binding affinity between hScrib and E6. Furthermore, this enhanced affinity was lost after treatment with λ -PPase. Consistent with this increased binding affinity, phosphorylated hScrib was more susceptible to E6-induced degradation. Mass spectrometry analysis has identified the potential phosphorylation site on hScrib following osmotic shock which is located in the C-terminus of hScrib. Interestingly, this site is found to be an ERK-phosphorylation motif and using specific kinase inhibitors, we show that JNK/ERK signaling is in part responsible for the phosphorylation of hScrib following osmotic shock. These studies demonstrate that the hScrib/hDlg cell polarity complex is regulated through MAPK signaling pathways; one of the consequences of which being enhanced targeting by the HPV E6 oncoproteins.

O-23.05 DOWN-MODULATION OF THE P53-NOTCH1 PATHWAY BY HPV-16 E6

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The E6 protein of HPV-16 has the ability to inhibit keratinocyte differentiation. Notch1 is a determinant of keratinocyte differentiation and functions as a tumor suppressor in mammalian epidermis. We have previously identified Notch1 gene as a novel p53 target and shown that E6 down-regulates Notch1 gene expression through p53 degradation in normal human epithelial cells. The p53 family member, p63 plays a pivotal role in epithelial development. One of its isoform, DeltaNp63alpha which lacks the main transactivation domain is predominantly expressed in the basal layer of stratified epithelia and has a functional link to proliferative potential or stemness. Over-expression of DeltaNp63alpha has been found in a number of human squamous cell carcinomas including those of head and neck, cervix, and lung, although its functional relevance in tumorigenesis remains elusive. Here, we show that DeltaNp63alpha represses Notch1 gene expression by counteracting p53 activity. Chromatin immunoprecipitation analysis revealed p63 binding to the p53responsive elements in the Notch1 promoter and its replacement by p53 upon genotoxic stress. Knock-down of p63 expression by short hairpin RNA (shRNA) caused induction of Notch1 and differentiation marker expressions as well as decreased proliferation of normal human keratinocytes, while over-expression of DeltaNp63alpha suppressed Notch1 expression and enhanced clonogenic growth. Expression of E6, p53 shRNA, or Notch1 shRNA significantly rescued the phenotype caused by p63 silencing, indicating that the cytostatic effect induced by p63 loss is at least partially attributable to Notch1 function. Our results therefore suggest that down-modulation of Notch1 by p53 inactivation or DeltaNp63alpha over-expression can lead to maintenance of proliferative potential, which may contribute to tumorigenesis through dysregulation of Notch1-mediated differentiation program.

O-23.06

IDENTIFICATION OF THE NUCLEAR TRANSPORT SIGNALS OF HPV16 E7 ONCOPROTEIN

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Background: The high risk HPV16 E7 has targets and functions in the nucleus and cytoplasm. Our previous studies suggested that HPV16 E7 enters the nucleus via a novel Ran-dependent pathway independent of nuclear import receptors. This is in agreement with E7 not having a classical nuclear localization signal (NLS) or any characterized NLS.

Methods: In order to map the NLS of HPV16 E7 we used 1) transfection assays with enhanced green fluorescent protein (EGFP) and 2xEGFP fusion plasmids containing E7 or E7 domains followed by fluorescence microscopy analysis of the intracellular localization, and 2) in vitro nuclear import assays with GST-fusion proteins containing either E7 or E7 domains.

Results: Analysis of the localization of EGFP and 2xEGFP fusions with E7 and E7 domains in HeLa cells revealed that 16E7 contains a novel NLS in the N-terminal domain (aa 1-37). Interestingly, treatment of transfected HeLa cells with two nuclear export inhibitors, Leptomycin B and ratjadone, changed the localization of 2xEGFP-E7(38-98) from cytoplasmic to mostly nuclear. These data suggest the presence of a leucine-rich nuclear export signal (NES) and a second NLS in the C-terminal domain of E7 (aa 38-98). Mutagenesis of critical amino acids in the putative NES sequence (IRTLEDLLM) changed the localization of 2xEGFP-E7(38-98) from cytoplasmic to mostly nuclear suggesting that this is a functional NES. Nuclear import assays with GST-16E7(44-98) revealed that it can enter the nuclei of digitonin-permeabilized cells in the presence of Ran-GDP or RanG19V-GTP as previously shown for the GST-16E7. In addition, the nuclear localization of 16E7 was found to be independent of its interaction with pRB or of its phosphorylation by CKII.

Conclusion: In this study we found that HPV16 E7 has two NLSs and a leucine-rich NES. This is consistent with E7 having functions in the nucleus and cytoplasm.

O-23.07

CUTANEOUS HPV38E7 INTERACTS WITH HUMAN EEF1A AND MODULATES ITS FUNCTIONS

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The role of the beta cutaneous human papillomavirus (HPV) in the development of non-melanoma skin cancers has not been clearly demonstrated. Our laboratory has previously demonstrated that the cutaneous HPV38 E6E7 can immortalize human keratinocytes in vitro, and that transgenic mice expressing E6E7 of HPV38 exhibit a high susceptibility to chemical-induced skin cancer. Mechanistically, HPV38 E6E7 induces an accumulation of specific forms of p53 that mediate the expression of its own inhibitor delaNp73, in contrast to the mucosal HPV16 E6 which targets p53 for degradation. To further delineate the role of viral oncoproteins in HPV38-mediated carcinogenesis, we performed GST-pull down assay followed by mass spectrometry to identify and characterize cellular proteins that interact with the oncoprotein E7.

We found that HPV38E7 interacts with human eukaryotic elongation factor 1A (eEF1A), both in vitro and in vivo. Similar data were obtained in independent experiments with HPV5E7 as well. HPV38E7 associates with the two isoforms of eEF1A, eEF1A1 and eEF1A2. Immunofluorescence assays indicated that HPV38E7 colocalizes with eEF1A. Remarkably, expression of HPV38E7 or eEF1A induces a robust actin remodeling and redistribution across the cytoplasm, events often associated with tumour initiation and progression. When expressed together, HPV38E7 induces eEF1A aggregation and modulates its effects on actin reorganization. Thus, interaction of HPV38E7 and eEF1A and their role in regulating actin remodeling is biologically relevant, and this could account for HPV38-mediated cellular immortalization and transformation.

O-23.08

HPV-16 INFECTION ALTERS CELLULAR MICRORNA EXPRESSION IN SCCHN

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Background: Most of the 500,000 annual new cases of squamous cell carcinoma of the head and neck (SCCHN) worldwide result from alcohol consumption and/or tobacco use. However, ~30 percent of SCCHN contain HPV DNA, which correlates with a better prognosis and significant decrease in mortality. MicroRNAs (miRNAs) are small, endogenously-encoded ssRNA molecules that function as post-transcriptional negative regulators of gene expression.

Objectives: We investigated the expression profiles of cellular miRNAs using HPV-16-positive and HPV-negative SCCHN cell lines and oropharyngeal tumors, and normal oral mucosa.

Results: MiRNA microarray data showed a global up-regulation of miRNAs in HPV-positive and HPV-negative SCCHN compared to normal oral mucosa. We found 66 up- and 14 down-regulated miRNAs in all SCCHN cell lines compared to normal oral mucosa. Up-regulated miRNAs included members of the miR-17~93 family of clusters, which are over-expressed in many cancers. MiR-145, which is down-regulated in many cancers, was down-regulated in SCCHN cell lines. The HPV-positive cell lines showed 3 up- and 9 down-regulated miRNAs compared to the HPV-negative SCCHN cell lines, indicating a possible role in HPV-associated SCCHN. MiR-363 was up-regulated, while miR-181a was down-regulated in HPV-positive SCCHN cells. MiR-218, which we have shown to be down-regulated in HPV-positive cervical cancer cells, was also down-regulated in HPV-positive SCCHN cells. To elucidate the role of HPV in SCCHN in vivo, oropharyngeal tumors were analyzed. There were 11 up- and 1 down-regulated miRNAs in SCCHN tumors compared to normal oral mucosa. We found 6 down-regulated miRNAs in HPV-positive oropharyngeal tumors compared to HPV-negative tumors.

Conclusions: This data indicates a role for both SCCHN and HPV-16 infection in cellular miRNA dysregulation. MiRNA(s) of interest will be further studied to elucidate potential gene targets. Exploring the possible role of HPV in altering cellular miRNAs in SCCHN may identify mechanisms involved in the development of these cancers.

SESSION 24

VIRAL PROTEINS: STRUCTURE AND FUNCTION

PROGRAMME

Oral presentations

THE 25TH INTERNATIONAL PAPILLOMAVIRUS CONFERENCE MAY 8-14 2009, MALMÖ, SWEDEN

TIME	NUMBER	TITLE	ROOM	
2009-05-13				
SESSION 24: VIRAL PROTEINS: STRUCTURE AND FUNCTION K1-3				
14.00-14.11	O-24.01	HPV18 E2 PROTEIN STABILITY IS CELL-CYCLE DEPENDENT S Bellanger, C L Tan, W L Nei, P He, F Thierry		
14.11-14.22	O-24.02	POST-TRANSLATIONAL MODIFICATION REGULATES HPV16 E1^E4 PROTEIN STRUCTURE AND FUNCTION J Khan, P McIntosh, C Davy, S Hinz, J Doorbar		
14.22-14.33	O-24.03	MODIFICATION OF HPV18 E1^E4 BY PROTEOLYSIS,CDK AND PKA PHOSPHORYLATION. A Pugh, G KNIGHT, I BELL, S ROBERTS		
14.33-14.44	O-24.04	UP-REGULATION OF P18INK4C EXPRESSION BY HPV E6 VIA P53-MIR-34A PATHWAY X Wang, Z-m Zheng		
14.44-14.55	O-24.05	INTERACTION OF THE E8^E2C REPRESSOR-PROTEIN WITH THE CELLULAR CHD6 PROTEIN J Fertey, T Iftner, F Stubenrauch		
14.55-15.06	O-24.06	USE OF A LUCIFERASE FUSION TO STUDY HPV E2 DEGRADATION D Gagnon, S Joubert, H Sénéchal, A Fradet - Turcotte, S Torre, J Archambault		
15.06-15.17	O-24.07	INTERACTION OF BETA PAPILLOMAVIRUS E2 TETHERING PROTEINS WITH MITOTIC CHROMOSOMES A McBride, A Poddar, V Sekhar		

O-24.01 HPV18 E2 PROTEIN STABILITY IS CELL-CYCLE DEPENDENT

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The papillomavirus type 18 E2 protein has previously been shown to be degraded through the ubiquitin-proteasome pathway. However, very little is known about the ubiquitin-ligase(s) responsible for its degradation. We have previously found that, despite very strong interactions with Cdh1 and Cdc20, two co-activators of the Anaphase Promoting Complex/ Cyclosome (APC/C), high-risk HPV E2 proteins are not degraded through the APC/C ubiquitin-ligase.

Here we show that HPV18 E2 stability is cell-cycle dependent, E2 being degraded at the G1/S transition. The Skp1/Cu1/ Skp2 (SCFSkp2) ubiquitin-ligase is known to regulate the stability of several proteins at the G0/G1 and G1/S transitions as well as during S-phase, such as the Cdk inhibitor p27. We show here that knock-down of the Skp2 gene expression by siRNA strongly stabilizes E2 in asynchronous cells and at the G1/S transition. We also show that E2 interacts with both Skp1 and Skp2 in co-immunoprecipitation assays, whereas stable E2 deletants do not bind to these components of the SCF. Altogether these data indicate that the SCFSkp2 ubiquitin-ligase complex plays an important role in E2 degradation, especially when the cells reach the G1/S transition.

O-24.02

POST-TRANSLATIONAL MODIFICATION REGULATES HPV16 E1^E4 PROTEIN STRUCTURE AND FUNCTION

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Post-translational modifications, such as phosphorylation, multimerisation and cleavage are a unique way in which to control the behaviour of a protein. Recently published data by Wang et. al. has pinpointed a number of phosphorylation sites within 16E1^E4. The T57 phosphorylation of 16E1^E4 is of particular interest as it induces a structural change within 16E1^E4 and promotes 16E1^E4 stability and keratin binding. Further data by McIntosh et. al. describe the formation of amyloid-like fibres by 16E1^E4 proteins that contain both deletions within the N-terminus and an intact C-terminus. We show here that 16E1^E4 is N-terminally truncated by calpain, a cysteine protease found in differentiating epithelia. These truncated forms of 16E1^E4 are similar to those found in transient transfections, and in a HPV16 organotypic raft model system that supports the complete life cycle. Further characterisation of the truncated form of 16E1^E4 has been undertaken by N-terminal sequencing and mass spectrometry and indicates a cleavage event after lysine-14 and/or glycine-17. In vitro experiments show calpain cleavage of 16E1^E4 to lead to fibre formation. A single mutation within the leucine cluster can prevent cleavage from occurring and the consequences of this are currently being investigated on the virus life cycle. It appears that there are a number of tightly controlled post-translational modifications of 16E1^E4 that regulate different functions at specific stages of the HPV16 life cycle. We believe that the loss of T57 phosphorylation in the upper epithelial layers leaves the 16E1^E4 protein susceptible to cleavage and fibre formation. Fibre formation of truncated 16E1^E4 is thought to facilitate the cross linking of 16E1^E4 bound-keratin which eventually leads to filament reorganization, an event suggested to be important for virus release. Additional cleavage fragments may have functions separate to fibre formation, which may be essential for the productive phase of the HPV16 life cycle.

O-24.03 MODIFICATION OF HPV18 E1^E4 BY PROTEOLYSIS,CDK AND PKA PHOSPHORYLATION.

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Posttranslational modification of E4 includes phosphorylation, proteolysis and oligomerization. Our previous work has shown that proteolysis is a key regulatory mechanism of HPV1 E4 function; E1^E4 functions are attenuated by proteolysis and an N-terminal truncation polypeptide encodes a distinct function (G2 arrest), whilst complex formation between the different species mediates a novel function (cellular DNA replication inhibition).

HPV18 DNA amplification in human foreskin keratinocytes (HFK) correlates with accumulation of full-length E1^E4 protein and several smaller species lacking N-terminal sequences. Since E1^E4 expression is necessary for HPV18 genome amplification, the smaller proteins may contribute to its role in the replication cycle. We have mapped key elements necessary for proteolysis to leucine-rich sequences in HPV18 E1^E4, a region known to be required for an association with the keratin cytoskeleton. Mutations that attenuate E4 proteolysis but preserve the keratin interaction have been introduced into HPV18 genomes. Following transfection into HFK, mutant genomes were maintained as episomes. No significant differences concerning copy number were evident between cells containing wild-type or mutant genomes. The affect of these mutations on differentiation-dependant vegetative functions is under investigation.

HPV18 E1^E4 is phosphorylated in vivo and is a substrate for multiple kinases including cyclin-dependent kinases (cdk) 1 and 2, and PKA, but not MAPK. By using mass spectroscopy and site-directed mutagenesis, Thr23 has been identified as a cdk1/2 phospho-acceptor site, and PKA phosphorylates E1^E4 at Ser58. HPV18 E1^E4 mutants containing alanine or aspartic acid substitutions at Thr23 and Ser58 were generated and expressed in epithelial cells. These mutant E4 proteins maintain an association with the keratin cytoskeleton, and are able to sequester cyclin A to the cytoplasm, indicating that Thr23 and Ser58 phosphorylation do not have a significant role to play in these E1^E4 functions.

O-24.04 UP-REGULATION OF P18INK4C EXPRESSION BY HPV E6 VIA P53-MIR-34A PATHWAY

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Binding of p53 to miR-34 promoter activates the expression of tumor suppressive miR-34a. Our previous study demonstrated that oncogenic HPV infection down-regulates miR-34a expression through degradation of p53 mediated by E6. In searching for miR-34a targets, we found that miR-34a mainly down-regulates p18Ink4c, a CDK4/6 inhibitor induced by E2F transactivation. HPV18+ HeLa cells with ectopic miR-34a expression or by E6 siRNA knockdown-induced expression of miR-34a had a substantially reduced expression of p18Ink4c in a dose-dependent manner, but had no effect on 16Ink4a, another member of CDK4/6 inhibitor family. Further investigation showed an increased p18Ink4c level in cervical cancer tissues by western blotting as compared to normal cervix. By immunostaining of tissue arrays, an increased expression of p18Ink4c was found in 71.8% of cervical cancer tissues, but only in 8.3% of normal cervical tissues. As a cell cycle regulatory protein in normal cells, the increased p18Ink4c expression by E6-induced p53 destabilization and miR-34a reduction in cervical cancer would lead to suppress cell cycling and proliferation. However, viral E7 expressed in cervical cancer cells inactivates pRB and disassociates E2F from pRB, resulting in disruption of normal G1 check-point. Thus, an increased p18Ink4c in the cervical cancer cells would not affect the cell growth. To test this hypothesis, we knocked down p18Ink4c expression by siRNA both in HPV18-positive HeLa cells and HPV-negative HCT116 cells, a colon cancer cell line that contains an intact G1 check-point. As we predicted, knocking down p18Ink4c expression promoted the growth of HCT116 cells, but had no effect on HeLa cells. These data indicate that an increased p18Ink4c expression in cervical cancer tissues could not lead to tumor suppression when the cancer cells lack a G1 check-point and can be used as a new biomarker for cervical cancer diagnosis and prognosis.

O-24.05

INTERACTION OF THE E8^E2C REPRESSOR-PROTEIN WITH THE CELLULAR CHD6 PROTEIN

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Background: The HPV E2 and E8^E2C proteins are generated from the E2 ORF and regulate transcription, replication and partitioning of papillomavirus genomes. It has been described for E2 that these activities are mediated by the interaction with host cell and viral proteins. In contrast, very little is known about interaction partners of E8^E2C.

Objectives: The identification of host cell proteins interacting with the HPV31 E8^E2C protein.

Methods and Results: A Gal4-E8^E2C construct was used to screen a B-cell lymphoma cDNA library by a yeast-2-hybrid assay. We found an N-terminal fragment of CHD6 (chromo helicase DNA binding domain 6) as a potential interaction partner which has been suggested to act as a transcriptional regulator. A corresponding CHD6 cDNA fragment cloned from HPV18-positive cells was used for in vitro translation and found to specifically interact with MBP- E8^E2C. Also E8^E2C Proteins of HPV16 and HPV18 interacted with the CHD6 fragment suggesting a conserved interaction. Deletion analyses revealed that the E2C domain is responsible for the interaction. MBP-E8^E2C interacted with the 300 kDa full-length CHD6 protein in cell extracts and vice versa MBP-CHD6 interacted with E8^E2C in cell extracts. We have identified a mutant in the C-terminus of E8^E2C that no longer binds to CHD6. This mutant displayed reduced transcriptional repression on HPV18 URR and synthetic E2BS luciferase reporter plasmids.

Conclusions: Several binding assays revealed a conserved interaction between CHD6 and the DNA-binding domain of E8^E2C. As the loss of CHD6 binding to E8^E2C resulted in a reduced transcriptional repression activity, CHD6 may contribute to repression by E8^E2C.

O-24.06

USE OF A LUCIFERASE FUSION TO STUDY HPV E2 DEGRADATION

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Background: The E2 protein of human papillomavirus (HPV) binds to specific sites in the viral genome to regulate its transcription, replication and maintenance in infected cells. Like most regulatory proteins, E2 is rapidly turned over. Objectives: To study the rapid degradation of HPV E2 and identify factors involved in this process, we set out to develop a

high-throughput assay to quantify the expression and stability of E2 in vivo. Methods: The assay we developed is based on a translational fusion of E2 to Renilla luciferase (RLuc). Using this Rluc-E2 fusion protein, the steady state levels of E2 could be quantified by measuring the amounts of associated luciferase activity

fusion protein, the steady state levels of E2 could be quantified by measuring the amounts of associated luciferase activity and its degradation measured by following the decrease in enzymatic activity occurring after a block of translation with cycloheximide.

Results: Using this method, the E2 proteins from a low-risk (HPV11) and a high-risk (HPV31) HPV type were found to have short half-lives of 60 min in C33A cervical carcinoma cells and to be ubiquitinated and degraded by the proteasome. Analysis of mutant proteins showed that the instability of E2 is independent of its DNA-binding and transcriptional activities but is encoded within its transactivation domain (TAD), the region that binds to the cellular chromatin factor Brd4 to regulate viral gene transcription. Overexpression of Brd4, or of its C-terminal E2-interaction domain, was found to increase the steady-state levels and stability of wild type E2 but not of E2 mutants defective for binding Brd4.

Conclusions: These results indicate that the stability of E2 is increased upon complex formation with Brd4 and highlight the value of the luciferase assay for the study of E2 degradation. Other factors influencing the proteasomal degradation of E2 will be presented.

O-24.07 INTERACTION OF BETA PAPILLOMAVIRUS E2 TETHERING PROTEINS WITH MITOTIC CHROMOSOMES

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Papillomavirus are stably maintained in the basal layer of the epithelium by tethering their genome to the host chromosomes during mitosis. This ensures maintenance and partitioning of the viral genome after each cell division. The viral E2 protein tethers the viral genome to the host chromosomes but different papillomaviruses interact with different chromosomal regions and targets. The tethering mechanism has been best characterized for BPV-1, where the E2 protein tethers the viral genome to mitotic chromosomes in complex with the cellular bromodomain protein, Brd4. In contrast, the beta-papillomavirus HPV-5 and HPV-8 E2 proteins bind as large speckles at the pericentromeric region of mitotic chromosomes and this does not require the Brd4 protein. More specifically, they target the short arm of acrocentric chromosomes and interact with the repeated ribosomal DNA genes found in this location. The C-terminal DNA binding domain and a short peptide from the hinge region are necessary and sufficient for interaction with mitotic chromosomes. Defining the molecular interaction of the E2 proteins with mitotic chromosomes will enable us to design anti-viral therapies to inhibit such interactions and eliminate viral genomes from infected host cells.

POSTER ABSTRACTS SESSION 24

POSTER SESSION III TUESDAY 20.00: ODD NUMBERS

POSTER SESSION IV

TUESDAY 21.00: EVEN NUMBERS

P-24.08

INVOLVEMENT OF PAPILLOMAVIRUS E2 PROTEIN IN CELLULAR GENE EXPRESSION

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Background. Papillomavirus E2 protein regulates viral transcription and is required for viral DNA replication and stable genome maintenance. E2 is a sequence-specific DNA-binding protein that recognizes a 12 bp palindromic DNA motif with a consensus sequence 5'-ACCGNNNNCGGT-3'. E2 can interact with cellular transcription factors and bind transcriptional regulatory elements in the cellular DNA thereby potentially affecting expression of cellular genes. Examples of genes that have been shown to be regulated by E2 include human telomerase reverse transcriptase, matrix metalloproteinase 9, SF2/ASF and β 4-integrin.

Objectives. Our aim was to study the papillomavirus E2 protein involvement in cellular gene expression regulation. We identified the abundance and placement of papillomavirus E2 specific DNA binding sites in the human genome and studied the role of some of these sequences in E2 dependent cellular transcription regulation.

Methods. E2 responsive transcription regulation was examined in U2-OS and HaCat cell lines using luciferase assay and real-time PCR. Experiments were conducted with both high-risk (HPV18) and low-risk (HPV11) human papillomavirus E2 proteins.

Results. Human genome contains over three thousand copies of papillomavirus E2 protein specific DNA motif, but only 753 are located in repeat free regions. E2 binding sites occur less frequently than is expected from genome nucleotide content. Additionally, most sites are suboptimal for HPV E2 binding. Our experiments show that some of the genomic sequences containing at least two E2 binding sites within 500 bp can act as E2 responsive transcription regulatory elements in transient transcription assays. E2 can also induce changes in expression levels of genes containing those sites in the genomic context.

Conclusions. Papillomavirus E2 proteins can alter cellular gene expression through binding to specific E2 recognition sites in the human genome. Number and structure of these sites appears to be under negative selection pressure.

P-24.09

HPV1 E1^E4 INHIBITS SRPK1 PHOSPHORYLATION OF SR PROTEINS

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In papillomavirus infections, viral regulatory proteins are expressed during the early stage of infection in cells of the undifferentiated basal and parabasal layers. Expression of viral structural proteins occurs in the differentiated cell layers. The coordinated expression of viral genes is regulated by transcriptional and posttranscriptional mechanisms. Serine-arginine (SR) proteins are a group of arginine-serine (RS) domain-containing proteins known to regulate pre-mRNA splicing, export and translation of mRNAs. SR proteins including ASF/SF2 associate with cis-acting regulatory elements in the papillomavirus genome that regulate splicing, and the phosphorylation state of SR proteins is important for their role in regulating late HPV gene expression.

SR proteins are phosphorylated by multiple kinases including members of the SR protein kinase (SRPK) family. We have shown that E1^E4 proteins of the cutaneous virus HPV1, and the anogenital viruses HPV16 and HPV18, associate with SRPK1, and that SRPK1 is sequestered to E4 inclusions in granular cells of HPV1 warts.

To address whether E1^E4 proteins alter SRPK1 activity, GST-tagged E1^E4 proteins of HPV1, 16 and 18 were titrated into in vitro kinase reactions containing His-tagged SRPK1 and GST-tagged ASF/SF2 proteins. HPV1 E1^E4 was a potent inhibitor of SRPK1 activity in these assays, while HPV16 and 18 E1^E4 failed to inhibit SRPK1-mediated phosphorylation of ASF/SF2. Maintenance of the association between SRPK1 and E1^E4 is necessary for inhibition, since a deletion HPV1 mutant defective in SRPK1-binding did not inhibit ASF/SF2 phosphorylation. The ability of E1^E4 to inhibit SRPK1 kinase activity is not restricted to ASF/SF2, rather this inhibition impairs SRPK1-mediated phosphorylation of several other SR proteins, including SRp20, SRp75 and 9G8. Our data indicate that HPV1 E1^E4 inhibits SRPK1 phosphorylation of SR proteins in vitro and that this E1^E4 function might act to regulate both host and virus gene expression.

P-24.10

PAPILLOMAVIRUS E2 PROTEIN INTERACTS WITH AND STABILIZES CELLULAR DAXX PROTEIN.

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Background: Papillomaviruses are DNA viruses capable of establishing a persistant infection in mammalian epithelial cells. The E2 protein plays a crucial role in the viral life cycle by regulating the expression of viral genes, genome replication and maintenance in infected cells. In order to fulfill its roles, E2 interacts with different cellular proteins and nuclear compartments. E2 of bovine papillomavirus type 1 (BPV-1) is attached to cellular chromatin by Brd4 throughout the cell cycle. The cellular protein Brd4 is required for E2-mediated transcriptional activation which is efficiently down-regulated by the C-terminal domain of Brd4 (Brd4CTD).

Objectives: Initially, we explored how Brd4CTD affects the nuclear distribution of E2. However, we ended up describing a novel interaction.

Methods: The co-localization of E2 and Daxx was observed by immunofluorescence analysis. The subnuclear distribution of E2 and Daxx was examined by biochemical fractionation. Interaction of E2 and Daxx was analyzed by co-immunoprecipitation and Western blot. The effect of E2 expression on Daxx was determined by FACS analysis. The effect of Daxx on papillomavirus DNA replication was analyzed by Southern blot and on E2-mediated transcription in dual-luciferase assay.

Results: The expression of Brd4CTD relocalized BPV-1 E2 and the E2 proteins of human papillomavirus type 18 (HPV-18) and 11 (HPV-11) into distinct nuclear foci which are part of detergent-resistant high salt insensitive nuclear matrix fraction. In these foci E2 proteins of BPV-1, HPV-18 and -11 colocalized with Daxx, a cellular protein involved in apoptosis and transcriptional regulation. We detected an interaction between E2 and Daxx proteins and observed a stabilizing effect of E2 expression on endogenous Daxx. Overexpression of Daxx did not have a significant effect on E2 in papillomavirus DNA replication. However, it affected E2-mediated transcription.

Conclusions: E2 proteins interact with and stabilize endogenous Daxx, suggesting that Daxx may have a role in E2mediated activities.

P-24.11 HYPOXIA-INDUCIBLE FACTOR, HIF-1A, IS INDUCED BY HUMAN PAPILLOMAVIRUS ONCOPROTEINS

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[Backgrounds] During tumor development, cells are exposed to a hypoxic environment. A key regulator of the cellular response to hypoxic environments is the transcriptional factor hypoxia-inducible factor 1 (HIF-1), which induces the expression of a number of proangiogenic factors. It is however, unclear what association, if any, there is between HIF-1 levels and expression of human papillomavirus (HPV) proteins. [Objectives and Methods] We investigated the interactions between HPV proteins and HIF-1 α , the major regulatory subunit of HIF-1, under conditions of normoxia and hypoxia. [Results and Conclusions] Our studies indicate that cells containing either HPV 31 or 16 genomes exhibited increased activation of HIF-1 α under hypoxic conditions, but not in normoxia. Both the E6 and E7 oncogenes were able to induce enhanced expression of HIF-1 α with a corresponding activation of some but not all HIF-1 α downstream targets. Our studies further indicate that HPV31 E7 associates with both HIF-1 α and p53 in cells, and this association results in reduced interaction of the HIF-1 α /p53 complex with MDM2. These findings shed light on the mechanisms by which HPV contributes to the angiogenesis aspect of the tumor phenotype and describes a novel role of the E7 oncogene in the virus-host interaction.

P-24.12

INTERACTION BETWEEN HPV VLPS AND HEPARIN

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Background: In vitro studies have clearly established that heparan sulfates (HS) are the primary cell attachment receptors for HPV virions and that interaction with HSPG requires an intact VLP. Little was known about the viral sequences contributing to the interaction with HS until recently, with contradictory results indicating that both a conserved domain at the c-terminus part of the L1 protein and a conformational cluster of lysine residues at the surface of HPV-33 virions interact with HS.

Objectives: The aim of this study was to confirm which L1 sequences interact with HS and whether neutralizing antibodies inhibit the binding of heparin to VLPs.

Methods: L1 deletion mutants lacking the 9 and 31 c-terminal amino acids for both types 16 and 31 were produced using recombinant baculoviruses. The wild type and deleted VLPs produced in insect cells were used to investigate the binding of VLPs to heparin. In addition, we investigated the interaction of HPV MAbs with heparin binding using the HPV-31 model.

Results: All wt and deleted mutant VLPs bound to heparin but not the L1 proteins obtained by denaturation of VLPs. Heparin binding to VLPs was not inhibited by some strong neutralizing antibodies whereas it was strongly inhibited by H31.D24, a non-neutralizing MAb. This is in agreement with the fact that this epitope contained one of the 3 lysine residues identified as interacting with HS.

Conclusions: The results obtained confirm that heparin binds to a conformational structure present at the surface of VLPs, but not to the C-terminal part of the L1 molecule. The results also suggest that inhibition of VLP binding to HSPG is not one of the mechanisms of HPV neutralization, although heparan sulfates are strong inhibitors of HPV infection.

P-24.13

HPV-16 E6 AND E7 ALTER NF-KB ACTIVATION IN CERVICAL CELLS

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Background: The NF-kB family of transcription factors mediates the host response to stress. NF-kB can stimulate cell growth and survival, and activation may contribute to malignant development. Several viruses have evolved mechanisms to activate NF-kB, and activation can play a role in viral transformation.

Objectives: We examined whether HPV-16 E6 and E7 proteins alter NF-kB activity in epithelial cells cultured from human cervix.

Methods: Secondary cultures were infected with retroviruses encoding HPV-16 E6, E7, or E6/E7. As a control, cells were infected with retroviruses encoding the empty vector. NF-kB activity was determined by transfection with an NF-kB reporter gene and performing a dual luciferase assay.

Results: E6 and E7 acted differently. E6 significantly increased basal NF-kB activity and E7 significantly decreased activation. When cells were treated with TNF-alpha to strongly induce NF-kB, E6 enhanced and E7 inhibited TNF-induced activation. Coexpression of E6/E7 generally reduced NF-kB activation, although our results were variable. The p65 subunit of NF-kB was predominantly localized in the cytoplasm, and E6/E7 slightly decreased both cytoplasmic and nuclear staining. The p50 subunit of NF-kB contributed to immortalization, cells were infected with HPV-16 E6/E7 retroviruses and then stably transfected with either (1) the IkB-alpha mutant that inhibits function, (2) the p65 subunit that increases activation, or (3) an empty vector as a negative control. Unexpectedly, the dominant negative IkB-alpha mutant stimulated colony formation and immortalization.

Conclusions: These results suggest that stable expression of HPV-16 E6/E7 causes variable reduction of NF-kB in cervical epithelial cells, and that reduction of NF-KB contributes to growth and immortalization of human cervical cells in vitro.

P-24.14 MODIFICATION OF HPV-16 MINOR CAPSID PROTEIN L2 BY SUMO PROTEINS

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Background: Human papillomavirus (HPV) genome is encapsidated by two structural proteins, a major structural protein L1 and a minor structural protein L2. Recently, it became known that L2 is involved also in the initial steps of the infection as well as in the generation of infectious virus particles. Conjugation of the small ubiquitin-like modifier (SUMO) to target proteins regulates numerous biological processes. It has been shown that sumoylation modulates the function of many transcription factors and other proteins, including some viral proteins (e.g. HPV E2). Objective: To identify and characterize sumoylation of HPV L2 protein.

Methods: Sumoylation in vivo was studied by using U2OS and 293 cell lines co-transfected with SUMO-1, -2 and -3 proteins and wild type HPV-16 L2 or different HPV-16 L2 lysine substitution mutants. Modification of HPV-16 L2 by SUMO proteins was characterized by western blot analysis and immunofluorescence microscopy.

Results: In vivo experiments revealed that HPV-16 L2 is strongly sumoylated by SUMO-2 and SUMO-3, and to smaller extend also with SUMO-1. Fluorescence microscopy showed distinct nuclear colocalisation of HPV-16 L2 with all three SUMO isoforms. Mapping studies identified lysine 35 of the SUMO motif (K35 mutant) as the principal residue for conjugation of SUMO to HPV-16 L2, but there is at least one other lysine involved in the sumoylation. Expression level and intracellular localization of HPV-16 L2 K35 mutant were not notably changed. In terms of functional relevance of SUMO modification, we observed stabilization of HPV-16 L2 protein in cells co-transfected with SUMO proteins.

Conclusions: Results on HPV L2 modification with SUMO proteins suggest that sumoylation plays a role in the regulation of papillomavirus L2 protein and in the modulation of the L2 functions at the post-translational level.

P-24.15 EFFECTS OF HPV-16 EARLY PROTEINS ON TROPHOBLASTIC CELLS

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Background: The trophoblastic cell represents the main functional unit of the placenta. It proliferates, migrates, and invades the maternal tissue in a way that is similar to malignant tumors. Nevertheless, these processes are tightly controlled by stringent spatial and temporal confines. Therefore, the trophoblastic cell, as 'a well-behaved tumor', represents an ideal model system to investigate several oncogenic processes. Several studies reported that HPV viruses could infect trophoblasts during pregnancies. Surprisingly, HPV can replicate in vitro in trophoblasts. Higher HPV infection frequency has been reported to be associated with some spontaneous abortion and gestational trophoblastic diseases.

Objectives: In this study, we have studied the impacts of HPV-16 early proteins, mainly E5, E6 and E7, on the viability, adhesiveness, migration and invasion of trophoblastic cells.

Methods: Molecular biology techniques, confocal microscopy, growth, migration and invasion assays.

Results: Our results showed that the hydrophobic E5 protein is localized in many interne membranes compartments of the transfected trophoblast. E5 affects the viability of transiently and stably transfected trophoblastic cells. The viability seemed to be restored or even increased in the presence of E6 and E7. These observations were also confirmed by transfection in C33a cells, the HPV-negative human cervical carcinoma cell line. In addition, E5 decreased the adhesiveness of the trophoblastic cells to the support and to the endometrial cells. Cells expressing metastasis E6, E7 and in less extend E5 favour chemotaxic migration and matrigel invasion compared to the cells expressing the LacZ control. These effects are also observed when early proteins are expressed under the control of their own viral promoter (LCR). Our findings show that HPV-16 early proteins can affect the adhesiveness, the migration and the invasion of trophoblastic cells, key properties involved in placentation and metastasis.

SESSION 25

PROPHYLACTIC VACCINES, BASIC SCIENCES

PROGRAMME

Oral presentations

THE 25TH INTERNATIONAL PAPILLOMAVIRUS CONFERENCE MAY 8-14 2009, MALMÖ, SWEDEN

TIME	NUMBER	TITLE	ROOM
2009-05-13			
SESSIC	ON 25: PRO	OPHYLACTIC VACCINES (BASIC SCIENCES)	K1-3
15.20-15.31	O-25.01	IN VIVO MECHANISMS OF PROTECTION BY VLP AND L2 VACCINATION J Schiller, RC Kines, C Thompson, S Jagu, R Roden, D Lowy, P Day	
15.31-15.42	O-25.02	STRUCTURAL AND IMMUNOLOGIC ANALYSIS OF DIFFERENTLY MODIFIED HPV16 L1 CAPSOMERES L Schädlich, B Gerlach, T Senger, N Mücke, C Klein, I Bravo, M Müller, L Gissmann)
15.42-15.53	O-25.03	HPV16 ANTIBODY EPITOPES FROM VACCINE RECIPIENTS AND FOLLOWING NATURAL INFECTIONS GC Wipf, JJ Carter, LA Koutsky, D Galloway	
15.53-16.04	O-25.04	ANTIBODY RESPONSES IN ORAL FLUID AND SERUM FOLLOWING HPV VACCINATION JJ Carter, A Rowhani - Rahbar, Se Hawes, Jp Hughes, La Koutsky, d Galloway	
16.04-16.15	O-25.05	RECOGNITION PATTERN OF NEUTRALIZING AND NON-NEUTRALIZING ANTI-L2 ANTIBODIES M Müller, A Bolchi, M Tommasino, S Ottonello, I Rubio	
16.15-16.26	O-25.06	CHIMERIC L1/L2 VIRUS-LIKE PARTICLES (VLP) AS POTENTIAL BROAD- SPECTRUM HPV VACCINES C Schellenbacher, R Roden, R Kirnbauer	

O-25.01 IN VIVO MECHANISMS OF PROTECTION BY VLP AND L2 VACCINATION

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Using the murine model of cervicovaginal pseudovirus (PsV) infection, we have investigated the mechanisms of in vivo protection by vaccination with HPV16 L1 VLPs or an L2 fusion polypeptide composed of aa 11-88 from HPV1, 5, 6, 16, and 18. As expected, VLP vaccination conferred type-restricted protection, while L2 also protected against heterologous viral challenge. PsV localization in vivo was studied in unvaccinated and vaccinated mice. In unvaccinated mice, PsV first attach predominantly to the basement membrane (BM), undergo a conformational change that exposes the L2 aa17-36 cross-neutralization epitope, and are then transferred to the epithelial cell surface and internalized. These results differ from in vitro studies, where PsV undergo furin cleavage and exposure of the L2 aa17-36 epitope while on the cell surface. After VLP vaccination, PsV are not found in vivo at the BM and do not expose the L2 epitope. Instead, they are associated with the epithelial cell surface but are not internalized. As expected for type-restricted protection, the deposition pattern of heterologous PsV is unaffected in HPV16 L1 VLP-vaccinated mice. Anti-VLP polyclonal sera act similarly in vitro, preventing extracellular matrix (ECM) binding, but allowing cell surface binding without internalization. In L2 polypeptidevaccinated mice, the PsV bind to cervicovaginal BM at 4 hours similarly to unvaccinated mice. However, at later time points in L2-vaccinated animals, neither homologous nor heterologous PsV can be detected on the BM or epithelial cell surface. The in vivo results differ from in vitro observations, where L2-antibody engagement causes detachment of PsV from the cell surface and accumulation of PsV on the ECM. Thus, ECM and BM differ with respect to their interactions with capsid/L2 antibody complexes. Furthermore, VLP and L2 vaccination induce dramatic alterations in PsV localization, an encouraging result for L2 vaccination prospects.

O-25.02

STRUCTURAL AND IMMUNOLOGIC ANALYSIS OF DIFFERENTLY MODIFIED HPV16 L1 CAPSOMERES

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HPV L1 capsomeres can be purified in high amounts from E. coli and are considered an economic alternative to the current vaccines against HPV infections. However, a previous report showed that HPV 16 capsomeres -- generated from an L1 protein with cys ala modifications at position 175 and 428 -- are much less efficient than VLPs in triggering antibody responses.

Since several modifications of L1 have been shown to allow capsomere formation we have compared the immunogenicity of eight differently modified L1 proteins purified as capsomeres from E.coli. Despite the almost identical structures observed by electron microscopy and confirmed by sedimentation analysis, the proteins differed remarkably in immunogenicity. We suggest that this difference can be attributed to the assembly properties of the capsomeres; the ability of the proteins to assemble into stable larger particles prior to or during immunization correlated strongly with higher immunogenicity. However, an assembly step prior to immunization did not increase the immunogenicity of the L1 proteins in vivo. Importantly, injection of mice with one of the analyzed L1 proteins, the L1 Δ N10, resulted in antibody titers equal to those seen after immunization with VLPs. Our data demonstrate that the modification of the L1 protein can affect immunogenicity and that certain L1 capsomeres can be as immunogenic as VLPs and thus do represent a cost-effective alternative to VLP-based prophylactic HPV vaccines.

O-25.03

HPV16 ANTIBODY EPITOPES FROM VACCINE RECIPIENTS AND FOLLOWING NATURAL INFECTIONS

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BACKGROUND: The solvent accessible surface of HPV capsids is composed of amino acids of the L1 protein that vary among types. Several regions (or loops) have previously been identified as composing epitopes important for binding and neutralization. Vaccinated individuals tend to have higher titer antibody responses than people naturally infected with HPVs. We wanted to determine if epitopes recognized by sera from vaccinated individuals differed from epitopes recognized by sera from individuals with HPV16 infections. METHODS: Sera were obtained from previous studies conducted in Seattle and only included female subjects who had previously tested HPV16 or HPV31 seropositive. One study was a HPV natural history study and the second a HPV vaccine trial. Epitope swap substitutions were made in HPV 16L1 by replacing the loops or epiope residues with the homologous HPV 31 L1 sequences. All five surface loops were substituted, plus two variable regions at the C-terminus and several point mutants. All L1 proteins were expressed as GST-L1 fusions and binding assays were performed on a Luminex platform allowing for simultaneous testing of fusion proteins with serum in a single well. RESULTS: Preliminary data from 19 sera (10 natural infection and 9 vaccinated women) indicated that sera from vaccinated women had significantly higher cross-reactivity to 31L1 (P = 0.002) and higher reactivity to denatured 16L1 (P=0.004). Among women with natural infections, mutation of any of the loops reduced reactivity by approximately 75%. Among sera from vaccine recipients, mutation of the loops had considerably less effect, supporting the more cross-reactive character of these responses. CONCLUSIONS: These experiments indicate a difference between antibodies produced following vaccination and after natural HPV16 infections.

O-25.04 ANTIBODY RESPONSES IN ORAL FLUID AND SERUM FOLLOWING HPV VACCINATION

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BACKGROUND: Oral fluid sampling is simple, noninvasive, and painless, however, immunoglobulin G levels are lower than in serum. We sought to assess the potential utility of using oral mucosal transudate (OMT), collected by an OraSure* device, for detection of human papillomavirus type 16 (HPV-16) specific antibodies among women who had received a prophylactic HPV-16 L1 virus-like particle vaccine. METHODS: Vaccine recipients (139) and placebo controls (137) who had participated in a phase IIb randomized controlled vaccine trial (1998 – 2004) were enrolled in an extended follow-up study conducted in Seattle (2006 - 2008). Serum and OMT specimens were collected and tested using GST-L1 fusion proteins on a Luminex* platform. To optimize sensitivity, a subset of OMT samples were tested twice using different protocols. The incubation time for antigen coated beads with OMT fluid was 1 hour at room temperature for the first test and overnight at 4 C for the second. The sensitivity and specificity of the tests were compared by receiver operating characteristic analysis and the more sensitive test used for testing all sera and OMT samples. RESULTS: Overnight incubation improved assay sensitivity. Using the overnight incubation procedure on OMT collected 8.5 years after enrollment in the monovalent vaccine trial, the average median fluorescence intensity (MFI) among the placebo recipients was 440.2 (95% confidence interval [CI]: 370.5 - 509.9) compared with an MFI of 2,208.2 (95% CI: 1,598.4, 2,818.0) among the vaccine recipients. Although 97.8% of the sera samples from the vaccine recipients tested positive, only 47.5% of OMT samples were positive. Six months after administration of the licensed quadrivalent vaccine, OMT anti-HPV-16 reactivity rose 9.8-fold among the monovalent vaccine recipients, with all OMT specimens testing anti-HPV-16 positive. CONCLUSION: Oral fluid testing has potential as an alternative method to venipuncture in monitoring future HPV vaccination programs.

O-25.05

RECOGNITION PATTERN OF NEUTRALIZING AND NON-NEUTRALIZING ANTI-L2 ANTIBODIES

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Two commercial vaccines against HPV16 and HPV18 (Cervarix and Gardasil) are available and protect against about 70% of all HPV high risk infections. Recently, the N-terminal part of the HPV L2 protein was reported to contain several epitopes able to induce the production of neutralizing antibodies with cross-protection abilities (Gambhira et al., 2007; Kawana et al., 1999). Using bacterial thioredoxin as a scaffold, we were able to increase the immunogenicity of the L2 neutralizing epitopes. However, testing sera from mice immunized with Trx-L2 fusion proteins, we observed a discrepancy between sero-reactivity directed against the epitopes and neutralizing activity indicating that most of the antibodies recognizing the rather defined L2 epitopes are in fact non-neutralizing. To determine the recognition patterns for non-neutralizing, neutralizing and cross-neutralizing antibodies, we isolated a panel of 44 monoclonal antibodies against different epitopes of L2 protein. Among them, four monoclonal antibodies were characterized as neutralizing antibodies and two of them are also able to cross-neutralize a broad range of papillomavirus (HPV 58, 31, 18, 45, 27, 57 and BPV-1). The antibodies were characterized by peptide ELISA and the epitopes able to induce neutralization and cross-neutralization were identified. The identification of the epitopes in L2 able to induce cross-protection against several papillomavirus types might allow vaccine approaches specifically evoking neutralizing antibody responses.

O-25.06

CHIMERIC L1/L2 VIRUS-LIKE PARTICLES (VLP) AS POTENTIAL BROAD-SPECTRUM HPV VACCINES

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Papillomavirus L1 VLP vaccines provide enduring, high-titer and type-restricted antibody responses. In contrast several N-terminal peptides of L2 induce low-titer antisera that also cross-neutralize heterologous types. The aim was to more completely characterize neutralizing epitopes within N-terminal HPV16 L2 in the context of chimeric L1/L2 VLP, to potentially improve L2-immunogenicity by surface-display on highly-ordered particles. Nine overlapping peptides (aa 2-22, 13-107, 18-31, 17-36, 35-75, 75-112, 115-154, 149-175, 172-200) comprising N-terminal 200 aa of HPV16 L2 were genetically engineered for expression by BPV1 L1 surface loop. Additional chimeras comprising insertion of HPV31 L2 peptide 17-36 into BPV1 L1, and HPV16 L2 17-36 into HPV16 L1 are currently evaluated. Chimeric proteins were baculovirus expressed and purified. Two NZW rabbits were immunized in Freund's adjuvant with each native or SDSdenatured particles. Established immunogens were further administered using alum-MPL adjuvant and into Balb/c mice. Sera were analyzed by L2 peptide-ELISA and pseudovirion neutralization assays. Except for L1 displaying HPV16 L2 peptides 35-75 and 13-107 recombinant proteins assembled into VLP. By peptide-ELISA immune sera revealed titers up to 60,000, indicating immunogenic epitopes in all surface displayed peptides beside 2-22. Sera to chimeras 75-112, 115-154 and 13-107 neutralized homologous HPV16 with titers up to 1000. Antisera to chimeras 2-22, 149-175 and 172-200 were non-neutralizing for HPV16. Insertion of previously described L2 epitope 17-36 (RG-1) induced broadly neutralizing antisera to divergent high-risk HPV 16/18/31/45/52/58, low-risk HPV11 and beta-type HPV5, with titers ranging from 50 to 10,000. Alum/MPL adjuvanted immunogen induced a similar neutralization pattern, in both rabbit and mice, albeit less robust with 100-fold lower titer. Native VLP induced higher titers than denatured particles. Immunization with chimeric L1 VLP displaying L2 peptides in adjuvant applicable for human use can induce broad-spectrum antibody responses to mucosal high-risk, low-risk and beta papillomaviruses.

MOLECULAR MARKERS & HPV TESTING METHODS

SESSION 26

PROGRAMME

THE 25TH INTERNATIONAL PAPILLOMAVIRUS CONFERENCE MAY 8-14 2009, MALMÖ, SWEDEN

TIME NUMBER TITLE

ROOM

2009-05-14			
SESSI	ON 26: MO	LECULAR MARKERS & HPV TESTING METHODS	SCANIA
08.30-08.40	O-26.01	DIAGNOSTIC HPV16 RNA PATTERN DISCRIMINATING MILD AND SEVERE CERVICAL LESIONS M Schmitt, V Dalstein, T Waterboer, C Clavel, L Gissmann, M Pawlita	
08.40-08.50	O-26.02	CAN IMMUNOHISTOCHEMISTRY FIX THE DIAGNOSTIC INTERPRETIVE PROBLEMS WITH CERVICAL BIOPSIES? M Stoler, K Atkins, M Galgano, W Brix, S Nassau, P Castle	
08.50-09.00	O-26.03	E6/E7 MRNA AND DNA TESTS FOR DETECTION OF ≥CIN II S Ratnam, F Coultee, D Fontaine, J Bentley, N Escott, P Ghatage, G Holloway, E Bartellas, N Kum, C Giede, V Gadag, A Lear	
09.00-09.10	O-26.04	COMPARISON OF MEASURES OF VIRAL PERSISTENCE TO PREDICT SUBSEQUENT DISEASE J Gage, M Schiffman, D Solomon, P Castle	
09.10-09.20	O-26.05	ABSOLUTE RISK OF SUBSEQUENT CIN3+ ACCORDING TO DIFFERENT HPV TYPES S Kjær, C Munk, K Frederiksen, J Junge, T Iftner	
09.20-09.30	O-26.06	AMPLICOR HPV DETECTION IN THE ASCUS-LSIL TRIAGE STUDY N Wentzensen, P Gravitt, D Solomon, C Wheeler, PE Castle	
09.30-09.40	O-26.07	EVALUATION OF ABBOTT HIGH RISK HPVDNA ASSAY IN PRIMARY HPV- SCREENING B Hammas, J Dillner, C Wahlström	
09.40-09.50	O-26.08	P16INK4A IN CERVICAL CYTOLOGY AND HISTOLOGY: SYSTEMATIC REVIEW AND META-ANALYSIS I Tsoumpou, M Arbyn, M Kyrgiou, N Wentzensen, G Koliopoulos, P Martin - Hirsch, V Malamou - Mitsi, E Paraskevaidis	
09.50-10.00	O-26.09	DIGENE HPV GENOTYPING LQ-TEST: HIGH-THROUGHPUT GENOTYPING USING XMAP TECHNOLOGY D Geraets, DAM Heideman, M de Koning, PJ Snijders, DCJG van Alewijk, CJLM Meijer, Lj van Doorn, W Quint	

DIAGNOSTIC HPV16 RNA PATTERN DISCRIMINATING MILD AND SEVERE CERVICAL LESIONS

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Persistent infections with high-risk human papillomaviruses HPV), mainly HPV type 16, can cause malignant transformation of the human cervical epithelium and the development of cervical cancer (CxCa). A rapid and precise diagnosis of the precancerous lesions by conventional cytology or HPV DNA tests remains difficult and often leads to overtreatment.

To differentiate mild from severe HPV16 induced lesions by RNA patterns, we quantitatively analyzed the HPV16 transcriptome of 80 HPV16 DNA-positive cervical scrapes diagnosed by cytology as NIL/M (normal, n=25), LSIL (low-grade squamous intraepithelial lesion, n=24), HSIL (high-grade lesion, n=24) or CxCa (n=7), with a novel nucleic acid sequence-based amplification (NASBA)-Luminex assay.

Using a combination of four marker transcripts, 100% of CxCa and 67% of HSIL cases were correctly identified as severe, and 74% of LSIL and 92% of NIL/M samples as mild cytological grade.

We identified a novel diagnostic HPV16 RNA pattern for grading of cervical lesions with a potentially high diagnostic value for the primary screening of cervical cancer precursors and the triage of cervical lesions. A new integration-independent model for HPV16-induced CxCa development is proposed.

O-26.02

CAN IMMUNOHISTOCHEMISTRY FIX THE DIAGNOSTIC INTERPRETIVE PROBLEMS WITH CERVICAL BIOPSIES?

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Background: Inaccuracy in biopsy diagnosis has significant clinical implications. Clearly, whatever is biopsied should be interpreted correctly in order to direct the correct management. Given magnitude of biopsy interpretative variation it is unlikely that a pathologist's judgment to order additional tests will adequately address the problem as compared to a model where adjunctive stains are required on every case.

Design: All cervical biopsies in 1-year will be evaluated to establish the magnitude of diagnostic variability amongst 10 pathologists. An independent masked algorithmic panel review will establish a gold standard diagnosis. Kappa statistics for inter-observer variation vs. the gold standard will be calculated. Independently the sensitivity and specificity of 3 immunohistochemical (IHC) assays as potential mandatory diagnostic adjuncts; p16, Ki-67 and HPV L1 will be evaluated. Masking to all H&E diagnoses will allow calculation of the sensitivity and specificity of each stain relative to the diagnostic gold standard.

Result: In this interim analysis of over 770 biopsies, the overall agreement amongst pathologists was 73% with the gold standard, corresponding to a moderate level kappa (0.58). In contrast the kappa for IHC interpretation was similar or better (0.56-0.88). For the distinction of CIN vs. NON-CIN, the specificity of p16 ranged from 81-97%, for Ki-67, 70-99% and for L1, 97%. In contrast, the sensitivity for CIN1 at the best performing cutoff was for p16-53%, Ki-67-56% & L1-32%. However, for CIN 2/3, the sensitivities were similarly p16-81/100%, Ki-67-84/98% and L1-32/16%.

Conclusion: H&E diagnostic variation is significant and IHC interpretations are less variable. However, none of the adjuncts are adequate for resolving the CIN vs. NON-CIN interface as over 40% of adjudicated diagnoses can be missed by these IHCs. Despite this, p16 and Ki-67 are quite promising for the distinction CIN2+ vs. <CIN2.

E6/E7 MRNA AND DNA TESTS FOR DETECTION OF ≥CIN II

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Background: Testing for HPV E6/E7 expression is likely to have greater clinical specificity than testing for HPV DNA. A multicentre study in Canada is assessing E6/E7 mRNA-based tests in comparison with DNA-based tests.

Objectives: The clinical sensitivity/specificity of HPV mRNA and DNA-based tests for the detection of \geq CIN II was assessed in colposcopy referral and routinely screened populations. Methods: The APTIMA® HPV Assay (Gen-Probe) and PreTect HPV-Proofer® (Norchip) tests were used for E6/E7 mRNA testing, the HC2® (Qiagen) and AMPLICOR® (Roche) tests for DNA testing, and the LinearArray® test (Roche) for genotyping. Histology confirmed \geq CIN II served as the gold standard.

Results: APTIMA and PreTect were assessed in comparison with HC2 and AMPLICOR in 478 referral patients, with 107 having \geq CIN II. In this population, the % clinical sensitivity/specificity was: 91.6/61.7 for APTIMA, and 74.5/83.0 for PreTect; this compared with 90.7/50.1 for HC2, and 96.3/43.4 for AMPLICOR. APTIMA and PreTect were further assessed in comparison with HC2 in 831 referral patients, with 240 having \geq CIN II. In this population, the % clinical sensitivity/ specificity was: 94.6/46.7 for APTIMA, and 77.9/75.0 for PreTect; this compared with 95.0/38.1 for HC2. APTIMA was further evaluated for its clinical specificity in comparison with HC2 in 1120 routinely screened women, with 7 having \geq CIN II. In this population, the sensitivity was 100% for both APTIMA and HC2. Among those with \leq CIN I, a lower proportion tested positive by APTIMA than HC2 (138 vs 174; p <0.05). The cross-reactivity rate, based on LinearArray, was lower for APTIMA compared with HC2 and AMPLICOR.

Conclusions: The APTIMA mRNA test showed a higher clinical specificity, while maintaining equal or slightly lower clinical sensitivity compared with the HC2 and AMPLICOR DNA tests, respectively. The PreTect mRNA test showed a higher clinical specificity but a lower sensitivity.

O-26.04

COMPARISON OF MEASURES OF VIRAL PERSISTENCE TO PREDICT SUBSEQUENT DISEASE

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Background: Detection of persistent carcinogenic HPV infection is associated with increased risk of cervical precancer and cancer. HPV persistence as measured by genotype-specific testing might better indicate risk for precancer than repeatedly testing Hybrid Capture 2 (hc2) positive.

Methods: We examined the risk of histologic CIN3+ at 18-24 month visits among 1,055 women enrolled in ALTS who underwent enrollment colposcopy but had less than a CIN2 diagnosis in the first 12 months. We compared hc2 positivity at enrollment, 6 and 12 months with sustained HPV PCR positivity at all three visits for at least one type (type-specific persistence).

Results: Thirty-eight (3.6%) women had complete hc2 and PCR results for enrollment, 6- and 12 month visits and were diagnosed with CIN3+ at 18-24 months. Although the risk of CIN3+ for repeatedly testing hc2 positive was similar to the risk for type-specific persistence (9.4% of 351 vs. 9.4% of 202, p=.99), hc2 detected a larger fraction of CIN3+ (86.8% vs. 50.0%, p<.01). Risk of CIN3+ following persistent HPV16 infection was greater than the risk given persistence for other carcinogenic types (21.6% of 37 vs. 8.0% of 174, p=.01). The negative predictive value (reassurance against having CIN3 at 18-24 months) was extremely high if any hc2 test in the first 12 months was negative (99.3%, 95% CI 97.0-99.0). The corresponding negative predictive value provided by type-specific testing was lower, only 97.8%, 95% CI 96.8-98.8, p=.02).

Conclusions: Among women who underwent enrollment colposcopy but had <CIN2, repeatedly testing hc2 positive had the same positive predictive value for CIN3+ compared to HPV type-specific testing but identified more CIN3+ women overall. Persistent HPV16 infection corresponded to greater risk for CIN3+ compared with other carcinogenic HPV types. A possibly useful test might distinguish HPV16 from the pool of other carcinogenic HPV types.

ABSOLUTE RISK OF SUBSEQUENT CIN3+ ACCORDING TO DIFFERENT HPV TYPES

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BACKGROUND. The majority of women in screened populations who are high-risk HPV positive will have a concurrent normal cytology. It is clinically important to establish the subsequent risk of high-grade lesions in these women OBJECTIVES. To assess absolute risk of subsequent CIN3+ in initially cytologically normal women with concurrent HPV infection.

MATERIAL. We have followed two cohorts: 1) From 1991 to 1993 we established a prospective study of 11,088 women. Two years later, 8656 of these women were re-examined and again, cervical swabs were obtained. In the present analysis, we included 7120 women having normal cytology at the second examination. 2) In another cohort study we collected liquid-based cytology samples from 40,382 Danish women. Samples from both cohorts were HPV-tested (HC2) and genotyped using the LiPa test. The women were followed passively through linkages with the Pathology Data Bank (containing information on all cervical cytological/histological examinations). Currently, the cohorts have been followed for up to respectively 13 years and 5 years. We estimated cumulative incidence rates for CIN3+ according to HPV type.

RESULTS.Cohort 1: Among cytologically normal women being HPV16 positive, 27% (95% CI: 21.1-31.8) developed CIN3+ after 12 years of follow-up. The corresponding risks were respectively 19% (HPV18), 14% (HPV31), and 15% (HPV33). The absolute risk of a subsequent CIN3+ following infection with other HR HPV (i.e. HPV 16, 18, 31, 33 negative) was much lower, 6.0% (95% CI: 3.8-8.3). The risk for CIN3+ at 12 years following two times positivity for HPV16 was 47% (95% CI: 34.9-57.5).

CONCLUSION. HPV16 was out-standing in terms of potential carcinogenicity. Also cytologically normal women with HR types such as HPV18,31,33 had a high risk of subsequent high-grade cervical lesions, but they were all much lower than for HPV16. Results for cohort 2 will be presented.

O-26.06

AMPLICOR HPV DETECTION IN THE ASCUS-LSIL TRIAGE STUDY

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Background: HPV DNA testing has been approved for the management of women with abnormal cervical cytology. Recent studies have demonstrated that HPV testing is a viable option for primary cervical cancer screening. Currently, there is only one FDA-approved HPV DNA detection assay (hybrid capture 2).

Objectives: We analyzed the performance of Amplicor for detecting carcinogenic human papillomavirus infections and cervical precancer in women with an atypical squamous cells of undetermined significance (ASCUS) Pap and compared the results to Hybrid Capture 2 (hc2) in the ASCUS and LSIL triage study (ALTS).

Methods: Baseline specimens collected from women referred into ALTS based on an ASCUS Pap result were prospectively tested by hc2 and retrospectively tested by Amplicor (n=3,277). Following receiver-operator-characteristics curve analysis, Amplicor performance was analyzed at three cutoffs (0.2; 1.0; 1.5). Paired Amplicor and hc2 results were compared for the detection of 2-year cumulative cervical intraepithelial neoplasia (CIN) grade 3 and more severe disease outcomes (CIN3+) and for the detection of 13 targeted carcinogenic HPV types.

Results: Amplicor at the 0.2 cutoff had a higher sensitivity for the detection of CIN3+ (95.8% vs 92.6%, p=0.01) but a much lower specificity (38.9% vs. 50.6%, p<0.001) than hc2. Amplicor at the 1.5 cutoff had an identical sensitivity for the detection of CIN3+ (92.6%) and a slightly lower specificity (47.5%), p<0.001). The positive predictive value of hc2 was higher at all Amplicor cutoffs, while referral rates were significantly lower (53.2% for hc2 vs. 64.1% at the 0.2 cutoff and 56.0% at the 1.5 cutoff, p<0.001). Amplicor was more analytically specific for detecting targeted carcinogenic HPV types than hc2.

Conclusions: Amplicor at the 1.5 cutoff had comparable performance to hc2. While Amplicor missed more disease related to non-targeted types, hc2 was more likely to miss disease related to targeted types.

EVALUATION OF ABBOTT HIGH RISK HPVDNA ASSAY IN PRIMARY HPV-SCREENING

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Background: HPV16 and 18 stand out as having higher risk than other high risk(HR) types. Methods that can readily identify these types are therefore important.

Objective: Longitudinal evaluation of the Abbott RealTime HR HPVDNA assay using a randomised primary HPV-screening trial with 4-year follow-up (Naucler et al,NEJM,2007:357:1589)

Methods: The Abbott assay detects HPV 16, HPV 18 and a group of 12 other HR genotypes (HPV31/33/35/39/45/51/5 2/56/58/59/66/68) and is performed in an automated closed system(m2000) that includes DNA extraction. The samples used had previously been tested with AmplicorTM PCR, Hybrid Capture IITM (HCII) and GP5+/6+PCR (Wahlström et al, J.Med.Virol.,2007:79,1169). Samples were selected at random from the general population and from women who developed CIN2 or worse (CIN2+) during follow-up.

Results: Population prevalence for HPV16 was 2,4%, for HPV18 0,5% and for other oncogenic HPV types 8,1%. The population prevalence of any HPV positivity with the Abbott assay was 10,5% (22/209 tested), which is lower than AmplicorTM PCR (16,3%), similar to GP5+/6+PCR with AmpliTaqGold (12,0%) and higher than HCII (6,2%). Among women who developed CIN2+, 38,6% were positive for HPV16, 7,6% for HPV18 and 51,8% for the other oncogenic HPV types, while 10,7% were positive in >1 test (16/18/other). Overall longitudinal sensitivity for CIN2+ during 4 years of follow up was 87,3% for the Abbott test, 80,0% for HCII, 89,8% for GP5+/6+PCR and 93,4% for AmplicorTM PCR. When the time from HPV testing to CIN2+ diagnosis was <0,5 years, the sensitivity of the Abbott test for CIN2+ was 92,7% compared to 100%, 97,6% and 89,0% with GP5+/6+ PCR, AmplicorTM PCR and HCII, respectively.

Conclusions: The Abbott RealTime HR HPV DNA assay has acceptable sensitivity for CIN2+ at a reasonable HPV population prevalence and has improved ability to predict CIN2+ risk by distinguishing between HPV16/18 and other oncogenic HPV types.

O-26.08

P16INK4A IN CERVICAL CYTOLOGY AND HISTOLOGY: SYSTEMATIC REVIEW AND META-ANALYSIS

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Background: P16INK4a is identified as a biomarker for transforming HPV infections. It could act as an adjunct to current assessment of cervical smears and biopsies, allowing the identification of those women with ambiguous results that require referral to colposcopy and potentially treatment. This review represents an attempt to collect, systematically present and analyse the existing evidence on possible clinical applications of p16INK4a immunostaining in cytological and histological samples from the uterine cervix.

Material and Methods: We conducted a systematic review of all studies that evaluated the role of p16INK4a in cytological or histological specimens from the uterine cervix. We also estimated the pooled average proportion of samples that were positive for p16INK4a in cytology and histology, stratified by the grade of the lesion. Random effects models were used for pooling and analysis for 95% confidence interval (CI). Interstudy heterogeneity was assessed with the Cochran's Q test

Results: Sixty-one studies were included. The proportion of cervical smears overexpressing p16INK4a increased with the severity of cytological abnormality. Among normal smears, only 12% (95% CI: 7-17%) were positive for the biomarker compared to 45% of ASCUS and LSIL (95% CI: 35-54% and 37- 57% respectively) and 89% of HSIL smears (95% CI: 84-95%). Similarly, in histology only 2% of normal biopsies (95% CI: 0.4- 30%) and 38% of CIN1 (95% CI: 23- 53%) showed diffuse staining for p16INK4a compared to 68% of CIN2 (95% CI: 44- 92%) and 82% of CIN3 (95% CI: 72- 92%).

Conclusion: Although there is good evidence that p16INK4a immunostaining correlates with the severity of cytological/ histological abnormalities, its reproducibility is limited due to insufficiently standardized interpretation of the immunostaining. After a consensus is reached regarding the evaluation of p16INK4a staining, the biomarker needs to be evaluated in various clinical settings addressing specific clinical questions.

DIGENE HPV GENOTYPING LQ-TEST: HIGH-THROUGHPUT GENOTYPING USING XMAP TECHNOLOGY

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Background: Eighteen mucosal HPV types have been classified as high-risk (HR) or probably HR (i.e., HPV16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82). Recent evidence suggests a different oncogenic potential among HR HPV types (most importantly HPV16, 18 and 45), indicating the importance of HPV genotyping following universal HPV testing.

Objectives: The goal of this study was to evaluate the performance of the novel xMAP bead-based digene HPV Genotyping LQ Test (digene LQ Test) for the genotyping of 18 (probably) HR HPV genotypes, in comparison to the established inhouse reverse line blot (RLB) assay using PCR products generated with the clinically validated GP5+/6+-PCR test.

Methods: For this purpose, GP5+/6+-PCR amplimers generated from 434 digene High Risk HPV HC2 DNA Test (HC2)-positive and 95 HC2-negative cervical smears were used. Both the digene LQ Test and the RLB assay were performed on the same amplimers.

Results: Overall, the digene LQ Test showed an equivalent detection rate of HR HPVs compared to the RLB assay ($\kappa = 0.875$) in the series of GP5+/6+-PCR amplimers from the 434 HC2-positive samples. At the genotyping level (i.e. comparison for 18 (probably) HR HPV types), both tests were also highly concordant (overall $\kappa = 0.956$). The digene LQ Test following GP5+/6+-PCR showed positivity for one or more (probably) HR HPV type(s) in 86% of the HC2-positive women.

Conclusions: The high-throughput digene HPV Genotyping LQ Test showed a high genotyping agreement with the established RLB assay. Consequently, this novel assay following GP5+/6+-PCR could well be used in large cervical screening programmes as a reflex test for women who are HC2-positive to identify the genotypes of HR HPV infections in a high-throughput setting.

POSTER ABSTRACTS SESSION 26

POSTER SESSION III TUESDAY 20.00: ODD NUMBERS

POSTER SESSION IV

TUESDAY 21.00: EVEN NUMBERS

IMPROVED SPECIFICITY OF HYBRID CAPTURE PROBES

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Background: Hybrid Capture technology is a robust and sensitive method used to detect the presence of viral or bacterial DNA in clinical specimens. The technology relies on the hybridization of complementary RNA probes to target DNA and proprietary DNA:RNA hybrid-specific antibodies to capture and detect the target by means of an amplified chemiluminescent signal. Long in vitro transcribed RNA is the standard probe for this technology, and provides excellent clinical sensitivity. However, in applications where the target genomes are highly homologous to other related genomes, discriminating between these genomes can be challenging.

Objectives: We aimed to develop a set of probes for the Hybrid Capture technology, that still enabled sensitive detection of a set of target genomes, while also achieving excellent specificity against even very similar related species.

Methods: 15 high-risk human papillomavirus (hrHPV) types were chosen as our model system, to test simultaneous detection of multiple targets that could be distinguished from highly homologous low-risk HPV types. Individual synthetic oligoribonucleotides (synRNA) were bioinformatically designed to bind only to specific regions of the target genomes, and tested with Hybrid Capture technology.

Results: A synRNA probe mixture of 2,007 short type-specific probes for the detection of 15 hrHPV types was able to detect 5,000 copies/assay of each target genome. There was no significant cross-reactivity against up to 10^8 copies of 24 individual low-risk HPV types. This synRNA probe set has analytical sensitivity and dynamic range similar to that of in vitro transcribed probes. Compatibility with HPV positive and negative clinical specimens was demonstrated.

Conclusions: We demonstrate excellent analytical specificity of Hybrid Capture technology through the use of bioinformatically designed synthetic RNA probes. While HPV genomes were used as the model for this system, synRNA probes could be designed for the capture and detection of any DNA target.

P-26.011 MULTIPLEX GENOTYPING OF HUMAN CUTANEOUS A, M AND N PAPILLOMAVIRUSES

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Cutaneous papillomaviruses of the genus α (species 2 and 4), μ and n are associated with benign plantar, common, and flat skin warts both in the general population and in organ transplant recipients. Moreover, some of these types have also been detected in head and neck, and anogenital lesions. Recently, we have developed Multiplex HPV Genotyping (MPG), a reproducible, sensitive and highly specific high-throughput procedure for the identification of all 15 high-risk, three putative high-risk and the 6 most prevalent low-risk genital HPV genotypes in a single reaction (Schmitt et al., 2006, 2008, J. Clin. Microbiol).

Based on this work, we designed a new assay for the genotyping of cutaneous HPV types 1, 2, 3, 6, 7, 10, 27, 28, 29, 40, 41, 57, 63, 77, 91 and 94. Upon amplification of HPV L1 gene sequences by a novel broad-spectrum PCR, the biotinylated products were subsequently detected with 16 type-specific oligonucleotide probes covalently coupled to distinct sets of fluorescence-labelled polystyrene beads (Luminex technology).

In total, 10 forward and 8 biotinylated backward primers targeting the L1 region enabled the sensitive amplification of these types with 10 to 1,000 viral genome copies in the presence of 100 ng of human placental DNA. Additionally, a beta-globin PCR and hybridisation probe were integrated into the α , μ and n cutaneous genotyping assay without affecting the analytical sensitivity of the HPV PCR. Detection by Luminex proved to be highly specific. Results of a validation study comprising DNA from 71 skin warts will be presented.

PERFORMANCE OF APTIMA HPV ASSAY IN REFERRAL POPULATION

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Background-The performance of the APTIMA HPV (AHPV) Assay, which detects E6/E7 mRNA from 14 high-risk (HR) HPV types, was compared to the Qiagen Hybrid Capture 2 (HC2) assay in a Referral sample set. AHPV/HC2 discordant results were resolved by testing with a reverse-transcription PCR (RT-PCR) sequencing assay specific for E6/E7 mRNA from 14 HR HPV types. Methods-A RT-PCR sequencing method was developed to use residual PreservCyt liquid-based cytology (LBC) samples. In feasibility testing using referral samples, very good agreement between RT-PCR and AHPV assays was obtained (Kappa = 0.84; 95%CI: 0.66-1). A larger set of LBC Referral samples with available cytology (N=121) and histology (N=41) information was then tested with the AHPV, HC2 and RT-PCR assays. Results-Compared to HC2, the AHPV assay recorded fewer positives in ASCUS, LSIL and HSIL samples, with positive agreements of 59% (95%CI: 43.3-73.7%), 62.2%(95%CI: 46.5-76.2%), and 94.1% (95%CI: 71.3-99.9%), respectively. Of the 36 HC2+/AHPV-discordants in these categories, 100% were negative in the RT-PCR assay. For the 33 samples with abnormal histology diagnosis (CIN1+), the AHPV assay had 96.6% positive agreement (95%CI: 82.5-99.9%) and 100% negative agreement (95%CI: 39.8-100%) with HC2. For the one HC2+/AHPV- sample identified (= CIN1), RT-PCR was negative. Both AHPV and HC2 assays detected 100% of CIN2+ samples (n=19). Conclusion-Use of RT-PCR sequencing to evaluate discordant results between the RNA-based AHPV assay and the DNA-based HC2 assay confirms the accuracy of the lower reactive rate of AHPV in low-grade cytology- and histology-diagnosed Referral samples. Compared to HC2, these results support an equivalent sensitivity and an improved specificity of the AHPV assay for detecting HPV-induced cervical disease.

P-26.013

MULTIPLE HPV INFECTIONS AND GENOTYPE ATTRIBUTION IN CERVICAL CANCER PROGRESSION

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Background: Determining the causal attribution of HPV genotypes to cervical disease is important to estimate the effect of HPV vaccination and to establish a type spectrum for HPV-based screening.

Objectives: We analyzed the prevalence of HPV infections and their attribution to cervical disease in a population of 1670 women referred to colposcopy for abnormal cytology at the University of Oklahoma.

Methods: HPV genotyping was performed from cytology specimens using the Linear Array assay that detects 37 HPV genotypes. We used different methods of type attribution to revised cervical disease categories.

Results: We found a very high prevalence of multiple HPV infections with up to 14 genotypes detected in single specimens. In all disease categories except for cancers, there was a significant trend of having more infections at a younger age. We did not see type interactions in multiple genotype infections. HPV16 was the most frequent genotype at all disease categories. Based on different attribution strategies, the attribution of vaccine genotypes (6, 11, 16, 18) ranged from 50.5% to 67.3% in cancers (n=107), from 25.6% to 74.8% in CIN3 (n=305), from 15.2% to 52.2% in CIN2 (n=427), and from 6.6% to 26.0% in <CIN2 (n=708). In the HSIL cytology group (n=651), attribution ranged from 26.1% to 64.7%.

Conclusions: The attribution of vaccine types to HSIL was substantially higher compared to the lower cytology categories. The potential range of HPV genotype attribution is wide at the disease categories <CIN2 to CIN3. Genotyping from cervical lesions and analyzing viral oncogene expression may improve estimates of HPV genotype attribution.

FTA CARD FOR CERVICAL CELL COLLECTION FOLLOWED BY HPV TYPING

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BACKGROUND: Many HPV tests rely on viral genome detection by nucleic acid hybridization or PCR from extracted DNA. FTA elute micro cardTM (Whatman) enable the collection, transport and archiving of nucleic acids from several biological sources. The sample matrix consists of paper chemically treated with bactericidal, fungicidal, and virucidal activity, to protect the DNA from degradation. Indicating FTA elute card incorporates a color indicator, identifying the location of the sample deposit by means of a color change.

OBJECTIVE: We have optimized a simple, cost-efficient, method based on these FTA elute micro cards TM for collection of cervical epithelial cell samples, suitable for subsequent analysis of human papillomavirus (HPV) by real-time PCR. METHOD: Cervical cells are collected with a standard cytobrush and applied to the FTA card. Cells are retrieved from the card by collecting 6 punches (3 mm ø) and the DNA eluted with dH2O. By using HPVIR real-time PCR assay for genomic DNA quantitation and detection of high-risk HPV this method was first optimized by comparing 50 cervical cell samples applied to the regular FTA elute micro card TM with DNA isolated from the cytobrush. This method was then validated in 106 cervical cell samples using the indicating FTA elute micro card TM.

RESULT: The agreement between FTA and cytobrush samples was excellent (94%) (Kappa = 0.88).

CONCLUSION: The indicating FTA elute micro card TM represent a suitable medium for collection of cervical cell samples. The method is especially attractive when specimens have to be transported to a central laboratory for diagnosis of HPV. The procedure for DNA retrieval and molecular typing of HPV is amendable to automation, making it suitable for large scale testing of samples from follow-up screening of women.

P-26.015

DISTRIBUTION OF HPV GENOTYPES IN CERVICAL SPECIMENS FROM DANISH WOMEN

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Background: Human papillomavirus infection is an important public health problem. Genotypes 16 and 18 are associated with ca. 70% of cervical cancers; however, the frequency of other genotypes among patients with cervical HPV infections is high.

Objectives: The aim of the study was to determine the distribution of HPV genotypes among our patients.

Methods: Cervical specimens were analysed for HPV DNA using a microarray (ClinicalArraysHPV, Genomica, Spain), that allows for simultaneous detection of 35 different HPV genotypes (HPV-6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 68, 70, 71, 72, 73, 82, 82, 83, 84, 85, and 89).

Results: We here present the HPV genotype distribution among our patients in general and for a subset of women diagnosed with CIN2+. We also show how the frequency of multiple types varies according to age. Women under 30 years of age were more often infected with multiple types than older women, and certain types were more frequently found in women of younger age. Women with CIN2+ were mostly detected with one or more of the five most common high risk types: HPV-16, HPV-18, HPV-31, HPV-33, and HPV-51; however, high risk types HPV-58, HPV-52 and HPV-45 were also frequent. Conclusions: Multiple infections with HPV are extremely common among women in all age groups and for women with or without the diagnosis of CIN2+.

HPV DNA GENOTYPES AND HPV-33 SEQUENCE VARIATIONS IN MONGOLIA

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Objective: Mongolia is one of the countries with high prevalence of cervical cancer. According to the statistical data of last 5 years, the incidence of cervical cancer has registered as 30.6 cases per 100.000 women with age 15 and above in average. The prevalence of high risk HPV genotypes and their variations are correlated to HSIL and invasive cervical cancer.

Materials and methods: HPV DNA chip microarray kit (Bio-Core, Korea) was used for detection of HPV types in 66 invasive cervical cancer patients. In 13 HPV-33 positive cases LCR, E6 and E7 gene variants were determined by direct sequencing.

Results: In overall 61 cases (92.4%) were HPV DNA positive. Among them 80% (49/61) were single infections, 20% (12/61) were co-infections of high or low risk types. HPV-16 was the most common (54%) type followed by HPV-58 (13%), HPV-18 (11.5%), HPV-33 (11.5%) and HPV-39 (4.9%). HPV-31, 35, 52, 59 types were detected in two cases and HPV-26, 43, 61 were detected in one case, respectively. In four cases we could not determine HPV types due to the specificity of the test kit used.

Four variants of HPV-33 were detected by the sequencing analysis. The LCR17-E6-6- E7-0 variant was in 10 out of 13 samples (76.9%). The LCR0-E6-6-E7-0, LCR5-E6-6- E7-0 and LCR17-E6-0-E7-0 (MT17-0-0) variants were detected in one case, respectively.

Discussion: The HPV-16 was the most common genotype among Mongolian woman with cervical cancer followed by HPV-58. This is the similar pattern as in other Asian countries. We have detected three novel sequence variations of HPV-33 in Mongolians that were not reported previously.

P-26.017

QUALIFICATION OF LASER-CAPTURE-MICRODISSECTION TO INCREASE SPECIFICITY OF HPV DETECTION

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Background: Accurate assignment of human papillomavirus (HPV) genotypes to cervical lesions in clinical biopsies is crucial in vaccine efficacy studies. Laser-Capture-Microdissection (LCM) is a technique for isolating specific cell populations from a tissue section and is compatible with a variety of tissue types and staining methods (Haematoxylin Eosin, P16).

Objective: To qualify the LCM technology for the detection of HPV DNA in cervical epithelial cells of CIN lesions and cervical carcinoma in formalin-fixed, paraffin-embedded biopsy specimens.

Materials and Methods: LCM (P.A.L.M., Zeiss) of selected cells from either HE or P16 stained tissue sections were analyzed by the broad-spectrum HPV SPF10 PCR/ LiPA25 HPV genotyping system version 1. HPV genotyping results obtained from LCM-isolated lesions were compared to those obtained from whole tissue sections. The precision, sensitivity, accuracy and specificity of the methodology were analyzed in a series of qualification experiments.

Results: Precision and sensitivity of the LCM technique was determined based on repeated LCM experiments on cultured HPV16 or HPV 18 positive NIKS cells. The sensitivity was 100% when ~8-16 NIKS cells were captured, containing ~60-80 HPV copies. Sensitivity, accuracy and specificity of the HPV LCM/PCR procedure were also determined on clinical samples. The sensitivity was influenced by a variety of technical and biological parameters. LCM areas as small as 8000 µm2 were generally HPV positive, and could consistently be assigned to a HPV genotype matching a genotype detected in total biopsy analysis.

Conclusion: LCM on HE- and P16-stained CIN biopsy sections, using a previously validated HPV DNA PCR testing algorithm reveals that the LCM/PCR technique can be reliably applied in cervical biopsy specimens, and offers a far more accurate method compared to the whole tissue section analysis for assignment of a HPV type to a CIN lesion.

SIMULTANEOUS MLPA ASSESSMENT OF HPV-TYPE, LOAD, INTEGRATION AND CHROMOSOMAL ABERRATIONS.

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Background: Oncogenic human papillomavirus (HPV) is the most important risk factor for cancer of the uterine cervix. Viral load has been associated with persistence of infection, while integration of HPV into the host cell genome is associated with transition to invasive disease. Viral integration is frequently correlated with loss of viral E2 and gain of the telomerase-related genes TERC and TERT.

Objective: To develop a new, rapid, and sensitive multiplex ligation-dependent probe amplification (MLPA) assay for the simultaneous analysis of viral load, integration, and copy number gain of TERC and TERT in HPV 16/18 related lesions. Methods: The performance of the assay was tested for HPV loads ranging from 0.2-200 copies per cell, and for percentages of integration ranging from 0%-100% in model systems as well as clinical samples. The model systems included plasmid mixtures as well as the HPV positive cell lines SiHa, HeLa, and CaSki. The clinical samples consisted of cytological (n > 90) as well as histological samples (n > 70) comprising different (pre)malignant stages. The MLPA data from the clinical samples were compared to the results obtained with the E2/E6 quantitative PCR and two HPV typing assays.

Results: In samples with low viral load MLPA analysis revealed that: 1) viral integration can be reliably determined when more than 20% of the virus is integrated, 2) gain of the telomerase-related genes in the cell lines was in accordance with data reported in the literature and 3) in the clinical samples a higher sensitivity and specificity compared to the other tested assays was obtained.

Conclusions: Our study demonstrates that by means of a single MLPA-reaction viral type, load, integration, and gain of TERC and TERT can be reliably determined. Simultaneous assessment of these parameters may improve risk assessment for patients suspected for HPV infection.

P-26.019

E6 BASED RAPID DIAGNOSTIC TEST FOR CERVICAL PRE-CANCER AND CANCER

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In developing countries, cervical cancer is a leading cause of cancer related death of women, due to the lack of implementation of screening tests for cervical pre-cancer and cancer. A screening test for low resource settings should be simple, rapid, and cost effective, and ideally, such a test would be informative regarding HPV oncogenic activity.

Expression of both HPV E6 and E7 oncoproteins is essential for cervical cell transformation to occur. Results from a collaborative clinical study conducted previously by AVC, PATH, and the Chinese Academy of Sciences demonstrated a correlation of E6 oncoprotein positivity with both severity of current cervical histopathology and risk for progression. Hence, E6 oncoprotein promises to be an appropriate marker of HPV oncogenic activity.

Arbor Vita Corporation (AVC), in collaboration with PATH, has developed a rapid diagnostic test ("E6 Strip Test") that detects E6 oncoprotein from cervical swabs. The test uses a recombinant PDZ domain protein for specific capture of high-risk HPV-E6 oncoprotein, taking advantage of the finding that high-risk HPV-E6 proteins, but not low-risk HPV-E6, bind to cellular PDZ domains.

The current prototype detects E6 oncoprotein of HPV types 16, 18, and 45 on one test strip using a pooled detector system. The final test will enable detection of E6 oncoprotein of the seven HPV types most prevalent in cervical cancer. Analytical sensitivity of the current prototype is <10 pg E6 oncoprotein. Performance data of the E6 Strip Test prototype from a pilot study using a set of clinical cervical swab samples of confirmed pathology (normal, CIN1, CIN3, cancer) will be presented.

DOES RNA TESTING DETECT CLINICALLY SIGNIFICANT HPV INFECTION?

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Background: There is evidence to suggest that the detection of oncogenic transcripts: E6/E7 may be more accurate for the identification of clinically significant cervical HPV infection compared with DNA (L1) based assays. However more clinical data are needed to support this contention.

Objectives: To compare the clinical sensitivity and specificity of the APTIMA[®] HPV RNA based assay (AHPV, Gen-Probe Incorporated) with the Hybrid Capture 2 DNA based assay (HC2, Qiagen Ltd). Although our study is designed to be longitudinal (in order to assess prospective sensitivity and specificity) - we present the initial, cross-sectional data.

Methods: Women attending two NHS colposcopy clinics in two city hospitals in the UK were invited to participate. Liquid based cytology (LBC) samples were collected and tested via the 2 HPV assays described. Biopsies were taken where clinically indicated and clinical sensitivity and specificity of each assay for disease (defined as CIN2 or worse) were calculated.

Results and conclusions: A total of 532 women have been recruited to the study so far. At time of abstract submission, 387 LBC samples (from 385 women) have been tested by both assays and have associated, confirmed pathology results – analyses are based on this subset.

Overall, concordance between the two tests was 91%; 95% for CIN2+ and 88% for CIN1 or less. Sensitivity and specificity of the AHPV for CIN2 or worse were 97% and 60% respectively. By comparison, sensitivity and specificity of the HC2 for CIN2 or worse were 95% and 52% respectively. These preliminary data suggest that APTIMA and HC2 assays show equivalent sensitivities for the detection of CIN2 or worse (in a high-prevalence population) with the APTIMA displaying slightly higher specificity. Further data and associated 95% CIs will be presented.

P-26.021

CLINICAL PERFORMANCE OF THE ABBOTT REALTIME HIGH RISK HPV ASSAY

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Objectives: The fully automated Abbott RealTime High Risk (HR) HPV assay detects fourteen HR HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68), and types HPV 16 and HPV 18 in a single reaction, eliminating the need for HPV 16/18 reflex testing. This study evaluates clinical performance of the Abbott RealTime HR HPV, and Hybrid Capture 2 (HC2) assays in a colposcopy referral population.

Methods: Abbott RealTime HR HPV assay is performed on the m2000 System that automates DNA isolation, PCR plate assembly, amplification, detection and result reporting. It amplifies the conserved L1 region of HPV targets and a beta globin sequence with modified GP5+/6+ primers and internal control primers, respectively. The detection of HPV 16, HPV 18, Other HR HPV (non-HPV 16/18) and beta globin is achieved with target-specific probes labeled with different fluorophores allowing these signals to be distinguishable in a single well. In this study, 851 cervical specimens from a colposcopy referral population were collected in PreservCyt and were tested by the Abbott RealTime HR HPV and HC2. The clinical sensitivity and specificity for detection of HR HPV and for detection of disease (CIN2+) were determined for both assays.

Results and Conclusions: The sensitivity for detection of disease (CIN2+) was 97.3 % with RealTime HR HPV and 96.0% with HC2. The specificity in this colposcopy referral population was 36.0% with RealTime HR HPV and 38.2% with HC2. For detection of HR HPV, the sensitivity was 98.5% with RealTime HR HPV and 93.5 % with HC2, and specificity was 98.6% with RealTime HR HPV and 90.5% with HC2.

VAGINAL HPV INFECTIONS AND GENOTYPE DISTRIBUTION IN 744 DANISH WOMEN

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Objectives: Use of microarray technology (MA) in clinical Human Papiloma Virus (HPV) diagnostics offers simultaneous diagnostic capability along with genotyping of specific genotypes. Methods: HPV-DNA microarray analysis was performed on 744 individual, clinical vulvar and vaginal samples from women with indications consistent with HPV infection using a low-density HPV MA system (Genomica S.A.U., Spain) containing specific probes for 35 mucosal HPV types.

Results: Of the 744 vulva and vaginal samples, overall 38% were positive for one or more HPV types. The age group of <25 years had the highest incidence of HPV infections with 49% of all samples being positive for HPV, whereas women 25-30 years, women 31-45 and women >45 had incidence rates of 46%, 28% and 30%, respectively. Most commonly seen HPV genotype was type 16 (21%-27%) followed by type 53 (11%-19%) across the age groups. Genotypes 6 and 11 were not represented in the top 5 most common HPV types in any of the groups, and type 18 was only seen in the top 5 amongst women >45 years (5.8%). Women <25 years had the highest incidence of multiple HPV infections (47%), whereas 17% of the women age >45 years were found to have 2 or more simultaneous HPV genotypes. The most commonly diagnosed HPV type 16 was predominantly found as a single infection (61% single infections versus 39% multiple infections). However, the multiple infections including HPV 16 showed co-infection with HPV 51 (34%), HPV 53 (29%), HPV 31 (20%) and HPV 18 (17%), indicating that specific co-infection patterns can be discerned from a broader study, eventually leading to better risk estimates.

Conclusion: Use of HPV-DNA MA technology greatly improves the diagnostic power with regard to determining single as well as multiple HPV infections associated with vulvar and vaginal disease.

P-26.023

LABEL-FREE BIOSENSORS: A FUNCTIONALIZED NANOPIPETTE FOR ANTIGEN-ANTIBODY DETECTION

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We have developed a sensitive, label-free biosensors adapting our nanopipette technology to detect Human pappilomavirus (HPV) proteins such as HPV's E6, E7 and L1. The dimensions of our nanopipette, comparable to or slightly bigger than those of proteins and

macromolecules, make it suitable for sensing antibody/antigen in general. Molecular interaction taking place on the nanopipette tip is transduced to electrical signals based on changes in size, electrical charges and structures of the nanoscopic pore region.

Here we demonstrate protein detection of HPV E6 and E7 oncoproteins at low concentration levels and with a dynamic range of more than x decades. The multi-analyte ability, sensitivity, scalability, and ease of use of the nanopipette-based protein assay technology make it a strong contender for versatile and sensitive molecular diagnostics in both research and clinical settings.

OPTIMISATION OF HPV DETECTION FROM DIFFERENT CLINICAL SAMPLES

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Background and objectives: Human papillomaviruses(HPV) are the aetiological agents of certain benign and malignant tumours of skin and mucosae, the most important of which is cervical cancer, however, the incidence of ano-genital warts, HPV-anal cancer and oro-pharyngeal cancers are also rising. We have directly compared three of the most commonly used primer sets in detection of HPV from different clinically diagnosed samples, to ascertain an optimal detection protocol. Study design: We compared the PGMY09/11, MY09/11 and GP5+/6+ primers in direct comparative PCRs of 35 clinico-pathologically diagnosed samples of genital warts, vulval biopsies and cervical brushings from 30 patients. All negative samples were subsequently tested using the previously reported PGMY/GP system and products directly sequenced for confirmation and typing.

Results: HPV detection rates for cervical samples showed that the primers GP5+/6+ (80%) and MY09/11 (90%) compared favourably with the PGMY09/11 (100%) primers. Genital wart samples showed inadequate detection rates with MY09/11 (64.3%) and GP5+/6+ (64.3%) compared to PGMY09/11 (92.9%). Detection of HPV from vulval biopsies were inadequate with all three primer sets, MY09/11 (63.6%), GP5+/6+ (54.5%) and PGMY09/11 (54.5%) performing poorly. Subsequent nested PCR with PGMY/GP demonstrated that HPV was indeed present at low copy number, and direct sequencing confirmed genotype.

Conclusions: PGMY09/11 primers are the preferred primer set for primary PCR screening with different clinical samples. MY09/11 and GP5+/6+ may still be used (particularly for cervical samples) but demonstrate lower detection rates. A nested PCR approach (i.e. a PGMY/GP system) is mandatory for all initially negative samples, with subsequent confirmation by genotyping

P-26.025

EVALUATION OF THE PERFORMANCE OF NOVEL HPV 4 ACE TEST

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Background: Detection and selective genotyping of human papillomavirus (HPV) is crucial for discriminating women who are at high-risk for progression to cervical cancer from whom are likely to regress.

Objectives: The aim of this study was to compare the novel HPV detection method; HPV 4 Auto-Capillary Electrophoresis (ACE) test with Hybrid Capture (HC) 2 assay for the detection of high-risk HPVs. In addition, we compared the HPV 4 ACE test with the polymerase chain reaction (PCR) HPV Typing Set test for the detection of HPV 16 and 18 genotypes. Methods: 199 cervical swab samples obtained from women with previous abnormal Pap smear were used for above 3 HPV

tests. HPV 4 ACE test used newly developed dual priming oligonucleotide system to minimize the risk of non-specific priming. It can detect HPV 16 and HPV 18 genotypes and also identify other 11 high-risk HPVs.

Results: HPV 4 ACE test and HC 2 assay showed substantial agreement for the detection of high-risk HPVs (85.4%, kappa=0.71). HPV 4 ACE test also showed substantial agreement with the PCR HPV Typing Set test in the detection of HPV 16 and HPV 18 genotypes (89.9%, kappa=0.65). In correlation with cytological results, the sensitivity and specificity of HPV 4 ACE test and HC 2 assay were 92.9% versus 92.9% and 48.1% versus 50.8%, respectively when high grade squamous intraepithelial lesion was regarded as an abnormal cytology.

Conclusion: A novel HPV 4 ACE test has proven to be a valuable tool for the detection of high-risk HPVs and for genotyping of HPV 16 and HPV 18.

HPV GENOTYPE PREVALENCE AMONG AUSTRALIAN WOMEN PRIOR TO VACCINE IMPLEMENTATION.

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Background. Infection with high-risk (HR) HPV is the major causative factor for carcinoma of the cervix. Vaccines targeting specific oncogenic-type HPV infections have been produced, and regimes implemented in Australia to reduce incident infections. Objectives. To determine the prevalence of HPV genotype infections prior to vaccination in Australian women by ascertaining genotype distribution across: age group; Indigenous status; Pap smear status; and region of residence.

Methods. Women attending for routine Pap smear (April 2005 - Feb 2008) were invited to participate (N = 2500: 18-40y; 500: >40y). Aliquots of Thin-Prep specimens were genotyped using the Roche HPV LINEAR ARRAY test.

Results. HPV prevalence and genotype distribution were stratified by age group, Indigenous status, state/region and Pap prediction. Overall, the three most detected genotypes were HPV16 (14.3%), 51 (8.7%) and 53 (8.1%). There was no significant difference in HR-HPV positivity between Indigenous (33.0%) and non-Indigenous (29.9%) women <40y (P = 0.100). Indigenous women had higher rates of HPV detection (49.6%) than non-Indigenous women (42.0%) for <40y (P < 0.001). There was no difference in prevalence of vaccine-specific genotypes by Indigenous status among women <40y. HPV positivity, for HR and vaccine preventable types (6/11/16/18), decreased with age.

Conclusions. The cross-sectional prevalence of HR-HPV infection is high in women undergoing Pap smear testing, regardless of Indigenous status. HPV infection is very common in sexually active Australian women and extremely common in young women. HPV16 and/or 18 were found in over 40% of HR-HPV positive women, suggesting vaccination should significantly reduce infection with these oncogenic types, thereby of benefit to Australian women. The association of other risk factors such as smoking status, contraceptive use and area of residence is being evaluated and will be presented.

P-26.027

IMMUNOASSAYS FOR DETECTION OF HPV E6/E7 ONCOPROTEINS IN CERVICAL CANCER

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Overexpression of E6 and/or E7 oncogene is required for degradation of p53 and pRb, to result in malignant transformation of cervical epithelium. Detecting HPV infection by nucleic acid methods could not differentiate the diagnosis of LSIL from HSIL, nor CIN lesions from non-transforming latent or remissive viral infection. Thus, there is a need to develop immunoassays for direct detection of HPV oncoproteins in various precancerous or cancer specimen. The objectives of this study are to 1). detect HPV E6 and E7 proteins expressed in cervical cancer cell lines using polyclonal and monoclonal antibody we generated specific to the HPV oncoproteins. 2). develop immunoassays for detection of HPV E6, E7 oncoproteins using pap smear samples (liquid based). 3). compare the results of HPV DNA test, immunoassays, and clinical diagnosis (histology or pap smear results). Our preliminary results show that HPV E6 and E7 oncoproteins are detected from cervical cancer cell lines such as HeLa (HPV18), Caski (HPV16), but not in CA33 (HPV negative) by western blot using the specific HPV antibody we generated. EIA data indicate direct detection of HPV E7 oncoprotein from various stages of cervical scrape samples. Compared EIA results to PCR, high correlation was found in these two methods. Immunocytochemistry (ICC) assay also demonstrates the overexpression of E6 and E7 oncoproteins present in the nuclear and/or cytoplasm of abnormal cells from various stage of liquid based cervical scrape samples. Therefore, various formats, high throughput, user friendly immunoassays can be further developed.

HPV-GENOTYPING WITHIN NTCC: IS IT POSSIBLE TO INCREASE HPV-TEST SPECIFICITY?

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Background: Hybrid Capture 2 (HC2) is targeted to detect infection by HPV-types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68.

Objectives. Defining the proportion of HC2 positive women negative to the targeted HPV-types.

Methods. In NTCC trial women in experimental arm were tested by HC2. During a first phase the ThinPrep and in a second phase Standard Transport Medium (STM) were used. Residual cells were stored at -20°C. On samples HC2-positive we performed PCR by GP5+/GP6+ consensus primers and typing by reverse line blot hybridisation. Reciprocally adjusted Odds Ratios (ORs) for the presence of HPV-types targeted by HC2 were obtained by multiple logistic regression.

Results. Of 2921 samples 2208(75.6%) were found to be positive at line blot for HC2-targeted HPV-types. 23 further cases with infection by HC2-targeted types were detected by sequencing and 38 by reverse line blotting after nested PCR. We detected only high-risk HPV types not targeted by HC2 in 272 samples (9.3% of HC2 positives), only low-risk or unidentified HPV types in 91(3.1%) and no HPV in 289(9.9%). The most common non-HC2 HPV-types detected in absence of HC2-types were HPV66, 53, 70, 67, 82 and 73, representing 4.4%, 1.9%, 1.3%, 0.9%, 0.4% and 0.3% respectively. The probability of detecting an HC2-targeted HPV-type significantly increased with increasing RLU (p<0.001) and was higher with STM than with ThinPrep (OR 1.42;95%CI 1,18-1.72). Some 5.5% of CIN2+ identified among studied HC2-positive women were in women without HC2-types detected (8 CIN2 and 3 CIN3+). On the other hand, if women without HC2-types were not referred PPV would have increased from 6.9% to 8.3%.

Conclusions. Using the STM and applying higher RLU as cut-off will decrease the probability of a false positive HC2-test.

P-26.029

SELECTION OF MABS DETECTING E7 IN SOLUTION AND FIXED CELLS

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Background: The E7 oncoprotein is expressed early in the malignant transformation of cervical epithelial cells and is considered a promising marker for early detection of cancer. For diagnostic use, detection of E7 with immunocytochemistry (ICC) could be a complement to the currently used Pap smear technique. Ultimately, the antigen could be detected in lysed cell scrapes by an immunoassay, replacing the need for cytology based methods, and/or for sero-immunological monitoring of treatment and relapses. Both approaches would require sets of highly specific immunoreagents.

Objectives: To establish highly specific monoclonal antibodies (MAbs) for detection of HPV16 and HPV18 E7 in fixed cells using ICC and for development of immunoassays detecting the antigen in solution.

Methods: Balb/c mice were immunized with soluble and formalin-fixed E7 protein and B-cells were fused to myeloma cells according to standard techniques. Hybridomas were screened with regard to the intended use. For immunoassays, MAbs were screened for reactivity to soluble E7 and MAbs with low koff values were selected using Quatz Crystal Microbalance (QCM) technology. Potential sandwich pairs were chosen by epitope mapping with phages displaying overlapping E7-fragments. Selection of ICC MAbs was done by testing for reactivity against immobilized formalin-fixed antigen and formalin-fixed E7 positive cancer cell lines.

Results: Sandwich immunoassays with high sensitivity for HPV16 and HPV18 E7 were constructed with MAbs selected against antigen in solution. The assays were highly specific with no cross-reactivity to low-risk HPV 1, 6 or 11 E7. Several MAbs selected on fixed recombinant antigen showed specific staining of E7 positive cancer cell lines in ICC.

Conclusion: MAbs detecting E7 in ICC or ELISA were established, by choosing appropriate immunization and screening methods. MAbs selected for one of the applications usually showed poor functionality in the other, further stressing the importance of using hybridoma screening methods resembling the intended use.

A MODIFIED PCR SYSTEM FOR DETECTION OF MULTIPLE HPV-TYPES

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Background: Human papillomavirus (HPV) infection is a necessary cause of cervical cancer and cervical dysplasia. Accurate and sensitive genotyping of multiple oncogenic HPVs is essential for a multitude of both clinical and research uses.

Objective: We developed a modified general primer (MGP) PCR system with 5 forward and 5 reverse consensus primers. Methods: The MGP system was compared to the classical HPV general primer system GP5+/6 using a proficiency panel with HPV plasmid dilutions as well as cervical samples from 592 women with low grade cytological abnormalities. Results: The reference method (GP5+/6+) had the desirable high sensitivity (5 copies/PCR reaction) for 5 oncogenic HPV types (HPV 16, 18, 56, 59, and 66). The MGP system was able to detect all 14 oncogenic HPV types at 5 copies/PCR reaction. In the clinical samples, the MGP system detected a significantly higher proportion of women with >2 concomitant HPV infections than did the GP5+/6+ system (102/592 women as compared to 42/592 women). MGP detected a significantly increased number of infections with HPV 16, 18, 31, 33, 35, 39, 42, 43, 45, 51, 52, 56, 58, and 70 than GP5+/6+ did. Conclusions: The MGP primers allow a more sensitive amplification of most of the HPV-types that are established as oncogenic and had an improved ability to detect multiple concomitant HPV infections.

P-26.050

EVALUATION OF A NEW SCREENING BIOMARKER PANEL

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Primary HPV screening is an emerging concept, which may fundamentally influence cervical cancer screening, making possible to establish new high throughput automated screening methods in primary screening. Following this path, the combination of HPV testing with screening biomarkers could be the next major milestone. With the advent of a fully automated, low cost, biochemical screening method both the overall quality of the cervical screening programs could be improved and also the programs could be more accessible for women, especially in low resource countries.

We have developed a new gene expression, HPV combination panel for cervical screening. The genes were identified using TaqMan^{*} Custom Array on 300 patient sample, detecting expression of 190 preselected genes in duplicates (the overall number of screening PCR reactions were 114,000). Mathematical modeling revealed a classificatory algorithm which identified 8-10 marker genes, which were capable to produce ROC area better than 0.9 and classify the samples with high precision. The method was validated on a colposcopic referral population using PreservCyt liquid-based cytological specimens, giving 95% specificity and 70% sensitivity for CIN1+ histology. For CIN2+ (excluding CIN1 cases) the specificity and sensitivity is only slightly changed.

EVALUATION OF NOVEL MAB FOR THE DETECTION OF DYSPLASTIC CELLS

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Background: The sensitivity of a single Pap-smear for the detection of dysplasia and cervical cancer is poor. The detection of proteins characteristically over-expressed as a consequence of HPV-induced carcinogenesis can be of diagnostic value. We have identified a novel proliferation-associated marker, NET-1/C4.8, which is over-expressed in a subset of cervical precancers (CIN2/3) and in a high percentage of cervical squamous cell carcinomas. This protein is a member of the tetraspanin family whose typical structure reveals four transmembrane domains delimited by two extracellular regions. Tetraspanins are known to associate with interaction partners in membrane microdomains that provide a scaffold for the transmission of external stimuli to intracellular-signalling components

Objective: To evaluate monoclonal antibodies directed against C4.8/NET-1 for the detection of dysplastic cells in monolayer preparations (ThinPreps).

Methods: Cervical scrapes were obtained from women participating in regional cervical cancer screening programs. Of each cell suspension two monolayer preparations (ThinPreps) were prepared according to standard protocol (Cytyc Corporation). One was Pap-stained for routine cytology, the second slide was divided into two halves: One halve was stained for p16 over-expression (CINtec Cytology, MTM, Heidelberg), the other halve was stained for C4.8/NET-1 expression. p16 over-expression was used as an indicator for severe dysplasia. For each patient the HPV status and if indicated the histological diagnosis were also available.

Results/Conclusion: Two antibody clones directed against C4.8/NET-1 specifically stained dysplastic cells. In the majority of cases C4.8/NET-1 expression correlated perfectly with p16 over-expression. Discrepant cases showed either no p16 over-expression but C4.8/NET-1 expression or vice versa. To assess the usefulness of C4.8/NET-1 as a putative diagnostic/ progression marker, all of the above mentioned clinical parameters are relevant and need to be considered. Evaluation is still ongoing.

P-26.052

SIMULTANEOUS P16/KI-67 EXPRESSION AS HIGHLY SPECIFIC MARKER OF CERVICAL DYSPLASIA

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Background: Over-expression of p16 protein in cells with an intact cell-cycle regulation results in cell-cycle arrest. In contrast, p16 has been shown to be strongly over-expressed in cervical dysplastic cells which have undergone HR-HPV mediated cell-cycle dysregulation. As the co-expression of antiproliferative (p16) and proliferation markers in cells with intact cell cycle regulation should mutually exclude each other, we hypothesized that the co-localization of both detectable p16 and Ki-67 expression within the same cervical cell in cytology preparations may be used to identify cervical dysplasia without the need for morphological interpretation of immuno-reactive epithelial cells.

Objectives: Various clinical evaluation studies have been initiated to assess the performance of an immuno-cytochemical dual staining protocol simultaneously detecting both p16 and Ki-67 over-expression in cervical cytology specimens.

Methods: A dual staining immunocytochemistry protocol based on a newly developed reagent set for the simultaneous staining of slide specimens has been established and validated for its use on alcohol-fixed cervical cytology preparation. Using liquid-based cytology specimens from subjects attending Pap cytology based screening as well as from patients referred to colposcopy due to abnormal Pap cytology results, the sensitivity and specificity of the dual stain testing for the identification of high-grade CIN is evaluated.

Results: Preliminary data from the various studies indicate a high level of sensitivity and specificity of cervical cytology specimens positively tested for the presence of individual cells simultaneously expressing both p16 and Ki-67 biomarkers. Detailed results from the studies will be presented.

Conclusions: The detection of cells double-immunoreactive for both p16 and Ki-67 may allow to easily distinguish dysplastic cervical epithelial cells from non-dysplastic cells occasionally showing single expression of one of the biomarkers under normal physiological conditions, without the need for morphology interpretation.

P-26.053 HIGH-THROUGHPUT SAMPLE PROCESSING ON A NEW HYBRID CAPTURE® TEST PLATFORM

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Introduction: Specimens in PreservCyt^{*} (PC) medium are a challenge in the current HC2 assay due to the volume of sample required (4 mL) and the low throughput of manual sample conversion protocol. A prototype sample prep instrument was developed to produce up to ten 96-well plates of extracted DNA in less than 5 hours for subsequent analysis in a Next-Generation hybrid capture Assay (NGA, currently under development). The first fully processed plate through the system takes 59 minutes with subsequent plates available every 24 min. Our objective for this study was to compare manual nucleic acid extraction method from 1 ml of PC samples with the high-throughput automation method for recovery, carryover, throughput and reproducibility of HPV DNA processed.

Methods: In this study a prototype sample-prep instrument with proprietary DNA-extraction chemistry was used to automate the manual process of sample conversion. HC2-positive PC specimens were spiked into pooled HC2-negative specimens and replicate aliquots were processed in the sample prep processing instrument or manually. Recovery was determined by comparative HC2 signal output from each method.

Results and Conclusions: In two separate runs of a PC positive clinical pool the percent signal recoveries from the automated method were 99% (9% CV) and 93% (6% CV). Results from negative pools run through the automated system were also comparable to those manual method results: the RLU/CO of the manually processed clinical pools compared to automated-processed pools from three separate runs was comparable with good CV. In carryover measurements, the average RLU/CO difference between negative specimens that were processed before and after a high positive were minimal. Intra-plate and inter-plate reproducibility was very good with low CV. Ten plates of individual clinical PC specimens were successfully run through the automated system in a continuous fashion in just over 4.5 hours.

P-26.054

INNO LIPA AND LINEAR ARRAY FOR HPV IN ARCHIVAL TISSUE

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Background: With the recent introduction of the human papillomavirus (HPV) vaccine, it is important to accurately monitor HPV genotypes present in high grade lesions and cancers that have been associated with HPV. Detection of HPV genotypes in archival paraffin embedded biopsies poses a challenge as several factors influence quality of DNA, impacting the ability to amplify and further detect intact DNA regions. A sensitive and accurate method of detection of HPV genotypes in paraffin embedded biopsies is therefore important in understanding presence of high-risk HPV in such tissue. Objective: To evaluate and compare two commercially available HPV genotyping test i.e. the SPF10-INNO LiPA HPV gentoyping test (SPF10) and the Roche Linear Array HPV genotyping test (LA) for detection of HPV genotypes present in high grade vulvar intraepithelial neoplasia (VIN) and invasive vulvar cancer biopsies. Method: Overall, 21 histologically confirmed high grade VIN and 29 invasive vulvar cancer biopsies between 1 January 1996 - 31 December 2005, from women residing in the Northern Territory of Australia were obtained. Paraffin embedded biopsies were utilized to extract DNA followed by HPV genotyping on SPF10 and LA. Comparsion was limited to 25 HPV genotypes detected by both assays. Results: Initial LA testing adequately amplified 21 (42%) biopsies. Subsequent testing on SPF10 amplified all 50 (100%) biopsies. SPF10 detected 43 high risk HPV (HR HPV) infections compared to 21 infections on LA (P < 0.001). SPF10 detected 32 HPV 16 infections compared to 19 HPV 16 infections on LA (P= 0.009). Overall agreement between both assays for detection of HR HPV and HPV 16 was 97% ($\kappa = 0.656$) and 74% ($\kappa = 0.741$). Conclusion: SPF10 has greater sensitivity for detection of HPV genotypes in vulvar paraffin embedded tissue.

HPV GENOTYPE DISTRIBUTION ACCORDING TO SEVERITY OF CERVICAL NEOPLASIA.

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Objective. To study the HPV DNA genotype distribution and E6/E7 mRNA expression according to the severity of cervical neoplasia.

Methods. Included in the study were 643 Norwegian women (median age 35, range 17-76 years). Histology revealed CIN2 in 135, CIN3/ACIS in 484 and invasive carcinoma in 13 cases. HPV was detected with the L1 based PCR test Linear Array (Roche)(LA) which differentiates 37 HPV genotypes and PreTect HPV-Proofer (Norchip AS) which detects E6/E7 mRNA full-length transcripts from HPV16, 18, 31, 33 and 45.

Results. By LA, HPV16 was detected in 44.3% (285/643) followed by HPV31(14.8%), HPV33(12.3%), HPV52(9.8%) and HPV18(9.0%). HPV16 was more common among women with CIN3+ as compared to CIN2, odds ratio 1.96 (95% CI 1.30- 2.96) after adjustment for age (>30 years, yes/no). As a contrast, the adjusted odds ratios for having CIN3+ versus CIN2 among women positive for HPV39, 51, 52, 58 or low risk genotypes were, for all genotypes, less than 1.0 (from 0.37 (95% CI 0.21-0.66) to 0.58 (95% CI 0.36-0.94)). By using HPV-Proofer, the adjusted odds ratio of having CIN3+ versus CIN2 for HPV16 was 2.79 (95 % CI 1.80- 4.30). HPV18 and HPV45 were not associated with severity of cervical neoplasia, neither with HPV-Proofer nor LA. Multiple HPV infections were detected in 45.7% by LA. The most common pattern was co-infections with HPV16 and HPV31. By using HPV-Proofer, multiple HPV infections were detected in 5% of the women and the most common was co-infection with HPV16 and HPV33. Presence of multiple HPV infections was not associated with the severity of cervical lesion.

Conclusions. HPV16 was associated with more severe cervical lesion, with a two-fold estimated higher risk for CIN3+ versus CIN2 when the LA was used to detect HPV16 and almost three-fold higher risk when E6/E7 expression was used for detection of HPV16.

P-26.056

HPV16 LOAD AND INTEGRATION STATE AS BIOMARKERS FOR ≥CIN2?

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Background: High-risk HPV viral load and integration state have been suggested as clinically relevant risk markers for high-grade cervical intraepithelial neoplasia (CIN) and carcinoma.

Objective: This retrospective case-control study assessed HPV16 load and integration state as biomarkers for \geq CIN2 lesions in HPV16-positive women in a routine liquid-based cytology setting.

Methods: Triplex qPCR for HPV16 E6, E2 and β -globin was performed to determine the HPV16 load and the E2/E6 ratio, as a surrogate for integration, for 238 samples with a negative histological endpoint and 272 samples with a \geq CIN2 endpoint.

Results: Our analysis showed a significantly higher HPV16 load in the case group (median 79.12 E6/cell versus 43.21 E6/ cell for controls), which appeared to be completely attributable to the high viral load of samples with invasive carcinoma as histological endpoint (median 576.6 E6/cell). The E2/E6 ratio proved to be significantly lower for the cases (median 0.86 versus 0.94 for controls). However, the presence of HPV integration was established in a considerable amount of control samples (141/238), supporting the theory that HPV integration occurs very early in the development of cancer.

Conclusions: The intrinsic heterogeneous nature of the cervical cytology samples caused a substantial overlap of the HPV16 load and the E2/E6 ratio between controls and cases. This precluded the determination of cut-off values for risk prediction, as such limiting the clinical applicability of viral load and integration state as biomarkers for cervical disease.

MLPA AND FISH: RELIABLE APPROACHES TO IDENTIFY HPV-INTEGRATION IN PARAFFIN-TISSUE.

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Background: Oncogenic human papillomavirus (HPV) is an important risk factor for cervical and oropharyngeal cancers. Viral load has been associated with persistence of infection, while integration of HPV16 and 18 into the host cell genome is associated with transition to invasive disease. Viral integration is frequently accompanied with loss of the viral E2 gene. However, data on viral integration frequencies differ markedly, probably as a result of the applied methodology or used material.

Objective: To evaluate the reliability of a novel multiplex ligation-dependent probe amplification (MLPA) assay to detect viral integration based on a change in the viral E2/E6 copy number ratio and to compare the results with other integration detection methods.

Methods: DNA was isolated from paraffin embedded material of 18 HPV-positive carcinomas, of which viral integration data were available (amplification of papilloma virus oncogene transcripts [APOT] analysis on RNA from frozen tissue). Multicolor fluorescence in situ hybridization (FISH) was applied to identify 1) HPV integration as punctate nuclear signals and 2) co-localization with APOT-derived chromosomal loci of integration.

Results: Viral integration was detected by MLPA in 14 out of 18 tumors. In the remaining cases episomal HPV was identified, most probably due to high viral load and/or absence of deletion within the MLPA E2 target sequences. FISH confirmed integration in 13 out of 18 tumors and was inconclusive in the remaining cases (harboring low viral loads). Co-localization to APOT-identified loci was proven in 8 out of 10 cases.

Conclusions: MLPA and FISH confirm APOT results in most tumor cases and are a reliable alternative to the APOT assay for detection of HPV integration in paraffin embedded tissue materials.

P-26.058

COMPARING MRL TYPE-SPECIFIC MULTIPLEX HPV PCR WITH INNO-LIPA GENOTYPING ASSAY

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Background: Real Time type-specific Multiplex HPV PCR assays were developed by Merck Research Laboratories (MRL) to detect HPV DNA in samples collected for efficacy determination of the quadrivalent HPV (Types 6, 11, 16, 18) L1 VLP vaccine, GARDASIL^{*}. Multiplex (3 HPV-ORFs) or Duplex assays (2 HPV-ORFs) were developed to detect ten additional high-risk HPV types.

Objective: We evaluated concordance between MRL type-specific Multiplex HPV PCR and INNO-LiPA Genotyping assays for 14 HPV types common to both methods.

Methods: Genital swab and thinsection specimens were tested for presence of L1/E6/E7 ORF sequences of HPVs 6, 11, 16, 18, 31, 45, 52, and 58 and E6/E7 ORF sequences of HPVs 33, 35, 39, 51, 56 and 59 in MRL type-/gene-specific realtime Multiplex HPV PCR assays and for L1 sequences from 28 HPVs available via INNO-LiPA genotyping. Analysis was conducted on the 14 types common to both assays. Agreement rates were calculated using McNemar's exact p-value and Kappa tests for comparison.

Results: Overall agreement rates between assays were >90% for swabs and >95% for thinsection specimens. Significant differences (McNemar's p<0.05) in detection were observed for HPV6, HPV16, HPV18, HPV35, HPV39, HPV45, HPV56, HPV58, and HPV59 in swabs and for HPV45, HPV58 and HPV59 in thinsections. Where McNemar's was <0.05, discordance was due to detection of more HPV positives by MRL type-specific Multiplex PCR assays. For Kappa evaluations, only HPV59 in swab specimens resulted in poor agreement classification (κ =0.213) between assays.

Conclusions: Overall percent agreement for detection of fourteen HPV types between MRL type-specific Multiplex HPV PCR and INNO-LiPA Genotyping assays was good. Differences in positive sample detection favored MRL Multiplex HPV PCR suggesting increased precision of HPV DNA detection by MRL type-specific Multiplex HPV PCR assays.

EVALUATION OF A NEWLY DEVELOPED GENOARRAY HPV GENOTYPING ASSAY

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Background: Carcinogenic human papillomavirus (HPV) testing has been advocated in addition to cytology for triage of equivocal cytology in women.

Objectives: We evaluate the performance characteristics of a newly developed PCR-based HPV GenoArray test (Hybribio) for the detection of HPV infections by comparing results with those obtained using Roche Linear Array (LA) HPV genotyping test and DNA sequencing.

Methods: 317 cervical samples including 101 cancers, 99 high-grade (HSIL) and 93 low-grade squamous intraepithelial lesions (LSIL) and 24 normal cervices were used. HPV GenoArray (GA) test detects 21 HPV genotypes including 13 high-risk types. Comparison analysis was limited to those common HPV genotypes detected by both genotyping tests.

Results: There was no significant difference in overall detection of positive HPV between three detection methods, 91.8% for both GA and LA and 89.4% for DNA sequencing. The overall agreement between GA and LA was 96.9% with a kappa value of 0.83, indicating an excellent agreement. The agreement for detection of carcinogenic HPV was 89.1% by GA and DNA sequencing, and 96.8% with a kappa value of 0.85 (p=0.565) by GA and LA tests. A good agreement (kappa=0.676, p=0.489) was observed by both genotyping tests in detection of multiple HPV infections, 50.8% and 53% by GA and LA testes, respectively. 63.4% samples showed concordant HPV genotypes by two genotyping tests, while 34.1% samples showed compatible results (correspondence for some genotypes). Only eight samples (2.5%) showed no similarity between tests (discordant). The overall intertest comparison agreement for most of individual carcinogenic HPV types was considered good (k=0.61-0.80) to excellent (k=0.81-1.0), excepted HPV51 and HPV56 showed moderate agreement (k=0.41-0.6).

Conclusions: The GA genotyping test appears to be an accurate and sensitive method for detection and genotyping of HPV infection. It has the potential to be a useful screening test in clinical and epidemiological studies.

P-26.060

A NOVEL QUANTITATIVE RT-PCR BASED ASSAY FOR 13 HR-HPV DETECTION

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Objectives: Many studies evaluated HPV quantification as a potentially relevant information for a better management of high-risk HPV (HR-HPV) infections. This study evaluates an innovative quantitative real-time PCR assay for the detection of 13 HR-HPV DNA (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68), developed by Bio-Rad.

Methods: A strategy on sequence identity of E1, E6 and E7 viral genes has been chosen to design primers and probes. A single reference is used to normalize a global viral load value for the 13 detected HR-HPV, according to a patented mathematical algorithm. An Internal Control (IC) and a House Keeping Gene (HKG) are used to evaluate the presence of inhibitors and cell adequacy. The analytical performance is evaluated on HPV plasmids dilutions with human genomic DNA in transport medium. DNA is extracted with an optimised protocol using a commercial DNA extraction kit (DSP virus[™] Qiagen). The different targets are co-amplified and quantified on a real-time thermal-cycler (CFX96[™], Bio-Rad).

Results: All 13 HR-HPV types were identified with an equivalent analytical sensitivity. Amplification was highly specific of each target. No cross-detection was observed with low-risk HPV. The HR-HPV titration range was estimated to 8log. IC and HKG amplification was validated whatever the HR-HPV input within the PCR mix. Intra-assay and inter-assay reproducibility were evaluated.

Conclusions: This HR-HPV DNA quantification system (specimen collection device, standardized DNA extraction and real-time quantitative PCR assay) shows promising performances in terms of analytical sensitivity, specificity, reproducibility and reliability. IC and HKG allow a robust validation. Clinical studies are now required to validate the performance of this Bio-Rad assay for reflex HPV testing or primary screening. This standardized global viral load may also prove to be a useful biomarker to predict HR-HPV infection outcome. Complete analysis of the results will be presented.

NEW BIO-RAD QUANTITATIVE RT-PCR ASSAY COMPARED TO HC2 IN SMEARS

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Objectives: Many high-risk HPV (HR-HPV) tests are currently available, all of them giving qualitative results. The purpose of this study was to evaluate an innovative Bio-Rad quantitative real-time PCR assay for the pool detection of 13 HR-HPV DNA (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) on cervical smears and to compare the data with HC2 results. Methods: 749 cervical sampling has been prospectively obtained from patients attending for routine screening: a first

scrape was taken with ThinPrep device and dedicated to cytology examination and HC2 testing; a second scrape was taken with the Bio-Rad sampling device and dedicated to Bio-Rad HPV assay. The DNA extraction step used an optimized DSP virus[™] kit (Qiagen) protocol, after addition of an exogenous Internal Control (IC). The RT-PCR co-amplified the 13 HR-HPV along with the IC and a cellular housekeeping gene (HKG), on a Bio-Rad CFX96 thermal-cycler. A new patented mathematical algorithm using a single point quantification allowed the calculation of the global 13 HR-HPV content. HPV genotyping was performed in case of discordant results.

Results: Complete data were available for 741 samples, of which 7.8% harboured cytological abnormalities. HR-HPV positivity was 12.69% for the Bio-Rad assay at the threshold of 34Ct and 14.44% for HC2, respectively. Analytical sensibility and specificity were respectively 72.90% and 97.48% with a total agreement of 93.93% compared to HC2.

Conclusions: This new Bio-Rad quantitative real-time PCR assay shows promising results in a clinical setting. IC and HKG amplification allow a robust validation of the results. Moreover, this method offers an interesting global 13 HR-HPV viral load quantification, which could prove to be a useful biomarker to predict HR-HPV infection outcome. Finally, this assay could be used as a valuable tool for primary screening, triage or post-conization follow-up.

P-26.062

HPV GENOTYPING AND HIGH GRADE LESION PREDICTABILITY IN ABNORMAL CYTOLOGY

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Objective: This study aimed to determine the positive rate and type-specific detection of high risk (HR) human papillomaviru (HPV) in women with cytologically normal liquid-based Pap smear result in repeated tests after abnormal Pap smear or positive HR-HPV.

Materials and Methods: Of women referred for abnormal cytology or positive HR-HPV during routine screening from 2006 February to 2008 November, repeated liquid-based Pap smear and HPV genotyping test using HPV DNA chip were simultaneously performed as their follow-up. Histologically confirmed cervical diseases were retrospectively recorded, if performed.

Results: A total of 221 women (47.1 ± 10.8 years) had cytologically normal in repeated Pap smear. Overall, positive rate of HR-HPV was 30.8% (n=68), and HPV 16 was most commonly detected genotype (n=10, 14.7%) including multiple infection (n=4). Cervical diseases were histologically confirmed in a total of 58 subjects, which were 52 of chronic cervicitis, 2 of cervical carcinoma in situ (CIS), 2 of condyloma accuminatum and one endocervical polyp. As expected, two subjects with CIS in particular were positive for HPV 16.

Conclusion: The positive rate of HR-HPV in women with cytologically normal in repeated Pap smear are much higher than already known positive rate in those with normal in routine screening cytology. The added benefit of HPV genotyping test to liquid-based cytology should be evaluated as a follow-up tool of normal Pap smear results shown after previously abnormal cytology, if these finding are confirmed in larger study.

CLINICAL UTILITY OF THE PAPILLOCHECK ASSAY; A PILOT STUDY

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Objective; To conduct a pilot study of the Papillocheck HPV genotyping assay on cervical samples. The Papillocheck system is based on amplification of a portion of the E1 gene followed by hybridisation to type specific probes immobilised onto a micro-array chip.

Methods; Stored archival samples of cervical cells from 400 women obtained during the course of the ARTISTIC trial were available for this study. Following nucleic acid extraction using the Biomerioux EasyMag automated system, DNA was amplified and detected using the Griener Papillocheck assay. Cytology and HC2 High Risk (HR) HPV results were available on all samples. In addition Reverse Line Blot (RLB) assay and Histology results were also available on a large number of these women.

Results; Preliminary results obtained from testing 341/400 samples showed that of 39 cytology normal/HC2 positive women 27 (69%) contained a HR type by Papillocheck; of 138 borderline cytology women 58(42%) contained a HR type by Papillocheck compared with 52 (37.6%) testing HC2 positive; of 120 mild/moderate cytology women 91(75.8%) contained a HR type by Papillocheck whilst 92 (76.6%) were positive by HC2 and in 35 women with severe cytology 32(91.4%) contained HR types by Papillocheck compared to 33 (94.2%) testing positive by HC2. In addition, amongst 16 samples found to be HC2 positive but which were negative by RLB only 3 contained a HR type by Papillocheck. Data will be presented on all 400 samples including RLB and consequent histology results available.

Conclusions: Although only comparatively few samples have been tested it would seem that whilst equal clinical sensitivity to the HC2 assay has been demonstrated the Papillocheck assay has improved specificity. If this observation is confirmed in a larger study it would result in fewer women being needlessly referred for colposcopy.

P-26.064 AUTOMATED DNA PURIFICATION OF PRESERVCYT° FOR HYBRID CAPTURE° 2 ASSAY

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Objective: Cervical specimens collected in PreservCyt^{*} (PC) media are routinely used for cervical cancer screening programs. Further, PC specimens are most commonly used with the digene High-Risk HPV hc2 Test ^{*} (hc2. The current hc2 PC sample conversion protocol requires 4 mL of PC specimen, which is centrifuged and denatured and one-half of the sample is used in the hc2 assay. In this study, we describe an automated novel QIASymphony[™] instrument protocol for DNA concentration and purification from 2 mL PC clinical specimens for use in the hc2 test.

Methods: 243 residual cervical samples were screened using the standard hc2 manual conversion method using 4 mL sample aliquots. The novel automated QIASymphonyTM platform utilized 2 mL, aliquots of the same set of PC samples. Hc2 manual conversion method lysates and QIASymphonyTM lysates were tested using hc2 test and the results were compared. Conclusions: The automated QIASymphonyTM method performed with 2 mL of PC, showed a strong correlation with the standard 4 mL hc2 manual conversion method (219/243 (90%) results agreed; κ =0.800). Our study indicates that the automated QIASymphonyTM method and standard hc2 manual conversion methods yield comparable hc2 results. The QIASymphonyTM method shows promise for current and future HPV screening applications because it requires a lower sample volume (2 mL versus 4 mL), minimizes user-dependent variability, and, is less labor-intensive. In addition, the QIASymphonyTM method purifies DNA, which could be used in a variety of downstream assays.

DEVELOPMENT OF A HIGHLY SENSITIVE HUMAN PAPILLOMAVIRUS GENOTYPING DNA CHIP

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OBJECTIVES: Test of human papillomavirus (HPV) is a useful adjunctive tool of Pap smear to screen cervical cancer. We have developed a novel HPV genotyping DNA chip arrayed by multiple oligonucleotide probes of both L1 and E6/E7 gene sequence of 42 types of anogenital HPV.

METHODS: Consensus PCR products of L1 and E6/E7 gene sequences of HPV are hybridized to arrayed probes on the HPV chip and HPV genotypes are identified by fluorescence scanner. We have comparatively analyzed the value of HPV DNA chip and DNA sequencing in 100 cervical cancer tissues.

RESULTS: Overall, 98 cervical cancer tissues were found to harbor DNA sequences of high-risk type HPVs, of which 88 (89.8%) were detected by PCR-sequencing of L1 alone, 98 (100%) by PCR-sequencing of both L1 and E6/E7, and 98 (100%) by HPV DNA chip, respectively. All of the genotypes of HPV detected on sequencing analysis were also found on DNA chip analysis. HPV DNA chip was superior to direct DNA sequencing in detection of mixed infection.

CONCLUSIONS: These results suggest that HPV DNA chip analysis in the present study is highly accurate for detection and genotyping of HPV and may have potential value as a robust, high-throughput screening test of uterine cervix cancer.

P-26.066

A MULTIPLEX TAQMAN PCR ASSAY WHICH IDENTIFIES 21 HPV GENOTYPES

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BACKGROUND. Molecular-based assays for genital HPV detection have an expanding role in the diagnosis of these infections, either as a supplement to cytology or when incorporated into a primary screening algorithm. With the increasing use of assays which detect and identify multiple HPV genotypes it is becoming evident that mixed HPV infections are common in genital samples. Because of competition between high and low copy number targets in multiplex reactions, it is possible a low-risk HPV genotype in high copy number may mask the presence of a high-risk one at a low copy number. This competition is also a hazard when products from a consensus PCR are used for sequencing reactions to identify genotypes. OBJECTIVES. This study sets out to assess an inexpensive, multiplex, tandem real-time assay targeting HPV E6 genes which detects and identifies 21 high and low-risk HPV types while reducing the effects of target competition in mixed infections. METHODS. The assay incorporates an internal control of sample extraction and PCR inhibitor removal and also measures the number of human cells in the sample. The assay was compared with a nested MY09/11 - GP5+/GP6+ PCR followed by DNA sequencing of the PCR products. RESULTS. The multiplex tandem assay detected 66 mixed infections from 439 random samples tested and also detected 21 additional samples with high-risk HPV types compared with the nested PCR. CONCLUSIONS. This multiplex assay represents an economical, rapid and sensitive method for the detection and identification of high-risk mucosal HPV types and reduces the chance of failure to detect high risk HPV types in the presence of high copy numbers of low-risk types.

HIGH CONCORDANCE BETWEEN DIGENE HPV GENOTYPING RH TEST AND RLB

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Background: Eighteen mucosal HPV types have been classified as high-risk (HR) or probably HR (i.e., HPV16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82). Recent evidence suggests a different oncogenic potential among HR HPV types (most importantly HPV16, 18 and 45), demonstrating the importance of HPV genotyping assays. Several HPV detection and genotyping assays are available, but there is considerable lack of uniformity in the accuracy to detect clinically relevant HR HPV infections.

Objectives: The goal of this study was to evaluate the performance of the novel digene HPV Genotyping RH Test (digene RH Test), a nitrocellulose strip-based reverse hybridization assay using type-specific probes for the 18 (probably) HR HPV genotypes, in comparison to the established in-house reverse line blot (RLB) assay using PCR products generated with the clinically validated GP5+/6+-PCR test.

Methods: A selection of GP5+/6+-PCR amplimers generated from 493 digene High Risk HPV HC2 DNA Test (HC2)-positive and 95 HC2-negative cervical smears was used. Both the digene RH Test and the RLB assay were performed on the same amplimers.

Results: Overall, the digene RH Test showed an equivalent detection rate of HR HPVs compared to the RLB assay ($\kappa = 0.886$) in the 493 HC2-positive samples analysed. At the genotyping level of the 18 types, both tests were also highly concordant (overall $\kappa = 0.946$, individual κ range 0.721-1.000). The digene RH Test following GP5+/6+-PCR showed positivity for one or more (probably) HR HPV type(s) in 87% of the HC2-positive women.

Conclusions: The strip-based digene HPV Genotyping RH Test showed a high genotyping agreement with the established RLB assay. Accordingly, this novel assay following GP5+/6+-PCR could serve as a reflex test in a clinical setting for women who are HC2-positive to identify the classified HR HPV genotypes.

P-26.068

HPV GENOTYPE CORRELATION BETWEEN COBAS® 4800 AND LINEAR ARRAY TESTS

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Background: The Linear Array HPV genotyping teat (LA) has been shown to perform similarly to hybrid capture 2 (hc2) in the identification of CIN3 among women with an abnormal Pap. The prototype cobas 4800 is a highly automated system that performs, sample preparation, real-time HR-HPV amplification and simultaneous detection of 12 HR-HPV genotypes in a single pool, with separate detection of HPV16, HPV18, as well as the human beta globin gene, all in single tube.

Objectives: We tested the correlation in detection of HPV16, HPV18, and HPV31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 by the cobas 4800 HPV test with the Linear Array HPV genotyping test.

Methods: A total of 195 cervical samples were collected from women during follow-up of Pap abnormalities. HPV genotyping results obtained from these samples using the LA and cobas4800 HPV test were compared for positive and negative correlations with kappa analyses.

Results: Valid results were obtained from 195 samples. 191/195 were concordant for the presence or absence of HPV16 (98% concordant, kappa 0.93), 194/195 were concordant for HPV18 (99% concordant, kappa 0.92) and 171/195 were concordant for the other HR-HPV genotypes (88% concordant, kappa 0.75)

Conclusions: The cobas4800 HPV test is a highly automated sample extraction, HPV amplification and HPV genotype detection system. The prototype cobas 4800 HPV genotyping results have a very high correlation to those obtained using the validated Linear Array HPV genotyping test.

EPIGENETIC MARKERS FOR HPV-INDUCED DYSPLASIA AND CERVICAL CARCINOMA

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Background: The sensitivity of a single Pap-smear for the detection of dysplasia and cervical cancer is poor. By implementation of an HPV-DNA test in a primary screening setting the sensitivity approaches 100%. However, the specificity of the HPV-DNA test for the detection of CIN is inadequate, particularly for women 30 years of age and younger.

Objective: To define molecular markers highly specific for CIN and cancer in cervical cancer screening programs. Methods: Cervical carcinogenesis is a multi-step process which also involves gene silencing. A common mechanism for silencing is hypermethylation of cytosines in CG dinucleotides of CpG islands, regions with high GC content predominantly located in the promoter region of genes. Hypermethylated genes can be detected with high sensitivity and specificity by the use of real-time methylation-specific PCR (MS-PCR). DNA isolated from cervical scrapes is chemically treated, leaving methylated cytosines unchanged, while unmethylated cytosines are converted to uracil.

Results: Based on cDNA array analyses we have selected 100 candidate genes which are consistently down-regulated in the course of carcinogenesis. For these genes we have successfully established CpG island-specific MS-PCR using primers allowing the amplification of methylated DNA only. Specificity for methylation is given the highest priority, and only genes which show methylation in less than 20% of HPV-negative or HPV-positive cervical scrapes without evidence of dysplasia fulfil the inclusion criteria for being considered as part of an epigenetic signature for dysplasia/cancer.

Conclusion: Promoter regions of several candidate genes were found to be methylated with high frequency in cell scrapes taken from cervical carcinoma and CIN2/3. By comparison HPV-negative or HPV-positive cervical scrapes without evidence of dysplasia were methylated only sporadically and at a very low level. It will be a challenge to define a subset of hypermethylated genes which can differentiate between HPV-positive women with and without dysplasia/cancer.

P-26.070

EVALUATION OF AUTOMATED ABBOTT REALTIME HR HPV ASSAY

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Backround: Abbott RealTime HR HPV assay is a qualitative real time-PCR based assay for the detection of 14 high risk HPV DNA in cervical cells collected in liquid media. The assay can differentiate between the infection by HPV 16, HPV 18 and non-HPV 16/18 types through the distinct fluorescent labels on the type specific probes.

Methods: The RealTime HR HPV assay, performed on the fully automated m2000 system, was compared with Linear Array, Hybrid Capture 2 and PapilloCheck on 180 of archived ASCUS plus samples.

Results: The study demonstrated excellent overall agreement,;95.0% with Linear Array, 92.2% with Digene HC2, and 93% with Papillocheck. One significant advantage of the RealTime HR HPV assay is its ability to identify type16 and 18 in one test. To that end, correlation of type 16, 18 and other HR HPV results with Linear Array and PapilloCheck were also assessed in this study. Agreement for samples containing type 16 was 97.8% with Linear Array and 98.3% with Papillocheck; Agreement for samples containing type 18 was 100% with Linear Array and 99.4% with Papillocheck; Agreement of samples containing other HR HPV types was 90% with Linear Array and 89% with Papillocheck. With the Abbott RealTime HR HPV assay 103 samples were HR HPV positive. Of these 103, 24 (23.3%) were HPV 16 only, 15 (14.5%) were HPV16 plus other HR HPV, 5 (4.9%) were HPV 18 only, 2 (1.9%) were HPV 18 plus other HR HPV, and, 57 (55.3%) were other HR HPV.

Conclusions: This study demonstrated that ; 1) TheAbbott RealTime HR HPV assay closely correlated with other assays . 2) The automation and ability to identify type 16 and 18 make this a very attractive option for HPV testing in laboratories and potentially provides improved patient management

PENILE CARCINOMA: HISTOLOGICAL CLASSIFICATION, P16INK4A, P53 AND HPV DETECTION.

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Background: About a third of penile squamous cell carcinomas (SCC) showing warty or basaloid pathological features are considered to be HPV related. In cervical neoplasia, as a consequence of HPV infection, p16ink4a overexpression can be detected by immunohistochemistry.

Objective: In this study we explore p16ink4a as a marker for HPV DNA presence in major subtypes of penile carcinomas.

Methods: Hematoxylin-eosin stained paraffin sections corresponding to 207 penile tumors were reviewed by a panel of pathologists and diagnosed according to the WHO classification. HPV DNA was amplified by PCR with SPF-10 broad spectrum primers followed by DEIA and genotyping by LiPa 25 (version 1). P16ink4a (mtm, Heidelberg) staining was carried out using standard immunohistochemical procedures and tested blinded to HPV status. p16ink4a was considered positive when a diffuse and continuous strong staining throughout the tumor or at basal layer was present. Patchy or discontinuous staining was considered negative. p53 immunohistochemical detection is being performed and results will be presented.

Results: Seventy-one penile SCC were HPV positive (34.3%) being HPV 16 the most frequent type (63.4%). HPV detection according to major histological subtypes was: 13/26 (50%) in warty SCC, 18/25 (72%) in basaloid SCC, 19/88 (21.6%) in conventional SCC, 4/16 (25%) in papillary carcinoma and 0/12 (0%) in verrucous carcinoma. P16ink4a did not show good correlation with HPV detection with a sensitivity of 60,6%, which was particularly low in conventional SCC (31.6%).

Conclusions: HPV is most frequently found in basaloid SCC, followed by warty SCC and conventional SCC. The low sensitivity of P16ink4a in our series precludes its use as research-triage tool for HPV detection in penile invasive carcinomas, especially in conventional SCC.

P-26.111

VALIDATION OF TWO NOVEL UNBALANCED WHOLE ARM-TRANSLOCATIONS IN CERVICAL SMEARS

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Background: Persistent infection with a high risk human papillomavirus (HR-HPV) type is a prerequisite for the development of cervical cancer (CxCa). This process is accompanied by numerous genetic alterations including chromosomal aberrations. Recently, we identified two novel unbalanced translocations, der(10;14) and der(7;21), in HPV-immortalized cells. Der(10;14) could also be detected in approximately 40% of low grade cervical intraepithelial lesions (CIN1), 80% of CIN2/3 and 60% of CxCa and der(7;21) in about 20% of CIN1, 53% of CIN2/3 and 47% of CxCa.

Objective: The aim of this project was to establish a protocol for the detection of these chromosomal translocations in cervical smears as a basis for further diagnostic evaluation.

Methods: Cervical smears from 31 patients (healthy controls (n=15), CIN1 (n=6), CIN2/3 (n=7) and CxCa (n=3)) were collected and stained according to standard procedures for "Papanicolaou" (Pap)- staining. Cytological images of the areas of interest were made and the respective XY-coordinates were recorded. Coverslips were then removed using xylene and the slides were prepared for fluorescence in situ hybridization (FISH). After interphase-FISH procedure images from the identical areas of the slides (according to XY-coordinates) were taken.

Results and Conclusions: Signal distribution characteristic for the above translocations could be detected in some of the nuclei of Pap-stained dysplastic cells. Both translocations were detected in three CIN2/3 and two CxCa. Furthermore in one CIN2/3 and in one CxCa only the der(10;14) was found. None of the cytologically normal Pap-smears or normal cells in CIN or CxCa showed one of the aberrations. The diagnostic/prognostic value of these translocations for cervical cancer screening will be assessed in further studies.

P-26.112 P16INK4A MRNA LEVELS MEASURED BY TAQMAN-QPCR CORRELATE TO CYTOLOGIC DIAGNOSIS

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Introduction: The incidence and mortality of cervical cancer has been remarkably reduced by cytologic screening. This technique has been pushed to its limits but could be improved by addition of biomarkers associated with high grade lesions. Qualitative detection of p16 has been shown to be a valid approach for detection of high grade CIN. The quantification of p16 protein levels in Pap smears remains a challenge. Quantitative Real-Time PCR is a feasible alternative to estimate protein expression based on mRNA abundance. Here we present a novel TaqMan assay for p16 RNA levels in Pap smears and tumour specimens.

Methods: High-risk HPV negative Pap smears diagnosed as Pap II were compared to High-risk HPV positive tested Pap II, Pap III and VI, as well as to CIN from cervical biopsies. The amount of p16 mRNA relative to ß-actin was investigated and related to cytologic results.

Results: Our highly efficient (> 95%) and sensitive (< 10 copies) TaqMan - assay has a wide dynamic range (10e+0 to 10e+8 p16 copies). In clinical samples and cell lines p16 mRNA was detectable and increased with severity of Pap diagnosis. There was no difference between HPV negative and positive Pap II smears. In Pap III a slight increase was seen. Significantly higher amounts of p16 mRNA were found in Pap IV smears and even higher in cervical cancer biopsies. Conclusion: Quantification of p16 mRNA levels could improve diagnostic results.

P-26.113

P14ARF, BCL-2, P53 EXPRESSION IN HPV(+) AND HPV(-) PATIENTS.

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Human papillomavirus (HPV) is commonly known to be present in pathologically changed cervical tissue. Prolonged infection with high oncogenic HPV types leads to precancerous lesions and cervical cancer. However, infection with high oncogenic HPV types does not determine feasibility of neoplasmatic progression.

Recent studies suggest usefulness of immunohictochemical staining in neoplasmatic progression risk assessment. The aim of the study was to evaluate whether expression of p14ARF, bcl-2 and p53 could be utilized in diagnosing patients with high-risk HPV (HR-HPV) infection.

A total of 92 women were included in the study, 20 of them, classified as controls, had normal cytology and were HPV negative. All pathological cases had abnormal cytology evaluated with the Bethesda 2001 system (45=ASCUS, 12=LGSIL, 15=HGSIL). Also all cases were genotyped for high oncogenic risk HPV types (Amplicor) and were evaluated histopathologicaly. Afterwards p14ARF, bcl-2 and p53 staining was preformed.

In HR-HPV positive cases degree of cytological pathology correlated significantly with the expression of p53 staining (p<0,07), where more than 20% of cells were positively stained. There was no correlation with histopathology results.

In HR-HPV positive cases degree of cytological pathology correlated significantly with the expression of p14ARF staining (p<0,07), where more than 50% of cells were positively stained. There was also no correlation with histopathology results.

In HR-HPV positive cases degree of cytological pathology correlated significantly with the expression of bcl-2 staining (p<0,01), where positive staining was observed in basal cells. Also in patients with HR-HPV infection significant correlation between histopathology result and bcl-2 expression was observed (p<0,003), where basal cells were stained.

Our results show that performing histopathological evaluation and immunohistochemical staining of the cervical tissue samples may give more accurate information about the degree of cervical neoplasia than histopathological evaluation and HPV genotyping only.

HUMAN PAPILLOMAVIRUS TYPE 16 E6 VARIANTS IN MSM POPULATION

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Objectives: Human papillomavirus type 16 (HPV 16) has intra-type variants and some are related with enhanced oncogenic potential.

The objective of the present study is to examine the polymorphisms in E6 HPV16 region, among HIV-positive and - negative men who had sex with men (MSM) living in Madrid (Spain).

Methods: Out of a total of 411 anal samples from MSM recruited from January 2005 to December 2007, 102 HPV16 anal samples were selected for E6 region analysis. The DNA extraction was made using an automated extractor (Qiagen) and HPV typing was carried out by Linear Array[®] HPV Genotyping test (Roche Molecular Systems). A specific set of primers designed to amplify the E6 region was used for PCR. HPV16 classes and subclasses were identified by direct sequencing. Multiple sequence alignments were performed with MegAling program (DNASTAR, Madison USA). The sequence as published by Seedorf (HPV-16R) that belongs to European lineage was used as a reference.

Results: From the 102 anal samples characterized as HPV 16 type, 90 valid E6 sequences were obtained. Of these 45 (50%) were from HIV seropositive men, 36 (40%) from seronegative HIVmen and 9 (10%) from men with unknown HIV status. The frequency distribution of HPV 16 variants was: 80 (88.9%) belonged to European lineage, being 39 (43.3%) Ep-350T, 30 (33.3%) Ep-350G, nine (10%) Ep-350G with additional changes and two (2.2%) E-C109G; four samples (4.4%) was AA classes (two Aaa, one AAc and one AAc/C271), two sequences (2.2%) were Af2 subclasses and four (4.4%) belong to the North American lineage.

Conclusions: We describe the distribution of HPV 16 intra-type variants in MSM living in Spain, being the European variants the most frequently detected. The European variants with additional changes are presented only in men HIV-positive. None of samples was characterized as Asian HPV 16 variant (As).

P-26.115

THE QIAGEN HPV 16/18/45 PROBE SET APROACH: 3RD RESULTS

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Objective: Today HPV-Infection is accepted to be one of the major risk factors to develop cervical cancer. Nevertheless, generally the related precancerous cervical lesions are successfully suppressed by the t-cell system. Yet, persistent infections with hr HPV DNA types are associated with the development of cervical intraepithelial neoplasia (CIN) and may cause a progression to invasive cervical cancer. HPV hr positive cervical smears are retested with the Probe Set (PS), a test "for research use only", that detects the hr HPV Types 16, 18 and 45. Cytological results are correlated with histology. Does the use of the Probe Set test offer a benefit for the patient treatment with reference to their clinical outcome?

Material & Methods: The study has started in October 2007. We established two groups: a control group of 109 women who attend the regular screening for cervical cancer and a "risk group" of 478 women who are HPV high risk (hr) positive. Initial HPV testing is based on HC2 test. It was ensured that all of the collected smears of the "risk group" were positive for hr HPV-DNA. Smears are evaluated according to the Munich nomenclature. Follow up is conducted according to the german gynecological guidelines.

Results: Average age of the risk group is 34.9 years (PS+) and 34.6 years (PS-). The average age of the control group is 28.1 years (PS+) and 32.6 years (PS-). 245 smears have demonstrated signs of cervical lesions and 233 are within normal limits. 310 of the hr infections were positive for the Probe Set test (133 morphologically inconspicuous / 177 demonstrated signs of cervical lesions). 168 of the hr infections were negative for the Probe Set test (100 morphologically inconspicuous / 68 demonstrated signs of cervical lesions). In the control group 15 samples have contained HPV-HR DNA: 9 were PS+.

P-26.116 PERFORMANCE OF VLP ELISAS FOR HPV16 & 18 ANTIBODY DETECTION

G Keating, S MacNamara, J Nolan

HPV16 and HPV18 Virus-Like Particles (VLPs) were expressed in a baculovirus system using Sf9 insect cells and purified using a variety of techniques, including precipitation and column chromatography.

The purity and intregrity of VLP preparations was assessed using a range of methods, including TEM, SDS-PAGE and immunoblotting with antibodies to conformational epitopes.

VLPs were immobilised on microtitre plate wells and used to produce ELISAs for detection of antibodies to HPV16 and HPV18.

The performance of the assays was assessed using a panel of characterised samples, and assay specificity, sensitivity and reproducibility were evaluated.

These commercial prototype assays can detect circulating antibodies to high risk HPV subtypes in reponse to vaccination and natural infection. The availability of reproducible commercial kits for HPV16 and HPV18 antibody detection would facilitate standardisation of ongoing seroepidemiology and vaccine monitoring studies.

P-26.118 CLINICAL IMPACT BETWEEN ABERRANT CYTOLOGY AND NEGATIVE HUMAN PAPILLOMAVIRUS INFECTION

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Background: Since the development of cytology-based cervical cancer screening using the Pap smear in the mid 20th century, Pap smears have been implemented for secondary prevention of cervical malignancy. Human papillomavirus (HPV) has been recognized as a necessary cause of cervical malignancy.

Objectives: We investigated the rate and clinical relevance of abnormal cervicovaginal cytology in patients with negative human papillomavirus test.

Methods: In this study, we evaluated 2477 women who had cervicovaginal cytology and HPV testing simultaneously in our institution between January 2006 and April 2006. High-risk Hybrid Capture II (HC-II; Digene, Gaithersburg, MD) was used in conjunction with cytology. The results were compared concurrent or subsequent HC-II, cytology, or biopsy results.

Results: Of 2477 women, 61 (2.46%) had abnormal cytologic results with negative HPV results (41, conventional smear with Papanicolaou method; 20, liquid based cytology). Fourty-three patients (70.5%) had ASCUS (atypical squamous cells of undetermined significance), 10 (16.4%) had LSIL (low grade squamous intraepithelial lesion), 4 (6.6%) had HSIL (high grade squamous intraepithelial lesion), and 4 (6.6%) had AGC (atypical glandular cells). Twenty five patients had repeated HPV testing and 5/25 (20%) had positive HPV results on repeated test. Nine patients had persistent abnormal cytologic results on follow up cytology test. Subsequent colposcopy directed biopsy results showed 11.5% (7/61) patients had high grade lesion (CIN2/3, cervical intraepithelial neoplasia). Of 7 with high grade lesion, 3 had repeated HPV test and results turned to be negative in all cases.

Conclusions: High grade lesions may be identified in abnormal cytology with HPV negative results. Although the rate is very low, in patients with discordant cytology and HPV testing, colposcopy or repeating test should be considered.

HR-HPV VIRAL LOAD, P16INK4A - PREDICTIVE OF PERSISTENCE INFECTION

R URSU, UNIVERSITY, IASI, ROMANIA

L IANCU, UNIVERSITY, IASI, ROMANIA

Background: Even with one effective vaccination strategy, eradication of HPV infection can be difficult because of asymptomatic infections and chronic carrier state. Ideally is to detect HPV infection in early stages, using specific biomarkers.

Objectives: 1.to establish corelations HR HPV genotypes viral load - CIN grade, and corelations between physical state of HPV integration - CIN grade

2. to asses clinical utility of HPV viral load detection by Real Time PCR

3. to asses P16INK4a expression as specific biomarker in cervical (pre)cancer lesions

Method: Literature synthesis. Strategy: full text articles in data bases, using associations between: HR HPV - VIRAL LOAD – CIN – REAL TIME PCR. The analyzed data bases: AIDS&Cancer Research, Cochrane Library, Nature, Springer, PubMed.

Results:

1. 28 articles fulfilled our criteria, published between 2000 - 2008. 19/28 found that there was a direct relations between the viral load of HR HPV and CIN grade (the higher the viral load, the more severe lesion was found in those patients), 7 articles didn't found any relations between the viral load and severity of CIN lesions, and 2 articles found that viral load increased from normal to LSIL (e.g. 537.5 copies/cell), followed by sudden decrease in HSIL (e.g. copies/cell).

2. 3/9 articles who correlated the physical status of HPV integration with the CIN grade, found integrated form of HPV in CIN1 grade, in proportion who varied: 10 - 72%.

3. viral load of HR HPV 16 was expressed in different measure units: copies/50 ng DNA, copies/100 cells , copies/µl, copies/103 cells.

4. 39/40 articles found a positive correlation between p16INK4a and lesion grade or HR HPV presence.

Conclusions: there is a need for more studies to asses the utility of viral load determination and physical status analyzing. Overexpression of p16INK4a may be an indicator of pathogenic activity of HR HPV.

P-26.120

CYTOACTIV[®] - INTERNATIONAL MULTICENTER STUDY OF 5000 EARLY DYSPLASTIC LESIONS

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Background: We and others have shown the prognostic significance of HPV L1 capsid protein detection with cytoactiv on HPV high risk associated mild and moderate dysplastic lesions.

In short HPV L1 capsid protein negative early dysplastic lesions are significantly more likely to progress to histologically confirmed CIN 3 lesions than are L1 positive cases.

Objective : The aims of this prospective, international multicenter study are

1.)to validate the prognostic relevance of immunocytochemical HPV L1 capsid protein detection for early dysplastic lesions, using cytoactiv.

2.) to evaluate the impact of different preparation techniques (conventional Pap smear versus FDA approved Liquid based Cytology) on the sensitivity of L1 detection and its prognostic significance.

Material and Methods : Until June 2008 study centers located in Germany, USA, Sweden, Italy, Swizerland, Greece, Russia and Australia contributed 5000 randomly selected cases of HIV negative, non-pregnant, non HPV L1 vaccinated women reported as LSIL (internationally) or as group IIID (Germany), with subclassification into mild (LSIL) or moderate (HSIL) dysplasia.

Study centers in Arm A used a DNA method to confirm the HPV high risk association of the lesions, study centers in Arm B did not perform HPV testing.

Follow up will be until June 2010.

Results and Conclusion : Preliminary data of the multicenter study after a follow up period of 12-24 month will be presented.

INCREASED DIAGNOSIS OF CIN WHEN P16 IS COMBINED WITH HISTOLOGY

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Background: The diagnosis of CIN has high interobserver variability. This is a problem for the treatment threshold of CIN 2,3. Studies suggest that p16 IHC improves diagnostic accuracy. Here we investigated the impact of combining p16 with H&E on the detection of CIN 2,3 by experienced pathologists.

Design: 740 biopsies from 2,447 colposcopies were utilized. Slides were stained with H&E and p16 and then interpreted independently by two experts who initially made a diagnosis using H&E alone and then several weeks later made a diagnosis based on both the H&E and p16 slides. p16 was graded as negative, focally positive, and diffusely positive.

Result: There was very good agreement when grading p16 staining; kappa = 0.887 at diffusely positive. At a CIN 1+ cutoff, agreement was very good using H&E alone (kappa = 0.848) or H&E plus p16 (kappa = 0.867). Although agreement was good at a CIN 2+ cutoff using H&E (kappa=0.66), agreement improved using H&E plus p16 (kappa=0.731). Using H&E alone, 47 biopsies were consensus CIN 2,3 whereas using H&E plus p16, 62 biopsies were classified as CIN 2,3. Although this represents a non-significant increase in CIN2,3 (p=0.163), it represents a 32% increase cases classified by both as CIN 2,3. All of the additional cases were high-risk HPV positive and 4 had been diagnosed as normal by at least one of the pathologists.

Conclusion: Use of H&E plus p16 IHC produced a 32% increase in the number of biopsies concurrent in the diagnosis of CIN 2,3. Although this increase was non¬significant in this study, it may be clinically meaningful since a diagnosis of CIN 2,3 is the threshold for therapy. Furthermore, the magnitude of the impact of p16 IHC is likely underestimated because of the overall high levels of agreement between the experienced pathologists compared to general practice.

HPV INFECTION IN MALES

SESSION 27

PROGRAMME

THE 25TH INTERNATIONAL PAPILLOMAVIRUS CONFERENCE MAY 8-14 2009, MALMÖ, SWEDEN

TIME

2009-05-14

NUMBER TITLE

ROOM

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11.11-11.22	O-27.02	CORRELATES OF PERSISTENT INCIDENT GENITAL HPV INFECTION IN YOUNG MEN Y Arima, J Partridge, R Winer, S-K Lee, Q Feng, Jp Hughes, M Stern, S O'Reilly, La Koutsky			
11.22-11.33	O-27.03	HPV IN DANISH MEN. ASSOCIATION WITH CIRCUMCISION-STATUS AND GENITAL WARTS S Kjær, C Munk, B Nøhr, A Nielsen, K Liaw, T Iftner			
11.33-11.44	O-27.04	PROBABILITY OF HPV TRANSMISSION AMONG NEWLY-FORMED COUPLES A Burchell, J Hanley, P Tellier, F Coutlée, E Franco			
11.44-11.55	O-27.05	CONDOM USE IS ASSOCIATED WITH LOWER HPV PREVALENCE IN MEN CM Nielson, RB Harris, A Nyitray, EF Dunne, KM Stone, A Giuliano			
11.55-12.06	O-27.06	INTERNATIONAL HPV INCIDENCE AMONG MEN AGES 18-70 YEARS A Giuliano, JH Lee, WJ FULP, LL Villa, E Lazcano, M Papenfuss, D Smith, M Abrahamsen			
12.06-12.17	O-27.07	CONDOMS PREVENT INCIDENT ANAL HUMAN PAPILLOMAVIRUS INFECTION IN MEN P Chin - Hong, E Vittinghoff, D Benet, S Buchbinder, M Da Costa, T Darragh, F Judson, B Koblin, K Mayer, J Palefsky			
12.17-12.28	O-27.08	HIGH ANAL HPV PREVALENCE IN MULTINATIONAL SAMPLES: THE HIM STUDY A Nyitray, D Smith, L Villa, E Lazcano - Ponce, M Abrahamsen, A Giuliano			

O-27.01 OUADRIVALENT HPV VACCINE EFFICACY IN MEN HAVING SEX WITH MEN

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BACKGROUND: In males, anogenital infection with human papillomavirus (HPV) can lead to neoplastic lesions, including genital warts, penile, perineal, perianal, and anal cancer. No data are currently available showing a benefit of HPV vaccination against the incidence of infection or genital disease in men who have sex with men (MSM).

OBJECTIVE: We examined the efficacy of the quadrivalent HPV (6/11/16/18) vaccine in MSM against HPV6/11/16/18related external genital lesions (EGL) (warts, penile/perineal/perineal/perineal intraepithelial neoplasia, and penile/perineal/ perianal cancer), as well as anogenital persistent infection and DNA detection.

METHODS: Data are from 602 MSM aged 16-26 who were randomized to receive vaccine or placebo at enrollment, month 2, and month 6. Serum was collected at enrollment and at months 7, 24, and 36 for analysis anti-HPV antibodies. Subjects underwent detailed anogenital exams as well as sampling from the penis, scrotum, perineal/perianal region and anal canal at enrollment, month 7 and at 6-month intervals afterwards. Efficacy analyses were performed in a per-protocol population seronegative at day 1 and DNA-negative from day 1 through month 7 to the relevant vaccine HPV type. Median follow-up was 1 year (post-dose 3).

RESULTS: Vaccine efficacy against HPV6/11/16/18-related EGL was 79.0% (95% CI: <0. 99.6) (1 vaccine case versus 5 placebo cases). Efficacy against the incidence of HPV 6/11/16/18 persistent infection and DNA detection at any visit was 94.4% (95% CI: 64.4, 99.9) (1 vaccine case versus 18 placebo cases), and 48.8% (95% CI: 11.6, 71.2) (21 vaccine cases versus 42 placebo cases), respectively. At month 7, 96.5%, 97.4%, 94.2%, and 89.5% of subjects receiving vaccine seroconverted for HPV6, 11, 16, and 18, respectively.

CONCLUSION: The quadrivalent HPV vaccine is effective in reducing the burden of anogenital HPV infection and EGL in young MSM. Further follow-up will provide additional cases that will increase the confidence of these observations.

O-27.02

CORRELATES OF PERSISTENT INCIDENT GENITAL HPV INFECTION IN YOUNG MEN

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Background: Identifying correlates of HPV persistence in men will help develop strategies to reduce transmission. Objectives: Determine the proportion of incident HPV infection that persists in men and identify correlates of persistence.

Methods: 428 male university students 18-20 years old were followed between 2003-2008 in Seattle, USA. Subjects completed a biweekly sexual behavior diary and genital samples (shaft, glans, scrotum, and urine) were collected for HPV genotyping by PCR-based assay (38 types) every 4 months, with a maximum follow-up of 3 years. Incident infection was defined as first detection at any genital site of a specific HPV type after ≥ 1 visit where that type was not detected. Persistent infection was defined as incident HPV infection followed by 2 consecutive visits with detection of the same type (-+++). Transient infection was defined as incident infection followed by 2 consecutive visits without detection of that type (-+--). Multivariate logistic regression with generalized estimating equations was used to identify correlates of persistent HPV infection (cases) versus transient infection (controls).

Results: Among 97 men with incident HPV infection, 270 type-specific patterns were documented. 43 men experienced 72 persistent infections (26.7%) and 70 men experienced 144 transient infections (53.3%). Not included in analysis were 54 incident infections (20.0%) with either a single repeat detection (-++-) or episodic detection (-+-+). Compared to testing negative in the respective genital site, incident detection of HPV DNA in the shaft (adjusted OR=7.22; 95%CI=2.43-21.46) or urine (adjusted OR=3.11; 95%CI=1.39-6.94) was associated with persistence. Circumcision, age, ever smoking, ever anal intercourse, HPV-16, and multiple types were not associated with persistence.

Conclusions: About 1 in 4 incident \overrightarrow{HPV} infections persisted for ≥ 8 months in this male population. Persistence was associated with initial detection of HPV DNA in penile shaft or urine specimens.

O-27.03

HPV IN DANISH MEN. ASSOCIATION WITH CIRCUMCISION-STATUS AND GENITAL WARTS

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T Iftner, Dept. of Medical Virology, University of Tübingen, Tübingen, Germany

BACKGROUND: The natural history of HPV infection in men has been less intensively studied than in women, but there is an increasing interest in understanding HPV and HPV-related diseases in men.

OBJECTIVES: To assess the importance of circumcision and other risk factors for the prevalence of HPV in men, and to describe the HPV type distribution in circumcised and non-circumcised men, and in men with and without genital warts.

MATERIAL:During a 14 months period we included 2,437 men (18-65 years) from different employment groups at military barracks in Denmark, including e.g. military personnel, medical personnel, mechanics and office workers. During a clinical examination we assessed circumcision status and presence of genital warts, and obtained penile swabs, which were subsequently tested for HPV using Hybrid Capture 2 (HC2), high-risk (HR) and low-risk (LR) probe. All samples were also tested using a PCR-based assay, LiPa. Finally, the participants answered a questionnaire.

RESULTS: A total of 543 men (22.3%) were HC2 positive, covering 17.8% HR HC2 positive and 10.8% LR HC2 positive. The HPV prevalence using LiPa was 41.9%. The detection of HR HPV (HC2) was higher among non-circumcised men (18%) compared to circumcised (12%), and was also higher in men with genital warts (52%) than in men without (16%). Among HPV positive men with genital warts, nearly 80% were positive for HPV 6 (~70%) or HPV 11 (~10%). The corresponding numbers for men without genital warts were 9% and 1%. In the multivariate analysis, circumcision was significantly associated with decreased risk of HR HPV after adjusting for age, number of partners, time since last intercourse and genital warts.

CONCLUSION: Risk of HR HPV infection is reduced in circumcised men. The full risk factor analysis across different categories of HPV (any HPV, HR- and LR-HC2 positivity and LiPa positivity) will be presented.

O-27.04 PROBABILITY OF HPV TRANSMISSION AMONG NEWLY-FORMED COUPLES

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E Franco, *McGill University*, *Montreal*, *Canada*

Background: The transmission probability (β) is one of the key parameters that determine infection spread in a population.

Objective: To estimate the HPV transmission probability per partnership (βp) and per act of intercourse (βa).

Methods: The HITCH Cohort Study (HPV Infection and Transmission among Couples through Heterosexual activity) enrolls new couples (maximum = 6 months). Women aged 18-24 attending a university or junior college in Montreal, Canada and their male partners are eligible. Self-collected vaginal swabs and clinician-obtained swabs of epithelial cells from the penis and scrotum were tested for DNA of 36 HPV types. We analyzed enrolment data from 156 couples in whom at least one partner was HPV+, and interpreted positive type concordance as a proxy measure of transmission.

Results: Men and women reported a median of 5 lifetime partners. Couples had engaged in vaginal sex for a mean of 3.6 months (median 59, mean 73 acts). The mean number of types present in couples was 3.3 (range 1-11), of a total of 517 observations of a specific type. Forty-one percent (210/517) were concordant. The overall transmission probabilities were 42.1% per partnership (β p, 95%CI 36.4%, 48.0%) and 0.80% per act (β a, 95%CI 0.64%, 1.00%). Among the 27 couples who had engaged in 25 acts or fewer, the per-partner β was lower (β p: 29.4%, 95%CI 19.3%, 42.1%) but the per-act β was higher (β a: 2.32%, 95%CI 1.45%, 3.72%). Probabilities were higher among couples who never used condoms (n=14; β p: 59.2%, 95%CI 38.7%, 76.9%; β a: 1.20%, 95%CI 0.68%, 2.12%) than among those who always used them (n=27; β p: 24.2%, 95%CI 14.0%, 38.6%; β a: 0.49%, 95%CI 0.25%, 0.95%).

Conclusions: HPV is highly transmissible. These data support some but not complete protection with condoms. Accurate estimates of β will help to better forecast the impact of prevention strategies, including vaccines.

O-27.05

CONDOM USE IS ASSOCIATED WITH LOWER HPV PREVALENCE IN MEN

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Background: Reported associations between condom use and HPV infection in men have been inconsistent.

Objective: To evaluate the associations between frequency of condom use during vaginal sex and HPV detection by anatomic site in a cross-sectional study of asymptomatic men.

Methods: We tested 463 men ages 18 to 40 in two U.S. cities for HPV at 6 anogenital sites and in semen. HPV testing by PCR and reverse line blot genotyping for 37 types was conducted. Men completed a self-administered questionnaire, including questions regarding number of partners and the frequency of condom use during vaginal sex with any partner in the past 3 months ("always", ">half the time", "<half the time", "<half the time", and "never"). Among 393 men who reported ≥ 1 female partner in the past 3 months, the proportions of men with HPV detected overall and at each anatomic site by frequency of condom use were calculated. Logistic regression was used to examine associations between level of condom use and detection of HPV, after adjustment for confounders. Effect modification by number of recent partners was evaluated using stratified analysis (1 vs >1 partners).

Results: The proportion of men with HPV detected at any site ranged from 37.9% among men who "always" used condoms to 53.9% among those who "never" did (ptrend=0.008). Always using condoms (vs less frequent use) was associated with lower odds of HPV detection at any site (adjusted odds ratio [AOR]: 0.50, 95% confidence interval [CI]: 0.30-0.83). Associations were significantly different between strata of recent number of partners (AOR for 1 partner: 0.69, CI: 0.38-1.25 vs AOR for >1 partner: 0.22, CI: 0.08-0.58; p for interaction=0.05).

Conclusions: Consistent condom use, particularly among men with multiple partners, was strongly associated with lower HPV prevalence in men.

O-27.06

INTERNATIONAL HPV INCIDENCE AMONG MEN AGES 18-70 YEARS

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M Abrahamsen, H. Lee Moffitt Cancer Center, Tampa, US

Introduction: Human Papillomavirus (HPV) infections cause disease in men and women, and HPV transmission from males to females influences cancer risk in females. Unfortunately we know little regarding the natural history of these infections in men. The purpose of this study was to describe the overall, age specific, and country specific incidence of HPV infections in men.

Methods: HPV incidence was estimated among 1160 men ages 18-70 years who reside in Brazil, Mexico, and the US. Men were examined at baseline and every six months, with a mean follow-up of 12.6 months. Samples obtained from the coronal sulcus, glans penis, shaft, and scrotum were combined and analyzed for presence of HPV DNA using the Roche Linear Array method.

Results: The rate of new HPV infections was 5.0% per month with the highest rates observed for HPV types16, 51, 52, and 84. The incidence of a new HPV infection was 5.2, 1.2, 6.0, and 2.4 per 1000-person months for HPV types 6, 11, 16, and 18 respectively, while the 12 month cumulative risk of acquiring any of these 4 vaccine types was 13%. A significantly higher rate of non-oncogenic HPV infection acquisition was observed among Brazilian men compared to men in Mexico and the US (p<0.01), and Mexican men had a significantly lower acquisition rate for oncogenic infection (p<0.05). Overall, the rate of acquiring any, oncogenic, and nononcogenic new HPV infections did not vary by age group (18-30, 31-44, 45-70 yrs.) However, acquisition of a new HPV 6 infection was inversely associated with age.

Conclusion: In conclusion, 18-70 yr old men had a relatively high rate of HPV infection acquisition.

O-27.07

CONDOMS PREVENT INCIDENT ANAL HUMAN PAPILLOMAVIRUS INFECTION IN MEN

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Objectives: Data regarding the efficacy of male condoms in preventing HPV transmission is conflicting. We determined the impact of condoms in decreasing incident anal HPV and anal dysplasia in sexually active men who have sex with men (MSM).

Methods: This multisite prospective study recruited 1409 HIV-negative MSM. During each twice-yearly visit, we assessed anal HPV infection by PCR, obtained anal cytology and collected behavioral data including condom use. We used generalized estimating equation regression analyses to account for repeated measures in each participant and to identify predictors of incident HPV infection and anal dysplasia.

Results: Of 1409 MSM followed for up to 36 months, 40% reported always using condoms for receptive anal sex (RAS). At baseline 60% were HPV-infected, 15% had low-grade squamous intraepithelial lesions (LSIL) and 5% had high-grade SIL (HSIL). The incidence of anal HPV was 57 per 100 person-years (py), LSIL: 13 per 100py and HSIL: 5 per 100py. In univariate analyses, less frequent condom use during RAS was associated with incident anal HPV infection and incident LSIL, but not incident HSIL. After adjustment for sexual activity, substance use and age, a higher proportion of condom use in the previous 6 months was associated with lower incident anal HPV infection (P<0.001). There was moderate evidence for condom use in preventing incident LSIL (P=0.07), but no evidence for condom use in preventing incident LSIL (P=0.80).

Conclusions: Condoms protect against incident anal HPV infection and likely LSIL in a population with a high prevalence of disease. Given increasing rates of HPV-associated anal cancer and limited treatment options for anal HPV disease, condom use should be encouraged as a strategy to prevent anogenital HPV acquisition and could bolster messages for its use in HIV prevention.

O-27.08

HIGH ANAL HPV PREVALENCE IN MULTINATIONAL SAMPLES: THE HIM STUDY

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Introduction: An increase in anal cancer incidence in men from western countries highlights the importance of gathering data about anal canal HPV prevalence in men. There are no reports of anal HPV prevalence in cross-national, community samples of men.

Methods: Linear array genotyping for 37 HPV types was conducted for anal canal samples of 1200 men ages 18-70 years from São Paulo, Brazil; Cuernavaca, Mexico; and Tampa, USA. Eligibility included no history of genital warts or anal cancer, no HIV diagnosis, and no current STD diagnosis. Exfoliated cell samples between the anal os and the dentate line of the anal canal were obtained with a saline-wetted Dacron swab.

Results: Median age was 30 years. In Brazil, 25.4% of men were positive for anal HPV. In Mexico 10.1% were positive and in the US 22.2% were positive. In the combined population 19.4% had anal HPV. Oncogenic HPV types were present at the anal canal in 13.4% of men. Among men who reported no sex with other men, 13.3% were positive for anal HPV. Among men who reported sex with other men, 43.2% were positive for anal HPV.

Conclusions: At all three study sites, anal HPV was a common infection in men; however, prevalence in Brazilian and US men was twice as high as prevalence in Mexican men. In the combined population, prevalence of oncogenic genotypes at the anal canal was common. Anal HPV infection was also common among men who acknowledged no lifetime sex with other men; however, prevalence was three times higher among men who acknowledged sex with other men. This finding suggests a need for more research to understand the natural history of anal HPV in men.

POSTER ABSTRACTS SESSION 27

POSTER SESSION V THURSDAY 10.00

CIRCUMCISION AND SEROLOGICALLY DETERMINED HPV INFECTION IN A BIRTH COHORT

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Circumcision has been reported to affect infection with human papillomavirus (HPV) in men, but results are inconsistent. In spite of this uncertainty, protection from HPV infection and subsequent lower risk of cervical cancer has been suggested as a reason to circumcise boys.

We have undertaken the first population-based study of the relationship between circumcision and serological evidence of HPV infection. We followed males in a cohort born in Dunedin, New Zealand in 1972/73, from age 3 to 32 years. Seropositivity for the oncogenic HPV-16 and 18, and the non-oncogenic types 6 and 11 at age 32 were studied in relation to circumcision status at age 3. Information about family factors and sexual behaviour was used to adjust for potential confounding.

Seropositivity to any of these types was associated with lifetime number of sexual partners (p= 0.03), and lower moral-religious emphasis of the family of origin (p<0.001).

Circumcision did not appear to be protective. The adjusted odds ratio (95% confidence interval) for seropositivity among the circumcised compared to the uncircumcised for HPV-16 and/or 18 was 1.4 (0.85-2.2), for HPV-6 and/or 11 was 1.1 (0.43-2.8), and for any of these types, 1.4 (0.89-2.2).

These data do not support the hypothesis that circumcision provides major protection against HPV acquisition by men, but do not rule out the possibility that circumcised men have less persistence of HPV infection which may result in some reduction in risk of transmission to women.

P-27.10

OUTCOMES FOLLOWING INCIDENT HPV 6/11/16/18 DNA DETECTION IN YOUNG MEN

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Objective: To examine clearance, persistence and progression following incident external genital HPV 6/11/16/18 DNA detection in young men.

Methods: The study population consisted of men 15-27 years-old undergoing penile, perianal/perineal and scrotal swab PCR-testing for HPV 6/11/16/18 at approximately 6-month intervals for up to 3 years in the placebo-arm (n=2,038) of a clinical trial of a quadrivalent HPV vaccine. Intra-anal swabs were additionally collected for men having sex with men (MSM). Outcomes following incident HPV DNA detection (n=424) were estimated using standard and modified Kaplan-Meier methods. Incident HPV types were correlated with types detected in biopsy specimens in assessing disease development, with fractional adjudication for lesions infected with multiple HPV types.

Results: Within 12 months following incident HPV 6-or-11 DNA detection (n=154), 22.7% of heterosexual (HM) men were estimated to develop genital warts positive for HPV 6 or 11 as compared to 40.7% of MSM (p < .05). None of the HM (n=183) or MSM (n=45) with incident HPV 16-or-18 DNA were estimated to develop genital warts positive for these HPV types. HPV-16 was more likely to persist (50.2%) at 12 months than HPV-18 (36.8%), HPV-6 (27.0%) or HPV-11 (17.0%) (p<.05) (in the absence of genital warts), with 12-month clearance rates without wart development of 49.7%, 63.1%, 48.3% and 51.6%, respectively. However, without truncation at wart development, HPV-6 (58.7%) and-11 (71.2%) were more likely to clear than HPV-16 (p<.05).

Conclusions: MSM were observed to be more likely to develop genital warts following incident external genital HPV 6 or 11 DNA detection than HM. This finding may relate in part to broader anogenital contact (e.g., external and intra-anal), or additional study surveillance procedures performed (e.g., rectal exam, anal cytology), in MSM.

MEN HAVE A HIGHER HPV VIRAL LOAD THAN WOMEN

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Background: Human papillomavirus (HPV) infection is associated with genital cancers in both women and men. Whereas much is known about HPV and cervical cancer in women, data on male genital HPV infections are limited. This study examined HPV prevalence and viral load in human immunodeficiency (HIV) seropositive (HIV+) and HIV seronegative (HIV-) women and men. Method: Participants were 257 HIV+ women, 290 HIV- women, 143 HIV+ men and 354 HIVmen. High-risk (HR) HPV viral loads in cervical and penile cells were determined by hpVIR real-time polymerase chain reaction. . Results: The prevalence of HR-HPV infection was significantly higher in HIV+ women and men (53%, 132/257 and 59%, 85/143 respectively) compared with HIV- women and men (21%, 60/290 and 32%, 112/354, respectively, P<0.0001 for both women and men). Both HIV+ women and men showed a significantly higher prevalence of multiple (>1) HR-HPV genotypes (49%, 65/132 and 60%, 51/85 respectively) compared to HIV- women and men (28%, 17/60 and 33%, 37/112 respectively, P=0.007 for women and P=0.0002 for men). HIV- men were found to have a significantly higher prevalence of HPV infection compared with HIV- women (32% 112/354; 21% 60/290; respectively P=0.002). HIV- men were also found to have significantly higher penile HR-HPV viral load per cell compared with HIV-women (median 3 in men compared with median 1 copies per cell in women, P=0.001). Assessing HPV-16, HIV-men were found to have significantly higher viral load/cell compared with HIV- women (median 5.6 copies per cell in men; median 0.4 copies per cell, P=0.02). No difference was found in HR-HPV prevalence or viral load of HIV+ men compared with HIV+ women. Conclusion: HIV- men had a significantly higher HPV prevalence and viral load compared to women indicating that males are important HPV carriers and could be at risk of developing penile cancer.

P-27.12

PREVALENCE OF COLPOSCOPY-DETECTED PENILE LESIONS IN KENYAN MEN

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Background: Human papillomavirus (HPV)-associated penile lesions in men may increase the risk of HPV transmission to their female partners. Prevalence data on HPV-associated penile lesions are needed from less-developed countries, including Africa, with a high burden of cervical cancer.

Objectives: To determine the prevalence of colposcopy-detected penile lesions (HPV-associated flat lesions; papular lesions; pearly penile papules) and clinically-diagnosed genital warts among young men from Kisumu, Kenya.

Methods: Visual inspection of the penis was conducted using a colposcope, after application of 3% acetic acid, at the 24 month visit for a subset of men participating in a randomized controlled trial of male circumcision. Visual inspection was conducted from May 2006 to October 2007. All photos were double-read for quality control.

Results: Of 276 participating men, 159 were circumcised and 117 were uncircumcised. The median age of men was 22 years (range 20–26). HPV DNA prevalence in penile exfoliated cells from the glans/coronal sulcus was 38.2%. The prevalence of papular lesions and HPV-associated flat lesions after acetic acid application was 33.8% and 16.2%, respectively, on the glans or foreskin, or glans and foreskin remnant of circumcised men. Pearly penile papules were detected in 62.9% of participants. Genital warts were uncommon, with one case among an uncircumcised participant. Compared to men who were circumcised, men who were uncircumcised had a higher prevalence of flat lesions (27.0 versus 8.3%;p<0.0001), but not papular lesions (23.3 versus 41.5%;p=0.002) nor pearly penile papules (61.5 versus 63.9%;p=0.7). Socio-demographic risk factors for ascertained penile lesions, as well as HPV positivity and viral load data will be presented.

Conclusions: The highest prevalence of aceto-white flat lesions was found on the glans and foreskin among uncircumcised men. Circumcision may reduce the point prevalence of HPV-associated flat lesions, which may ultimately reduce male to female HPV transmission.

HPV DNA IN THE FINGERNAIL TIPS OF YOUNG MEN

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Background: DNA from alpha-genus HPV types has been detected in fingernail tip specimens obtained from sexually active young men.

Objectives: Compare overall and type-specific HPV DNA incidence in fingernail tip and genital site specimens.

Methods: A cohort of 18-20 year old, heterosexual male university students was enrolled. Clinical exams were performed every 4 months and sexual history diaries were completed bi-weekly. The maximum follow-up was 3 years. Cell samples were collected from fingernail tips and genital sites (penile shaft and glans, scrotum and urine) and tested for 38 alphagenus HPV types using a PCR-based assay. HPV DNA incidence in fingernail tips and genital sites was compared using Cox regression.

Results: 434 men completed 2255 visits. The majority of subjects were white (85.6%) with an average age of 18.9 years. Incident detection of HPV DNA (any-type) in fingernail tip and genital specimens was 24.9/100 person-years and 38.9/100 person-years, respectively (Hazard Ratio 0.60; 95% CI 0.50, 0.72). HPV-16 and HPV-84 were the most common types detected at both sites. All types were detected less frequently in fingernail tip specimens than in genital specimens. Of 245 cases of incident type-specific HPV DNA detected in fingernail tip specimens, 176 (72%) had incident type-specific HPV DNA detected in fingernail tip specimens, 176 (72%) had incident type-specific HPV DNA detected after the same type was first detected in a genital specimen, 67 (38%) were detected after the same type was first detected in a genital specimen and 13 (7%) were detected before the same type was first detected in a genital specimen.

Conclusions: About 25% of enrolled men had incident alpha-genus HPV DNA detected in fingernail tip specimens. Nearly three-quarters of men with incident type-specific HPV DNA detected in fingernail tip specimens had the same type detected in genital specimens.

P-27.14

COST-EFFECTIVENESS OF ANAL CANCER SCREENING IN HIV-POSITIVE MEN

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Background: Histology is the gold standard for the detection of anal intraepithelial neoplasia (AIN) and can be obtained by high-resolution anoscopy (HRA) with directed anal canal biopsy. Anal cytology (Pap testing) and oncogenic HPV detection are potential screening tests in addition to HRA but the cost-effectiveness of these tests is unknown. Objective: To assess the cost-effectiveness of HRA, anal cytology and/or anal HPV detection in screening for high grade anal dysplasia (AIN 2/3). Methods: 401 HIV+ males were part of the TRACE anal cancer screening study: they had concomitant Pap, HPV and HRA examinations. Compared with usual care (i.e. no screening), a decision analytical model was used to determine the cost-effectiveness of three anal cancer screening strategies: 1) the direct use of HRA, 2) abnormal cytology followed by HRA and 3) a positive oncogenic HPV test followed by HRA. The model included different definitions of abnormal cytology (HSIL, LSIL or ASCUS) and the combined use of cytology and HPV testing. The outcome was the number of AIN 2/3 cases detected. Costs were estimated from institutional data and sensitivity/specificity of cytology and HPV tests were obtained from the TRACE study. Results: The costs per procedure for HRA, cytology and HPV testing were $\notin 170, \notin 80$ and $\notin 85$, respectively. The direct use of HRA was the most cost-effective strategy. It detected 98 cases of AIN 2/3 and cost $\notin 1,090$ /case. Equally effective screening included a positive HPV test followed by HRA but its cost was $\notin 1,353$ /case. Conclusion: In HIV+ gay men the direct use of HRA is the most cost-effective strategy for detecting AIN 2/3. The higher cost per use for HRA was offset by the high sensitivity and low specificity of other screening tests.

P-27.15 INCIDENCE OF WARTS AND LESIONS IN MEN: THE HIM STUDY

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Background: Little is known about the natural history of genital warts and lesions in men. Objectives: To assess the incidence and HPV type distribution of genital warts and lesions in men. Methods: We examined 2607 men ages 18-70 in the US, Brazil and Mexico for newly acquired warts and lesions. The presence of HPV DNA was detected using the Roche Linear Array method. Results: The overall incidence rate for warts and lesions was 2.5 (95% CI=1.9-3.2) and 0.6 (95% CI=0.3-0.9) per 1000 person-months, respectively. Among the 4 current vaccine types, the rate of acquiring warts with detectable HPV6 was 1.3, followed by HPV types 11 (0.5), 16 (0.3), and 18 (0.04) per 1000 person-months. The incidence rate for lesions with HPV types 6, 11, and 16 was 0.1, 0.1 and 0.04 per 1000 person-months, respectively. HPV18 DNA was not detected in any lesions. In men who tested positive for any HPV infection on their adjacent healthy skin at baseline, the incidence rate of warts was doubled compared to men who tested negative (3.2 vs.1.5 per 1000 person-months). Compared to men with fewer than 5 female sex partners in their lifetime, men with 5 or more female partners had higher incidences of both warts (1.4 vs. 3.1 per 1000 person-months) and lesions (0.5 vs. 0.7 per 1000 person-months). The highest rate of acquisition of warts was observed in US men (3.9 per 1000 person-months), whereas the rate of lesions was highest in Mexico (0.8 per 1000 person-months). Warts were most common among men ages 18-26 (3.3 per 1000 person-months), but men ages 41-70 had the highest rate of lesions (0.8 per 1000 person-months). Conclusion: The incidence rate was higher for warts than lesions. HPV6 and HPV11 were most common in warts.

P-27.16 IMMUNOGENICITY OF QUADRIVALENT HPV (TYPES 6/11/16/18) VACCINE IN YOUNG MEN

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BACKGROUND: In males, anogenital infection with human papillomavirus (HPV) can lead to neoplastic lesions including genital warts, penile, perineal, perianal, and anal cancer. The quadrivalent HPV (types 6/11/16/18) vaccine is highly immunogenic in adolescent and adult females. Data are sparse concerning HPV vaccine immunogenicity in males; however, previous analyses have suggested that antibody responses to quadrivalent HPV vaccination in adolescent males were non-inferior to those of females.

OBJECTIVE: This analysis examined quadrivalent HPV vaccine-induced serum anti-HPV 6, anti-HPV 11, anti-HPV 16, and anti-HPV 18 responses in young men.

METHODS: Data presented are from a randomized, double-blind, placebo-controlled trial wherein 4,065 young men aged 16-26 years were administered quadrivalent HPV vaccine or placebo at enrollment, month 2, and month 6. Serum was collected at enrollment and at months 7, 24, and 36 for analysis of vaccine type anti-HPV antibodies via competitive Luminex immunoassay (cLIA). Seroconversion was determined from the percentage of vaccinated subjects with cLIA geometric mean titers above a predefined cutoff value (20, 16, 20, and 24 mMU/mL for HPV 6, 11, 16, and 18, respectively). Analyses were conducted in a per-protocol population seronegative at day 1 and DNA-negative from day 1 through month 7 to the relevant vaccine HPV type.

RESULTS: At month 7, vaccine-induced serum antibody titers for HPV 6, 11, 16, and 18 were 447.0 mMU/mL (95% CI: 422.1, 473.5), 624.2 mMU/mL (95% CI: 594.4, 655.6), 2402.5 mMU/mL (95% CI: 2,270.6, 2,542.0), and 402.2 mMU/mL (95% CI: 380.2, 425.6), respectively. Antibody titers for subjects receiving placebo were below the limit of detection. By month 7, 98.9%, 99.2%, 98.8%, and 97.4% of subjects seroconverted to HPV 6, 11, 16, and 18, respectively.

CONCLUSION: In summary, the immunogenicity of quadrivalent HPV vaccine in men is comparable to that of women, as measured by cLIA titer and seroconversion percentage.

HPV PREVALENCE, GENOTYPES AND CLINICAL OUTCOME IN PENILE CANCER

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Although penile cancer is a rare malignancy, it causes severe morbidity, mortality and physiological stress. Penile cancer has many similarities with cervical cancer and seems to have a strong link to high-risk types of HPV. In the literature, the prevalence of HPV in penile cancer varies between 42 and 81,5 %. The large variation might depend on methodological differences, differences among populations and small study groups.

Here, the aim was to investigate HPV in a large, well-characterized material and to correlate data to clinical outcome.

The prevalence and genotype of HPV was studied in 236 patients diagnosed with penile cancer. Using real-time PCR with type-specific primers, HPV types 16, 18, 31, 33, 35, 45, 51, 52, 56, 58, 6 and 11 were investigated.

HPV DNA was found in 90% of the tumors. The most common types were HPV 16 (66%), 18 (33%), 11 (11%), 6 (5%) and HPV 35 (3.8%). Of these, HPV 16, 18 and 33 are found to represent a high risk for cervical cancer. Multiple infections were present in 32%. Co-infection with HPV 16 and 18 was the most frequent combination but multiple infections with 3-4 HPV types were also present. Correlation to clinical data is in progress.

We present an investigation on HPV prevalence in the largest clinical material described. Our results show that the prevalence is higher than earlier described. Our data also suggest, in accordance with previous studies, that HPV 16 and 18 are the main types involved in the progression to penile carcinoma. Cervical cancer low risk types such as HPV 6 and 11 are found in 16% of penile cancer. Multiple type HPV infections are more common than described earlier in the literature.

An evaluation of HPV type as a predictor of clinical outcome will be presented.

P-27.18

MALE CIRCUMCISION AND PENILE HPV: SYSTEMATIC REVIEW AND META-ANALYSIS

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Background: The relationship between male circumcision (MC) and genital HPV infection and HPV related lesions in men is controversial.

Objectives: The main objective of the study is to assess the association between MC and penile HPV and genital warts using published data and taking into account potential sources of heterogeneity and publication bias.

Methods: A MEDLINE search using combinations of selected keywords were used to identify published studies with data on circumcision status in men with and without HPV infections. A meta-analysis was performed of published studies from 1971 through October 2008. Pooled estimates of the OR were calculated with both fixed and random effects model assumptions. Heterogeneity between studies was tested using the Q statistic. Publication bias was evaluated using the funnel plot and the Egger's and Begg's tests.

Results: So far we have included 18 studies that involved a total of 6,778 circumcised and 5,181 uncircumcised men. We found that MC is associated with a statistically significant reduced risk of detecting penile HPV and related lesions, with an overall OR of 0.62 (95%CI: 0.47-0.81). The inverse association was much stronger and statistically significant for penile HPV DNA detection (OR=0.53, 95%CI: 0.39-0.73) than for genital warts (OR=0.89, 95%CI: 0.59-1.33). Since studies showed a great variability in methodological issues, further stratified analyses by design variables will be shown in detail. The final results to be presented will include data from additional studies published after October 2008.

Conclusions: Our meta-analysis shows a consistent and robust inverse association between being circumcised and detection of penile HPV, suggesting a protective effect of MC on penile HPV. Given the consistent protective effects also found with HIV, MC could be considered an option in adult men, particularly those in high-risk countries that cannot afford prevention strategies to control HIV, HPV and their related diseases.

HPV INFECTION AMONG MEN PARTICIPATING IN A CLINICAL TRIAL

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Background: Recent studies have demonstrated a high burden of HPV-related disease in males. Protocol-020 was designed to evaluate the efficacy of Gardasil^{*} in men (heterosexual men [HM] and men-having-sex-with-men [MSM]). Efficacy against any HPV6/11/16/18-related external genital warts and penile/perineal/perianal intraepithelial neoplasia in the per-protocol population was 90.4% (95%CI:69-98).

Objectives: Baseline data from the males enrolled in this trial provide useful information on the prevalence of genital HPV infection in a large, international population. We investigated the baseline prevalence of 14 common HPV types and seroprevalence of the 4 vaccine-HPV-types

Methods: We enrolled 4,065 men aged 16-26. Subjects underwent detailed anogenital exams and sampling from the penis, scrotum, anal (MSM cohort only) and perianal region. Infection in any one of these specimens classified the participant as HPV postive for this analysis. Samples were tested for 14 HPV types (6/11/16/18/31/33/35/39/45/51/52/56/58/59). Anti-HPV6/11/16/18 antibodies were measured using a competitive Luminex[®] assay which measures a single neutralizing epitope for each HPV type.

Results: HPV6/11/16/18 DNA and/or antibodies were found in 17.3% of subjects (12.2% by DNA; 7.6% by serology). HPV6, 11, 16, and 18 DNA prevalence was 4.7%, 1.4%, 5.1%, and 2.8%. Seroprevalence was 4.4%, 1.5%, 2.3%, and 1.1%. Multiple vaccine-HPV-types were detected in 3.5% of subjects, with 2.6%, 0.6%, and 0.2% positive to 2, 3 and 4 vaccine-HPV-types, respectively. In the overall cohort, 25.5% of subjects were positive by DNA to ≥ 1 of 14 tested HPV types. Infection with HPV16 was most common (5.1%), followed by types 6 (4.7%), 56 (4.7%), and 51 (4.5%).

Conclusion: In summary, a substantial proportion of the study population was positive to at least one common HPV type, while multiple vaccine type infections were relatively low. A high proportion of men in this age group are expected to benefit from quadrivalent HPV vaccination.

P-27.20

SMOKING AND HUMAN PAPILLOMAVIRUS (HPV) IN MEN: THE HIM STUDY

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Background: Smoking may be associated with HPV infections by suppressing immune function. Smoking is an established co-factor in HPV-associated cancers among women, but the effects of smoking on HPV infection in men is not well described. We conducted a cross-sectional analysis using data from the on-going HIM Study to assess the role of smoking and HPV infection in men. Methods: Men were recruited from Brazil, Mexico and the US. Following exclusions, selfreported epidemiological covariates were available on 2,518 men; HPV status, assessed by PCR, was available on 1,986 men. HPV infection was grouped by: 'Any type HPV', 'Oncogenic HPV', and 'Non-oncogenic HPV'. Descriptive and multivariable logistic regression analyses were performed. Results: Mean age of participants was 32.7 yrs, 45.6% were 'White', 35.3% were circumcised, and 55% had \leq 9 lifetime female partners. Those with HPV data, 64.5% were positive for any HPV, 31.9% for oncogenic HPV, 38.5% for non-oncogenic HPV, and 35.5% tested negative. 21% of HPV negative men were current smokers compared to 24.9% in any HPV (P=0.14), 27.2% for oncogenic HPV (P=0.03), and 35.5% nononcogenic (P=0.65). HPV negative men also smoked fewer cigarettes/day and years smoked. There were no statistically significant associations between smoke status or amount smoked and risk of HPV overall or when stratified by country or age. However, when we stratified by number of lifetime female partners and compared to never smokers, current-(OR=1.55; 95% CI 1.02-2.36) and light smokers (OR=2.04; 95% CI 1.13-3.71) with fewer lifetime female partners (\leq 9) were at a statistically significant increased risk of oncogenic HPV. Conclusions: Current smoking may be associated with oncogenic HPV infection in men with fewer sexual partners. Future longitudinal analyses may reveal an association between smoking and HPV clearance and/or persistence.

PENILE CANCER – INCIDENCE IN DANISH MEN 1978 - 2003

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BACKGROUND: Penile cancer is a rare cancer constituting less than 1% of all male cancers. Depending on the histological type distribution of the cancer around 40-50% of all penile cancers have been shown to be associated with HPV, especially HPV type 16. If the two prophylactic vaccines against HPV16 and HPV18 also protect men against infection, the burden of HPV-related disease – including penile cancer - in men will be important to establish.

OBJECTIVE: To take advantage of the unique Danish Cancer Registry and report the incidence of penile cancer in a largely uncircumcised population in the period 1978-2003. Furthermore, to study the distribution of different histological types.

METHODS: All penile cancers reported to the Danish Cancer Registry from 1978 to 2003. Age-specific and agestandardized incidence rates were calculated. Trends over time will be evaluated.

RESULTS: Almost 1100 penile cancers were identified in the 26 year time period. The overall age-standardized (World Standard Population) incidence rate for the whole time period was 1.01 per 100,000. The incidence seemed to remain unchanged in the time period (0.97/100,000 in 1978-83 and 1.11/100,000 in 1999-2003). The median age at diagnosis was 69 years. No cases were observed under the age of 20 years, and the incidence peaked in men older than 85 years. No major changes were apparent in the age-specific incidence rates over time. Finally, nearly 95% of cancers were squamous cell carcinomas.

CONCLUSION: Reports from other countries have reported a decreasing incidence of penile cancer. However, in the Danish male population, which is mainly uncircumcised, the incidence remained stable over time at around 1/100.000 in the period 1978 - 2003. If a vaccine against HPV also protects men, the potential for reducing the incidence of penile cancer is apparent. However, it will take several decades before this effect can be observed.

P-27.22

HPV IN HIV + MEN WITH GENITAL WARTS IN AFRICA

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BACKGROUND: Genital warts account for 5% of the male sexually transmitted infections (STI) presenting in Johannesburg clinics. A HPV vaccine, protecting against type 6, 11, 16 and 18 has been developed. Little data exists regarding the HPV types in penile warts of HIV-infected men in Africa.

OBJECTIVE: To determine HPV types and epidemiological associations in men with genital warts.

METHODS: Nurses collected demographic data and swabs from the prepuce, penile shaft and genital warts areas of the penis for HPV genotyping from 74 HIV seropositive men with penile warts. Three samples were not available for analysis. HPV genotyping was performed using the Linear Array HPV Genotyping Test (Roche, USA). Effects of condom usage, multiple sexual partners, age, CD4 count and circumcision status on HPV results were estimated using chi square tests for proportions, t tests for means and other non-parametric tests where appropriate.

RESULTS: 90% (64/71) of the men were receiving antiretroviral therapy with median age of 36.2 years. 69% (49/71) clients were uncircumcised. 85% (60/71) tested positive for HPV; 35% (25/71) and 34% (24/71) of the men had HPV types 6 and/or 11 respectively. 7% (5/71) participants had both 6 and 11. Of those who tested positive for the oncogenic types 16 /18, 10/71 (14%) were positive for type 16 only, 5/71 (7%) were positive for type 18 only, 8/71 (11%) were positive for both. There were no associations between HPV type and age, condom use, circumcision status or CD4 count.

CONCLUSION: Our study shows a high prevalence HPV types 6 and/or 11 for total prevalence of 69% in HIV-infected men with penile warts. Given the poor availability of treatment for genital warts in the SA clinics, a quadrivalent vaccine for men would have a significant benefit.

ASSESSING DIFFERENCES IN MEN WHO SELF-REPORT HPV TEST RESULTS

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Background: HPV is responsible for anogenital warts and cancers among both males and females. Males play an important role in HPV transmission; however, there is very little research that examines males' cognitive and emotional responses to an HPV test result. Men are not routinely tested for HPV; however, an ongoing natural history study of HPV in men provides a unique opportunity to assess the psychosocial impact of an HPV test result and whether men accurately report their test results.

Objectives: To describe demographic, cognitive and emotional differences between men who self-reported their HPV test results accurately and those who self-reported inaccurately.

Methods: Males (ages 18-70) participating in an HPV natural history study completed a theoretically grounded computerassisted-survey-instrument that assessed their cognitive and emotional reactions to an HPV test result. Surveys were completed 2-4 weeks after receiving their test results. Descriptive statistics and chi-squares were tabulated.

Results: Of the 205 males, most were young (66% were 18-25 years), white (69%), single (74%), and had some college credit (54%); 42% were HPV+ and 58% were HPV-. Overall, 81% accurately reported their HPV test results; of those, 74% of HPV+ and 87% of HPV- men accurately reported their test results (p = 0.01). Significant differences (p < 0.05) were found by marital status, Hispanic ethnicity, insurance status, emotional responses and those who reported defensive/avoidance responses. There were no significant differences by age, education, HPV knowledge, partnership status, perceived threat of HPV-related outcomes, disclosure of test results to partners, or vaccine intentions.

Conclusions: Some differences were found between men who self-reported their HPV test results accurately and those who self-reported inaccurately. Further research is needed to help ascertain why men inaccurately self-reported their HPV test results and how this inaccuracy affects primary and secondary HPV prevention efforts.

P-27.24

HPV IN PENILE CARCINOMA. CASES FROM CENTRAL AND EAST EUROPE

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Background: Penile cancer (PC) is a rare disease. Strong association between HPV infection and development of PC has been identified. Prevalence of HPV found in PC ranged from 30-80% depending on characteristics of detection methods and tumor type.

Objective: To determine the prevalence and the distribution of HPV genotypes in PC samples from the Central and East Europe.

Methods: We report 81 cases of penile carcinoma collected in the files of Pathology Departments in Pilsen, Czech Republic and Moscow, Russia. The patients ranged in age from 33 to 89 years. All presented with tumors involving the glans and/or shaft measuring 0.5-5 cm. Microscopically, 46 tumors were conventional squamous cell carcinoma (SCC), 20 conformed to the so-called mixed type, 6 were warty SCC, 1 verrucous, 2 papillary, and 1 basaloid, 1 sarcomatoid. The remaining 4 cases evidenced SCC in situ. HPV was analyzed from formalin-fixed paraffin-embedded (FFPE) tissues in 50 cases using PCR with primers CPSGB and SPF10 primers located in E1 and L1 region of HPV genome, respectively. Genotyping was performed by sequencing analysis and reverse hybridization (LiPA).

Results: Overall, HPV was detected in 36/50 cases. HPV 16 was the most common type , detected in 25 cases, of which 17 were conventional SCC, 6 mixed SCC, 1 papillary SCC, and 1 warty. HPV 18 was detected in 1 case of papillary SCC. Other HPV detected included both HR and LR types, particularly 6, 33, 45, 51, 54, 56, 58, 66, and 70.

Conclusion: The majority (72%) of penile carcinoma samples from Central and East Europe contain HPV DNA, with the highest frequency of HPV type 16. The PCR system with primers targeting both L1 and E1 region of HPV genome is suitable for HPV DNA detection in FFPE samples. Quality of FFPE material limits the sensitivity of detection.

P-27.25 SEXUALLY TRANSMITTED INFECTIONS IN ASYMPTOMATIC MEN FROM THE HIM STUDY

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Sexually transmitted infections (STI) have large impact in sexually active population due to its complications. A considerable number of STIs are asymptomatic or missed by routine laboratory tests. We sought to determine the prevalence of Chlamydia trachomatis, Neisseria gonorrhoeae, Herpes simplex type 2 (HSV-2) and Syphilis in asymptomatic men from an international epidemiological study of the natural history of HPV in Men (HIM study). The study population consisted of men who were between 18-70 years, were residents in Sao Paulo, Cuernavaca and Tampa, negative for clinical signs or symptoms or currently being treated for any sexually transmitted disease. Men who reported previous genital or anal warts, as well as penile or anal cancers were ineligible. At study entry, men were asked to provide a urine sample that was sent for detection of C. trachomatis and N. gonorrhoeae by PCR (Cobas Amplicor *, Roche, USA). In addition, Syphilis and HSV-2 antigens were searched in sera samples from the same individuals. Results are reported from 2190 Men included in the study from 2005 to 2008. Prevalence of HSV-2 infections was 21.1% among Brazilians, 11.3% in men from Tampa and 8.4% in Cuernavaca. The Brazilian site also showed the highest prevalence of Syphilis (3.2%), followed by Mexico (0.8%), while no cases were reported at the American site. Concerning bacterial STIs, the following frequencies were observed: Chlamydia trachomatis: USA, 1.6%; Mexico, 1.5%; and Brazil, 1.2%; Interestingly, Neisseria infections were only found among Brazilians (3.2%).

Prevalence of common STIs is relatively frequent among asymptomatic men from three different communities in the US, Mexico and Brazil. Overall, higher rates of STIs were observed among Brazilian men compared to men in Mexico and the US. HPV DNA data will be presented.

P-27.26

HUMAN PAPILLOMAVIRUS IN LIQUID CYTOLOGY IN MEXICAN MALES

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BACKGROUND. Human papillomavirus (HPV) is sexually transmitted and males are implicated in the epidemiological chain of infection, and can be asymptomatic carriers, transmitters or victims of HPV infection. High oncogenic risk HPV types such as 16 and 18, are also related to penile cancer. OBJECTIVE. Determine the frequency of HPV infection in liquid based cytology samples from the mucose of the urinary meatus of asymptomatic and sexually active males from Chilpancingo, Gro. Mexico that attended the Cytopathology Laboratory of the Universidad Autonoma de Guerrero from September 2007 to February of 2008. METHODS. Thirty-seven males aged 20-58 years old, with a mean age of 36 were included in the study. HR-HPV presence was determined by PCR-RFPLP, liquid based cytology (LiquiPREP) was done with Papanicolaou staining. RESULTS. Eighteen normal cytologies were diagnosed (48.6%) and 19 (51.4%) cytologies with koilocytic changes characteristic to HPV infection were found. Using PCR we found 33 cases positive for viral DNA, the 13 cytologies with koilocytic changes were positive by PCR. Four different genotypes were found (16, 6, 62 y 81). HPV 16 was the most frequently found (13.5%). CONCLUSIONS. The detection of koilocytic changes from HPV infection by liquid based cytology in combination with PCR-RFLP constitute an excellent tool for the early diagnosis of HPV infection in asymptomatic males.

P-27.27 CIRCUMCISION STATUS AFFECTS GENITAL HUMAN PAPILLOMAVIRUS INFECTION IN MEN

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Background. There is evidence that partners of uncircumcised men have an elevated risk of cervical cancer and that uncircumcised men have an increased risk of penile cancer. We recently demonstrated that compared to circumcised men, uncircumcised men have a higher prevalence of HPV infection of glans penis.

Objective. Investigate the role of circumcision status in the acquisition and clearance of genital HPV infection in men.

Methods. HPV acquisition and clearance were examined in a longitudinal cohort study of 445 men. At baseline and at 2-month intervals, interviews were conducted and cell specimens were obtained for HPV DNA detection from genital subsites including glans penis, penile shaft, and scrotum.

Results. Overall, we observed no difference in the acquisition of HPV infection by circumcision status, but differences in the duration of HPV infection were observed. A significantly increased risk of HPV acquisition in the glans (RR=1.72; p=0.01) was observed for uncircumcised men who have sex with men (MSM). HPV took longer to clear in the glans of uncircumcised men (RR=0.70; p=0.01).

Conclusions. Our results provide evidence that uncircumcised men may be at greater risk of HPV-associated disease sequelae due to increased persistence of infection specifically in the glans penis. We previously observed a higher prevalence of HPV infection in the glans penis of uncircumcised men. The present analysis demonstrates that this higher prevalence is attributed to the longer duration of infection in the glans penis of uncircumcised men rather than to a greater rate of acquisition of infection. Differences in the ability to clear genital HPV infections by circumcision status appear to be site-specific. Persistent HPV infection in men may increase the risk of transmission of HPV to sexual partners and the risk of development of penile cancer.

P-27.28

ANALYSIS OF P53 POLYMORPHISM AND HPV DETECTION IN PENILE CARCINOMAS

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BACKGROUND: Several studies have tried to clarify the relationship between HPV related carcinomas and the p53 polymorphism Arg72Pro. These studies have been performed on cervix, lung and oral carcinomas. In previous studies we have observed a high incidence of penile carcinomas in our population and a high incidence of HPV detection (72%) in this population.

OBJECTIVES: The aim of this study was to study the frequency of this polymorphism in penile carcinomas and its possible correlation with HPV detection, in an effort to clarify if there is a genetic condition in our population that may influence its high incidence of this carcinoma.

METHODS: Samples from 49 patients with diagnosis of penile carcinoma and 100 healthy subjects (control group), were analyzed with specific PCR and SCCP techniques, for the detection of the Arg72Pro polymorphism. Detection of HPV DNA from patients were analyzed using consensus primers My09/11 and Gp5+/6+.

RESULTS: There was a significant difference in the frequency of the polymorphism between the group with carcinoma and those in the control group: Arg/Arg genotype, 8.15% vs. 67%; Pro/Pro or Arg/Pro genotypes 91.85% vs. 33%.

In the group with penile lesions 25% of the subjects Arg/Arg were HPV +; 82% of the subjects with Arg/Pro genotype were HPV+.

CONCLUSIONS: The Arg/Arg genotype is the most frequent genotype found in our control population (67% of the group). In contrast, the study group appears to be closely related with the presence of the Arg/Pro heterozygosis (91,85% with Arg/Pro genotype; no genotype Pro/Pro was detected).

Based on these findings, it is suggested that, in our population, p53 Arg/Pro heterozygosity could act as a potential risk factor for penile tumorigenesis.

ACKNOWLEDGEMENTS: This work has been supported by grants FIS PI07-0406 and FISCAM PI2006/37. JM Godínez is supported by a FISCAM fellowship MOV-2006-JI/04

P-27.29 HISTOPATHOLY OF PENILE LESIONS AND HPV FROM HIV POSITIVE MEN

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There is little information about the clinical and histopathological features of HPV infection in the male anogenital region. We therefore thought to correlate histopathological results and HPV DNA detection in a group of men residing in Sao Paulo, between 18-60 years, of whom 72 were HIV-seropositive and 72 were negative for HIV. The study was conducted from February 2004 until March 30, 2005, and was approved by the Institutional IRB. Each subject underwent urological examination and peniscopy, aiming the detection of clinical lesions. One hundred and two biopsies were performed in 17 aceto-white lesions and 19 warts observed in HIV-positive men and 29 aceto-white lesions and 37 warts observed in HIV negative men, partners of women referred to the clinic due to an abnormal cytology result. As compared to HIV-negative men, a higher frequency of HPV DNA positivity was observed in HIV-positive in both warts and aceto-white lesions which included acute and chronic inflammation, dysplasia of low and high grade, papillomatosis and diffuse parakeratosis. HPV 6 and 11 were the most frequent types detected in warts. However, aceto-white lesions were less HPV positive than histologically confirmed warts. Histopathological features including dysplasia, cytopathic viral changes and papillomatosis differ significantly between warts and acet-white lesions. Further studies to understand the characteristics of such lesions in the penis and its association with HPV infection are warranted.

SESSION 28

BEST POSTER LECTURES

PROGRAMME

SCANIA

THE 25TH INTERNATIONAL PAPILLOMAVIRUS CONFERENCE MAY 8-14 2009, MALMÖ, SWEDEN

TIME	NUMBER	TITLE	ROOM
2009-05-14			

SESSION 28: BEST POSTER LECTURES

- 13.30-13.40 O-28.01 BEST POSTER AWARDS
- 13.40-13.50O-28.02BEST POSTER AWARD LECTURE NUMBER 1
to be annonunced13.50-14.00O-28.03BEST POSTER AWARD LECTURE NUMBER 2
to be annonunced

SESSION 29

LATE-BREAKING NEWS

PROGRAMME

oral presentations

K1-3

THE 25TH INTERNATIONAL PAPILLOMAVIRUS CONFERENCE MAY 8-14 2009, MALMÖ, SWEDEN

TIME	NUMBER	TITLE	ROOM
2009-05-14			
SESSIC	SCANIA		
14.00-14.10	O-29.01	CROSS-PROTECTIVE EFFICACY OF CERVARIX™ AGAINST ONCOGENIC HPV TYPES BEYOND HPV-16/18. R Skinner, D Apter, SN Chow, C Wheeler, G Dubin, for the HPV PATRICIA Study Group	
14.10-14.20	O-29.02	RAPID DECLINE IN WARTS AFTER NATIONAL QUADRIVALENT HPV VACCINE PROGRAM C Fairley, J Hocking, M Chen, B Donovan, C Bradshaw	
14.20-14.30	O-29.03	DEFICIENCY IN THE FANCOMNI ANEMIA PATHWAY SENSITIZES MICE TO HPV-ASSOCIATED HEAD AND NECK CANCER J W Park, K Strati, P Lambert	
14.30-14.40	O-29.04	ARE 2 DOSES OF HPV VACCINE ADEQUATE IN GIRLS ? S Dobson, M Dawar, D Scheifele, T Kollmann, S McNeil, S Halperin, J Langley, M Dionne, M-E Picard, C Sauvageau, M Krajden, F Marra, D Miller, D Money, M Naus, G Ogilvie, J Singer, J Bettinger, E Young	
14.40-14.50	O-29.05	CERVICAL CANCER AMONG IMMIGRANTS IN SWEDEN, FROM 1968 THROUGH 2004 F Azerkan, K Zendehdel, P Tillgren, E Faxelid, P Sparén	
14.50-15.00	O-29.06	FINAL PHASE III EFFICACY ANALYSIS OF CERVARIX™ IN YOUNG WOMEN J Paavonen, P Naud, J Salmerón, C Wheeler, S-N Chow, D Apter, H Kitchener, X Castellsague, J Teixeira, SR Skinner, J Hedrick, U Jaisamrarn, G Limson, SM Garland, A Szarewski, B Romanowski, F Aoki, T Schwarz, WAJ Poppe, X Bosch, D Jenkins, K Hardt, T Zahaf, D Descamps, F Struyf, M Lehtinen, G Dubin	

2009-05-12

SESSION 16: HPV AMONG THE HIV INFECTED

 15.17-15.30
 O-29.07
 TYPE-SPECIFIC CERVICO-VAGINAL HPV INFECTION INCREASES RISK OF FEMALE HIV ACQUISITION

 K.K. Smith - McCune, S. Shiboski, Z. M. Chirenje, T. Magure, J. Tuveson, Y. Ma, M. DaCosta, A.-B. Moscicki, J. Palefsky, R. Mutasa, T. Chipato, A. van der Straten, G.F. Sawaya, ,

CROSS-PROTECTIVE EFFICACY OF CERVARIX[™] AGAINST ONCOGENIC HPV TYPES BEYOND HPV-16/18.

R Skinner, Vaccine Trials Group, Telethon Institute for Child Health Research, and Sydney University Discipline of Paediatrics and Child Health, Children's Hospital at Westmead, Westmead, Australia; **D Apter**, Family Federation of Finland, Sexual Health Clinic, Helsinki, Finland; **SN Chow**, Department of Obstetrics and Gynecology, College of Medicine and the Hospital, National Taiwan University, Taipei, Taiwan; **C Wheeler**, Departments of Molecular Genetics and Microbiology and Obstetrics and Gynecology, University of New Mexico Health Sciences Center, Albuquerque, NM, USA; **G Dubin**, GlaxoSmithKline Biologicals, King of Prussia, PA, USA; for the HPV PATRICIA Study Group

Objectives: The AS04-adjuvanted HPV-16/18 vaccine, Cervarix[™], GlaxoSmithKline, shows high prophylactic vaccine efficacy (VE) against cervical intraepithelial neoplasia (CIN)2+ associated with HPV-16/18. We evaluated cross-protective VE against CIN2+ associated with oncogenic HPV types beyond 16/18.

Methods: In this study (NCT00122681), women aged 15-25 years were randomized (1:1) to receive HPV-16/18 vaccine (n=9,319) or control (n=9,325) at months 0-1-6. Cervical samples were collected every 6 months for HPV DNA typing; gynecological and cytopathological examinations were performed every 12 months. CIN2+ analyses considered detection of HPV-DNA in the lesion independently of other types. VE results are reported for the Total Vaccinated Cohort (TVC)-naïve (a TVC subset including women who received ≥ 1 vaccine dose, with normal cytology, seronegative for HPV-16/18 and DNA negative for 14 oncogenic HPV types at baseline (Mean [SD] follow-up: 39.5 [8.99] months).

Results: VE (96.1% CI; p-value) against CIN2+ was: 100% (82.2, 100; p<0.0001) for HPV-31/45, 68.2% (40.5, 84.1; p<0.0001) for the 5 most frequent oncogenic types (HPV-31/33/45/52/58), 68.4% (45.7, 82.4; p<0.0001) for the 10 most frequent oncogenic types (HPV-31/33/35/39/45/51/52/56/58/59), 66.1% (37.3, 82.6; p<0.0001) for A9 species (HPV-31/33/35/52/58) and 77.3% (36.0, 93.7; p=0.0009) for A7 species (HPV-39/45/59/68). Cross-protection was further substantiated by VE against individual HPV types including 31&45. Overall VE against CIN2+ associated with 14 oncogenic types, including vaccine types (HPV-16/18/31/33/35/39/45/51/52/56/58/59/66/68) was 77.7% (63.5, 87.0; p<0.0001).

Conclusions: Vaccination of HPV-naïve women with the AS04-adjuvanted HPV-16/18 vaccine provides significant crossprotective efficacy against CIN2+ associated with non-vaccine oncogenic HPV types. This extended protection could contribute to additional & clinical meaningful reductions in the overall incidence of cervical cancer & pre-cancer.

O-29.02

RAPID DECLINE IN WARTS AFTER NATIONAL QUADRIVALENT HPV VACCINE PROGRAM

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Background: Australia provided free quadrivalent human papillomavirus (HPV) vaccine to 12-18 year old girls in a schoolbased program from April 2007, and to women 26 years through general practices from July 2007.

Objective: To determine if the Australian HPV vaccination program has had a population impact on presentations of genital warts.

Methods: The proportion of new clients with genital warts at Melbourne Sexual Health Centre (MSHC) from January 2004 to December 2008.

Results: 36,055 new clients attended MSHC between 2004-2008 and genital warts were diagnosed in 3,826 (10.6%; 95% confidence intervals(CI): 10.3-10.9). Clinical prevalence ratios (RR), and 95% CIs were calculated for the proportion of new clients with genital warts for 2004-2007 combined compared to 2008. The proportion of new clients with genital warts was significantly lower in 2008 than 2004-7 for men (RR=0.82 (95% CI, 0.75-0.90)) and women (RR=0.62 (95% CI, 0.54-0.72)). Analysis of subgroups found only women <28 years (RR=0.52 (95% CI, 0.44-0.63)) and heterosexual men (RR=0.83 (95% CI, 0.74-0.92)) but not homosexual men (RR= 0.93 (95% CI, 0.73-1.17) or women \geq 28 years(RR=0.91 (95% CI 0.70-1.17)) not had a significant fall in genital warts in 2008 compared to 2004-7. From January to December 2008 there was a significant decline in the monthly presentations for warts among women <28 years (p for trend=0.03).

Conclusions: Our data suggest a rapid and marked reduction in the incidence of genital warts among vaccinated women may be achievable through an HPV vaccination program targeting women, and supports some benefit being conferred to men. A reduction in genital wart diagnoses in heterosexual but not homosexual men is consistent with reduced heterosexual transmission of HPV as a result of female vaccination.

DEFICIENCY IN THE FANCOMNI ANEMIA PATHWAY SENSITIZES MICE TO HPV-ASSOCIATED HEAD AND NECK CANCER

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Fanconi anemia (FA) is a rare autosomal recessive disease characterized by congenital abnormalities, progressive bone marrow failure and cellular sensitivity to DNA cross-linking agents. FA patients have an increased risk for squamous cell carcinomas (SCCs) at sites of predilection for infection with high-risk human papillomavirus (HPV) types, including the oral cavity and the anogenital tract. A number of cellular genes (FA genes) comprise the FA pathway, which is involved in the repair of damaged DNA. This FA pathway is activated in cervical SCCs, at least in part, due to the high-risk HPV E7 oncoprotein, which can induce genetic instability. Therefore, it has been hypothesized that the activated FA pathway contributes to the repair of genetic damage induced by E7. This hypothesis might explain why FA patients are predisposed to HPV-associated cancers. To determine the importance of the FA pathway in modulating E7's oncogenic abilities, we crossed K14E7 transgenic mi ce, expressing the HPV16 E7 oncogene, to FancD2 knockout mice to establish K14E7/ FancD2-/- and K14E7/FancD2+/+ mice and monitored their susceptibility to head and neck cancer. To induce head and neck cancers, mice were treated with a chemical carcinogen, 4-nitroquinoline-1-oxide, at a dose previously determined to induce cancers in only a subset of K14E7 mice. At the endpoint tumor burden was measured in the oral cavity and the esophagus. K14E7/FancD2-/- mice had a significantly higher incidence of head and neck tumors compared to K14E7/ FancD2+/+ mice. No tumors were found in non-HPV transgenic, FancD2-/- or FancD2+/+ mice indicating that a deficiency in the FA pathway was specifically sensitizing mice expressing the E7 oncoprotein to tumors. We conclude that defects in the FA pathway increases the sensitivity of mice to HPV-induced cancers of the head and neck. This finding may help to explain why FA patients are predisposed to HPV associated head and neck cancers. Add itional studies currently in progress are designed to assess whether defects in the FA pathway likewise sensitize mice to HPV-associated cancers of the female lower reproductive tract (cervix and vagina).

O-29.04

ARE 2 DOSES OF HPV VACCINE ADEQUATE IN GIRLS ?

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Background. Bridging immunogenicity studies suggest that pre-adolescents respond to HPV vaccines better than adults. Objectives: To determine if the antibody responses to HPV-16,18,6,11 are non-inferior at month 7, following a 2 dose paediatric regimen as compared to a 3-dose adult regimen of quadrivalent HPV vaccination (Q-HPV).

Methods: In this phase III, post licensure randomized controlled multi-centre trial, three groups were recruited. The three groups and treatment regimens were as follows:

Group 1) Healthy girls, 9-13 years old (n=259)–2 doses Q-HPV vaccine at 0, 6 months; Group 2) Healthy girls, 9-13 years old (n=261)–3 doses of Q-HPV vaccine at 0, 2 and 6 month; Group 3) 16-26 years old(n=310) who were healthy, non-pregnant females with fewer than 5 sexual partners, no history of genital warts or CIN and not previously vaccinated with HPV – 3 doses of Q-HPV vaccine at 0, 2 and 6 months. Blood at Months 0, 7 was evaluated using Merck Competitive Luminex ImmunoAssay (cLIA) to assess serum antibody concentrations to HPV-16, -18,-6 and -11. An Analysis of Variance (ANOVA) to test differences in the Geometric Mean Titres (GMTs) was performed. Non-inferiority of any treatment arm was declared if lower bounds of the 95%CIs of GMT ratios were greater than 0.5.

Results: GMT Ratios (95% CI) were: Group1/Group3 Group 1/Group 2 Group 2/Group 3

Anti-HPV	16:2.10(1	.62,2.73)	0.96(0.74	,1.24)	2.20(1.69,2.8	35)
Anti-HPV	18:1.84(1	.47,2.31)	0.70(0.56	,0.88)	2.62(2.09,3.2	29)
Anti-HPV	6:2.37(1.)	78,3.14)	1.17(0.88,	1.56)	2.02(1.52,2.6	7)
Anti-HPV	11:1.86(1	.53,2.25)	1.11(0.92	,1.35)	1.67(1.38,2.0)2)
		• • •				

Conclusions: Following a 2 dose paediatric regimen, antibody responses to HPV-16,18,6,11 were non-inferior at month 7, as compared to 3-dose regimens.

CERVICAL CANCER AMONG IMMIGRANTS IN SWEDEN, FROM 1968 THROUGH 2004

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Background: Due to great variation in the prevalence of Human Papilloma Virus infection and other risk factors of cervical cancer worldwide, migrant studies may help further the understanding of the aetiology and improve prevention of cervical cancer. Objectives: Our aim was to study the risk of invasive cervical cancer among immigrant women. Methods: We followed 758,002 immigrants from different countries who resided in Sweden between 1968 and 2004. Age-standardised incidence rates (ASRs) of immigrants were compared with that in their countries of origin. Poisson regression models estimated the relative risks of cervical cancer among immigrants, overall and stratified by age at migration and follow-up time, compared to Swedish-born women. Results: Overall 1,991 of 19,542 observed cases of cervical cancer occurred among immigrants. Generally they had lower ASRs than in their countries of origin, with the exception of Nordic immigrants. Compared to Swedish-born women, we observed a higher relative risk of cervical cancer among immigrants overall (RR=1.13, 95% CI 1.08-1.18), and particularly among women from Denmark (RR=1.8, 95% CI 1.6-2.1), Norway (RR=1.7, 95% CI 1.5-1.9), and Central America (RR=2.5, 95% CI 1.3-4.9), while the relative risks were lower in immigrants from Eastern Africa (RR=0.2, 95% CI 0.1-0.6), South Central Asia (RR=0.4, 95% CI 0.2-0.6), and South Western Asia (RR=0.5, 95% CI 0.4-0.7). Conclusions: Follow-up time and age at migration were important effect modifiers for cervical cancer risks. We suggest targeted prevention toward high risk immigrants, specifically older women, in the first 10 years after arrival into their new homeland

O-29.06

FINAL PHASE III EFFICACY ANALYSIS OF CERVARIX™ IN YOUNG WOMEN

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Background: The human papillomavirus(HPV)-16/18 AS04-adjuvanted cervical cancer vaccine (Cervarix^{**}, GlaxoSmithKline) has shown high and sustained efficacy against oncogenic HPV infections and cervical intraepithelial neoplasia(CIN)2+. An interim analysis of this Phase III, double-blind, randomized study(NCT00122681) has been reported; we present the results of the final event-driven efficacy analysis. **Objectives:** The primary objective was to evaluate vaccine efficacy(VE)against HPV-16/18 CIN2+. Secondary and exploratory objectives included VE against CIN2+ associated with any oncogenic HPV types, CIN2+ overall(i.e., irrespective of HPV type detected in the lesion) and safety. **Methods:** 18,644 women 15-25 years(total vaccinated cohort; TVC) received HPV-16/18 vaccine(n=9,319)or control(n=9,325)at Months 0,1,6. Efficacy analyses were performed in the According-To-Protocol cohort for Efficacy (ATP-E; vaccine=8,083; control=8,069; mean [SD] follow-up: 34.9 [6.41] months after dose three), the TVC and the TVC naïve (a subset of the TVC that included subjects who had normal cytology, were seronegative for HPV-16/18 and DNA negative for 14 oncogenic HPV types at baseline; vaccine=5,822; control=5,819). Safety was assessed in TVC. **Results:** VE(96.1%CI)against HPV-16/18 CIN2+ in the ATP-E was 92.9% (79.9;98.3) in the pre-defined primary analysis, 98.1% (88.4;100) in an analysis that assigned probable HPV causality in lesions containing multiple HPV types; and was 98.4% (90.4;100) in TVC naïve. Overall VE against CIN2+ was 30.4% (16.4;42.1) in the TVC (regardless of baseline cyto/sero/DNA status) and 70.2% (54.7; 80.9) in the TVC naïve. Individual cross-protection against CIN2+ associated with HPV-31 and HPV-45 was observed in the TVC. Rates of adverse events (including serious adverse events and medically significant conditions) were generally similar between groups. **Conclusions:** Cervarix[®] showed high efficacy against HPV-16/18. The vaccine demonstrated a favorable safety profile.

TYPE-SPECIFIC CERVICO-VAGINAL HPV INFECTION INCREASES RISK OF FEMALE HIV ACQUISITION

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Background: Sexually transmitted infections (STIs) increase risk of HIV infection. We sought to evaluate whether detection of cervico-vaginal human papillomavirus (HPV) increases risk of HIV acquisition independent of other common STIs. Methods: In a trial of diaphragm/lubricant gel for HIV prevention, 2040 HIV-negative Zimbabwean women were followed for a median of 21 months and tested quarterly for 29 HPV types (with L1 PCR primers) and HIV (antibody testing on blood samples with DNA or RNA PCR confirmation). Infection with any oncogenic HPV was defined as having one or more of the following types: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 or 82v. Cox regression analyses were used to test for associations between HPV status and HIV acquisition.

Results: Participants had an average age of 28 years (range 18–49). A total of 88 women acquired HIV, for an HIV incidence of 1.9 per 100 woman-years. Baseline HPV prevalence was 24.5%; HPV58 (5.0%) and 16 (4.7%) were the most prevalent types. In multivariate models adjusted for baseline and time-dependent variables, concurrent infection with any oncogenic HPV (aHR 2.33; 95%CI 1.23-4.44) or with oncogenic type 53 (aHR 5.13; 95%CI 1.76-14.94) or 58 (aHR 3.18; 95%CI 1.22-8.34) were independently associated with HIV acquisition. Detection of any oncogenic HPV within the previous 6 months was also an independent predictor of HIV acquisition (aHR 1.89; 95%CI 1.05-3.40).

Conclusion: Recent infection with any oncogenic HPV, and concurrent infection with any oncogenic HPV or with type 53 or 58, significantly increased the risk of HIV acquisition in Zimbabwean female participants, independent of prevalent or incident STIs and other risk factors. These findings suggest that biologic mechanisms associated with oncogenic HPV infections may play a role in HIV acquisition.

POSTER ABSTRACTS SESSION 29

POSTER SESSION V THURSDAY 10.00

HPV-INDUCED CHANGES IN ANTIGEN PROCESSING MACHINERY COMPONENTS AND MHC-CLASS-I EXPRESSION

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Background: Clearance of HPV infection requires an effective T-cell-mediated response. Therefore, several therapeutic vaccine approaches have focused on HPV-derived T-cell-epitopes – so far with limited success. HPV employs numerous

immune evasion mechanisms, among which MHC-class-I down-regulation and repression of components of the antigen processing machinery (APM) most directly interfere with T-cell recognition of infected/transformed cells. Objectives: We aimed to assess effects of HPV-16 & -18 on elements of the APM using a diverse panel of keratinocyte cell lines including normal mucosal & skin keratinocytes, HPV-16-transformed cervical & oropharygeal lines, and HPV-18transformed skin & mucosal lines.

Methods: mRNA expression of 16 components of the APM was assessed by TaqMan-RT-PCR. Differences between normal and HPV-transformed cells were further investigated at the protein level. MHC-class-I expression was analyzed by flow cytometry.

Results: Proteasome components were found to be reduced in all cervical cancer cell lines. The immunoproteasome was more affected in frequently used "older" cell lines (CaSki, SiHa). These also showed a significant reduction of TAP-1 and -2, as did one oropharyngeal cell line. The endoplasmatic-reticulum-aminopeptidase-associated-with-antigen-processing (ERAAP) was reduced in all HPV-16-transformed cells. Hierarchical clustering analysis differentiated normal and transformed cells, interestingly also discriminating between cervical and oropharyngeal cells. By contrast, no consistent changes were observed in the HPV-18-transformed lines. Baseline MHC-class-I expression was unchanged in HPV-transformed cells compared to normal keratinocytes, but induction by IFN-gamma was impaired.

Conclusions: Specific components of the APM are down-regulated in HPV-16-transformed cell lines. MHC-class-I expression was comparable between normal and transformed cells in this panel of cell lines. We conclude that HPV-transformed cells should have sufficient MHC-class-I expression to effectively present peptides, but that the repertoire of presented peptides might be altered by the influence of HPV on the proteasome, immunoproteasome, ERAAP and TAP proteins. This altered peptide presentation should be considered when formulating T-cell-epitope vaccines.

P-29.09

GARDASIL® IMPACT ON CIN2-3/AIS IN SEROPOSITIVE WOMEN WITH HPV16/18 INFECTION

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Background: Gardasil is highly effective in preventing disease associated with HPV6/11/16/18 in naïve women. Objectives: The impact of Gardasil on development of CIN2-3/AIS in women with ongoing HPV16 or 18 infections prevaccination is reported.

Methods: 17,622 women aged 16-26 were enrolled in 1 of 2 randomized, placebo-controlled, efficacy trials (protocol 013 [FUTURE-I; n = 5,455] and protocol 015 [FUTURE-II; 12,167]). Vaccine (V) or placebo (P) was given at day 1, month 2, and 6. Women were tested for HPV6/11/16/18 DNA and antibodies at day 1. We focus on the subset of women who were seropositive and DNA positive to HPV16 or HPV18 pre-vaccination.

Results and Conclusions: In the combined studies, 430 V recipients and 460 P recipients were seropositive and DNA positive to HPV16 or HPV18 pre-vaccination. In this sub-population, the incidence of CIN2-3/AIS related to HPV16/18 was 6.8 and 6.4 per 100 person-years-at-risk in V and P recipients, respectively (efficacy of -5.6%, 95% CI: -45.0 to 23.2). In protocol 013 alone, a higher, but non-statistically significant, incidence was observed in the V arm for HPV16/18-related CIN2-3/AIS (10.9 V; 7.0 P). Analyses of the baseline characteristics revealed that in this sub-population of protocol 013 the rates of smoking (39.8% V; 28.2% P), HSIL (8.3% V; 4.1% P), and history of STI (35% V; 29% P), were higher among V recipients than P recipients. In contrast, the subgroup analysis of protocol 015 alone demonstrated similar incidence rates of CIN2-3/AIS in V (5.5) and P (6.2) recipients and the baseline demographic data of this sub-population of protocol 013 was likely due to the subject's baseline characteristics. Ultimately, however, population-based surveillance of vaccinated individuals beyond these clinical trials will be required.

HPV 16 E2 INDUCES P53 RELOCALISATION AND TRANSCRIPTION-INDEPENDENT APOPTOSIS

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The papillomavirus E2 protein is required for efficient viral replication and equal genome segregation. E2 binds to 4 sites in the viral long control region and represses transcription. The down-regulation of E6 and E7 transcription by E2 results in the stabilization of p53 and Rb and E2 thereby has an indirect effect on host cell proliferation. However, we and others have shown that the E2 proteins from HPV types associated with cervical cancer are also capable of directly influencing cell survival via a protein-protein interaction with p53. Binding of the HPV 16 E2 to p53 can induce p53-dependent apoptosis. Interestingly this E2-induced apoptosis does not require the transcriptional activity of p53. In addition, the binding of p53 to HPV 16 E2 inhibits HPV DNA replication. We have shown recently that mutations in this E2 protein that block binding to p53 alleviate the repressive effects of p53 on E2-dependent HPV DNA replication. These mutations in E2 also block E2-induced apoptosis. Here we use a panel of site-directed p53 mutants to investigate E2-induced apoptosis. We show that mutations in p53 designed to block the interaction with E2 inhibit E2-induced apoptosis although they have little or no effect on E2-independent apoptosis. We show that the HPV 16 E2 protein increases the cytoplasmic localization of wild-type p53. However, p53 mutants that fail to trigger E2-induced apoptosis are not relocalized to the cytoplasm by E2. These data strongly suggest that the E2-p53 interaction results in the cytoplasmic accumulation of p53 leading to transcription-independent apoptosis.

P-29.11

NEUTRALIZATION OF NATIVE HPV TYPES WITH ANTI-L2 EXTERNAL LOOP-TARGETING ANTIBODIES

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Human Papillomavirus (HPV) capsids are composed of 72 pentamers of the major capsid protein L1, and an unknown complement of L2 minor capsid proteins. Recent research suggests that an N-terminal "external loop" of L2 exists which extends through the inner conical hollow of L1 pentamers and is the target of neutralizing and cross-neutralizing antibodies in a variety of pseudovirion (PsV) types in addition to native HPV11. Unfortunately, a high resolution image of the L2 external loop is lacking. Here, we show that neutralization of 10 and 20-day native HPV16 virions with anti-L2 antibodies is temporally-dependent, suggesting that L2 epitopes are maximally exposed only in late-stage, 20-day virions. While maximum exposure of HPV16 L2 epitopes is temporally-dependent, this is not true for native HPV31, HPV18, and HPV45 10 and 20-day virions as they are, for the most part, equally susceptible/resistant to neutralization. In addition, these data reveal subtle differences in the neutralization profiles between native and PsV, have elucidated a region which contains the highest cross-neutralizing potential, and have produced a crude map of the L2 external loop in HPV16, HPV31, HPV18, and HPV45.

P-29.12 CLAUDINS IN VIN AND VULVAR SQUAMOUS CELL CARCINOMA

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Background: Claudins are a group of tight junctional proteins, found at epithelial and endothelial cell interphases. Currently 24 different types of claudins have been recognized. They are criterial for maintaining cell adhesions and polarity. They act as selective barriers regulating flow of water, ions and many macromolecules through paracellular spaces in tight junctions.

Vulvar intraepithelial neoplasia (VIN) has been thought to be a precursor of vulvar carcinoma. Vulvar squamous cell carcinoma is a multifactorial disease that develops from two different pathways; an human papilloma virus (HPV)-dependent pathway and

an HPV-independent pathway in which differentiated VIN III lesions may preceed invasive disease.

Increased expression of claudins have been previously shown in the early phase of carcinogenesis in cervical squamous intraepithelial neoplasia. There are no previous studies on claudin expression in vulvar intraepithelial neoplasia and invasive vulvar carcinoma.

Objectives: The purpose of this study was to evaluate expression of claudins 1, 3M (membrane-bound), 3S (cytoplasmic), 4, 5 and 7 in vulvar epithelial neoplasia (VIN I-III) and invasive vulvar squamous cell carcinoma.

Methods: Paraffin tissue sections from 74 vulvar neoplasms (12 VIN I, 12 VIN II-III and 50 vulvar carcinoma) were examined by immunohistochemistry for expression of claudins 1, 3M, 3S, 4, 5 and 7.

Results: Significant difference was found in claudin 3 M expression between VIN I, VIN II-III and squamous cell carcinoma (p=0.026). Expression of claudin 3M was significantly higher in VIN I compared to carcinoma (p=0.002). Claudin 5 did not show any staining in VIN I or VIN II-III and also in the carcinoma group positive expression was low.

In conclusion, the expression of claudin 3M may be associated with the progression from vulvar intraepithelial neoplasia to invasive vulvar squamous cell carcinoma.

P-29.13

UTILITY OF ENDOCERVICAL CURETTAGE IN 12,743 COLPOSCOPICALLY-GUIDED BIOPSY EXAMINATIONS

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Background: The utility of endocervical curettage (ECC) in colposcopically-guided biopsy examinations continues to be debated and often uniformly collected for disease ascertainment. ECC is routinely taken at all colposcopy exams at outpatient colposcopy clinics of the Calgary Health Region in Alberta, Canada, providing a unique opportunity to assess the benefit of ECC in almost 13,000 examinations.

Methods: We reviewed pathology reports for colposcopically-guided biopsy examinations conducted between January 1, 2003 and December 31, 2007. For exams where both ECC and biopsies were taken, we compared the final histopathology result for ECC with the final histopathology result for all other biopsy samples. Results were categorized as <CIN2, CIN3, CIN3 and cancer. Findings were stratified by the woman's age at the examination and compared using contingency analyses using McNemar's chi-square statistics.

Results: Among 12,743 colposcopy exams in women aged 16-95, 2,416 (19.0%) had a final histopathology result of CIN2+ for either ECC and/or biopsy (1,415 CIN2, 976 CIN3 and 25 cancers). Overall, greater disease was detected from biopsies compared to ECCs: 17.9% of biopsies were CIN2 or more severe (CIN2+) compared to 4.7% of ECC (p<.01). The vast majority of ECCs did not yield additional diagnostic information as ECC results were no worse than the biopsy results in 98.6% (n=12,568) of exams. Yet, if not for the routine inclusion of ECC, 16.0% of cancers, 5.1% of CIN3, and 6.2% of CIN2 would have been missed by biopsy alone. When compared with younger women, ECC in older women detected more CIN2+ that would have been missed by biopsy alone (4.6% in women aged \leq 40 vs. 7.5% aged 41-50 and 17.4% aged 51+, p<.01).

Conclusions: Most cases where ECC was performed with biopsy, it failed to benefit the woman, yet 5.4% of CIN3+ would have been missed without it.

P-29.14 USING HPV TRANSGENIC MICE TO MODEL ANAL CARCINOMA

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Background: Nearly 80% of anal cancer is associated with HPVs.

Objectives: We initially sought to determine expression patterns of E6 and E7-oncogenes in the anal tissue of our previously generated K14E6/E7-transgenic mice. Our ultimate goal is to establish an anal cancer mouse model using these mice.

Methods: To detect E7 protein, Western analysis of perianal tissue was performed. To monitor the influence of E6 and E7 on acute phenotypes of perianal epithelium, immunohistochemistry for minichromosome maintenance 7 (MCM7) and bromo deoxyuracil (BrdU) was performed on anal tissue harvested from transgenic and nontransgenic mice that were or were not subjected to radiation one day prior to sacrifice. To assess the susceptibility of HPV transgenic mice to anal cancer, transgenic and nontransgenic mice were treated topically with dimethylbenz[a]anthracene (DMBA) once a week for 20 weeks and sacrificed 28 weeks after treatment began.

Results: Western analysis detected E7 protein in anal tissue (E6 Westerns are being pursued). Anal tissue of E6/E7transgenic mice displayed heightened MCM7 staining consistent with viral oncogene expression. E6/E7-transgenic mice also displayed a statistically significant increase (p<0.01) in cells supporting DNA synthesis (BrdU-positivity) within the suprabasal compartment (24%) in comparison to nontransgenic mice (10%), similar to other epithelial tissues of E6/E7-transgenic mice. E6 and E7 can abrogate normal DNA damage responses. In the perianal tissue, DNA synthesis in irradiated transgenic mice did not decrease significantly (24% versus 20%) while in nontransgenic mice decreased DNA synthesis was observed (10% versus 2.6%). After DMBA treatment, 5/12 E6/E7-mice showed overt signs of tumors whereas 0/17 nontransgenic mice showed tumors.

Conclusions: K14E6/E7-transgenic mice display histological phenotypes consistent with oncogene expression in the perianus. Initial studies demonstrate HPV transgenic mice have heightened susceptibility to tumorigenesis of the anus.

P-29.15

IMMUNOGENICITY AND SAFETY OF HPV-16/18 AS04-ADJUVANTED VACCINE UP TO 7.3Y

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Background: Vaccines against HPV must provide long-term protection, as women remain vulnerable to HPV infection and potential development of related lesions throughout their life. We have previously reported sustained efficacy and immunogenicity of the HPV-16/18 AS04-adjuvanted vaccine up to 6.4 years. Further follow-up of Brazilian women from this study is continuing up to 9.5 years post first vaccination. Here, we report immunogenicity and safety data up to 7.3 years post first vaccination (109616/NCT00518336).

Objectives: Objective presented here is the evaluation of vaccine immunogenicity (seropositivity and serum antibody levels) and safety.

Methods: Women (15-25 years) with normal cytology and who were oncogenic HPV DNA-negative and HPV-16/18 seronegative (N=1113) were vaccinated in the initial study. From the Brazilian sub-cohort 433 subjects were included in an additional follow-up study (222 vaccine, 211 placebo). Total IgG antibody titres were measured by ELISA. Specific adverse events (AEs), including serious AEs, were collected throughout the study.

Results: 422 women were included in the according-to-protocol (ATP) for safety cohort, and 304 in the ATP for immunogenicity cohort. All women who received the vaccine remained seropositive for HPV-16 and HPV-18. Geometric mean titres for total IgG antibodies for both HPV-16 and HPV-18, having reached a plateau between Months 18 and 24 following vaccination, remained stable (383.4 and 251.0 EL.U/mL at the last interval [Months 83 to 88] for this analysis for HPV-16 and HPV-18, respectively). Levels remained \geq 13-fold above natural infection levels for HPV-16 and \geq 11-fold above for HPV-18. The safety profile of the vaccine was similar to that of placebo.

Conclusions: The HPV-16/18 AS04-adjuvanted vaccine offers high and sustained antibody levels and 100% seropositivity against both HPV-16 and HPV-18 up to 7.3 years after vaccination with a favourable safety profile. This is the longest follow-up reported for any licensed HPV vaccine.

HEPATITIS B SURFACE ANTIGEN-BASED VACCINE FOR HPV ASSOCIATED NEOPLASIA

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Due to improvements of delivery technology, DNA vaccines are gaining momentum as immuno-stimulators to induce protective and therapeutic T-cell responses to infectious agents and cancers in man. DNA vaccines themselves are quick and easy to construct and are easily manufactured.

In this study a DNA vaccine vectored by hepatitis B surface antigen (HBsAg) targeting Human Papillomavirus (HPV-16) tumour-specific-antigens E6 and E7, has been constructed. HBsAg per se is hugely immunogenic and assembles into virus-like particles. The vaccine is highly immunogenic C57BL/6 mice at extremely dose eliciting responses against both E6 and E7 epitopes, though issues of immunodominance arise. CTL responses are long lived and last up to to 23 weeks post vaccination. Protection against TC-1 tumour (HPV-16-associated) was achieved in 100% of mice. HPV-DNA was used as a therapy in TC-1 challenged mice and which evoked an increase in survival and decreased average TC-1 tumour volume. The E7-HBsAg-E6 construct has been expressed from sf9 insect cells by the Baculovirus expression system. The outcomes of this study have implications for the use of HBsAg as a generic vaccine delivery vehicle, as DNA or possibly VLP and more specifically, as a therapeutic vaccine targeting HPV-associated neoplasia.

P-29.18

MIRNAS ENCODED BY MUCOSAL AND SKIN HPV TYPES

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Background: MicroRNAs (miRNAs) play a pivotal role in the regulation of genes involved in diverse processes such as development, differentiation, and cellular growth control. Recently, several viral-encoded miRNAs have been discovered, most of them transcribed from double-stranded DNA viruses.

Objectives: MiRNAs expressed by HPV are suspected to play a role in regulating viral replication, viral-host interaction, pathogenesis and even tumorigenesis.

Methods: We selected in total eight HPV types; four cutaneous types HPV-5, HPV-8, HPV-20 and HPV-38 and four mucosal types HPV-6, HPV-11, HPV-16 and HPV-18. We computationally predicted their pre-miRNAs (hairpin RNA secondary structures) with RNAfold tool and compared their conservative mature miRNAs with currently known miRNA databases. To confirm our prediction for HPV-38 that expresses a miRNA conservative to miRNA let-7, a well-described and well conservative miRNA in many species, we tested non-melanoma skin cancer (NMSC) biopsies from renal transplant recipients that were HPV-38 positive, HPV-38 negative (but HPV-positive) and HPV negative.

Results: We predicted a total of 32 mature miRNAs for the eight HPV types, four of them from cuteneous HPV types and 28 from mucosal HPV types. Some miRNAs are common in the mucosal group but not in the skin HPVs. Our let-7 results showed that the predictied miRNA for HPV-38 was 10-fold higher expressed in HPV-38 positive skin samples compared to those in the HPV-38 negative (but HPV-positive) and the HPV negative samples, suggesting the increase is associated with HPV-38 infection.

Conclusions: Mucosal HPV types may encode some interesting miRNAs that relate to their infection and pathogenesis which promote further investigation. The predicated miRNA for HPV-38 was confirmed in the HPV-38 positive skin samples but its biological functions need to be verified.

HPV VACCINE IMPLEMENTATION IN MIDDLE AND LOW INCOME COUNTRIES

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Background: From 2007 to 2008, WHO held meetings in all WHO Regions to discuss the potential role of HPV vaccines in the context of cervical cancer control. Uniformly, Regions supported the need for improved cervical cancer prevention, including HPV vaccines as primary prevention, but highlighted vaccine price and affordability as main obstacles.

Objective: To assess the status of vaccine introduction and implementation strategies in low and middle income countries in each WHO Region.

Methods: We contacted WHO Regional offices, some country representatives, other organizations (PATH); conducted a search of literature and industry information to obtain data regarding HPV vaccine use, funding mechanism and delivery strategy in each WHO Region.

Results: In all WHO Regions, multiple low and middle income countries have licensed either the quadrivalent vaccine or bivalent vaccine, or both. Examples of vaccine implementation with public sector funding are available from a few countries in the AMRO region, and one country in the AFRO region. In several low income countries, HPV vaccine is being delivered through demonstration projects or via manufacturer donated vaccine. Private sector vaccine administration is ongoing in all WHO regions. Current vaccine delivery strategies include school and community based programs. A world map with examples of low and middle income countries administering the HPV vaccine in all Regions, combined with information summarizing delivery strategies will be provided.

Conclusions: HPV vaccine implementation in specific areas within low and middle income countries in WHO regions is progressing. However, scaling up demonstration projects or starting national programs may become more challenging because of the global financial crises. Current HPV vaccine price and affordability issues as well as funds needed for vaccine delivery may impact timelines of HPV vaccine introduction in many of these countries.

P-29.20

HPV ANTIBODIES IN A POPULATION-BASED COHORT OF WOMEN. CHILE 2001-2006

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Background This is the first study of HPV serology in the Chile, a country with an intermediate risk of cervical cancer. Objectives To measure antibodies against the most prevalent High Risk (HR)-HPV types among women.

Methods A population-based random sample of women 17-80 years of Santiago, answered a questionnaire and provided serum samples and cervical cytological smears in 2001 (n=1035) and 2006 (n=686). Antibodies against the L1 proteins of 8 HR-HPV types (16, 18, 31, 33, 35, 45, 52, and 58) were performed using a Luminex-based multiplex HPV serology assay. Results Women presenting antibodies for a HR-HPV were: 33.3% in 2001, 38.6% in 2006 (16% increase); 56.3% (n=607) of participants in both surveys had HR-HPV antibodies at least once, 2006 HR-HPV seropositivity ranged from 40.9% at age 30 years to 54.7% over age 60; 11.4% of women had antibodies against 2 or more HR types. HPV 16 L1 antibodies were present in 18.3% and 19.1% in 2001 and 2006 respectively; when antibodies against HPV 16 E6 or E7 proteins were included, prevalence of HPV 16 positivity reached 28.9% and 29.3% in 2000 and 2006. HPV 18 prevalence rose from 14.3% to 18.1% (a 24% increase in the period). Positivity to HPV 16 or 18 L1 antibodies was 30.8%, ranging from 23.8% to 41.2% at ages below 30 and over 60 years. The order by 2006 prevalence was: HPV 16(19.1%), 35(19.1%), 18(18.1%), 45(15.1%), 31(9.9%), 58(7.3%), 52(7.0%), 33(5.2%).

Conclusions We confirmed a very high exposure of this population to HR-HPV types, larger than expected based on its cervical cancer burden, suggesting a protective effect of the screening programs Nearly half of women at age 17-20 have already had contact with HR-HPV. This information should be considered in deciding age at vaccination.

EFFECT AND COST-EFFECTIVENESS OF VACCINATING WOMEN OVER 17 YEARS OLD

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Background: Most high-income countries have recommended routine school-girl HPV vaccination as a cost-effective preventive measure. However, recommendations for catch-up campaigns differ in terms of their upper age limit and whether to limit them to particular risk groups. The UK expert advisory committee on vaccination recommended a catch-up campaign for girls up to age 17, but suggested that vaccination could benefit individual women over that age.

Objectives: To investigate the effect and incremental cost-effectiveness of HPV vaccination beyond age 17 years old, either to all women, or to specific groups who may benefit more than others.

Methods: Evidence from epidemiological and sexual behaviour studies was used to extend previously published economic analyses of HPV vaccination in the UK. Models explored the potential impact of selection by sexual behaviour and of association between vaccine uptake and screening uptake. Results were compared to published economic models of vaccinating women over 17 years old in developed countries.

Results: The incremental cost-effectiveness of extending vaccination to women over 17 years old is affected by their existing indirect protection from vaccinating younger women, risk of having an existing infection at the time of vaccination, lifetime risk of acquiring a subsequent infection and relationship between vaccination and screening behaviour. In the UK, it only has a reasonable probability of being cost-effective if limited to women at low risk of having an existing infection. Extending vaccination to women in their 20s may not be cost-effective because of the lower remaining risk of infection after age 20.

Conclusions: The effect of vaccinating older women can be misjudged if account is not taken of herd immunity, lower vaccine effect in women with existing infections, remaining life-time risk of infection, and interaction with screening uptake. The choice of comparator is crucial for economic evaluations.

P-29.22

HPV 16&18 ANTIBODY COMPARISON: MERCK CLIA VS NEUTRALIZING ANTIBODY

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Background: Sera were collected at 0 and 7 months to test for antibody responses to HPV 16 & 18 from girls (446/828) enrolled in a 2 vs 3 dose Quadrivalent-Merck HPV vaccine trial.

Objective: To assess the correlation between Geometric Mean Titres (GMT) generated by an in-house HPV 16 & 18 pseudovirus (PsV) neutralizing antibody (NAb) assay expressing Red Fluorescent Protein (Buck et al. 2007) and Merck Competitive Luminex ImmunoAssay (cLIA) signals in this cohort, while remaining blinded as to the dosing regime. Methods: GMTs of NAb were measured based on 100% suppression of fluorescence in 293TT cells infected with 100 infectious units of HPV 16 & 18 PsV. These data were compared to the signals generated by the cLIA performed by the Merck Research Laboratory.

Results: For the 446 enrollees tested to date, at baseline 12 cases were HPV 16 (6 NAb/5 cLIA) & HPV 18 (6 NAb/4 cLIA) reactive. At 7 months all had seroconverted demonstrating GMT for HPV 16 NAb: Mean; Min; Median; & Max - 1:11,849; 1:160; 1:10,240; 1:230,960 and for HPV 18 - 1:3,828; 1:40; 1:5,120; 1:115,844 respectively. For the cLIA the corresponding signals for HPV 16: Mean; Min; Median; & Max - 6,836; 69; 7,196; 115,082 and for HPV 18 - 1,224; 17; 1,288; 62,222 respectively. The correlation coefficients between the two assays were 0.663 for HPV 16 and 0.870 for HPV 18.

Conclusions: Good correlation was observed between GMTs obtained by the NAb assay and the cLIA signals. The correlation coefficients were 0.663 for HPV 16 and 0.870 for HPV 18. This PsV-based NAb assay will allow independent serological validation of the cLIA generated signals to support this 2 vs 3 dose Q-Merck HPV vaccination trial.

P-29.23 PRELIMINARY CAREHPV[™] CLINICAL PERFORMANCE IN A POPULATION SCREEN IN KIGALI TOWNSHIP, RWANDA

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Introduction: Cervical cancer is the leading cause of cancer death in African women. One mission of Women's Equity in Access to Care and Treatment (WE-ACTx) is to improve access to health care including specific HIV and cancer services to reduce the incidence, morbidity, and mortality in Rwandan women. The careHPV[™] test, currently in late-stage development, is the product of a partnership between QIAGEN, Inc., and PATH (Seattle, USA) to produce a high-risk-HPV-DNA screening test that is accurate, affordable, and acceptable in resourceconstrained regions of the world. The foundation of the test is the Hybrid Capture* signal amplification chemistry, simplified and fortified to deliver robust, rapid results that facilitate same-day screening and treatment. Previously published results support the clinical utility of the careHPV test. Here we describe the final careHPV chemistry and present preliminary data on performance using pilot instrumentation in an ongoing clinical study in Kigali, Rwanda. Methods: Limit of detection of HPV 16 in the careHPV test was measured by serial dilution of HPV-16 plasmid target in careHPV collection medium. Three-week stability studies were performed on clinical specimens at elevated temperatures to mimic the conditions in Rwanda. All biologically labile kit components (RNA probe, capture antibody, detection antibodyenzyme conjugate, and substrate) were stabilized as part of the production process and monitored for stability over 18 months at 37 °C. In Rwanda, 2000 HIV-negative and 1000 HIV-positive women will be recruited to participate in a screening program over a 3-4 month period. Screening methods will include visual inspection with acetic acid (VIA), conventional cytology, and HPV screening using the careHPV test. Six nurses, three technicians and two medical doctors were trained in the use of the careHPV test by QIAGEN trainers, and in VIA by three medical specialists from the US and Uganda. Results: Limit of detection in the careHPV test was 1000 copies of HPV 16 DNA. Specimens collected in careHPV collection medium maintained stability through three weeks at 33 °C, with a positive agreement of 96% (95%CI = 85.7, 98.8) (n = 324). Kit reagents maintained 81-100% activity when stored for 18 months at 37 °C. Kit QC testing prior to release to the Kigali study site yielded mean S/N of 6.02 (95%CI: 5.84, 6.21). Three Rwandan technicians performing the careHPV test produced mean S/N of 5.02 (95%CI: 4.51, 5.53), with a range of 3.04-6.32. In early patient testing, the percent HPV positivity based on the careHPV test was 12.7% (32/251). Conclusion: Final careHPV chemistry and instruments using reconstituted reagents deliver analytical sensitivity and specificity that exceed design goals, and meet performance expectations for this cervical cancer screening study in Kigali. Reagent stability data support long-term storage of reagents during remote screening projects. After regulatory testing, submission, and approval, the test will meet the challenging needs of screening programs in resource-limited regions of the world that are adopting comprehensive public health HPV primary screening initiatives to reduce cervical cancer prevalence and mortality rates.

P-29.24

ABNORMAL CYTOLOGY, HPV, AND HISTOLOGY RESULTS: COTESTED POPULATION ANALYSIS

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Introduction: Women >30 having a Pap/HPV test (cotest) as a primary cervical screen have HPV results reported regardless of cytologic result. We evaluated relationship of HPV detection and abnormal cytology with histologic diagnoses of CIN 2,3+.

Materials and Methods: Women >30 (n = 14,742) having cytology of atypical squamous cells of undetermined significance (ASC-US) or more severe, high-risk (HR) HPV testing (Hybrid Capture 2), and histologic diagnoses were evaluated at the Kaiser Permanente Northern California Regional Lab during 2003-2007. Results were stratified by 5-year age groups from 30 to 70 and older. Women with HR-HPV-positive ASC-US and other abnormal cytology, independent of their HR-HPV results, had colposcopy

Results: There was a profound shift from more certain cytologic interpretations (high-grade squamous intraepithelial lesions [HSIL] and low-grade squamous intraepithelial lesions [LSIL]) to less certain (atypical squamous cells cannot rule out HSIL [ASC-H] and ASC-US) with age (ptrend < 0.001). For all abnormal cytology except HSIL, the percentage of women who tested HR-positive reached a nadir in the 50-54 or 55-59 age groups and rose again at older ages, with a concomitant increased risk of CIN2+. Among women with ASC-H (n = 880), the percentages of HR-HPV positive and CIN2+ were 83.7% and 51.6% in women age 30-34, 60.6% and 26.0% in women aged 50-54, and 83.3% and 41.7% in women aged 70 and older. HR-HPV was strongly associated with having CIN2+, CIN3+ and cancer among women with HSIL, ASC-H, atypical glandular cells (AGC), or LSIL. HSIL, ASC-H, and AGC were more strongly associated with CIN3 while LSIL and HR-HPV-positive ASC-US were more strongly associated with CIN2. Cancer was most commonly found in women with HSIL and AGC cytology.

Conclusions: Even among women with abnormal cytology, risk of CIN2+, CIN3+, and cancer was strongly associated with testing HR-HPV positive.

EXPRESSION OF HPV L1 CAPSID PROTEIN IN CERVICAL CYTOLOGY SAMPLES

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OBJECTIVE: The aim of the study is to investigate the expression rate of human papillomavirus(HPV) L1 capsid protein in cervical liquid-based cytology samples and correlate it with HPV genotype.

METHODS: 1,082 women who visit colposcopic clinic had been diagnosed by cytology and histology. Immunohistochemical stain for HPV L1 capsid protein and HPV genotypes by HPV DNA chip test were carried out on cervical liquid-based cytology samples. The HPV DNA chip kit contains 24 type-specific probes: 15 probes are from high-risk types and 9 probes are of low-risk types.

RESULTS: HPV L1 capsid protein was positive in 22% of normal, 55% of CIN I, 63% of CIN II, 38% of CIN III, and 9% of carcinoma. HPV L1 capsid protein and HPV 16 were positive in 31% of normal, 61% of CIN I, 63% of CIN II, 38% of CIN III, and 4% of cancer. HPV L1 capsid protein and HPV 18 were positive in 22%, 30%, 67%, 42%, 0% of normal, CIN I, CIN II, CIN III, and cancer, respectively.

CONCLUSIOS: Expression of HPV L1 capsid protein decreased with lesion progression from CIN I, II to CIN III and cancer. In CIN I/ II, L1 capsid protein and HPV 16 showed a higher positive rate than those with CIN III and cancer. HPV L1 capsid expression might be related to a disease regression in women with CIN I and CIN II.

P-29.26

HPV RESULTS GUIDE LSIL MANAGEMENT WHEN OBTAINED AS A COTEST

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Introduction: Women >30 having a Pap and HPV test (cotest) as a primary cervical screen have HPV results reported regardless of the cytologic result. We evaluated the relationship of HPV detection and histology in cotested women with LSIL cytology.

Materials and Methods: This analysis included all women >30 with LSIL conventional cytology, high-risk (hr) HPV test (Hybrid Capture 2 [hc2]) and histology results evaluated at the Kaiser Permanente (KP) Northern California Regional Lab during the years 2003-2007 stratified by age from 30 to >60. National recommendations during that period included referral of all women with LSIL to colposcopy, regardless of HPV test result.

Results: We evaluated 3,431 LSIL Pap results; 16.26% had CIN2+ and 5.25% had CIN3+. The percentage of LSIL testing hrHPV positive declined from a high of 90.53% in women aged 30-34 to a low of 70.41% in women age 55-59. The corresponding absolute risk for CIN2+ and CIN3+ was 19.21% and 6.34%, respectively, for women aged 30-34, and 11.24% and 3.55%, respectively for women 55-59. A negative HPV test defined a group at much lower risk regardless of age, with the risk for CIN2+ and CIN3+ for women with HPV negative LSIL declining to 7.77% and 3.88%, respectively, for women aged 30-34 and 0.00% for women 55-59. Sensitivity of hc2 for CIN2+ was 95.77% and for CIN3+ was 94.35%. Of the 5 cancers, one was hc2 negative, for a negative predictive value against cancer of 99.81%.

Conclusions: The percentage of hrHPV test positives in LSIL is too high at any age for reflex HPV testing to be useful. However, women with LSIL cytology screened by cotesting will have HPV results available, which when negative provides sufficient reassurance to repeat cotesting in one year in place of immediate colposcopy regardless of age.

EFFICACY OF GARDASIL AGAINST INFECTION AND DISEASE IN ADULT WOMEN

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BACKGROUND: Social changes in many countries over the past 30 years (delay in first marriage, etc) have increased the risk of HPV infection among adult women (~20-40 years old). Such women may benefit from prophylactic HPV vaccination.

OBJECTIVE: To evaluate the efficacy of quadrivalent HPV vaccine against high-grade cervical intraepithelial neoplasia (CIN2/3) or adenocarcinoma in-situ (AIS) (as well as persistent infection) in women aged 24-45.

METHODS: This study enrolled 3,819 24-45 year old women with no history of cervical biopsy in the past 5 years, LEEP, hysterectomy, or genital warts. Women received quadrivalent HPV vaccine or placebo at day 1, and months 2 and 6. Ancillary analyses against high grade disease were conducted in women who received 3 doses of vaccine/placebo within 1 year of enrollment and were naïve to the relevant HPV types at from day 1 to month 7 (per-protocol population [PPE]), and in women who received ≥ 1 dose of vaccine or placebo and were naïve to the relevant HPV type at study entry (naïve to relevant type population [NRT]). Adverse experiences were closely monitored.

RESULTS: Efficacy of quadrivalent HPV vaccine in the prevention of HPV6/11/16/18-related CIN2/3 or AIS was 75.2% (95% CI: <0,99.5) in the PPE population (4 placebo cases versus 1 vaccine case who was co-infected with HPV 51), and 25.4% (95% CI: <0,89.1) in the NRT population (4 placebo cases versus 3 vaccine cases, all of whom had co-infections). Efficacy against persistent HPV6/11/16/18 infection was 92.6% (95% CI: 76.9,98.5) in the PPE population and 75.5% (95% CI: 59.0,86.0) in the NRT population.

CONCLUSIONS: This ancillary analysis demonstrates that the quadrivalent HPV vaccine is effective in preventing HPV6/11/16/18-related persistent infection and CIN2/3 or AIS in susceptible women aged 24 to 45 years.

P-29.28

ABBOTT REAL TIME ASSAY FOR DETECTING HIGH RISK HPV GENOTYPES

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Objective; To conduct a pilot study of the Abbott Real Time High Risk (HR) HPV detection assay on cervical samples. This assay is designed to amplify and detect 14 HR HPV genotypes whilst simultaneously identifying infection with individual genotypes 16 and 18.

Methods; Stored archival samples of cervical cells from 411 women obtained during the course of the ARTISTIC trial were available for this study. Following DNA extraction using the Abbott M2000 SP system, amplification and detection were carried out on the Abbott M2000 RT system. In addition Reverse Line Blot (RLB) assay and histology results will be presented on a large proportion of these women.

Results; Of 52 cytology normal/HC2 positive samples 40 (76.9%) contained a HR type by Abbott; of 143 samples from women with borderline cytology 59 (41%) contained a HR type by Abbott compared with 53(37%) testing positive by HC2; of 140 samples from women with mild/moderate changes 105 (75%) were found to contain a HR HPV type by Abbott whilst 110 (84%) were HR HPV positive by HC2. In total, 39(97.5%) samples from the 40 women with severe cytology were HR positive by Abbott with 38(95%) testing positive by HC2. Full data will be presented on all samples including histology results and HPV typing results (Roche prototype Reverse Line Blot) where available.

Conclusions: This early data suggests that the Abbott Real Time HR HPV assay is similar in sensitivity to the Qiagen HC2 assay but exhibits greater specificity. In addition the ability of the assay to simultaneously identify those infections caused by HPV types 16 and 18 may prove clinically advantageous.

FOLLOW-UP OF WOMEN WITH HR-HPV INFECTION AND NEGATIVE CYTOLOGY

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BACKGROUND: Persistent HR-HPV infection is the necessary cause for cervical cancer. HR-HPV testing has higher sensitivity than cytology for identifying CIN2/3. An efficient management policy is required for women with HR-HPV infection, but negative cytology. OBJECTIVE: The aim of the study is to compare the outcomes of women with normal cytology and HR-HPV infection, with those with LSIL/ASC-US and HR-HPV. METHODS: From January 2000 to July 2008, women that fulfilled following inclusion criteria were prospectively recruited: 1) positive HR-HPV testing, 2) negative, ASC-US or LSIL cytology and 3) no previous abnormal smear (n= 535). Follow-up controls were scheduled every 6 months by cytology, colposcopy, and colposcopically directed biopsy or endocervical curettage if indicated and HR-HPV testing once a year. Patients were grouped according to their follow-up outcome: regression, defined as negative cytology and HR-HPV testing; persistence, LSIL cytology and/or histologycal CIN1, or persistent HR-HPV infection; and progression, CIN2/3 appearance. RESULTS AND CONCLUSION: Cytologies at enrollment were: normal in 118 women(22.1%), ASC-US in 70(13.1%) and LSIL in 347(64.8%). Mean age:33,9 years. No significant epydemiological differences were observed between the groups. Viral load (VL) of patients with negative cytology was lower than VL of women with ASC-US/LSIL (p 0.000). Outcomes at follow-up are shown in the Table.

Follow-up	Inicial diagnosis			
*	NEG	ASC-US	L-SIL	
Regression	% 37,3	30,0	38,6	The most remarkable result of our study was that patients
Persistence	% 53,4	57,1	54,5	with normal cytology but HR-HPV infection showed similar
Progression	% 9,3	12,9	6,9	progression risk than those with ASC-US/LSIL cytology.
(p=0,406)				

P-29.30

ANAL HR-HPV INFECTIONS AND E6/E7 MRNA EXPRESSION IN HIV-INFECTED PATIENTS

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Background: Anal cancer is an emerging disorder in HIV-infected patients and the causal role of HPV is object of intensive research.

Objectives: The aim was to compare HPV DNA and RNA testing in anal swabs and their significance in cancer risk evaluation.

Methods: HIV-infected outpatients, attending the Infectious Diseases Department (UCSC, Rome, Italy) were consecutively screened for anal dysplasia and cancer. Each patient was interviewed and underwent anoscopy, cytological test, biopsy of suspected lesions and anal swabs for HPV detection. DNA was detected by Hybrid Capture 2 (Digene) and typed by multiplex-PCR (HPV 6-11-16-18-31-33-45). E6/E7 mRNA expression (HPV 16-18-31-33-45) was detected by Nuclisen's EasyQ (BioMerieux).

Results: 141 patients were studied: 76.8% males, 42% men who have sex with men, median age 43 years; 90% were undergoing HAART, 72.4% had HIV-RNA <400 cp/ml, median CD4 count was 209 cell/l.

HPV-DNA was found in 68.8% of subjects, and 52.6% of positive showed multiple HPV types. HR-HPV were detected in 81% of HPV+ patients. Most prevalent genotypes were HPV 16 (61%), 18 (28.5%), 45 (26%), 31 (23.3%), and 33 (20.7%). E6/E7 mRNA was found in 39.1% of patients and in 70.4% of those with HR-infections.

Two biopsy-confirmed anal carcinoma and 9 AIN were detected. E6/E7 RNA expression was found in 10/11 cases with dysplasia/cancer). E6/E7 mRNA was detected in 44/66 patients with HR-HPV and negative cytology (44/130 of all with negative cytology, p<0.001). Overall sensitivity/specificity for dysplasia/cancer diagnosis of E6/E7 RNA detection were 91%/66%

Conclusions: HIV+ patients showed high prevalence of anal mixed HR-HPV infections with high rate of E6/E7 mRNA expression, especially in patients with dysplastic or neoplastic lesions. The results suggest that HPV RNA may be an early marker of persistent infections, with higher prognostic value than DNA testing. To assess this possibility a longitudinal study is in progress.

P-29.31 HIGH-RISK HPV TESTING FOR THE TRIAGE OF WOMEN WITH ASC-US

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Objective: We analysed the use of the HPV testing in the context of an organized cervical cancer screening programme in order to assess the compliance with the french guideline and the clinical relevance of the procedure.

Methods: Our organized screening programme registers the smears from Alsatian women aged 25 to 65 years as well as the subsequent diagnostic investigations thanks exhaustive data transmission from all cytopathology laboratories of the catchment area and information given by the practitioners who performed the smears. For this study we included the smears taken from November 1, 2006 to October 5, 2007.

Results: The cytopathology laboratories processed 177 824 Pap Tests of which 2 291 (1,3 %) were diagnosed as ASC-US. High-risk HPV testing using Hybrid Capture 2 method was performed on 626 specimens (27.3 %) and was positive on 174 (27.8 %) of them. Among these HPV-positive patients 11 (6 %) were lost to the follow-up, 40 (23 %) had a follow-up smear in transgression of the guidelines, 62 (36%) had a normal colposcopy and 61 (35 %) had a lesion which was CIN2+ in 28 % of the cases. Among the 452 HPV-negative patients 52 (11 %) had a colposcopy in transgression of the guidelines. The colposcopy and/or biopsy was normal in 41 cases (79 %) and showed a CIN1, CIN2 or CIN3 in 6, 3 and 2 cases. Unrecommended follw-up after HPV triage of ASC-US was observed in 92 patients (14.7 %), 23 % of HPV positives and 11 % of HPV negatives respectively (P<.001)

Conclusion: A significant amount of unrecommended follw-up after HPV triage of ASC-US calls for further education of practitioners in order to save cost and enhance safety for patients.

P-29.32 TOWARDS DEVELOPMENT OF L2 BASED HPV VACCINE

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Background: It is well demonstrated that minor capsid protein, L2, of human papillomavirus (HPV) is a promising candidate for the development of a broadly protective vaccine against cervical cancer. Several cross neutralizing epitopes have been identified so far in the N-terminal portion of L2 protein. Our investigations were aimed at understanding the immunogenicity of different regions of N-terminal portion of HPV 16 L2 or of multimers of tandem repeats of each region from different HPV types. Our earlier results showed that 11-88 and 11-200 regions of HPV L2 are the most immunogenic. Further results showing that fusion proteins comprising tandem repeats of specific regions of L2 from different HPV types elicit stronger immune response will be presented.

Objective: To identify the best candidate for the development of a HPV vaccine comprising of L2 multimers based on the immunological and physicochemical properties of different candidates.

Methods: Proteins comprising of tandem repeats of 11-88aa or 13-47aa regions from medically relevant HPV types were expressed in E.coli (Rosetta). The recombinant fusion proteins were purified using classical chromatographic methods suitable for a manufacturing scale. Purified proteins were vaccinated in rabbits and mice using Freund's adjuvant. Antibody responses were evaluated by HPV 16 ELISA and Pseudovirus neutralization assays. Results: All the multimers express in high levels in fermentor and were purified to 95% purity. The results show that there are subtle differences in some of their physicochemical characteristics, immunogenecity and cross neutralizing properties. These results will be presented. Conclusion: L2 multimeric proteins are ideal candidates for developing low cost HPV vaccine.

DELIVERY SYSTEM OF HPV PEPTIDE VACCINE IN MICE

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Object:Cervical cancer is the second most cancer in women worldwide and is nearly always associated with high risk HPV (16 and other types). The prevention and treatment of HPV infection are important to decrease the number of cervical cancer patients. Prophylactic and therapic HPV vaccines were developed worldwide. In the present study, we examined the simple and effective deliverly system of HPV peptide vaccine in mice. Methods:4-8 week old female C57BL/6J mice were divided into 6 groups. Mice in group A were injected with PBS with IFA. Mice in group B were immunized with synthetic HPV E7 49-57 peptide mixed with IFA by subcutaneous route. Mice in group C were immunized with E7 peptide mixed with IFA and AGA-100 by subcutaneous route. Mice in group D were immunized with E7 peptide with IFA by intravaginal route. Mice in group E were injected in all mice after immunization, and killed after a futher 35 days. Tumor weight and size in each mice were measured. Results:Mice immunized with HPV 16 E7 peptide via subcutaneous, intravaginal, and nasal route were protected against tumor growth.Conclusion:These results suggested that both systemic and mucosal immunization of HPV 16 E7 peptide vaccine induced tumor protection. In consequence, mucosal vaccine using peptide could be a candidate for HPV prophlatic and therapic vaccine as systemic vaccine.

P-29.34

DYNAMICS OF HRHPV IN A POPULATION-BASED SAMPLE OF CHILEAN WOMEN

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Background. This is the first longitudinal study of High-Risk (HR) HPV infection among Chilean women. Objectives. To describe the evolution of HRHPV infection in women from the general population in Santiago. Methods A population-based random sample of 604 women (17-80 years) answered a risk factor survey and provided 2 cervical specimens in 2000 and 2006. HPV DNA presence was assessed by GP5+/6+ PCR and Enzyme Immune Assay followed by reverse line blot genotyping to identify 44 HPV types.

Results. Between 2001 and 2006, HRHPV increased from 8.8% to 14.2%, (prevalence rate ratio (PRR) 1.6 95%CI: 1.2-2.3, p=0.002); highest increase was among women 40-59 years (6.9% to 15.9%, PRR 2.3. 95% CI: 1.4-3.8, p=0.0007). Increases below age 40 and over age 60 were not significant (9.9% to 12.1% and 11% to 14.6%, respectively). New HRHPV infections found in women below 30, from 30-39, from 40-59 and > 60 years were 13.4%, 5.3%, 11.7% and 3.7% respectively; corresponding figures for persistent HRHPV infections by age were: 15.8%, 40.0%, 26.1% and 75% respectively. HPV 18 increased more than 5 times (0.5% in 2001 to 2.8% in 2006 p= 0.0016 PRR 5.7 95% CI 1.7-19.2) and rose from the 10th to the 2nd rank, after HPV 16.The latter increased moderately (2.6% in 2001 to 3.1% in 2006, p= 0.2). From 2001 to 2006 occurred a significant increase in the number of women with >3 sexual partners (from 4% to 6.8% p<0.0003) and women with single marital status (from 7.9% to 14.4% respectively, p=0.03). The frequency of abnormal cytology findings was similarly low in both years (Ascus and higher 3.0%). Conclusions. HRHPV infection increased in the period from 2001 to 2006, particularly in middle age women. The main change in HPV genotype in the period involved a dramatic increase in prevalence of HPV 18.

P-29.35 THE CLINICAL PERFORMANCE OF THE CERVISTA HPV HR TEST

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Background: High-risk (HR) HPV testing is included in cervical cancer screening programs. Objective: This study evaluated the analytical and clinical performance of the Cervista HPV HR test for detection of HPV in cervical cytology specimens.

Methods: The Cervista HPV HR test was evaluated in a multi-center, prospective clinical study. DNA was extracted from residual ThinPrep[®] cytology specimens from approximately 4,000 women. All women with ASCUS or greater cytology results (~1,900) underwent colposcopic examination and biopsies were collected at the discretion of the physician. Cervista HPV HR test results were compared to colposcopy and histology results from a central pathology review panel. Residual DNAs were used for PCR and bi-directional sequencing and analytical performance was measured using those results. Additional analytical studies included reproducibility, accuracy, analytical sensitivity, analytical specificity/cross reactivity, and interfering substance studies.

Results: Sensitivity of the Cervista HPV HR test for detection of CIN2+ among women with ASC-US Cytology was 92.8% (95% CI: 84.1-96.9) and the NPV was 99.1% (95% CI: 98.1-99.6). Sensitivity for detection of CIN3 was 100% (95% CI: 85.1 – 100) and the NPV was 100% (95% CI: 99.4-100). The percentage of women with ASC-US cytology results that would be referred to colposcopy was 57.1%. The percent agreement with PCR/Sequencing was 86.1%. Analytical sensitivity ranged from 1,250 - 5,000 copies of HPV DNA/reaction depending on the HPV type. No cross-reactivity was observed to low-risk HPV types. The test produced reproducible results between days (within a site) and between 3 different testing sites. Conclusions: The Cervista HPV HR test has been clinically and analytically validated for detecting high-risk HPV types

for cervical cancer screening.

P-29.36

GENERATION AND PREVENTIVE EFFECTS OF HPV /CT-MOMP CHIMERIC DNA VACCINE

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Background: HPV and Chlamydia trachomatis (Ct) are the most common pathogens of STDs. Therefore, the effective chimeric vaccine both for HPV and Ct will help to control the STDs. Objectives: to study on the specific preventive effects in BALB/c mice immunized with the HPV /Ct-MOMP chimeric DNA vaccine. Methods: the multi-epitopes gene of Ct major outer membrane protein (MOMP) containing CTL, B cell and Th epitopes was selected, synthesized, and further fused to the N terminus of L1 of HPV6b gene, the chimeric gene HPV6bL1/ Ct MOMP was then cloned into the vector pcDNA3.1(+) as chimeric DNA vaccine. The control was made with the following 3 groups: pcDNA 3.1(+)/CtMOMP, pcDNA3.1(+) vector and PBS. The BALB/c mice were immunized through muscle injection at 0w, 2w, 4w respectively. ELISA, LDH release assays were used to measure the effects of humoral and cellular immune responses of the immunized mice respectively. The splenocytes intracellular cytokine were detected by FACS. Results: the serum IgG specific for both HPV6bL1 and Ct, and sIgA of reproductive tract were detected after immunization 2 weeks in mice, and at least, lasted 8 weeks; CTL activity specific both HPV6b L1 and the Ct were also detected at 5w, the humoral and cellular immune responses were significantly higher than that of the control groups, The IFN- γ from splenocytes increased in immunized group, while there is not significant difference of IL-4 and IL-10 between the immunized and control mice. When the mice were attacked by Ct strain at 1w after the last immunization, the reproductive tract inflammation of the mice immunized with chimeric DNA vaccine was observed delay, slight, short-duration compared with the control groups. Conclusions: The results suggest that HPV6bL1/Ct MOMP chimeric DNA vaccine is highly immunogenic, and capable of generating preventive effects against Ct in mice.

TTO APPROACH AND HPV-RELATED PATHOLOGIES: A MULTICENTER PILOT STUDY.

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Background. The economic evaluation of any Human Papillomavirus (HPV) vaccination strategy requires the measurement of clinical benefits expressed in Quality-Adjusted Life Year (QALYs) gained, to reflect both the increase in life expectancy, and the economic convenience associated with effective interventions. The goal of this pilot study was to investigate the feasibility of a method to quantify patients' preferences for health states affected by HPV-related pathologies in Italy. Methods. A multicenter, retrospective study was designed to enrol a cohort of females with a biopsy-confirmed diagnosis of Cervical Intraepithelial Neoplasia (CIN) 2/3 which had been managed in the previous 18 months. The value of utilities was calculated using the Time-Trade Off (TTO) method that rated both the experienced and perceived impact on quality of life of some relevant HPV-related pathology states including CIN 2/3, genital warts, and cervical cancer through standardized descriptions and structured electronic questionnaires (i.e. EQ-5D). An additional questionnaire on risk factors was administered.

Results. This pilot study evaluated 36 patients with a mean age of 37.19 (SD = 9.03) years. At the time of administration, the perceived quality of life was defined by patients as generally good with a value equivalent to 0.91 (sd=0.21). The average and standard deviation of utility results for each pathology state were reported as follows: CIN 2/3 0.73 (sd=0.22), genital warts 0.71 (sd=0.35), while the mean value for cervical cancer was 0.02 (sd=0.08). For the different risk factors were calculated the incidence data.

Conclusion. TTO is a feasible and appropriate technique for gathering patients' preferences and deserves implementation in larger populations. This pilot study provided early data concerning utilities associated with HPV-related pathologies in Italy and risk factor of disease. Women who previously had experienced precancerous lesions perceived genital warts as a more severe condition in comparison to CIN 2/3.

P-29.38

PERSISTENCE OF HPV-GENOTYPES AND SEROLOGY AMONG WOMEN WITH INCIDENT CIN

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Background: Persistent high-risk (HR) HPV is the most important predictive factor of cervical intraepithelial neoplasia (CIN). HPV antibody levels are low after natural HPV infection, and little is known about HPV-seroreactivity in women with incident CIN.

Objectives: A longitudinal analysis of HPV-genotypes and serology in women who developed CIN.

Methods: A cohort of 329 baseline pregnant women in the HPV Finnish Family Study were prospectively followed-up for up to 6.2 years (n=181), with cervical samples taken before delivery and at 2-, 12-, 24-, 36-, and 73-months, and serum samples at baseline and at 12-, 24- and 36 months. HPV genotyping was done using Multimetrix kit and HPV L1 antibodies were analysed by multiplex HPV serology (400 MFI cut-off).

Results: During the follow-up, 10 women (3,0%, 10/329) developed an incident CIN; 1 CIN1, 3 CIN2, and 6 CIN3. Persistent HPV infection was detected in 8/10 of these women; HPV16 in 6/10, HPV18 in 1/10, HPV31 in 2/10, HPV45 in 1/10, HPV58 in 1/10, and HPV59 in 1/10. HPV16 persisted for 7 years in 3 women who developed CIN1, CIN2, and CIN3. Persistent multiple HPV types were seen in 3 women (1 CIN1 and 2 CIN2). Of the 6 incident CIN 3 lesions, 4 were infected by single HPV type while 2 women had no persistent HPV detected. Seropositivity for HPV16 L1 was seen in 3 women who developed: 1 CIN1 and 2 CIN3. Two of them had persistent HPV16 infection. HPV16 antibodies were not detected in 2 women with persistent HPV16 infection.

Conclusions: Persistent HR-HPV infection was detected in 8/10 women who developed incident CIN lesions, HPV16 being the single most frequent genotype. HPV16 seropositivity was found in 3/9 women, indicating low or undetectable HPV antibody levels in women after natural HPV infection.

CPG(-110) METHYLATION OF IL-10 PROMOTER IN CERVICAL CARCINOMA CELL LINES

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Methylation frequency of the promoter proximal CpG(-110) of human interleukin 10 in cervical carcinoma cell lines Different epigenetic patterns characteristic for the different cellular components of neoplastic cervical lesions raise the possibility that DNA based markers identify the cellular composition of the lesion. High throughput methods for epigenetic pattern detection usually target genes and promoters with CpG islands and these methods can measure the average methylation level of the multiple CpG dinucleotides within the CpG island. On the other hand, several cytokines harbor scarce CpG dinucleotides in the promoter of which the methylation status of the proximal one determines whether or not the gene is permanently silenced. Previously, we have found lineage specific methylation at CpG(-110) of the human IL-10 promoter in cell lines of keratinocyte and cervical epithelial origin but not in cell cultures of lymphoid origin (Szalmas et al Eur. J. Cancer 2008 May;44(7):1030-8.).

Here, we report quantitating the methylation frequency of CpG(-110) in the IL-10 promoter in the above mentioned in vitro cultured cell types. We constructed a pair of Taqman probes corresponding to the bisulfit modified sequences of methylated and unmethylated CpG(-110). The methylation frequency of CpG(-110) in the T-lymphoid cell line Jurkat was <5%, while it was >95% in three cell cultures of moderate growth ability, namely primary human keratinocytes, SiHa and Caski cervical carcinoma cell lines. Hacat keratinocytic cell line, HeLa and C33A cervical cancer cell lines, all with fast growth ability had consistenly lower methylation frequency at CpG(-110) ranging from 62% to 77% in the in vitro cultured bulk populations. These results raise the question whether the same heterogeneous methylation frequency occurs in vivo or simply the replication of the epigenetic imprinting cannot keep pace with the fast in vitro cell proliferation.

P-29.40

HPV PREVALENCE, TYPE DISTRIBUTION AMONG 10-30 YEARS OLD GERMAN WOMEN

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Background: Knowledge about the prevalence of genital infection with HPV and the type distribution among young women at the age of 10-30 years in Germany is scarce. Such information is important to estimate the burden of HPV infection and the putative impact of vaccination of this group

Objective: The aim of the present study is to assess the prevalence and type distribution of HPV in young women from Germany and to examine risk factors for HPV infection.

Methods: We included women who visited their gynaecologist for a routine check in centres spread all over Germany. Women were sampled in pre-defined age intervals in a stratified manner. The participants went through a gynaecological examination where a conventional smear and a cervical swab for HPV testing were obtained. Finally, the women were interviewed about medical history and lifestyle factors. The cervical swabs were tested for high-risk (HR)-HPV by means of Hybrid Capture 2 (HC2), and genotyping was performed using the PCR-based LiPa test and the Papillocheck test.

Results: In total we included 1692 women in 77 participating centres. This total sample was distributed by age as follows: aged 10-16 (17.3%), 17-19 (20.7%), 20-22 (20.9%), 23-26 (20.7%) and 27-30 (20.4%). A total of 2.1% of women were PAP positive, with 0.4% Pap III, 1.7% PapIIID, 0.1% PAPIVa. The total HR HPV prevalence as tested by HC2 using the high risk probe was 19.1%. The observed age-stratified HR HPV prevalence was 9.2% (10-16 years), 20.3% (17-19 years), 24.0% (20-22 years), 22.0% (23-26 years) and 18.3% (27-30 years), respectively. The analysis of the genotyping results is currently performed.

Conclusion: The results show that HR-HPV infection is common in young German women. Vaccination of women in the age group of 10-30 years could therefore have a potential impact on the prevention of cervical disease.

WHO/UNFPA RESEARCH TO MAXIMIZE BENEFIT FROM HPV VACCINE FOR ADOLESCENTS

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Background: WHO advocates that HPV vaccine delivery will benefit from partnerships across health programmes and sectors. The introduction of HPV vaccines provides an opportunity to increase adolescents' use of other health interventions. WHO aims to operationalize the linkages between cervical cancer prevention, adolescent health, immunization, and the education sector.

Objective: This research study assesses the feasibility, acceptability, cost and means of delivery of the HPV vaccine with an adolescent-specific package of interventions.

Methods: WHO reviewed the scientific evidence in support of effective health interventions for delivery in concert with HPV vaccines: ongoing delivery approaches and vaccination recommendations for the age group; and review of adolescent-specific health commodities, services, information and screening interventions.

WHO is planning to implement this operational research in multiple countries.

Initially, a meeting was held in Mexico with representatives from MoH and key stakeholders of Colombia, Mexico, Panama, and Peru to discuss and review the operational research design, and the adolescent package in the context of their cervical cancer prevention strategy.

Results: From the literature reviews, a generic menu of interventions for adolescents was identified. This health package includes interventions that include information, screening, commodities, and referral, as well as promoting sexual and reproductive health.

During the meeting with Mexico, the four participating countries adopted and adapted the identified menu of adolescent interventions to their country situation. They have also decided on a vaccine delivery strategy that includes the adolescent package. Operational research will be conducted in these four initial countries.

Conclusions: It is expected that this package will increase coverage for selected health services for adolescents, ensure sustainability and coverage of HPV vaccine delivery, and strengthen the cervical cancer prevention program.

P-29.42

WOMEN'S KNOWLEDGE ON HPV AND VACCINATION IN PORDENONE PROVINCE, ITALY

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Background: Women's knowledge on HPV related genital lesions and vaccination is requested to obtain the expected success of the vaccination against HPV.

Objectives: to measure knowledge, attitudes and beliefs towards HPV and vaccination to optimize educational intervention on female population.

Methods: 212 consecutive women, aged 25 - 65 years, living in Pordenone province (Italy), with negative history for HPV related genital lesions were studied. They underwent to a screening pap smear at family planning clinic, from August, 1st, to October, 31st, 2008, when vaccination programme started. We submitted, anonymously, a semi-structured questionnaire to all recruited women, to check their knowledge about HPV infection, transmission of HPV, HPV related genital lesions, attitudes and beliefs about vaccination.

Results: Only 42% of the sample knows HPV related genital lesions, and 43% is aware that HPV infection is a common sexually transmitted disease. Up to 74.5% of women has learned, especially from mass media, about the vaccine, and 78% of this group knows that its efficacy is related to the oncogenic types included in the vaccine. Only 19.8% knows that one vaccine can also prevent genital condylomata. 71% of women heard about public vaccination programme, and 82.5% of them intended to have their daughter vaccinated.

Conclusions: This study confirms that there is a lack of information, or access to information, about HPV and that more needs to be done to rise awareness of HPV and HPV vaccination. 74.4% of women asks for more information and thinks that schools, gynecologist and family planning clinic should play a central role in the health education of teenagers. There is need for healthcare services and other agencies to play a more active role in publicizing, educating and informing patients about HPV related lesions and potential value of HPV vaccination.

LIONPROBE ASSAY FOR DIAGNOSTIC OF HIGH-RISK ONCOGENIC HPV GENOTYPES

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Human Papilloma Virus (HPV) is a DNA virus causing diseases that can vary from condiloma to neoplasic transformations associated to the expression of E6 and E7 genes. An early and trustworthy diagnostic is vital for correct patient management.

The objective of this work was the development of a high specific and sensitivity diagnostic assay for the screening of HPV genotypes classified as high-risk of neoplasic development. The developed molecular kit will support the physicians with a reliable, robust, specific, sensible and rapid tool for high-risk oncogenic HPV infection diagnostic.

The developed assay is based on a novel Real Time DNA amplification technique using specific hydrolysis primer-probes, LIONPROBES type (patented by BIOTOOLS.

LIONPROBES technique is based on the use of a DNA polymerase with 3'-5' exonuclease proofreading activity (Pfu) and a dually marked oligonucleotide probe, containing a fluorescent marker and quenching molecule which presents a mismatch at 3'end of the hybridization area with the target DNA. During amplification reaction the Pfu recognises the 3'mismatch and consequently excises the mismatched base pairs labelled with one of the labels. Once the 3'-end of the oligonucleotide is corrected amplification priming is enabled by the oligonucleotide. During each amplification cycle the fluorescence emerges as the result of the physical separation between the quencher and the fluorophore. This fluorescence signal is detected indicating the presence of oncogenic HPV genotypes in the analysed sample (types 16, 18, 31, 33, 35, 45 and 58). Additionally a melting curve analysis of the amplified labelled product confirm the specificity of the amplification that was validated using external run controls as well as real clinical samples genotyped by RFLP analysis and/or genic sequencing. The assay has a quantitative dynamic range between 500.000 copies/reaction to 50 copies/reaction and the co-amplification of a housekeeping human gene allows the relative quantification of oncogenic HPV genotypes.

P-29.44 UNMASKING HPV GENOTYPES AS IMPACT OF HPV VACCINATION

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Background: A number of countries have introduced a national vaccination programme using Gardasil or Cervarix to reduce the burden of cervical cancer and cervical intra-epithelial neoplasia due to HPV genotypes 16 and 18 in their communities, based on mathematical modelling of HPV transmission dynamics. However, the reduction in disease incidence may be less than expected due to unmasking; that is, the uncertainty about the HPV type causing disease in the case of individuals having infections with multiple HPV types. Models often use an oncogenic hierarchy to assign a single HPV type to an individual who is infected with multiple types in an observational study.

Objectives: A modelling study was conducted to explore the possible impact of the vaccine introduction on cervical cancer and cervical intra-epithelial neoplasia cases due non-vaccine types, including the possibility of unmasking.

Methods: A multi-type individual based model was developed to study the reduction of the incidence of disease due to oncogenic HPV types. Results were compared to a deterministic transmission dynamic model which used an oncogenic hierarchy to assign an HPV type to represent subjects in sample data who were infected with multiple types.

Results: For realistic parameter values attributing outcomes to the most oncogenic HPV type had only a small effect on results.

Conclusions: Infection by multiple HPV types and possible unmasking after vaccination should be considered in costeffectiveness analyses of HPV vaccine introduction, although in some cases using an oncogenic hierarchy may provide a good approximation.

HPV FROM DRY AND WET SELF-COLLECTED FLOCKED VAGINAL SWABS

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Background: Self collected vaginal swabs (VS) may be used for detecting high risk (HR) HPV in women reluctant to have cervical Pap screening. Some have concerns about handling specimen transport medium (STM). Objectives: To compare presence of HR HPV DNA in (a) self collected VS transported dry or in wet STM and (b) a cervical ThinPrep liquid based (L-Pap) sample. L-Pap samples were also tested for E6/E7 mRNA. Methods: Fifty women referred to colposcopy with an abnormal Pap collected a VS using dual flocked swabs (Copan Italia). One swab was placed into a dry tube and the other into STM. An L-Pap sample (Hologic ThinPrep), and a cervical biopsy where indicated were collected. Swabs and L-Pap were tested with Qiagen Hybrid Capture 2 (RLU/CO), and the L-Pap also by Gen-Probe APTIMA HPV (S/CO). In both tests, values between 1.0-2.0 were repeated. Results: Forty eight paired VS were in agreement (kappa=0.92). For both disparate results, the dry swab was negative, and the wet swab was borderline positive with normal biopsy. Swabbing to testing time ranged from 3-10 days, with no impact on positives (p=0.91) or signal values (p=0.66). RLU/CO for positives ranged from 2.3-1146.3 for dry and 1.2-1110.8 for wet swabs. For L-Pap, 5 pairs were discordant: 3 positive for DNA, negative for mRNA; 2 contained mRNA but no DNA. Sensitivity and specificity for CIN 2+ detection by dry and wet swabs, L-Pap DNA and mRNA were 0.58 and 0.55, 0.58 and 0.50, 0.91 and 0.46, 0.91 and 0.48 respectively. Conclusions: Self collected flocked VS transported dry may be an alternative to STM. Although the data shows that the sensitivity of vaginal self sampling was lower than L-Pap sampling, VS could provide an opportunity for HR HPV screening of women who avoid pelvic examination.

P-29.46

INCIDENCE OF ANOGENITAL WARTS AMONG STATUS FIRST NATIONS IN ALBERTA

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Background: Little is known about the epidemiology of HPV infection in Alberta First Nations. Objectives: The objective of the current study was to examine the incidence of anogenital warts, with a particular focus on status First Nations.

Methods: The province of Alberta maintains a publicly funded, universally available health care system. As part of this system, a population registry is maintain in which status First Nations are identified. All physician services are captured and maintained by the Ministry of Health and Wellness. All physician services with an ICD-9-CM code for viral warts due to HPV (078.1) and with a corresponding procedure code for their removal were extracted. Logistic regression was used to determine the odds of being diagnosed with HPV given First Nations status, adjusting for age and sex.

Results: A total of 25,949 individuals between 15 and 59 had been treated for anogenital warts in 2007. For First Nations and non-First Nations the highest incidence was in the 15 to 19 year age group. Overall and in each age-specific group, First Nations were less likely to be diagnosed (OR=0.40; 95% CI, 0.36, 0.44). Females were more likely to be diagnosed than males (OR=1.32; 95% CI 1.29, 1.35). There was an increasing likelihood of diagnosis with decreasing age. Relative to individuals between 40 and 60 years of age, individuals between 15 and 19 (OR=2.11; 95% CI, 2.04, 2.19), 20 to 29 (OR=1.68; 95% CI 1.63, 1.73), and 30 to 39 (OR=1.22; 95% CI 1.17, 1.26) had elevated odds.

Conclusions: Status First Nations appear to have a lower rate of anogenital warts. Their lower odds may be impacted by health seeking behavior or could reflect a lower infection rate. Both teens and women were more likely to be diagnosed.

ACIDIC MICROENVIROMENT STIMULI MODULATES HPV ONCOGENES, VEGF EXPRESSION AND AKT PATHWAY

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Persistent infection with a high risk type of human papillomavirus (HPV) is a necessary, but not sufficient cause for the development of cervical cancer. Other factors like coinfection with other virus or bacteria, smoking, hormonal and immunological status have been linked as possible cofactor in cervical cancer.

Nevertheless, studies on the mechanisms by which microenviroment regulate the expression and transcriptional regulation of HPV and subsequently affect specific cellular properties in cervical cancer cells, are lacking and were the subject of the present study

To test the influence of microenvironment Siha (HPV16), Caski (HPV16) and Hela (HPV18) cells were exposed to acidic medium (pH 5.5).

Signalling pathways involved in survival, proliferation and stress were analysed by Western-Blot, using antibodies against p38/MAPkinase Pi3K (p38, pp38, ERK 1/2and pERK1/2), NFkB (p65) and AKT (AKT, pAKT)pathways.

The expression levels of viral oncogenes and VEGF isoforms were quantified by Real Time-PCR.

VEGF levels were quantified by ELISA

Exposure of cervical cancer cells to acidic medium decreases viral oncogenes expression (E6 and E7).

For low levels of E6 and E7 expression we saw AKT. phosphorylation possibly involved in survival and proliferation.

Variation of viral oncogenes is accompanied by a variation in VEGF isoforms pattern, with an increase of the expression of VEGF 121 and 165, which result in an increase of total VEGF expressed.

Acidic pH affects viral oncogenes expression in cervical cancer cell lines, and thereby modulates AKT phosphorylation, and the production of VEGF, as well as it alternative splicing.

The others pathways doesn't show significative alterations.

P-29.49

CANCER SCREENING-THE FIRST STEP TOWARDS CURBING GHANA'S CANCER BURDEN.

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Background: Cancer deaths are over 7 million per annum, more than the combined deaths from HIV/AIDS, Tuberculosis and Malaria per year (WHO report 2003). Each year more than 11 million new cases are diagnosed, and more than 7 million people die from cancer (over 70% from low and middle-income countries). It is known that about 40% of cancers can be prevented through avoidance of certain risk factors.

Objective: To educate and promote cancer screening as first step towards early detection of cancers at eight health facilities in Accra.

Methodology: Eight (8) health facilities in Accra were selected for screening against breast, cervical and prostate cancers-Cocoa Clinic, Ussher Town Polyclinic, Ga Mantse Palace, Odawna Clinic, La Polyclinic, Tema General Hospital, Mamobi Polyclinic, and Adabraka Polyclinic.

The public was sensitized through a formal launch of month-long cancer awareness (2nd October- 2nd November, 2008) and other media campaigns.

A total of 1080 people participated in the programme (1007 females and 73 males).

Results: 1080 persons participated in the CSG screening programme- 1007 females, 73 males. Breast disorders were identified in 72 (about 8%) out of 924 females who went through breast examinations.

The highest turn-outs were at Ussher Town polyclinic (238) and Mamobi polyclinic (154) –both sited at very deprived communities. There were 22% and 11% breast disorders at Ussher Town and Mamobi respectively.

At Cocoa clinic 12 (25%) persons out of the total of 48 males recorded PSAs greater than 4.0.

Conclusion: Cancer screening for early detection of cancers should become part and parcel of the country's health delivery system. It is important that non-governmental organizations are adequately resourced to compliment the formal health care delivery system of the country.

Key: CSG - Cancer Society of Ghana

HPV GENOTYPE DISTRIBUTION IN SMEARS COLLECTED DURING ROUTINE GYNAECOLOGIC FOLLOW-UP.

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Introduction: HPV genotype distribution in specific cervical lesions has been widely studied but HPV overall prevalence and type-specific prevalence in smears collected during routine clinical practice remain poorly documented.

Material and method: HPV genotyping and smears were performed independently between 2000 and 2006 for a routine clinical follow up (primary screening and follow up) in the University Hospital of Nantes. We included each woman who had a cytological sample collected no more than 12 months before HPV genotyping. Pap Smears were classified according to Bethesda Classification (2004). PCR was performed with MY09/MY11 primers and genotyping by sequencing PCR product.

Results: A total of 1255 women were included. Mean age was 37.5 years (range 15-86). In 980 women, the cytological diagnosis was normal. Among women with an abnormal Pap smear (n= 275), 141 had an ASCUS, 98 an LSIL, and 36 an HSIL. Among all 1255 samples the proportion of HR HPV positive increased significantly according to cytological diagnosis severity from 8% in normal specimens to 21% in ASCUS, 49% in LSIL and 75% in HSIL (p<0.001). For the 980 women with normal cytology the proportion of HPV positive women varied significantly according to age, after 25 years old HR HPV positive were about 6.7%. HPV 16 was the most prevalent type in all groups of cytological diagnosis. HPV 53 appears as the second most common genotype in normal cytological samples but his prevalence decreases in HSIL to less than 4%.

Conclusion: The proportion of HR HPV positive increased significantly according to cytological diagnosis severity. HPV genotyping allows the diagnosis of HPV 16 who appears as the most commonly encountered genotype in cervical smears even when the diagnosis was normal. The prevalence of HPV 16 increases with increasing diagnosis severity hereby confirming that HPV 16 is more aggressive than other genotypes.

P-29.51

MULTIPLE HPV SERO-PREVALENCE AMONG FINNISH AND UGANDAN PREGNANT WOMEN

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Background: Although infections with multiple HPV types have been reported widely, more information is needed for occurrence of the different types.

Objective: To determine the prevalence of multiple HPV serotypes.

Methods: Cross-sectional seroprevalence study of antenatal clinic attendants. In Finland serum samples were randomly drawn from a subset of samples collected between 1995 -2007 for the Finnish Maternity cohort and in Uganda, samples were collected (between 2004-2008) from women enrolled after consenting. The serum samples were stored and analysed for HPV antibodies against seven HPV types; 6, 11, 16, 18, 31, 33, and 45 using direct VLP ELISA.

Results: The mean(SD) age for the multiple HPV infections was 23(3) and 25(5) years for Finnish and Uganda women respectively. Forty four percent of the women (1,439 of 3,252) in the Finnish sample had antibodies to at least one HPV type. Fifty percent of the HPV antibody positive women (721 of 1,439) had antibodies to more than one HPV type. Two hundred and sixty-five (18%), 123 (9%), and 333 (23%) had antibodies to two, three and four to six HPV types respectively. Fifty eight percent of the Ugandan sample (1,508 of 2,598) was positive for at least one HPV type. Forty nine percent of the HPV antibody positive women (739 of 1,508) had antibodies to more than one HPV type. Four hundred and two (27%), 185 (12%), and 152 (10%) had antibodies to two, three and four to six HPV serotypes types respectively. In both countries the most prevalent concomitantly occurring antibodies were directed against HPV types 16/31/33, 16/18 and 6/11

Conclusion: Multiple HPV infections as indicated by HPV antibodies to seven HPV types were highly prevalent in both Finnish and Ugandan women. Antibodies to a number of "African" HPV types (HPV52, 58, etc.) were, however, not yet tested.

FEATURES OF PAPILLOMAVIRUS INFECTIONS AT PERSONS YOUNG AND MIDDLE-AGED

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Background: Diagnostics and treatment of diseases, caused by human papillomavirus (HPV), draw attention of various experts in connection with sharp growth of spread in the population the given activator, significant it contagious and ability to transform epithelial cells which progressing results in a cancer. Latent form is typical for the HPV infection. Variety immunological infringements, features of HPV cause the big complexities in diagnostics, treatment and preventive maintenance of HPV infection.

The purpose: the characteristic of immunologic features of HPV infection and PCR results of HPV at persons young and middle-aged.

Materials and methods: it was testing 125 patients with clinical and latent forms of HPV infection. From them - 47 (37.6%) men and 78 (62.4%) women. Middle age - 32 years. Diagnosis of HPV infection was confirmed on the basis of colposcopic, cytologic, histologic and PCR methods. The clinical form of disease was revealed at 62 (49.6%) patients Latent form of HPV infection found out at 63 (50.4%) persons.

Results: HPV DNA have revealed among patients with latent and clinical forms of HPV infection prevalence of HPV-16 monotype (50.8 % and 50.0 %, accordingly), HPV-18 monotype infection were found out in 23.8 % and 21.0 %, accordingly and HPV types 16 and 18 - in 25.4 % and 29.0 % of cases, accordingly. At the analysis of immunologic parameters at patients with latent and clinical forms of HPV infection deficiency of CD3-, CD4-, CD25- and CD20-lymphocytes, hyperimmunoglobulinemia G and M, insufficiency of fagocytosis is revealed. And more expressed deviations are found in group of patients with the clinical form of disease.

Conclusions: at patients young and middle-aged with latent and clinical forms of HPV infection the prevalence of HPV 16 type alongside with deviations in the immune status, touching all parts of immune system is observed.

P-29.54

HPV IN SEMEN OF MEN PARTICIPATING IN ASSISTED REPRODUCTION PROTOCOLS

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Background: The extent of HPV infection among heterosexual men is of interest. Research has focused on the role of men in the transmission of HPV to women, but reported rates of HPV infection in men vary widely.

Method: Polymerase chain reaction (INNO-LIPA-HPV Genotyping, Innogentics, Gent, Beligum) was performed to detect HPV-DNA in 17 semen specimens of healthy men participating in assisted reproduction protocols.

Results: HPV-DNA was detected in 5 (29%) of 17 semen specimens (pt 9: HPV 66; pt 11: HPV 16, 51, 56, 74, 66; pt 16: HPV 31, 33, 44, 54, 52; pt 5 and pt 17: positive with unspecific typing)

Conclusion: Healthy men participating in assisted reproduction protocols appears to have a significant rate of HPV infection. The rate of transmission to their partners and to the pregnancy is unclear.

HIGH RISK HUMAN PAPILLOMAVIRUS DETECTION: MODIFIED PRIMER SYSTEM (MGP) CONTRIBUTION

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Background: a new modified general primer PCR system (MGP) allows a sensitive amplification of most of High Risk genotypes (HRg) of Human Papillomavirus (HPV). Objective: to compare MGP to MYGP and PGMY PCR based systems in the detection of HRg and probable HRg (PHRg). Methods: 3 protocols were carried out in 93 cervical samples: nested PCR MY09/11-GP5/6, MGP (both followed by DNA sequencing of amplificated products) and Linear Array HPV Genotyping Test (LA). Results: MGP system detected HPV in 55/93 samples (59.1%): HPV 16: 16 (17.2%), HPV 31, 18: 6 (6.4%) each, HPV 51: 5, HPV 52, 58: 3 each, HPV 45, 33: 2 each, HPV 35, 53, 56, 68: 1 each, only non HR-PHRg: 7. There were 2 coinfections and 1 non genotypable (ng) sample. Distribution of HR-PHRg was: ASC-US 9/39 (23.1%), LSIL 17/ested PCR MY09/11-GP5/629 (58.6%), HSIL-ASCH-AIS 15/18 (83.3%), carcinoma 6/7 (85.7%). The number of HR-PHRg and total HPV detected was: 47/57, 57/62, 104/163 for MGP, MYGP and LA, respectively. About the detection of HR-PHRg, 62 samples were positive with some method (62/93, 66.7%), and 44 of them (44/62, 70.9%) were concordant (at least one of the detected HR-PHRg was the same) by all methods. In 10 cases (10/62, 16.1%) MYGP and LA were concordant whereas MGP was negative (HPV 53, 59, 58, 66, 82) or detected a non HR-PHRg. 28 samples were negative for every tested method. For HPV 16, 2/18 samples were LA+/MYGP-/MGP- (other detected HRg or ng) and 1/18 was LA+/ MYGPng/MGP+. Regarding HPV 18, 1/8 samples was LA+/MYGP+/MGP-(other HRg) and 1/8 was LA-/MYGP+/MGP-. Conclusions: MGP system combined with sequencing was similar to the other evaluated methods in the detection of most HR-PHRg of HPV. The new protocol was useful for HPV 16 and 18 screening.

P-29.57

CONSERVATIVE TREATMENT IN STAGE IA CERVICAL ADENOCARCINOMA, ENDOCERVICAL TYPE

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Background: FIGO stage IA cervical adenocarcinoma, endocervical type, is a rare lesion, HPV related, rising close to the squamocolumnar junction. It is diagnosed occasionally in the conization performed for SIL/glandular lesions. There are no agreement about its treatment, and there are not reliable follow-up methods. Usually it is treated with demolitive surgery.

Objectives: We report four cases asked to maintain their fertility treated with conization only, with long follow-up.

Methods:From Jenuary, 1st, 1995- December, 31st, 2007 we performed 783 laser conization because of SIL/glandular lesions. We found six patients with microinvasive adenocarcinoma, endocervical type, without lymphvascular space invasion, clear margins (five stage IA1, one stage IA2). Two patients underwent simple hysterectomy, four asked for conservative treatment (three stage IA1 and one stage IA2, clearance from invasive lesion to the apex of the cone >10 mm. After informed consent they had follow up including pap smear, colposcopy, endocervical courettage, abdominal/pelvic ultrasound and abdominal CTscan at 12 months (IA2 case)

Results:We didn't find any residual disease in women treated with hysterectomy. The four patients treated with conization only are alive and free of disease at 23, 54, 68, 82 months. One out of four patient had AGC pap smear at 21 months, negative colposcopy: a new cone biopsy detected AIS, apex no clear; we proposed her hysterectomy, but she declined, and now she is under strict follow-up. Only two women, attempted to became pregnant without success.

Conclusions:Laser cone biopsy was safe in our serie. No invasive disease was found at follow-up in patients treated for stage IA adenocarcinoma, endocervical type, without LVSI, clear margins, clearance from lesion to the apex of the cone > 10mm. Anyway the desire of pregnancy have to be counterpoised with the low reliability of the follow-up and the risk of recurrent invasive disease.

DEVELOPMENT OF HPV-GENOTYPING, MRNA-EXPRESSION ASSAY AND APPROBATION ON BC SAMPLES.

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BACKGROUND. The problem of HPV association between HPV infection and oncogenic deseases diagnostics is still actual. The meaning of present genotype and oncogene expression status for the prediction of HPV associated lesion are shown in plenty investigations. But the problem of HPV role in urinary bladder cancer (BC) is now discutable. OBJECTIVE. To develop assay for analyzing HPV genotype and oncogene E7 expression and its approbation on BC samples. METHODS. DNA extraction was performed with modificated Boom method. Type specific primers and probes for 12 genotypes were selected for E7 gene. For RNA extraction we used Chomczynski thiocyanate-phenol-chloroform method, after we performed DNAse treatment with post inactivation by EDTA. RT PCR with obtained RNA was carried out with primers for E7 and GapdH as IC. RESULTS. Primers for genotype and 30 were HPV negative for 12 types. Because 28 weak positive samples might happen due to surgical instrument or other type of contamination, to prove real presence of HPV16 we examined E7 expression status. Among 28 weak positive samples on DNA 5 (13%) samples have shown weak E7 expression, among 2 DNA positives 2 (100%) have shown expression near 4 lg cop/ml.. CONCLUSION. Developed methods for identification of HPV 16 genotype and detection of HPV E7 RNA in combination help to prove the real HPV presence in BC and differentiate it from contamination of any origin

P-29.59

HPV GENOTYPES IN WOMEN LIVING IN ANGOLA, AFRICA.

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Background: HPV infection is a common STD that can be associated with cervical cancer when caused by high risk subtypes. Angola has a population of 4.37 million women with more than 15 years old who are at risk of developing cervical cancer. However, no reports concerning the HPV type prevalence in Angola, is available so far.

Aim: To identify HPV genotypes in archival abnormal Pap smears from women attended at the Luanda province in Angola, Middle Africa.

Methods: A total of 25 archival Pap smears were examined. Cytological abnormalities were classified according to the Bethesda classification system. DNA was isolated from cytology slides by lysis and protease digestion using the QIAGEN kit. HPV DNA was amplified by PCR to detect a segment of L1 region with the MY09/MY11 and GP5+/GP6+ primer sets. As a control, a 268 bp region of the human β -globin gene was amplified for each sample.

Preliminary Results: HPV DNA was detected in 32% (8/25) lesions and of these 62.5 % (5/8) were positive for high-risk types. Low risk HPV type (HPV 6) was identified in 25% (2/8) of the cases. High-risk HPV types found were: HPV 66 (12.5% in Low grade), HPV 58 (12.5% in High-grade), and HPV 16 (12.5% in ASCUS). Interestingly two of the samples (25%) contained HPV-83, an uncommon type (one was Low grade and one suggestive of HPV infection).

Conclusion: The present data indicates not only the presence of some high-risk HPV types commonly found in other parts of the world, but also the presence of a rare high risk type, the HPV 83, a fact that may be important to consider for the designing an adequate vaccine strategies in Angola.

Support: Ministry of Health of Angola; IOC-FIOCRUZ, Brazil

EFFECT OF HPV ONCOPROTEINS ON HUMAN MHC CLASS I PROMOTER.

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Cervical cancer is caused by infection by high-risk human papillomaviruses (HPV), most notably type 16 and 18. Cervical carcinogenesis is also dependent on the ability of HPV to mitigate the expression of host MHC Class I expression, and hence, eluded the CD8+ -mediated immuno clearance. However, the exact mechanism behind this escape strategy is not well defined. This study attempts to observe the influence of viral oncoprotein E6 and E7 on the activity of promoter for HLA-A1, which forms part of the human MHC Class I group of genes. The promoter activities were measured using a proprietary luciferase reporter expression system developed by Promega (USA). The system was designed to measure differential expression of the MHC Class I reporter, in response to decreasing expression of the HPV E6 and E7 gene in cervical carcinoma cell line. Activities of the E6 and E7 were decreased using HPV E2 gene, a viral regulatory element that was ectopically-expressed in the cell lines. This de-activation exercise however, does not yield satisfactory result as complete downregulation of the E6 and E7 was not achieved in this study. Nevertheless, activities of HLA-A1 promoter in cell lines that contained the partially-affected E6 and E7 demonstrate a strong upward trend. HLA promoter activity in cervical carcinoma cell line with full E6 and E7 expression records an average reading of 40.1464 RLU (Relative Light Unit for Luciferase), while the average reading in experimental subset that contains the affected E6 and E7 was 56. 4973 RLU. This roughly translates into 40% increase of HLA promoter activity. This finding is significant because it suggests a proactive role of viral E6 and E7 in the control of host MHC Class I promoter expression.

P-29.61

INFLUENCE OF HPV INFECTION FOR THE DEVELOPMENT OF CIN DISEASE.

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Background: Human papillomavirus (HPV) is the main cause for the development of cervical intraepithelial neoplasia (CIN) and cervical cancer. Molecular tests, that detect the presence of viral DNA, are important tools allowing to differentiate persistent from transient HPV infections.

Objectives: To evaluate the prevalence of HPV infections in women with the cervical cellular changes and to investigate the association between the HPV DNA copy number and the development of cervical disease.

Methods: Cervical cytobrush specimens were analysed from a group of 200 polish women that attended routine cervical screening. 178 of them had a history of cytological abnormalities or vulva warts. The control group was formed by 22 women with a normal cytology. All samples were analysed using quantitative Real-time polymerase chain reaction (PCR) assay to estimate the viral load of 8 of the most frequent oncogenic HPV types. For genotyping of 33 HPV types the PCR gel electrophoresis and restriction fragment length polymorphism (RFLP) methods were used.

Results: HPV infection was found in 78/200 (39%) women among all investigated patients. An infection was detected in 77/178 (43,3%) patients with abnormal cytology or vulva warts. 45 (25,3%) of them were infected with high risk HPV types, 22 (12,4%) with low risk HPV types and 10 (5,6%) patients had the HPV co-infection. Just 1/22 (4,5%) oncogenic HPV case (genotype 58) was identified in the control group. The dynamic range from 5x101 to 5x105 HPV copies per reaction for Real-time PCR test was obtained. HPV samples were also expressed as copies per scrape of cellular DNA.

Conclusions: The dominant HPV genotypes detected among the patients with cytological abnormalities were high risk types: 16 (16,9%) and 31 (14,3%), then low risk type 30 (11,7%). Measurement of HPV viral load and genotyping allow to distinguish clinically relevant from irrelevant HPV infections.

HR-HPVS INFECT DEEP GENITAL TRACT OF PROSTATE CHRONIC INFLAMMATION PATIENTS

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Background: Pathologies of human prostate represent one of the most important problems of the third millennium, both the oncologic ones such as the prostate adenocarcinoma as cause of morbidity and mortality in the world population and because the inflammatory ones are increasing in numbers especially in the lowest age classes, 20-40 years, with an important impact in terms of social, individual and health costs, with important impact also in life and fertility. In Italy chronic prostatitis have been proved as one of the emergent problems in young males in fertile age (Rizzo et al., 2002): characteristics are very low mean age, early symptoms abrupt , symptoms persist for years, possible impact of STDs microrganisms with long term sequelae like infertility and cancer.

Objective of our study was to evaluate the possible impact of HPV infection in these patients with chronic prostate inflammation in a population of patients from the Italian territor attending in 2008 our STDs center.

Methods: we have analysed for HPV-DNA presence 547 patients with symptoms of chronic prostatitis-chronic pelvic pain syndrome, CP-CPPS, and 1641 biological materials: total ejaculate-TE, first-void urine-FVU, post-prostatic massage urine-PMU by Inno-Lipa HPV Genotyping Extra (Innogenetics, Italy); 28 genotypes were tested, low risk-LR, high risk-HR and probably high risk-PHR.

Results: The prevalence rate for HPV infection was 33,4% with 183 positive patients. The prevalence in TE was 21,2% (116 pts), in FVU 18% (99 pts.) and 7,6% (42 pts.) in PMU. Concerning the genotypes detected out of the 161 typed in TE, 90 were HR (55,9%). In PMU the HR genotypes were 52,1% (46) and in FVU 69 out of 102 were HR HPV (68,3%).

Conclusion: impact of HR-HPVs in chronic prostatic pathologies seems to be proved by our study confirming that chronic inflammation due to virus persistance can impact also in prostate cancers.

P-29.63

COMPARATIVE EVALUATION OF IMMUNOGENICITY OF TWO PROPHYLACTIC HUMAN PAPILLOMAVIRUS VACCINES

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Background: Vaccine-induced protection against HPV-16/18 has been demonstrated for HPV-16/18 AS04-adjuvanted vaccine (Cervarix[™]; GlaxoSmithKline Biologicals) and HPV-6/11/16/18 vaccine (Gardasil[®]; Merck). It is unclear whether differences exist in vaccine-induced immune responses and whether they represent determinants of long-term protection, or protection against HPV types other than 16 and 18. Objectives: To compare immunogenicity one month after third dose (Month 7) of Cervarix[™] or Gardasil[®] in healthy women aged 18–45. Methods: In this study (NCT00423046), women (n=1,106) were stratified by age (18–26, 27–35, 36–45 years) and randomized (1:1) to receive Cervarix[™] (Months 0, 1, 6) or Gardasil[®] (Months 0, 2, 6). Neutralizing antibody responses (sera and cervicovaginal secretions [CVS]) were evaluated by pseudovirion-based neutralization assay (developed by NCI), and memory B-cell responses (sera) by ELISPOT assay. Results: In ATP cohort (seronegative/DNA-negative before vaccination for HPV type analyzed), GMTs of serum neutralizing antibodies were 2.3–4.8-fold higher for HPV-16 and 6.8–9.1-fold higher for HPV-18 with Cervarix[™] than Gardasil[®], across all age strata. In TVC cohort, irrespective of sero/DNA status before vaccination, Cervarix[™] induced significantly higher serum neutralizing antibody titers: p<0.0001 for each antigen in each age stratum. Positivity rates for neutralizing antibodies (CVS) were higher with Cervarix[™] (81.3% [95% CI: 67.4, 91.1]) than Gardasil[®] (50.9% [37.3, 64.4]) for anti-HPV-16; similarly for anti-HPV-18 (33.3% [20.4, 48.4] versus 8.8% [2.9, 19.3]). For women (ATP) without detectable B-cell response before vaccination, frequency of circulating antigen-specific memory B-cells in responders was 2.7-fold higher with Cervarix[™] than Gardasil[®] for HPV-16 and HPV-18 (p<0.0001). Both vaccines were generally well-tolerated, with high compliance (≥84%). Conclusions: Higher immune response was observed with Cervarix[™] than Gardasil®, which may represent a determinant of duration of protection against HPV-16/18. Long-term studies evaluating duration of vaccine efficacy are needed to assess the clinical relevance of observed differences in immune responses.

SEQUENCE VARIATIONS OF HUMAN PAPILLOMAVIRUS TYPE 58 ISOLATES CO-CIRCULATING AMONG KOREAN WOMEN

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Background: The prevalence of HPV types shows a marked geographical variation with HPV58 found in a relatively higher proportion of high-grade pre-cancers and cancers in Asia. Previous sequence data have focused mainly on HPV16 and 18 of which comprehensive data is now available and the current vaccine now cover these two types. However, sequence data for HPV58, a common type in Asia is still lacking. In this study we sought to generate this dataset which will provide sequence information to aid in the development of diagnostic tools and vaccines and to the understanding of its epidemiology and disease association. Methods: A total of 116 HPV58 DNA positive cervical samples collected from Korean women aged 23-80 (mean: 43, SD: 13.36) were included in this study. Forty women had normal cervices, 11 had ASCUS, 18 had LGSIL, 19 had CIN1, 7 had CIN2, 2 had HGSIL, 12 had CIN3 and 7 had ICC. Using PCR cycle sequencing, 6 ORFs (E2, E4, E5, E6, E7, and L1) and the long control region was sequenced. Results: The most variable genome region observed in HPV58 was the long control region, 9.1% (calculated by dividing the number of positions with nucleotide substitutions observed by the nucleotide length of the genome region), followed by E5 (6.5%), E4 (4.7%), L1 (4.6%), E7 (4.4%), E2 (3.4%) and E6 (2.0%). Altogether, 72 nucleotide changes were observed in the LCR, the three most frequent nucleotide changes was 7714 A->C, 69.8%, followed by 7266 C->T (50.9%) and 52 C->T (46.6%). Fifteen nucleotide changes were seen in E5, the most common three were 4033 C->T (94.0%) 3949 T->C (53.4%) and 3988 T->C (53.4%). For the E4 gene, 13 nucleotide changes were observed, the most common one was I54T (56.0%), then C81S (2.6%) and T28A (1.7%). For L1, 72 nucleotide changes were observed, the three most frequent were L150F (89.7%), I325M (89.7%) and 6434 T->C (55.2%). For E7, 13 nucleotide changes were observed, 744 T->G (94.0%), G41R (51.7%) and G63D (51.7%). Thirty-seven nucleotide changes were identified in E2 [S279A (100.0%), 3550 T->C (92.2%) and V282L (89.7%)], and finally for E6, nine nucleotide changes were observed [307 C->T (93.1%), K93N (3.4%) and 187 C->T (2.6%)]. Conclusion: This dataset has provided information on the sequence variability of HPV58 isolates co-circulating among Korean women. This information is essential for the design of PCR primers, and for the identification sequence signatures to facilitate more in-depth epidemiological and risk association studies.

P-29.65

CLINICAL PERFORMANCE OF THE CERVISTA HPV 16/18 TEST

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Background: Studies have demonstrated a higher incidence of cervical disease (>CIN2) in women positive for HPV16 or HPV18 compared to women with other high risk (HR) HPV types. Objectives: This study is the first prospective standardof-care study of the clinical performance of a commercially manufactured HPV 16/18 genotyping test in women with cytological abnormalities. Methods: ThinPrep[®] cytology specimens were collected from ~4,000 women undergoing routine cervical cancer screening, with ~50% having ASC-US or greater cytology. DNA was tested using the Cervista HPV16/18 Test and by PCR and bi-directional DNA sequencing as a reference method. Clinical performance was compared against colposcopy and histology results. Women with equivocal cytology results of atypical squamous sells of undetermined significance (ASC-US) were requested to undergo a colposcopic examination. Biopsy samples were collected from ~72% of the colposcopies. If histological data was absent, women who were not suspected of having any CIN lesions by colposcopy or did not have biopsy results were considered negative for the presence of disease. Results: Complete data sets (cytology, HPV16/18, HPV HR, PCR/sequencing, and colposcopy/biopsy results) were available for 1,312 subjects with ASC-US cytology, of which 69 had CIN2 or greater. Sensitivity of the Cervista HPV16/18 Test for HPV HR positive, CIN2+ and CIN3 in ASC-US subjects was 68.8% and 77.3% respectively. Specificity of the Cervista HPV16/18 Test for HPV HR positive, CIN2+ and CIN3 in ASC-US subjects was 69.3% and 67.3% respectively. NPV for CIN2+ and CIN3 was 97.8% and 99.5% respectively for subjects with ASC-US. Overall agreement with PCR/sequencing was 87.1%. The indeterminate rate was 1.1%. Conclusions: The clinical performance characteristics of the Cervista HPV16/18 Test suggest it may be an important tool for use in further triaging women testing positive for high-risk HPV for colposcopy referral.

GLOBAL HPV EPIDEMIOLOGY

SESSION 30

PROGRAMME

TIME	NUMBER	TITLE	ROOM				
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08.41-08.52	O-30.02	CUMULATIVE 2-YEAR HR-HPV PERSISTENCE AND ACQUISITION IN PERI- URBAN INDIAN WOMEN P Gravitt, H Vedantham, P Paul, B Kalpana, D Vidyadhari, P Sowjanya, G Ramakrishna, K Vijayaraghavan, K Shah					
08.52-09.03	O-30.03	SEROPREVALENCE OF HPV 6, 11, 16, 18 IN UNITED STATES Le Markowitz, M Sternberg, Ef Dunne, Er Unger					
09.03-09.14	O-30.04	AGE-SPECIFIC PREVALENCE OF PRECANCEROUS LESIONS OF THE CERVIX: GLOBAL REVIEW J Ting, DT Kruzikas, J Smith					
09.14-09.25	O-30.05	WORLDWIDE HPV TYPE-SPECIFIC PREVALENCE IN CYTOLOGICALLY NORMAL WOMEN (1995-2008) L Bruni, E Ferrer, M Diaz, KS Louie, G Albero, J Muñoz, X Castellsagué, F Bosch, S de Sanjosé					
09.25-09.36	O-30.06	HPV 16 AND 18 NEUTRALIZING ANTIBODY IN PRENATAL WOMEN M Krajden, K Karunakaran, S So, J Palefsky, R Sharma, A Yu, R Chow, S Dobson, G Ogilvie, M Petric					
09.36-09.47	O-30.07	HIGH-RISK HPV PERSISTENCE IN DANISH WOMEN FROM THE GENERAL POPULATION A Nielsen, S Kjær, C Munk, M Osler, T Iftner					
09.47-09.58	O-30.08	POPULATION-BASED SEROPREVALENCE OF HUMAN PAPILLOMAVIRUS IN CHINESE WOMEN J Ji, H Wang, W Chen, s Hu, M Esser, C. Velicher, JL Belinson, Rg Pretorius, J Smith, Y Qiao					

O-30.01

COMPARISON OF SERA AND DRIED BLOOD SPOTS FOR HPV SEROEPIDEMIOLOGY

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Background: Dried blood spots (DBS) on filter paper cards facilitate field handling in seroepidemiological studies especially in low laboratory infrastructure environments since they do not require centrifugation of blood samples and can be stored and shipped at ambient temperature.

Objective: To explore the feasibility of seroepidemiological studies based on DBS.

Methods: Using multiplex serology, a recently developed high-throughput method that allows the analysis of up to 1500 sera per day for antibodies to up to 100 different antigens simultaneously, we analyzed 985 pairs of DBS and serum samples from the same donors from a cross-sectional study conducted in Mongolia for antibodies to 25 different proteins from 4 different organisms: Human Papillomaviruses (HPV), Helicobacter pylori (H. pylori), Hepatitis C Virus (HCV), and JC Polyomavirus (JCV).

Results: Quantitatively measured antibody reactivities in serum and DBS were more comparable for high titre antibodies (H. pylori, HCV, JCV) than for low titre antibodies (HPV), as indicated by the slopes derived from linear regression analyses (median 1.09, range 0.77-1.21 and median 0.61, range 0.54-0.83 for high and low titre antigens, respectively). We developed a prevalence-based method to extrapolate seropositivity cut-offs previously established for serum to DBS and found very good and excellent correlation of DBS and serum results as indicated by median kappa values for the low and high titre antigens of 0.783 and 0.859 (range 0.553-0.858 and 0.779–0.918), respectively.

Conclusions: DBS provide a reliable alternative to serum samples for seroepidemiological studies and allow antibody determination for various pathogens.

O-30.02

CUMULATIVE 2-YEAR HR-HPV PERSISTENCE AND ACQUISITION IN PERI-URBAN INDIAN WOMEN

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D Vidyadhari, MIMS/SHARE India, Hyderabad, India
P Sowjanya, CDFD, Hyderabad, India
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K Vijayaraghavan, MIMS/SHARE India, Hyderabad, India

K Shah, Johns Hopkins University, Baltimore, USA

Objectives: To estimate high-risk (HR) HPV persistence and acquisition in a population-based sample of adult women living in peri-urban villages in Andhra Pradesh, India. Methods: 1032/2311 (44.7%) of women attending cervical cancer screening, including HPV DNA testing, were sampled a second time for HPV DNA testing either 1- (n=208) or 2- (n=829) years after baseline HPV testing using self-collected vaginal swabs. Baseline and follow-up samples were tested for HR-HPV using the Hybrid Capture 2 test. Results: Cumulative 2-year HR-HPV persistence was 32.2% (95% CI: 22.6, 43.1%) among the 87 women with HR-HPV detected at baseline. Among the 945 women who were HR-HPV negative at baseline, 44 acquired HR-HPV infection (4.7%; 95% CI: 3.4, 6.2). Persistence was less common in women <45 years (26.6%: 95% CI: 15.7, 37.4) compared to women 45 years and older (47.8%: 95% CI: 27.4, 68.2; p=0.06). HPV acquisition was similar in women <45 years (4.64%; 95% CI 3.12, 6.17) and women \geq 45 years (4.69%; 95% CI 1.85%, 7.54%; p=1.0). Conclusion: To our knowledge, this is the first study to look at crude natural history of HR-HPV in an Indian population. Our data suggest that only a fraction (25-50%) of HR-HPV detected in women targeted for cervical cancer screening in India will remain HPV positive 2-years later. The high clearance rate should be considered when evaluating suitability of HPV-based screen-and-treat programs in India. Type-specific analyses are in progress to further clarify the natural history of screen-detected HPV in this population.

O-30.03

SEROPREVALENCE OF HPV 6, 11, 16, 18 IN UNITED STATES

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Background: Population-based human papillomavirus (HPV) seroprevalence data can help define the epidemiology of this common sexually transmitted infection.

Objectives: To determine seroprevalence of HPV 6, 11, 16 and 18 in the United States.

Methods: We determined HPV 6, 11, 16, and 18 (HPV vaccine types) seroprevalence among 4303 persons age 14-59 years in the 2002-2004 National Health and Nutrition Examination Survey (NHANES). HPV antibody was determined using a competitive immunoassay. Demographic and behavioral variables associated with seroprevalence were analyzed.

Results: Seroprevalence of any HPV vaccine type was 32.5% (95% CI 28.7, 36.4) among females and 12.2% (95% CI 10.8, 13.8) among males (P<0.01). Among females, seroprevalence of HPV 6, 11, 16 and 18 was 17.0%, 7.1%, 15.6%, and 6.5%, respectively. Seroprevalence was lower in males for each type: 6.3%, 2.0%, 5.1%, and 1.5% (P<0.01). Among females, seroprevalence increased with age, peaking at age 25-29 years for HPV 6 (21.9%), 40-49 years for HPV 11 (11%), 30-39 years for HPV 16 (21.9%) and 40-49 years for HPV 18 (9.9%). Seroprevalence started to increase at older ages in males. Seroprevalence of HPV 11, 16 and 18 was <1% in both the 14-19 and 20-24 year age groups. Among females and males, respectively, 8.3% and 1.7% had antibody for any two types, 0.4% and 0% had antibody for all four types. Seroprevalence of any HPV vaccine type was higher in non-Hispanic blacks than in non-Hispanic whites or Mexican Americans. Factors independently associated with seropositivity among females were age, poverty level and lifetime sex partners. Among males, factors associated were age and lifetime sex partners.

Conclusions: These data, the first to describe population-based seroprevalence of HPV 6, 11, 16 and 18 before introduction of quadrivalent HPV vaccination in the United States, can inform HPV vaccine policy.

O-30.04

AGE-SPECIFIC PREVALENCE OF PRECANCEROUS LESIONS OF THE CERVIX: GLOBAL REVIEW

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Background: Age-specific data on the prevalence of cervical precancer across geographical regions may prove essential for future evaluation of HPV prophylactic vaccine effectiveness.

Methods: A systematic review of peer-reviewed publications was conducted to summarize worldwide data on the prevalence of high-grade and low-grade squamous intraepithelial lesions (HSIL/LSIL), cervical intra-epithelial neoplasia (CIN) 2/3 or 1, and atypical squamous cells of undetermined significance (ASCUS). Studies with 1,000 or more women with available data on age were included.

Results: Over 12,400,000 women were included from 108 eligible studies. Half of the studies were from Europe and the Middle East (37%) and North America (13%); 18% were from Asia or Australia; 18% from Central and South America; and 15% from Africa. Most studies used conventional (89%) and/or liquid-based (18%) cytology. The peak in age-specific prevalence of both HSIL and CIN-2/3 appeared to be at a relatively younger age in North America (<30 years), compared with 25 to 40 years in Europe, the Middle East, Africa, Asia, Central and South America. Age patterns of LSIL and CIN-1 generally declined after a peak in the younger age groups (20 to 30 years) or were relatively flat across age, although a few curves were characterized by elevated prevalence in both younger and older ages. Age-specific data on ASCUS were relatively limited in all geographical regions surveyed. Age-specific data on cervical precancer were also limited from Asia, Central and South America, and Africa.

Discussion: Variation in age patterns of CIN 2/3/HSIL across geographical regions are likely attributable to differences in age of screening initiation, sexual practices, and classification of histology and cytology. Observed age patterns of LSIL are generally consistent with those of HPV infection in women worldwide. These baseline data (in conjunction with HPV vaccination status) will be important for future long term HPV vaccine effectiveness evaluation.

O-30.05

WORLDWIDE HPV TYPE-SPECIFIC PREVALENCE IN CYTOLOGICALLY NORMAL WOMEN (1995-2008)

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Background: It is estimated that 10% of women with normal cytology (WNC) carry an HPV detectable cervical infection at a given time. However, this estimate derives from a broad range of prevalence surveys highly variable according to testing methods, the population tested, their age and geographical region.

Objectives: To update previous meta-analysis on overall and age-specific HPV prevalence and HPV type-specific distribution in WNC, including previously under-studied regions and providing more robust estimates to assess the burden of HPV infection worldwide and made them publicly available in the HPV information Centre (www.who.int/hpvcentre).

Methods: Systematic review of the literature through Pubmed database search. Reports on HPV prevalence published between January 1995 and November 2008 were retrieved with further search in references cited. Only studies using PCR or HC2 for HPV detection were included. Meta-analysis techniques were applied to estimate country and regional HPV prevalence estimates.

Results: 415,849 WNC from 178 studies comprising 56 countries were included. Crude overall prevalence was 11.4%(95%CI=11.3-11.5%) and 10.0%(9.9-10.1%) restricting the analysis to population-based studies and women from screening programs (N=350,234). The overall estimates by continent were: Africa 18.8%(17.9-19.7%), Latin America 17.1%(16.8-17.5%), Northern America 12.6%(12.3-12.8%), Europe 9.9%(9.8-10.0%) and Asia 10.7%(10.4-10.9%). The ten most frequent HPV types worldwide were HPV-16(2.5%), HPV-18(1.0%), HPV-31(1.0%), HPV-52(0.9%), HPV-51(0.8%), HPV-58(0.7%), HPV-56(0.6%), HPV-6/11(0.6%), HPV-39(0.5%) and 45(0.5%). Worldwide age-specific HPV distribution presented a U-shaped curve, with the highest prevalences under age 35, the lowest in a plateau within 35-55yrs, and a second peak after 55-65yrs variable between regions.

Conclusions: This meta-analysis on HPV prevalence on WNC is the most comprehensive to date and strengthens previous findings. HPV infection in women without cervical disease proves to be highly prevalent and mostly at expense of oncogenic HPV types. These estimates may be useful to assess the future impact of HPV vaccines in the general population.

O-30.06

HPV 16 AND 18 NEUTRALIZING ANTIBODY IN PRENATAL WOMEN

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Background: Neutralizing antibody (NAb) provides a definitive measure of natural or vaccine immunity but standardized assays are not available.

Objective: To implement a pseudovirus (PsV) based HPV 16 & 18 NAb assay (Buck et al. 2007) and assess seroprevalence in an age stratified cohort of prenatal BC women.

Methods: HPV 16 & 18 PsV were produced in 293TT cells transfected with plasmids containing L1, L2 and a reporter red fluorescent protein gene. PsVs were purified by ultracentrifugation and analyzed by electron microscopy, SDS-PAGE and Western Blot. NAb was quantified by mixing serial two-fold dilutions of serum with 100 infectious units of HPV 16 or 18 PsV, inoculating 293TT cells in duplicate and monitoring for the reduction of fluorescent cells. NAb was recorded as a 90% and 100% reduction and Geometric Mean Titres (GMT) were calculated for an age stratified cohort of 1020 prenatal women aged 15 to 39.

Results: Overall 211/1020 (21%) demonstrated NAb (90% neutralization) with a GMT mean, median, and range of 1:138; 1:160; 1:40-1:1280 to HPV 16 and 111/1020 (11%) demonstrated NAb with a GMT mean, median and range of 1:158; 1:160, 1:40-1:1280 to HPV 18. 49/1020 (5%) demonstrated NAb to both HPV 16 & 18. GMTs were similar across all age strata but the proportion demonstrating NAb was highest in the 20-24 age group, 20% HPV 16, 10% HPV 18, and 5% dual. The correlation coefficient between 90% and 100% neutralization for both HPV 16 & 18 was 0.964 and GMTs for 90% neutralization was consistently 2-fold higher.

Conclusion: Type-specific NAb from natural infection can be reliably measured by a PsV-based NAb assay. The seroprevalence of NAb in an age-stratified cohort of BC prenatal women was 21% for HPV 16 & 11% for HPV 18 (5% of demonstrated NAb to both types).

O-30.07

HIGH-RISK HPV PERSISTENCE IN DANISH WOMEN FROM THE GENERAL POPULATION

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BACKGROUND: To better understand the natural history of HPV it is important to examine each step in the process of cervical carcinogenesis - from the initial HPV infection to cervical high-grade lesions or invasive cancer. HPV persistence is a potentially critical step.

OBJECTIVES: The aim of the present study was to assess the type-specific HPV persistence rate and examine risk factors for persistence of high-risk (HR) HPV infections in a large cohort of Danish women.

METHODS: A population-based prospective cohort study of women (20-29 years) was established. Participants were interviewed and had two gynaecological examinations 2 years apart. Women with HC2 results at enrolment and follow-up visit were included in the analysis (N=7,418). Persistence was defined as being HPV positive for the same HR-HPV type at both examinations.

RESULTS: Overall, 4.2% of the women had persistent HR-HPV, accounting for 26.9% of the initially HPV positive women. HPV16, HPV58 and HPV31, all from species group alpha 9, were the most persistent HPV types. However, other HR-HPV types which rarely are detected in cancer cases were also likely to persist. In a multivariate analysis with simultaneous adjustment for a variety of factors, risk for persistence was increased in women with a history of genital Chlamydia infection and in women who ever had used oral contraceptives, whereas use of intrauterine devices decreased the risk for persistent HR-HPV infection. In addition, older age at enrolment and younger age at sexual debut were associated with an increased risk of HPV persistence. After adjustment, HPV16 detected at baseline significantly increased the risk of persistence when compared to other HR-HPV types, whereas no association was found with viral load.

CONCLUSIONS: Persistence was common among HR-HPV positive women, particularly in women positive to HPV16. Viral characteristics and lifestyle factors affected the risk of HR-HPV persistence.

O-30.08

POPULATION-BASED SEROPREVALENCE OF HUMAN PAPILLOMAVIRUS IN CHINESE WOMEN

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Y Qiao, Chinese Academy of Medical Sciences, Beijing, China

Background: Type-specific human papillomavirus (HPV) seroprevalence data have not been reported for different geographical regions of China.

Objective: To investigate cross-sectional seroprevalence of HPV 6, 11, 16, and 18 in Chinese women.

Methods: Population-based samples of women aged 16 to 54 years were enrolled from July 2006 to April 2007 in 3 rural (Xinjiang, Shanxi, and Henan) and 2 urban areas (Beijing and Shanghai) of China. Each consenting woman completed a questionnaire and provided a blood sample. HPV 6, 11, 16, and 18 serum IgG antibodies were detected using a competitive Luminex immunoassay (Merck Research Laboratory).

Results: The median age of 4,212 women with questionnaire and serology data was 37 (range 17-54 years). Prevalence of anti-HPV 6 or 11 was high in Xinjiang, the main residence of the Uyghur ethnic group (10.9%, 8.5%), while that of anti-HPV 16 or 18 was low (4.9%, 7%). Overall, age-specific data showed older women (35-54 years) had a higher prevalence of anti-HPV 16, 18 or 6 than younger women (15-34 years). Serum antibodies against HPV 16 or 18 were more likely detected in women with higher education (8.9% with secondary school or greater vs. 5.5%). HPV 6 or 11 seroprevalence was higher in smokers than non-smokers (13.7% vs. 9.7%). The prevalence of serological response to all four HPV types was higher in women with more than five lifetime sexual partners (34.5% versus 15.5% for <5 partners) and among women reporting husbands with extramarital sexual relationships (22.7% versus 13.3% reporting none).

Conclusion: As a marker of past HPV exposure, seroprevalence of individual HPV types ranged from 5% to 11%, with HPV type 6 being the most common. Differences in HPV seroprevalence by age, geography, ethnicity, and sexual behavior within China should be considered for optimal HPV prophylactic vaccination implementation.

POSTER ABSTRACTS SESSION 30

POSTER SESSION V THURSDAY 10.00

SEXUAL DEBUT AND PREGNANCY ARE RISK FACTORS FOR CERVICAL CANCER

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Early age at first sexual intercourse (AFSI) has long been associated with an increased risk of invasive cervical carcinoma (ICC). Age at first pregnancy (AFP) and ICC have been investigated less, although AFSI and AFP are strongly interrelated in most developing countries. A pooled analysis of case-control studies on ICC from eight developing countries (Morocco, Algeria, the Philippines, Thailand, India, Brazil, Colombia, Paraguay, and Peru) with 1864 cases and 1899 controls investigated the role of AFSI, AFP, and ICC risk. AFSI, AFP and age at first marriage were strongly interrelated and had similar ICC risk estimates. Compared to women with AFSI \geq 21 years, the odds ratio of ICC was 1.78 (95% CI: 1.48-2.13) among women with AFSI 17-20 years and 2.35 (95% CI:1.89-2.92) for AFSI \leq 16 years (p-trend<0.001). No statistical interaction was detected between AFSI and any established risk factors for ICC. The ICC risk was four-fold among those who reported AFSI and AFP at \leq 16 years. In the subset of women that provided information, the age gap difference between the woman's AFSI and their first male sexual partner, a surrogate estimate of HPV exposure at sexual debut, was an independent predictor of risk. These data confirm the independent effect of AFSI and suggests an additional increase in risk when early sexual initiation is followed by an early pregnancy.

P-30.10

HPV INFECTION IN WOMEN IN CONAKRY, GUINEA

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Background: Cervical cancer incidence in Western Africa is among the highest in the world.

Objectives and methods: To investigate infection with different human papillomaviruses (HPVs) in Guinea, we obtained cervical specimens from 831 women aged 18-64 years from the general population of the capital, Conakry, as well as from 77 locally diagnosed women with invasive cervical cancer. HPV was detected using a GP5+/6+ PCR-based assay.

Results: Among the general population, the prevalence of abnormalities was 2.6% by visual inspection of the cervix and 9.5% by liquid-based cytology (including 15 high-grade squamous intraepithelial lesions [HSIL]). 14 of 15 HSIL were visual inspection-negative. Overall HPV prevalence was 50.8% (17.7% multiple infections), and was relatively constant across all age groups. Single women, and those who reported 3 or more sexual partners, showed higher HPV prevalence than those married or with one sexual partner, respectively. HPV16 was the most common high-risk type, both among women with (13.9%) and without (6.7%) cervical abnormalities. Among 77 invasive cervical cancers, the most common HPV types were HPV16 (48.6%), 45 (18.6%) and 18 (14.3%). These three types were each more common, whereas other high-risk, low-risk and multiple-type infections were each less common, in invasive cervical cancer in comparison to HPV-positive women with normal cytology from the general population.

Conclusions: The disclosure of a very heavy burden of HPV infection and severe cervical lesions in Guinea calls for new effective interventions. Sixty-three percent of cervical cancers are theoretically preventable by HPV16/18 vaccines in Guinea, perhaps more if cross-protection exists between HPV16/18 and HPV45.

SEXUAL BEHAVIOUR & HPV-INFECTIONS AMONG WOMEN 18-29 YEARS; THE PRE-VACCINE ERA

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Background As prophylactic vaccines are being implemented to prevent specifically HPV 16 and 18 infections, shifts in prevalence in the post-vaccine era may occur.

Objectives This study assesses the dynamics of HPV infections, and may provide more insight in specific risk factors for acquiring genital HPV.

Methods This study has been conducted before nationwide introduction of HPV vaccination which will take place in the Netherlands in 2009. The study is part of a large prospective epidemiologic study performed among 2065 women aged 18 to 29 years. All women returned a self-collected cervico-vaginal sample and filled out a questionnaire regarding demographic characteristics and sexual behaviour at 0-3-6-9-12 months. This interim-analysis presents the results of 0 and 6 months. All HPV DNA-positive samples (by SPF10-DEIA) were genotyped using the LiPA HPV genotyping assay.

Results HPV prevalence among sexually active women was 19.5%. Low and high-risk HPV prevalence was 9.6% and 12.3%, respectively. After 6 months the cumulative HPV incidence was 12.4%. The clearance of one or more hr/lr-HPV infections was 63.6% and 70.8%, respectively. The 6 months persistence of hr-HPV was significantly higher than for lr-HPV (7.0% vs. 3.7%, p<0.001). Multivariate analysis at baseline showed that the number of lifetime sexual partners was the most powerful independent predictor of HPV positivity (p<0.001). Followed by type of relationship, frequency of sexual contact, age, and number of sexual partners in past 6 months. Multivariate analysis at 6 months, showed only age and partners in past 6 months to be significantly related.

Conclusions These unique epidemiological data on HPV in young women in combination with knowledge of sexual behaviour provide data on the natural course of HPV infections as well as factors influencing this process. Furthermore, it provides baseline data for research on possible shifts in HPV genotype epidemiology after the implementation of nationwide vaccination.

P-30.13

WORLDWIDE HPV GENOTYPE DISTRIBUTION IN 10,289 CASES OF CERVICAL CANCER

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Objective: To describe the HPV genotype distribution in invasive cancer of the cervix.

Material and methods: Paraffin embedded invasive cancer cases were collected from historical archives. HPV detection was done through amplification of HPV DNA by SPF-10 broad-spectrum primers PCR subsequently followed by DEIA and genotyping by LiPA25 (version 1). Samples were tested at HPV laboratories at ICO (Barcelona, Spain) and at DDL (Voorburg, The Netherlands). Quality controls between the two labs were occurring regularly. Countries in the study include Algeria, Argentina, Australia, Bangladesh, Bosnia-Herzegovina, Brazil, Chile, China, Colombia, Croatia, Czech Republic, France, Greece, Guatemala, Honduras, India, Italy, Japan, South Korea, Lebanon, Mexico, Mozambique, Netherlands, Nigeria, Paraguay, Peru, Philippines, Portugal, Spain, Taiwan, Thailand, Turkey, Uganda, USA and Venezuela. Results: Out of 10,289 cases of invasive cervical cancer, HPV detection and typing was successfully done on 8,714 cases. The five most common types detected as single types worldwide were HPV16(56.7%), HPV18(9.6%), HPV45(5.3%), HPV33(3.5%) and HPV31(3.5%). The first 3 HPV types were consistent with the exception of Europe where HPV33 was the third. The 4th and 5th HPV in ranking by region were as follows: America (HPV31&33), Europe (HPV45&31), Africa (HPV35&HPV31), Asia (HPV58&HPV52) and Oceania (HPV35& HPV39&HPV68 with the same relative contribution). In cervical adenocarcinomas the ranking was: HPV 16(46.9%), HPV 18(29.6%) and HPV 45(10.6%). Any other individual HPV type showed relative frequencies below 1%. HPV 16, 18 and both types combined accounted for 70.4% of the HPV positive cases. Multiple infections represented 6.0% of the HPV positive cases. Mean age of cases with HPV16, 18 or 45 (50.1, 48.3 and 47.2, respectively), were statistically lower than mean age of cases with other HPV types (54.8; p<0.05). Conclusion: The study confirms the consistent contribution of HPV 16 and 18 to cervical cancer around the world.

HPV PREVALENCE AND TYPE DISTRIBUTION IN SPAIN: THE CLEOPATRE STUDY

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CLEOPATRE Spain Study Group

Objective: Population-based prevalence data on HPV infection in Southern Europe is scarce. The objective of this study is to estimate the overall and age-stratified prevalence and type-specific HPV distribution in the Spanish general population of women attending cervical cancer screening.

Methods: Cleopatre is a population-based cross-sectional prevalence study conducted in 77 centres across Spain. Recruitment was conducted following a stratified sampling including the following age strata: 18, 19, 20-21, 22-25, 26-35, 36-45, 46-55, and 56-65 years. From June 2007 to May 2008, ThinPrep[®] liquid-based cytology samples were collected from women aged 18-65 years visiting their gynaecologist for cervical screening. Samples were sent to a central laboratory for cytology testing. Genotyping was performed on residual samples using the INNO-LiPA Extra line probe assay (Innogenetics).

Results: 3,256 women were included in the study of which 3,216 provided a valid cytology result. A total of 3,148 (97.9%) had normal cytology, 30 (0.9%) had ASCUS, 27 (0.8%) had LSIL, and 11 (0.3%) had HSIL. Genotyping is ongoing and type-specific data will be presented. The overall prevalence of single and multiple infections, and low- and high-risk HPV types will be described and analysed according to age group, cytological diagnosis, and Spanish autonomous community. Determinants of cervical HPV infection such as age, number of sexual partners, age at first sexual intercourse, marital and socio-economic status, screening usage, and contraceptive methods used among others, will be explored in univariable and multivariable analyses.

Conclusion: These data, in combination with those from the Cleopatre study performed in Denmark, will provide us with a better understanding of the wide spectrum of the epidemiology of HPV infection across Europe. The study will provide referent background HPV statistics to assess in the future the impact of HPV vaccines introduction on the overall and type-specific HPV prevalence in Spain.

P-30.15

POSSIBLE TRENDS IN HPV PREVALENCE AMONG YOUNG WOMEN IN COSTARICA

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Background: Changing patterns of sexual behavior may result in increases in HPV that may affect future cervical cancer burden. To investigate time trends in HPV prevalence and its determinants, we used data from two NCI-sponsored cohorts >10 years apart in Costa Rica.

Methods: In 1993-4, 1,259 women 18-25 years were recruited into the population-based Guanacaste Natural History Study (NHS) (approx. 93% response), and in 2004-5, 4,938 women from the same province and of the same ages were recruited into the community-based Costa Rica HPV Vaccine Trial (CVT) (approx. 30% of women identified via a census were eligible and participated). Women were interviewed about risk factors for HPV/cervical cancer, and specimens were collected for determination of HPV infection and cervical disease. HPV testing was performed by MY09-11 amplitaq gold PCR (NHS) and by SPF10/DEIA/LiPA25 and type-specific primers for HPV-16/18 (CVT). ORs and 95% CIs were computed using multivariate logistic models.

Results: Overall prevalence of oncogenic HPV types was 23.2% in 1993-4 and 35.4% in 2004-5 (pre-vaccination) (p<0.001). Increases were noted for HPV-16/31/35/39/45/51/52/56/59/66/ 68/73, while a decrease was noted for HPV-58. After adjustment for age, 18-25 year olds in 2004-5 were significantly more educated, had fewer pregnancies, used more oral contraceptives, were more likely to smoke and had more sexual partners than similarly aged women in 1993-4. Analyses are ongoing to determine what proportion of the difference in HPV prevalence observed between 1993-4 and 2004-5 can be explained by changes in behavior among the two cohorts.

Discussion: A significant increase in HPV prevalence among young women was detected between 1993-4 and 2004-5. These changes are possibly explained by an increase in risk factors for HPV in the later cohort. Limitations include differences in the PCR techniques used for HPV detection in the two studies and the fact that CVT is not populationbased.

PHYSICAL STATE AND CLEARANCE OF HPV16 INFECTION

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Background: Viral integration into the cellular DNA is frequently associated with viral disruption in the E1/E2 regions of HPV. Physical state is a promising molecular biomarker to detect the progression of cervical lesions, however little is known about its association with viral burden and persistence, a surrogate for risk of CIN2/3. Methods: We tested 380 HPV16 positive cervical brush samples from the Ludwig-McGill Cohort study for viral integration. Localization and degree of HPV16 integration was determined by PCR targeting different regions of varying length within the 5' and 3' portions of the E2 gene. In addition, the relative difference between E6 and E2 copy number obtained by Real-Time PCR was used to estimate the predominance of integrated over episomal forms (E2<E6: coexistence of integrated and episomal forms; and $E2 \ge E6$: episomal only). We assessed the association with clearance of HPV16 by integration status using Kaplan Meier analysis and multivariable Cox regression. Results: More than half (57%) of HPV16 positive samples tested contained both episomal and integrated HPV, and exclusively episomal or integrated forms were found in 19% and 24% of specimens, respectively. Viral burden, measured by E6 RT-PCR, was significantly lower in specimens with normal cytology, women harboring exclusively integrated HPV took longer to clear their infection than those harboring mixed or episomal forms (HR=0.64, 95%CI 0.35-1.17), independent of E6 viral load, age, co-infection and number of visits. Conclusion: Viral integration appears to be an early event in the natural history of cervical HPV and is associated with HPV persistence.

P-30.17

SYSTEMATIC REVIEW: PERINATAL TRANSMISSION OF HUMAN PAPILLOMAVIRUSES (HPV)

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Background: The perinatal epidemiology of HPV and its impact on newborns and children is not well understood although it is recognised that subclinical and clinical infections occur following perinatal transmission. Theoretically, HPV infections acquired in utero or postnatally could be linked to an increase in squamous carcinomas in children. Objective: The aim of this review was to quantify the probability of perinatal transmission of HPVs.

Results: Studies have reported widely varying rates of perinatal infection (in oral and genital mucosa) in newborns, with estimates ranging from 4% to 79% among infants born to mothers testing positive for HPV DNA during pregnancy. Different limitations were noted: (1) HPV testing was done soon after birth, perhaps reflecting surface contamination of the infant from infected maternal cells; (2) differences in techniques for the detection of HPV were used, with different analytical sensitivities; (3) sample sizes were very small and follow-up short. Interestingly, recent prospective studies with several time points confirmed that perinatal transmission does occur in a substantial number of infants and may persist. Conclusion: Perinatal transmission of HPV can not be ignored. Prospective cohorts in infants with a longer follow-up, extensive safeguards (to avoid maternal contamination) and the use of a sensitive sequencing method for HPV typing are needed to obtain relevant epidemiologic data.

P-30.18 ONCOGENIC HPV IN 25-65 YEAR OLD WOMEN WHO DATE ONLINE

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Background: The epidemiology of HPV infections in women \geq 25 is not well understood, and data on the risk of infection associated with new sex partners are lacking.

Objectives: To identify predictors of oncogenic HPV infections in high-risk 25-65 year old women.

Methods: To date we have recruited 252 women (median age=32) who reported using dating websites in the past year. Women were mailed kits for self-collecting vaginal specimens for PCR-based HPV testing (including 19 oncogenic types) at zero and four months. Sexual behavior questionnaires were completed with each self-collection. Generalized linear models were used to evaluate risk factors for baseline oncogenic HPV infections.

Results: The baseline prevalence of oncogenic HPV infection was 36.5%, and the most common types detected were 16 (8.7%), 53 (7.1%), and 18 (5.9%). In multivariate analysis, variables associated with an increased risk of oncogenic HPV infection included: reporting a lifetime number of male sex partners ≥ 12 (the median) (risk ratio [RR]=1.7,95%CI:1.1-2.4), reporting ≥ 2 concurrent partnerships in the past 8 months (RR=1.4,95%CI:1.05-2.0), and reporting having met a recent partner (in the past 8 months) via the internet (RR=1.4,95%CI:1.02-2.0). In addition, consistent condom use in the past 8 months was associated with a borderline statistically-significant decreased risk of infection (RR for always versus sometimes/never=0.5,95%CI:0.3-1.07). Twenty percent of 125 women returning a second sample tested positive for a new oncogenic type, and 59.5% of type-specific oncogenic infections were re-detected four months later.

Conclusions: The prevalence of oncogenic HPV infection was higher than expected in this population of high-risk women aged 25-65. Measures of both cumulative and recent exposure to HPV were associated with an increased risk of infection, suggesting a role for new acquisition in this age group. With ongoing data collection, we will further evaluate predictors of oncogenic HPV infections (including newly-detected and repeatedly-detected infections).

P-30.19

POPULATION-BASED HUMAN PAPILLOMAVIRUS 16/18/6/11 DNA AND SEROPOSITIVIY IN CHINESE WOMEN

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Background: To optimize HPV vaccination implementation on the population-level in China, data are needed on agespecific HPV 16, 18, 6 and 11 prevalence.

Objective: Evaluate age- and type-specific HPV 16/18/6/11 prevalence of DNA and serological antibodies in women from diverse geographical regions of China.

Methods: A cross-sectional, population-based study of women, aged 16 to 54 years, was conducted in 3 rural (Xinjiang, Shanxi, and Henan) and 2 urban areas (Beijing and Shanghai). From July 2006 to April 2007, women provided cervical cells for HPV DNA and liquid-based cytology (TriPath). High and low-risk HPV types were detected with HC-II (Digene), with genotyping of HPV-positive samples using Linear Array (Roche). HPV 16, 18, 6, and 11 serum antibodies were detected using a Luminex assay (Merck & Company).

Results: 4,206 women with data on both DNA and serum antibody results were included. Both HPV 16 and 18 DNA prevalence were relatively low at 16-24 years (2.5%,0.6%, respectively), peaked at 25-44 years (3.1%,1.0%), with a plateau at 44-54 years (2.7%,0.6%).

HPV type	16	18	16/18	6	11	6/11	16/18/6/11
PCR DNA %	2.9%	0.8%	3.6%	0.02%	0.3%	0.4%	3.8%
Serology %	6.3%	2.1%	7.8%	8%	3.2%	9.9%	15.8%
DNA &/or Serology %	7.8%	2.8%	9.8%	8%	3.4%	10.1%	17.5%

Most women were dually DNA and serum antibody negative: HPV 16 (92.2%), 18 (97.2%), HPV 16 & 18 (90.2%), 6 (92.0%), 11 (96.6%), 6 & 11 (89.9%), 16, 18, 6, & 11 (82.5%). Detailed age-specific data will be presented.

Conclusions: Future national HPV vaccination programs in China should optimally target younger women due to the increased exposure to HPV types 16, 18, 6 and 11 with increasing age, and given that cross-sectional data do not accurately reflect cumulative exposure to HPV infections over time.

HPV16/18 HIGH IN PRECANCER, LOW IN NORMAL CYTOLOGY IN CHINA

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Background: In China, few representative data are available on the population-based burden of HPV infection and cervical precancer/cancer among reproductive-aged women.

Objective: To ascertain the age-stratified prevalence of HPV DNA and cervical disease in women from diverse geographical regions of China.

Methods: From July 2006 to April 2007, a population-based study of women, aged 15 to 54 years, was conducted in 2 urban (Beijing and Shanghai) and 3 rural areas (Xinjiang, Shanxi, and Henan) of China. Consenting women provided cervical cells for HPV and Autocyte liquid-based cytology (TriPath). High-risk (HR) HPV DNA was detected with HC-II (Qiagen), in combination with the typing of HC-II positive-samples using the Linear Array PCR assay (Roche). Women with abnormal cytology or HC-II-positive results underwent colposcopy with multiple cervical biopsies.

Results: Of 4,215 participating women with data on cytological and HPV testing results, the mean age was 37.5 years (range 36.3 in Xinjiang to 38.9 years in Shanghai) among women screened, and 40.5 years among pathology confirmed \geq CIN-2 cases. The prevalence of \geq CIN-2 was 1.4% (11/774) in Shanghai, 1.1% (10/879) in Henan, 1.9% (17/883) in Xinjiang, 1.1% (9/795) in Beijing, and 2.5% (22/884) in Shanxi. The most common HPV types in \geq CIN-2 cases were HPV 16 (68.1%, 47/69), 58 (10.1%, 7/69), 31 (10.1%, 7/69), 18 (8.7%, 6/37), 33 (8.7%, 6/67) and 52 (7.2%, 5/69). HPV 16 and/or 18 prevalence was high in \geq CIN-2 cases (73.9%, 51/69), ranging from 60.0% (6/10) in Henan to 81.8% (9/11) in Shanghai. In contrast, HPV 16/18 prevalence was relatively lower among women with normal cytology (1.4%, 53/3,734), ranging from 0.5% (4/736) in Xinjiang to 1.8% (13/724) in Shanghai.

Conclusions: Based on this large population-based study from China, the proportion of \geq CIN-2 cases attributable to HPV types 16 or 18 is consistent with global data.

P-30.21

THE EFFECT OF OC AND MENSTRUAL CYCLE ON HPV PREVALENCE

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Background: The immune response in the female genital tract is likely to be subject to hormone-dependent variations during the menstrual cycle. Differences have been reported in the detection of Human Papillomavirus (HPV) with respect to a woman's last menstrual period (LMP). Additionally, HPV transcription and clearance is affected by longstanding oral contraceptive (OC) use.

Objective: To investigate the effect of the menstrual cycle, and/or the simultaneous use of OC on the point prevalence of HPV.

Methods: A population based cross-sectional study among 2065 women aged 18-29 years was conducted. All women returned a self-collected cervico-vaginal sample and filled out a questionnaire. For HPV detection broad-spectrum HPV DNA amplification was performed using a short-PCR-fragment assay (SPF10 Lipa).

Results: The point prevalence of HPV DNA in this sample was 19% (n=393), including multiple-infections. Low and high risk HPV prevalence were 9.1% and 11.8%, respectively. OC were used in 70.8% (n=1459) of all women. Data regarding the menstrual cycle were available in 88.3% of all women (n=1824).

Differences for hr-HPV prevalence for OC-users and Non-users, and for LMP, is not significant. Although there seems to be a linear trend, the combined effect of LMP and OC-use on the hr-HPV is not significant.

Conclusion: Hormonal contraceptives and the menstrual cycle do not influence the single point prevalence of HPV in young women when using a self sample and SPF10Lipa.

HPV PREVALENCE, TYPE DISTRIBUTION, AND RISK FACTORS AMONG TANZANIAN WOMEN

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Background: Knowledge about the prevalence of genital infection with human papillomavirus (HPV) and the HPV type distribution in Africa is relatively limited. Such information is important to estimate the burden of HPV infection among Tanzanian women.

Objective: The aim of the present study is to assess the prevalence and type distribution of HPV in women from respectively urban and rural areas of Tanzania, and to examine risk factors for HPV infection.

Methods: We included women from the general population in an urban area of Tanzania (DaresSalaam Region) and in rural areas (Pwani and Mwanza region). The participants went through a gynaecological examination where a conventional smear and a cervical swab for HPV testing were obtained. Following the application of aceto acid, visual inspection (VIA) was performed. Finally, the women were interviewed about socio-economic and lifestyle factors, had a blood sample taken, and were tested for HIV. The cervical swabs were tested for high-risk (HR)-HPV by means of Hybrid Capture 2 (HC2), and genotyping was performed using the PCR-based LiPa test.

Results: Presently 2567 women were included in the study, covering 1591 women from rural and 976 from urban areas. The study included women 15-82 years of age, the mean age being respectively 36.9 years (rural) and 40.9 years (urban). A total 3.3% of rural and 7% of urban women were VIA positive. Currently we have tested 1273 women for HPV. A total 111 women out of 562 (19.8%) in the rural area were positive for HR-HPV whereas this applied to 18.2% (129 out of 711) women from the urban area.

Conclusion: The preliminary results show that HR-HPV infection is common in Tanzanian women. The HPV type distribution and HPV positivity in relation to VIA result, cytology and lifestyle factors will be presented.

P-30.23

AGE-SPECIFIC HPV SEROPREVALENCE AMONG YOUNG FEMALES IN THE NETHERLANDS

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Objectives: To obtain insight into the age-specific seroprevalence (immunity) for HPV6, -11, -16, and -18 among girls aged 11 to 26 years, particularly among those aged 12-16 years who are targeted for HPV-vaccination.

Methods: HPV testing for antibodies to HPV6, -11, -16, and -18 was conducted in sera of 11-26-year-old girls (n=637) who participated in a study consisting of a representative sample of the general population. Sera were tested using a competitive Luminex assay with neutralizing monoclonal antibodies specific for each genotype. Associations between HPV seropositivity and demographics or sexual behaviour were studied with logistic regression. Seroprevalences were weighted by age, sex, ethnicity, and urbanization degree.

Results: The overall prevalence of antibodies against one or more HPV genotypes was 7.6%. For low-risk vaccine HPVtypes, 4.2% had antibodies against HPV-6 and/or -11, while 4.4% of the girls had antibodies against high risk HPV types 16 and/or 18. HPV seropositivity increased with age starting at the age of 16 years (median age of sexual debut). Between 21-26 years of age the overall HPV seroprevalence remained at a percentage of about 15%. Factors significantly associated with HPV seroprevalence were increasing age (OR 1.21; 95%CI 1.11-1.32) and ever having had an STI (OR 5.67; 95%CI 2.61-12.37). Girls who reported >1 sexual partner in the past 6 months had a 1.6-higher risk of being HPV seropositive compared to those who reported only one partner.

Conclusions: Girls aged 13-16 years seem to have similar benefit of HPV 16/18-vaccination compared to those aged 12 years. For the interpretation of the results, it has to be taken into account that about half of the individuals show an antibody response after infection. Results could serve as baseline seroprevalences. After implementation of routine HPV-16/18 vaccination and catch-up campaign, similar studies could be repeated to study the impact of vaccination.

HPV-16 VIRAL LOAD MEASUREMENTS AND DURATION OF INFECTION

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Background: Several studies have demonstrated that HPV16 viral burden may serve as a surrogate for persistent infection and thus help predict risk of cervical precancerous lesions. However, there is no a priori reason why the relationship should be type specific. Methods: To assess the role of viral load as a predictor of HPV clearance, we tested 418 cervical smears positive for HPV16 DNA from 224 women participating in the Ludwig-McGill cohort study using three different protocols: a Low-Stringency PCR (LS-PCR) using (GP5/6) consensus L1 primers that amplify over 40 known HPV types including HPV16, and Real-Time PCR (RT-PCR) targeting the HPV16-E6 and L1 genes. Viral load was converted to copy numbers/cell for each protocol using counts for human beta-globin gene. Using random-effects, generalized least squares model for repeated data, we assessed the type specific contributions to viral load counts by LS-PCR. We also calculated Pearson's correlation coefficients using log-transformed data to compare viral load measures by each protocol, and Kaplan Meier estimates to evaluate the protocol-specific viral load associations with HPV16 persistence. Results: The correlation between viral load measurements by E6 and L1 RT-PCR was high (r=0.90), although L1 RT-PCR tended to overestimate the viral load in specimens with low copy number by E6 RT-PCR. In contrast, the correlation between viral load measurements by L1 RT-PCR and LS-PCR was lower for samples with multiple HPV infections (r=0.54) and those with HPV16 only (r=0.70). Viral load estimates by LS-PCR were higher for HPV16 and related types, independent of other HPVs. Conclusion: We observed significant associations between viral load and HPV16 persistence regardless of which protocol was employed. Being less-resource intensive to perform than RT-PCR and able to detect most HPV types, LS-PCR is a robust and reliable method for viral load estimation and for predicting the likelihood of infection clearance.

P-30.25

TEST-RETEST RELIABILITY FOR MULTINATIONAL SEXUAL BEHAVIOR INTERVIEWS: THE HIM STUDY

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Background: the collection of sexual behavior data is central to the development of prevention strategies for sexually transmitted diseases. To create efficiencies in prevention programs, it may be helpful to gather sexual behavior data from multinational populations; however, the reliability of cross-national sexual behavior surveys using computer-assisted self-interview (CASI) methods is unknown.

Objectives: assess the test-retest reliability and item-refusal rates for 38 sexual behavior items of a computer-assisted self-interview used with 1069 men in Brazil, Mexico, and the US.

Methods: Refusal rates, kappa coefficients (κ), and intraclass correlation coefficients (ICC) were calculated for each country and then combined after weighting each country's reliability coefficient by the inverse of its variance. In addition, reliability coefficients were calculated to allow comparisons of men by age and by lifetime number of female sexual partners.

Results: the rate of refused questions in each country was, in general, very low with the lowest rate in Mexico and the highest in Brazil. Combined population reliability coefficients were substantial ($\kappa = 0.61-0.80$) or almost perfect ($\kappa \ge 0.81$) for all categorical items. Three discrete items asking for the number of sexual partners had lower reliability in Brazil (ICC < 0.61). The ICC of these questions increased to ≥ 0.79 when a small number of extreme outliers (≤ 2) were removed. With few exceptions, reliability coefficients were ≥ 0.61 regardless of age or number of female sexual partners.

Conclusions: with few exceptions, we found high test-retest reliability for a sexual behavior CASI used in three culturally and linguistically distinct countries.

HPV-TYPE DISTRIBUTION IN CERVICAL CANCER IN WALES AND SCOTLAND (UK)

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Background: Current data on HPV-type distribution are incomplete and methodology differs between studies. It is therefore important to collect data on the HPV-type distribution using a standardised protocol across Europe.

Objectives: As part of a pan-European, cross-sectional study, HPV-type distribution in a sample of two well-screened populations in Wales and Scotland diagnosed with invasive cervical cancer (ICC) and the ratio between squamous (SCC) vs adenosquamous (ADC) cases were assessed.

Methods: Archived cervical specimens collected from a convenience sample of women \geq 18 years from Wales (n=337) and from Scotland (n=266) with ICC were tested in DDL diagnostic laboratory (Voorburg, The Netherlands) using PCR-SPF10 LiPA25 version 1 methodology that detects 14 oncogenic HPV-types (HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) and 11 low-risk HPV-types (HPV-6, 11, 34, 40, 42, 43, 44, 53, 54, 70 and 74).

Results: HPV DNA was detected in 266/337 (78.9%) of ICC specimens collected in Wales, of which 255/266 (95.8%) were single infections. The most common types detected in single infections were HPV-16 (68.2%, CI:62.1-73.9) and HPV-18 (15.7%, CI:11.4-20.7). HPV DNA was detected in 237/266 (89.1%) of ICC specimens collected in Scotland, of which 213/237 (89.9%) were single infections. The most common types detected in single infections were HPV-16 (56.3%, CI:49.4-63.1) and HPV-18 (23.5%, CI:18.0-29.7). The ratio of ADC vs SCC was 1:4 in both Wales and Scotland.

Conclusions: For ICC, HPV-16 and -18 represented 83.9% and 79.8% in the studied sample population in Wales and Scotland, respectively. Based on worldwide HPV-type prevalence, this suggests that in these populations the likely impact of vaccination may be underestimated.

*SPF10 HPV LiPA25, version 1 and SPF10 HPV DEIA manufactured by Labo Biomedical Products (Rijswijk, The Netherlands) based on licensed INNOGENETICS SPF10 technology.

P-30.27

HPV INFECTIONS IN YOUNG VERMONT WOMEN WITH NEGATIVE CERVICAL CYTOLOGY

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Background: In this study we have investigated the prevalence and range of HPV genotypes present in cervical cytology samples diagnosed as negative for intraepithelial lesion or malignancy (NILM) to examine the exposure to HPV of young women in Vermont, USA.

Methods: NILM DNA samples were prepared from 214 women <20 (age range: 13-19 years) and from 167 women aged 20-24 years. HPV testing was performed by GP5+/6+ PCR (two alternative cycling strategies) and by PGMY09/11 PCR. HPV genotypes were identified by dot-blot hybridization and cycle sequencing.

Results: For the <20 age-group, a total of twenty-seven different genotypes were detected amongst the 38.8% that tested HPV positive; 52.1% of the 20-24 age-group tested HPV positive (P=0.01) for one or more of thirty-five different genotypes. High-risk (hr) HPV was found in 18.7% of women <20 and in 29.3% of women aged 20-24 (P=0.02). Low-risk HPV genotypes were identified in 25.7% of patients <20 and in 27.0% of patients aged 20-24 (P=0.82). Double or multiple infections were found in 11.7% of the teenagers and in 13.2% of women aged 20-24 (P=0.75). HPV16 and/or 18 was identified in 14.0% of women <20 and in 22.2% of women aged 20-24 (P=0.04). HPV6 and/or HPV11 were detected in 1.6% of the total sample (n=381).

Conclusion: These data demonstrate that exposure to a wide range of HPV genotypes is an early and common sub-clinical event: among women aged <25, 44.6% of cytologically normal cervical samples tested HPV positive, 23.4% for hrHPV, and 17.6% for HPV16/18. The findings support early vaccination policies, the development of additional hrHPV vaccines, and education promoting protective behaviors. Patient follow-up is required to determine the constancy of the infections detected, subsequent cytological diagnoses, and the clinical utility, if any, of screening NILM samples for HPV.

THE EPIDEMIOLOGY OF HUMAN PAPILLOMAVIRUS IN NORTHERN CANADA

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Cervical cancer rates are higher among aboriginal populations than among the general population in Canada. Since Human Papillomavirus (HPV) are highly associated with cervical cancer, it is essential to understand HPV epidemiology. in Northern Canada where aboriginal people account for a high percentage of the total population

The objective of this project is to determine the prevalence, distribution, and risk factors of type-specific HPV infections among women in northern Canada,.

Women living in the Northwest Territories (NT), Nunavut, and Yukon, at cervical cancer screening ages, and with no cancer history are included in the study. Cervical sample collection is incorporated into the routine sample collection for Pap testing. A questionnaire will collect socio-economic, demographic, and behaviour information of participants. HPV types are detected by using Luminex assay at the National Microbiology Laboratory of Canada. Pap test results, HPV types, and questionnaire data will be linked for analyses.

The prevalence and distribution of HPV type-specific infections and cervical dysplasia will be calculated with 95% confidence intervals. Multivariate regression will be used to explore the associations between type-specific HPV infections and cervical dysplasia as well as the associations between risk factors and type-specific HPV infections.

So far there are more than 7,000 samples from the NT and Nunavut have been tested for HPV types. The crude HPV positive rates are 26.9% and 33.9% of the NT and Nunavut, respectively. There are over 70% of the HPV positive samples from both regions are positive with high-risk types. The prevalence analyses will be completed in April 2009. More results will be available at the conference.

This project will contribute to the knowledge of HPV epidemiology among women in Northern Canada. The results may be useful for developing strategies to prevent HPV infections and reduce the burden of illness associated with high-risk HPV infections.

P-30.29

CERVICAL CANCER SCREENING PROGRAM AND COMMUNITY PARTNERSHIPS FOR HPV TESTING

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The role of HPV testing for surveillance and cervical cancer screening testing has gathered interest across Canada including the province of Manitoba following the introduction of HPV vaccination within the provincial school system. The Manitoba Cervical Cancer Screening Program is mandated to improve screening participation among women in Manitoba between the ages of 18 and 69. To improve access across the province, a walk-in, no appointment Pap clinic week was established in 2003. The Pap Clinic Week has grown from seven health clinic partners in 2003 to 124 clinic partners in 2008 successfully reaching women who are underscreened. Consistently, more than 35% of women who attend a walkin clinic have not had a Pap test in the previous five years. In 2006, a pilot project with three community health centres was conducted to evaluate the potential for HPV testing to be completed at independent clinical centres. In 2008, the Pap Clinic Week expanded to include linking HPV test results with pap and cancer registries and risk factor surveillance. This initiative resulted in 54 of the 124 clinics volunteering to participate in HPV research during the operation of their Pap clinic. The process of recruiting clinics, developing public consent forms and risk factor questionnaires, education of providers on HPV sampling techniques in the absence of technological infrastructure, and preliminary compliance and evaluation results from this project demonstrate that partnerships, technology, and self administered research documents can be achieved with great success. The lessons learnt from this project will inform the roll-out of a pan-Canadian HPV test, pap and vaccine 'registry-linkage' approach for HPV surveillance and how partnerships in research can be expanded to a large community of health care providers for the benefit of women in the population.

P-30.30 THE EUROPEAN PREVALENCE OF RECURRENT RESPIRATORY PAPILLOMATOSIS. (EURRPREVALENCE)

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Recurrent respiratory papillomatosis (RRP) is a relatively rare condition caused by HPV types 6 and 11. The mode of transmission of the HPV virus and subsequent development of RRP is uncertain. There is evidence supporting vertical transmission from mother to child. The development of childhood onset RRP is associated with firstborn children who are vaginally delivered by young mothers. Published prevalence varies10 fold between different studies from approximately 4 per 100 000 to 3.8 per million and while this maybe a reflection of true differences in disease prevalence it maybe due to study bias. The recent development and uptake of HPV vaccination targeting cervical cancer may have an effect on the prevalence of RRP. It is therefore important to have a reliable method of ascertaining changes in the prevalence of RRP. In order to monitor changes in the prevalence of RRP in Europe a multinational European Registry is proposed. The aim is to determine the incidence and prevalence rates of RRP paediatric and adult in participating countries and to aggregate

this data to allow a Europe wide estimate. To facilitate data collection a website will be developed which meets European standards for patient data collection. An agreed protocol with some local adaptation will be developed and ethical committee approval sought at primary centers. As the pattern of referrals is different in different countries the sampling strategy will be country dependant. The aim in all cases will be to arrive at a robust estimate of number of cases per 100 000 head of population.

The legislative and ethical framework governing the establishment of a pan-European RRP registry will be presented. Sampling strategies proposed for individual countries will be presented.

A registry is a viable method for monitoring changes in RRP prevalence.

P-30.31

RISK FACTORS FOR HPV DETECTION IN A PERI-URBAN INDIAN POPULATION

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Objective: To identify the determinants of HPV DNA detection in a peri-urban Indian population in Andhra Pradesh. Methods: All women living in Medchal Mandal who were age 25 years and older, had an intact uterus, and were not currently pregnant were invited to participate in a cervical cancer screening study at a local hospital, which included Pap smears, visual inspection with acetic acid (VIA), and HPV DNA testing by Hybrid Capture 2. Information regarding demographics, tobacco exposure and reproductive history were collected via interviewer-administered questionnaire.

Results: The overall HPV DNA prevalence was 10.5% (95% CI 9.2%, 11.8%), and was similar across 10-year age categories, with a slight but non-significant increase among women 50-59 years old. After multivariate adjustment, HPV prevalence was significantly higher among younger (p-trend=0.01) and less educated women (p-trend=0.02). Women living with a smoker (OR=1.46, 95% CI 1.10, 1.94) and women with a positive Pap smear (OR=2.40; 95% CI 1.70, 3.40) or cytologic diagnosis of trichomonas (OR=2.40; 95% CI 1.24, 4.64) also remained more likely to be HPV positive, while Muslim women remained significantly less likely to be HPV positive (OR=0.19; 95% CI 0.05, 0.78). Risk of HPV decreased with increasing age at shobadum except for women reporting age at shobadum >20 years (OR14-16=0.77; 95% CI 0.55, 1.07 : OR17-19=0.59; 95% CI 0.38, 0.93 : OR20+=0.98; 95% CI 0.56, 1.72, relative to ≤ 13 years).

Conclusion: There was little variability in HPV prevalence in this peri-urban Indian population, though lower educational level, tobacco exposure, later age of shobodum and cytopathologic trichomonas infection are associated with an increased risk of HPV detection, while Muslim religion was associated with a decreased risk of HPV. Possible influences of increasing age at shobadum in successive birth cohorts on the epidemiology of HPV in peri-urban India are under evaluation.

HPV PREVALENCE IN 467 GREEK WOMEN USING A MICROARRAY-BASED METHOD.

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HPV is the most common sexually transmitted infection with more than 40 types known to infect the anogenital mucosa. The prevalence of HPV infection is known to vary by location and different types of HPV seem to infect different populations.

The aim of this study was to analyse the prevalence of 35 most clinically relevant HPV types in a group of 467 women from Greece. The women had all been diagnosed with an abnormal pap test and/or colposcopy and were advised to proceed with the HPV DNA testing.

The HPV test was carried out in cervical samples using a commercially available kit, approved for in vitro diagnostic use. The kit enables the simultaneous detection of up to 35 different HPV types after a single PCR reaction. The technology is based on DNA hybridisation on low density microarrays.

Out of the total 467 samples, 52.9% tested positive for HPV DNA. The large proportion of negative samples indicates that cytological testing can often lead to misinterpretations and false positive results. Overall, among the HPV positive samples, HPV 16 was the predominant type (22.3%), followed by 53 (13.2%), then 6 (12,8%), 31 (10.4%), and 51 (9.2%). Interestingly, type 18, which is included in the two commercially available HPV vaccines, had a frequency of 7.2% in this group. Additionally, samples containing multiple infections represented a significant 38.4% of all positive cases.

Our study may be important in understanding the population variations that accompany infection with different types of HPV. The results of such studies from different countries will have an impact on the design and effectiveness of vaccinations programmes.

P-30.33

THE UNDER PRIORITISED BURDEN OF CERVICAL CANCER IN SUB-SAHARAN AFRICA

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Cancer of the cervix is the leading cause of cancer in women in sub-Saharan Africa (SSA). The magnitude of the problem has been under-recognised and under-prioritised compared to other competing health priorities such as the concurrent HIV/AIDS epidemic in the region, as well as the lack of epidemiological data and poor awareness, lack of human and financial resources, non-existent cancer service policies and political will to address the complex problem. Organized screening and early treatment programmes have been costly and difficult to implement and few benefit from these options in SSA. Preventable vaccines against human papillomavirus (HPV) infection are now available and may represent the first realistic opportunity to reduce the burden of cervical cancer in the next generations. However, the opportunity of these vaccines to have an effective impact in SSA will not materialise until they become affordable. Until then, basic groundwork is still needed in SSA, where data are scarce, to consider the most appropriate cervical cancer prevention strategies, including the implementation of HPV vaccine programmes. At the country-level, decision-makers will require evidence from (1) epidemiological studies on HPV burden, to estimate the impact of HPV vaccines and cervical cancer screening; (2) health systems and social science research, to analyse health system preparedness for the introduction of HPV vaccines; and (3) health economics and modelling analysis, to estimate the cost-effectiveness of population impact of introducing HPV vaccines and cervical screening. These three components will formulate a spectrum of information needed for decision-making and policy development for cervical cancer prevention. The presentation will describe the HPV African Research Partnership (HARP) among five African countries (Burkina Faso, Ghana, South Africa, Tanzania, and Uganda) that aims at collecting these three levels of comprehensive evidence to inform decision-makers in each of these countries on the most appropriate local cervical cancer prevention strategies.

PREVALENCE OF HUMAN PAPILLOMAVIRUS (HPV) ANTIBODIES IN PUEBLORRICO, ANTIOQUIA

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Background: Antibodies against HPV provides an estimate of population cumulative exposure. We conducted an HPV seroprevalence study in a rural town of Colombia. Objective: The aim was to examine the HPV 16, 18, 31 and 58 seroprevalence and identify factors associated with HPV genotypes antibodies. Methods: This is a population-based study in a randomly-selected, age-stratified sample of 15 years old or older women, living in a rural town of Antioquia, a state of the north-west region of Colombia. HPV antibodies were determined by HPV VLPs ELISA in 878 serum samples. Cut-off point to define seropositivity to each antigen preparation was three times the standard deviation to the mean of optical densities found in low risk, HPV DNA negative women. Unconditional logistic regression was used to estimate odds ratios (ORs). Results: The overall seroprevalence for the 4 genotypes was 27.50% (95%CI:24.60-30.5). The seroprevalence for HPV 16, 18, 31 and 58 was 17% (95%CI:14.60-19.60), 9.79% (95%CI:8.0-11.9), 11.40% (95%CI:9.45-13.7) and 12.50% (95%CI:18.20-23.50) respectively. Seropositivity to any HPV type was strongly associated with seropositivity to the other measured HPV types. Highest OR was observed for HPV 18 and 58 (OR:36.97, 95%CI:21.35-63.99) followed by HPV 18 and 31 (OR:31.82, 95% CI:18.74-54.01). Seropositivity to HPV16, 18, 31 or 58 increased as the time of years of sexually active life increased with highest probability of being seropositive for women with 31-35 years of sexually active life (OR:4,83, 95%CI: 1,25-18,68). In women without regular partners, more than one occasional sexual partner was associated with seropositivity to HPV 16 (OR:2.28, 95%CI:1.02-5.09) and 18 (OR:3.32, 95%CI:1.34-8.2). Women who smoke were less likely to have antibodies against HPV16, 18, 31 or 58 than non-smokers (OR=0.64. 95%CI=0.44-0.93). Conclusions:HPV seropevalence in this population is consistent with findings in previous reports. Years of sexually active life and smoking were determinants of HPV seropositivity.

P-30.36

HPV DNA DETECTED IN PERIPHERAL BLOOD SAMPLES FROM HEALTHY DONORS

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Background: The papillomaviruses require proliferating basal layer cells and must access the basal cell layer of the epidermis or mucosa to establish infection. It has long been believed that papillomavirus can not be spread to different body sites through blood, as papillomaviruses do not give rise to viremia. However, several recent studies have shown that HPV DNA can be found in peripheral blood, including peripheral blood mononuclear cells (PBMCs), sera, plasma and arterial cord blood.

Objectives: In light of these finding, we examined DNA extracted PBMCs from healthy blood donors in order to determine how common HPV DNA is in blood.

Methods: The 190 healthy male blood donors were recruited through the Australian Red Cross Blood Services in 2006 in Brisbane, Australia. All participants were Caucasian men between 18 and 91 years of age. Genomic DNA was extracted from peripheral blood cells. Specimens were tested for HPV-DNA by PCR using the broad range HPV-type primer pair FAP59/64. The positive samples were HPV-type determined by cloning and sequencing and obtained sequences were compared in GenBank.

Results: We found 8.4% (16/190) of our Red Cross blood donors to be positive, however most of the HPV types detected in the PMBCs were skin HPV types that are commonly found in asymptomatic infections of normal, healthy skin. High-risk HPV types that are associated with cancer development were detected in 1% (2/190) of the PMBCs.

Conclusions: HPV DNA can readily be found in PBMCs from healthy blood donors, which suggests that PMBCs can carry HPV in blood.

HPV-TYPE DISTRIBUTION IN INVASIVE CERVICAL CANCER IN DENMARK

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Background: Vaccination against HPV-16 and -18 with an adjuvanted cervical cancer vaccine is expected to result in a significant reduction of cervical cancer incidence over time. In order to estimate the potential effect, it is important to collect data on HPV-type distribution in invasive cervical carcinoma in different countries.

Objective: As part of a pan-European, cross-sectional study, HPV-type distribution in a sample of Danish women diagnosed with invasive cervical cancer (ICC) and the ratio between squamous (SCC) vs adenosquamous (ADC) cases were assessed. Results from a complementary study on HPV-type distribution in precancerous lesions are presented in a separate abstract.

Methods: Archived cervical specimens collected from women \geq 18 years with ICC were tested in DDL diagnostic laboratory (Voorburg, The Netherlands) using PCR-SPF10 LiPA25 version 1 methodology that detects 14 oncogenic HPV-types (HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) and 11 low-risk HPV-types (HPV-6, 11, 34, 40, 42, 43, 44, 53, 54, 70 and 74).

Results: HPV DNA was detected in 256 of the 266 (96.2 %) ICC specimens collected. Among the HPV-positive ICC specimens, a single HPV-type was observed in 237/256 (92.6%), with HPV-16 and -18 being the most prevalent types (62.0% and 14.3%, respectively). The HPV-type distribution in women with HPV-positive SCC specimens (205/266; 77.7%) was comparable to the distribution in women with ICC. In ICC specimens with multiple HPV-types, the most frequently reported combinations were HPV-16/18 and HPV-16/45. Ratio ADC vs SCC was 1:5.

Conclusions: Presence of HPV-16 or -18 was detected in 76.3% of a sample of Danish women with ICC. These data are in line with available global data.

*SPF10 HPV LiPA 25, version 1 and SPF10 HPV DEIA are manufactured by Labo Biomedical Products (Rijswijk, The Netherlands) based on licensed INNOGENETICS SPF10 technology.

P-30.38

HUMAN PAPILLOMAVIRUS PREVALENCE IN WOMAN ROUTINE EVALUATION, OURO PRETO, BRAZIL.

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Human papillomavirus (HPV) presents a double-stranded, circular DNA genome, 7.9 Kb, and the genotypes are classified into high, medium and low risk, according to their association with malignant progression. Previous studies showed the presence of HPV genome in several types of cancer, such as in uterine cervix, skin, conjunctive and upper respiratory tract, but there are very rare data about the virus prevalence in the population considered as clinically normal. Several risk factors have been considered, for example tobacco, alcohol consumption and environmental pollutants. Recently, some results associated with tumor development were obtained about bracken fern (Pteridium aquilinum), part of diet of some human populations in Ouro Preto, Brazil and in Japan. In this study we investigate the correlation of HPV infection, cervical cancer development, and environmental cofactors (P. aquilinum bracken fern), investigating the virus incidence in women attended at units of health for routine gynecologic analysis. We developed this study in Ouro Preto, Minas Gerais, Brazil. Cervical and peripheral blood samples were collected: the identification of viral DNA sequences was performed by PCR, RFLP and sequencing, 264 patients. Our preliminary results differ from former reports: 15% (39) were verified as presenting HPV(77% high risk, 21% HPV 16 and 42.1% multiple infection), 57.9% were bracken consumers. Further studies will be performed to evaluate the real bracken influence in the detected HPV incidence. These data support the hypothesis that the prevalence of HPV may differ according to the regions and that population-based studies are important before the vaccine approaches, comparing HPV results of the population in general and those of individuals with neoplastic lesions.

DEVELOPMENT AND APPLICATION OF A LUMINEX-BASED MULTIPLEX HPV GENOTYPING SYSTEM

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Background: An extensive population-based HPV surveillance programme has been initiated in the UK to monitor the impact of the national HPV immunisation programme.

Objectives: To develop a sensitive, high-throughput system for determining the presence of HPV genotypes within a variety of clinical sample types appropriate for the surveillance of target demographic groups, including: liquid-based cytology, vulval-vaginal swabs, penile swabs and urine.

Methods: A single-round multiplex PCR, incorporating a control PCR to assess specimen integrity, was developed to amplify HPV DNA, while Luminex xMAP[™] technology was employed for the resolution of specific HPV genotype(s). The empirical sensitivity was determined using a panel of HPV plasmids representing the 18 confirmed and putative oncogenic HPV types and the 2 HPV types most commonly associated with genital warts. The utility of this system for national surveillance studies was then examined using a range of clinical sample types.

Results: A multiplex HPV PCR was developed that could detect individual HPV genotypes with comparable sensitivity to the 'gold standard' Linear Array HPV Genotyping Test^{**} (Roche Molecular Systems). The system was then successfully validated against a range of clinical sample types commonly used for HPV surveillance studies, including: liquid based cytology samples (n=611), vulval vaginal swabs (176), penile swabs (137) and urine (400). Conclusions

The multiplex HPV PCR-Luminex Genotyping system is a sensitive, high-throughput tool appropriate for large-scale, molecular surveillance studies and the use of urine for such studies is a feasible alternative if more appropriate samples are not available.

P-30.40

CO-INFECTION PREVALENCE OF HPV IN HIGH RISK WOMEN IN SPAIN

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Introduction. The implementation of HPV vaccination justify the diagnoses and identification of these viruses. This study shows the preliminary results on identification of HPV in high risk exposed women, most of them sex workers in Spain. Objectives. Determine the prevalence of co-infections by different types of HPV in high risk exposed women to HPV infection.

Methods and materials. We screened 257 cervical samples from women related with high risk exposition to HPV. Samples were screened by HC2 (Digene Hybrid Capture[®]) which uses two different probes, one detecting HPV related with low risk of carcinogenesis and the other for high risk carcinogenic HPV. Positive samples by HC2 were subsequently analysed by microarrays (Clart HPV Genomica[®]) in order to identify HPV types involved. Nucleic acid automatic extraction was performed by boom method (Easymag BioMerieux[®]).

Results. Eighty two out of the 257 samples screened by HC2 were positive (31.9%) being 92.7% were of high risk and 7.3% of low risk. From those HC2 positive samples, 71 were typified using microarrays being the remainder negatives, 38 identified samples yield only one type of HPV (53.5%) while 33 samples had more than one type of HPV(46.5%). The distribution of HPV co-infections detected was as follows: 15 ouble co-infections, 7 triple co-infections and 11 samples with more than three types of HPV. The most frequent types were 58 (20%), 16 (16%), 70 (16%), 31 (16%), 33 (13%) and 18 (7%).

Conclusions. High prevalence and multiple co-infections of HPV in high risk women has been found according with other European studies. It is remarkable the small proportion of HPV 18. The role of others low risk HPV in co-infections with HPV of high risk in the natural course of the HPV infection needs to be elucidated.

HPV TYPE-DISTRIBUTION ON GENITAL WARTS IN CHINA: A MULTI-CENTER STUDY

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Objective: To determine HPV type-distribution across geographic areas in China, predict preventive impact of prophylactic HPV vaccines against LR-HPV relative diseases.

Methods: 1324 participants were recruited from 11 hospitals in metropolitan area and 8 hospitals in rural area, representing 7 geographic regions of China. 140-bp target fragment in L1 gene was amplified with MY09/11, MY09/GP5+ and GP5+/GP6+ using nested PCR. Amplicon was sequenced on ABI 310 DNA analyzer after separation by electrophoresis and purification by QIAGEN PCR purification kit. HPV types were confirmed by using BLAST 2.0 software server. Regarding multiple infections, target fragments were cloned into T-Vector from Takara. Five single clones of each individual were randomly selected for sequencing separately to ascertain HPV types.

Results: 407 of 1324 subjects were included into the interim analysis. The mean age of subjects was 32.1 years. Prevalence of HPV 6, 11, 16, 58, 59, 66, 67, 7, 81 and 87 were 40.3%, 41.5%, 11.5%, 0.5%, 0.5%, 0.2%, 0.2%, 0.7%, 1.0% and 0.2%. Multiple-infection was 4.7%. HPV 11, 6 and 16 are most three common HPV types in metropolitan (44.0%, 35.8% and 12.7%) and rural area (54.0%, 34.0% and 8.0%).

Conclusion: Potential impact of prophylactic HPV vaccines against low risk HPV type 6 and 11 is estimated to be high (81.8%) in China.

Key Words: Human Papillomavirus, Genital Warts

P-30.42

TOBACCO SMOKING/CHEWING AS RISK FACTORS FOR CERVICAL SQUAMOUS INTRAEPITHELIAL LESIONS.

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Tobacco exposure is a potential environmental cofactor of cervical cancer. In less industrialized countries, exposure to tobacco is more common through chewing than smoking among women. Our objective was to compare the association between tobacco smoking and chewing and the risk of multiple human papillomavirus (HPV) infections and cervical squamous intraepithelial lesions (SILs) in two populations with different tobacco exposure.

We studied 2,162 women from Côte d'Ivoire, West Africa, and 419

women from Finland, Northern Europe, with baseline data on cervical screening, HPV DNA status and smoking and

chewing habits.

The proportion of women who smoked and/or chewed tobacco was higher in Finland (36.8%) than in Co^te d'Ivoire (3.7%), where tobacco chewing (2.6%) was more common than tobacco smoking (1.4%). Having multiple HPV infections was common in HPV16 and/or 18-infected women (60.4% in Finland and 47.2% in Co^te

d'Ivoire). There was no increased risk of multiple HPV

infections among tobacco consumers. We found that women less than 30 years of age exposed to tobacco through smoking in Finland (OR: 2.2, 95% CI: 0.5–8.7) and chewing in Côte d'Ivoire (OR: 5.5, 95% CI: 2.1–14) had a moderately or highly increased risk of high-grade SIL, respectively. In the latter, the risk was statistically significant.

Our findings emphasize the need for health initiatives

targeted to prevent tobacco smoking or chewing among

women especially in less industrialized countries.

DISTRIBUTION OF HUMAN PAPILLOMAVIRUS TYPES IN CYTOLOGICAL NORMAL HONDURAN WOMEN

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BACKGROUND: The number of women infected with human papillomavirus (HPV) varies greatly across populations, as might the distribution of HPV types. Previous studies have shown a high prevalence of HPV infections in the general population in Honduras.

OBJECTIVES: We set out to estimate the age and genotype-specific prevalence of cervical human papillomavirus DNA in women with normal cervical cytology in a representative sample of the female population in Tegucigalpa, Honduras.

METHODS: A longitudinal study based on an age-stratified random sample of 500 women with normal cytological diagnoses attending screening clinics was carried out in 2008. A standardized protocol was used for cervical specimen collection and for identification of possible risk factors for infection. A 65-bp region of the HPV L1 gene was targeted for PCR amplification by using SPF10 primers, and 25 genotypes were detected by reverse-line blot hybridization of the amplicons.

RESULTS: Only 145 women aged 15-70 years without cytological abnormalities were included in this analysis. The overall HPV prevalence in this group of women was 27%. HPV prevalence was highest in women younger than 35 years of age. A second peak of HPV prevalence was observed in women aged 45 to 49 years of age. Cancer-associated HPV types were the most common in all age groups. Besides HPV types 16, 18, 39 and 45, types 51 and 52 were important contributors to the overall prevalence.

CONCLUSIONS: Our data indicate that the burden of prevalent HPV infection among females with normal cytology in Honduras is relatively high. Data on age-specific prevalence of human papillomavirus (HPV) infection in the Central American region are essential for the future implementation of HPV prophylactic vaccines for cervical cancer prevention.

P-30.44

ONCOGENIC HPV TYPES IN CERVICAL PRE-CANCER/CANCER IN CANADIAN WOMEN

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Background: Information on the prevalence of oncogenic HPV types in cervical pre-cancer/cancer is important for estimating the protective efficacy of HPV vaccines. Canada has a nationally sponsored school-based HPV immunization program. However, there is very little information on the prevalence of oncogenic types in cervical pre-cancer/cancer for Canadian women.

Objective: The prevalence of oncogenic HPV types in women with cervical pre-cancer/cancer was determined. Methods: The study population was comprised of colposcopy referral cases from 5 provinces. Colposcopy/histology was done as per routine practice. The LinearArray* test (Roche) was used for genotyping.

Results: Of 2565 cases genotyped, 610 tested HPV negative. Of the HPV+ cases, 1384 showed either no colposcopic/ histologic evidence of pre-cancers or only had CIN1. In this population, genotype 16 alone or in association with other oncogenic types (referred to as "16+") was detected in 27.4%, types "18+" and "31+" in ~ 7.5% each, with the other oncogenic types in smaller proportions. The remaining 571 HPV+ cases were diagnosed with \geq CIN2, with the prevalence of oncogenic types as follows: Genotype "16+" was detected in 62.3%, and genotype "18+" with the exception of type 16 in 7.2%. Genotype 31 was found as a monotype more frequently than type 18, and appeared to be the second most common if the proportion with type 18 in association with 16 was excluded. The other significant genotypes were 33, 39, 51, and 52, with each of these detected at ~ 3%.

Conclusions: This is the first major Canadian study to provide data on the prevalence of oncogenic HPV types in Canadian women with cervical pre-cancer/cancer. This data together with the known cross-protection of Gardasil vaccine provides strong evidence that the current Canadian HPV immunization strategy using this vaccine should reduce the future risk of cervical cancer in Canada by at least 70%.

HPV TESTING, II

SESSION 31

PROGRAMME

THE 25TH INTERNATIONAL PAPILLOMAVIRUS CONFERENCE MAY 8-14 2009, MALMÖ, SWEDEN

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11.40-11.50	O-31.05	TRIAGE OF ASC-US AND LSIL CYTOLOGY CASES BY P16 IMMUNOCYTOCHEMISTRY C BERGERON, D KARIN, K PETRA, T MARCUS, R RUDIGER
11.50-12.00	O-31.06	THE USE OF AN RNA-BASED HPV-TEST IN HPV TRIAGING SW Sørbye, S Fismen, T Gutteberg, ES Mortensen
12.00-12.10	O-31.07	RESULTS OF THE FRENCH APTIMA HPV SCREENING EVALUATION (FASE STUDY) J Monsonego, P Halfon, L Zerat, M Ricart, F Ruiz, k Syrjänen
12.10-12.20	O-31.08	A NOVEL BROAD-SPECTRUM PCR-LUMINEX ASSAY FOR IDENTIFICATION OF WART HPV M de Koning, J ter Schegget, M Kamp, B Kleter, Mcw Feltkamp, J Eekhof, JN Bouwes Bavinck, K Purdie, C Harwood, W Quint
12.20-12.30	O-31.09	HPV MRNA AND P16INK4A AS TRIAGE MARKERS IN CERVICAL CYTOLOGY M Reuschenbach, A Clad, C von Knebel Doeberitz, J Rahmsdorf, R Grote, L Wu, N Wentzensen, M von Knebel Doeberitz

O-31.01 THE WHO HPVLABNET INTERNATIONAL PROFICIENCY STUDY OF HPVDNA TYPING METHODS

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and the WHO HPV Laboratory Network

Background: Accurate and internationally comparable HPV DNA detection and typing methodology is essential both for evaluation of HPV vaccines and for effective monitoring and implementation of HPV vaccination programs. Therefore, the WHO HPV LabNet launched an international proficiency study of HPV DNA genotyping assays. Following announcement at the WHO HPV LabNet website, the responding laboratories were asked to perform HPV typing using one or more of their usual assays on 43 challenge samples composed of purified whole genomic plasmids of sixteen HPV types (HPV6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) in a background of human cellular DNA.

Proficient typing required detection in both single and multiple infections of 50 International Units of HPV 16 and HPV 18 DNA/ 5 l and 500 genome equivalents in 5 l for the other types, with at least 97% specificity.

Results: Fiftyone laboratories worldwide participated, with good regional representation and broad expertise. As several laboratories used >1 HPV typing method, a total of 82 data sets were received. Fourteen major HPV genotyping assays were used, with the Roche Linear array being the most commonly used method. Other major assays used were Lineblot/ Inno-LiPa, Clinical Array, type specific real time PCR, PCR-Luminex and different micro-array assays. For the most commonly used assays, analysis of assay robustness was possible. There were also several assays that demonstrated very high quality results but were used in only one or few laboratories. Altogether 53 data sets were considered as proficient typing. Performance of individual assays and assay groups will be presented. Global proficiency studies will be an important instrument towards establishing comparable and reliable HPV genotyping assay performance in worldwide labs.

O-31.02

NATIONAL QUALITY ASSURANCE PROGRAM FOR HPV-TESTING AND TYPING IN ITALY

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Background: Diagnosis of HPV infection is based on identification of viral DNA in clinical samples, which means that accurate diagnostic methods are requested. In Italy several studies for evaluation of HPV testing as primary screening test and epidemiological studies are in progress. If on one side introduction of HPV testing can increase efficiency of screening programs for early identification of pre-neoplastic and neoplastic lesions, from the other introduction of new diagnostic methods requires the standardization of the procedures.

Objective: Identification of HPV is complicated by: homologies between the viruses that infect the genital tract; differences between identification and genotyping methods; sampling and conservation of specimens. Aim of this study is to optimize a quality control program that should take all these criticisms in consideration.

Methods: All centers received from ISPO both synthetic samples at known concentration to evaluate the method' sensitivity and clinical samples to evaluate also the extraction method. Participants perform the analysis, then send results to reference center, that provide for statistical analysis and for a report with the performance score of each laboratory respect to the others.

Results and conclusions: 7 synthetic samples and 60 clinical samples were analyzed. Seven laboratories were involved in the HCII QC program; results indicate a high accuracy not also in terms of positive/negative result but also in semiquantitative results. The results of the four laboratory involved in the typing QC are more variable: 14 samples were perfectly concordant, for eight samples there was a concordance between three, while five were discordant.

This study allowed to evaluate feasibility of a QC program for HPV testing, compliance of laboratories, possibility of the use of standardized control samples, definition of the best procedures, efficiency of the whole analytical protocol, evaluation of sensibility and specificity of methods.

O-31.03

TRANSCRIPTOME PROFILING OF CERVICAL SWABS FROM PERSISTENTLY HPV16 INFECTED WOMEN

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Background: Persistent infections with carcinogenic HPV types 16 and 18 are a necessary precondition but not sufficient for cervical cancer. Using cervical swabs from a Danish cohort of younger women established in 1991-1993, persistently HPV16-infected women were identified by the LiPa genotyping assay. Within a median follow-up time of 11.1 years, 45% of the HPV16-persistently infected women developed moderate dysplasia, severe dysplasia, CIS or cancer.

Objectives: To identify potential markers for persistent HPV16 infections and predictive markers for progression, RNA from cervical swabs of these women and controls was isolated and subjected to transcriptome analyses.

Methods: RNA was isolated by a modified Qiagen RNAeasy extraction protocol. The cellular transcriptiome was determined with Affymetrix U133A 2.0 microarrays and then bioinformatically analysed. Differential expression of cellular and viral genes was confirmed by quantitative real-time PCR.

Results: Transcriptome analyses revealed statistically highly significant differences between HPV-negative women and women persistently infected with HPV16. Also within the group of women with persistent HPV16 infection, significant differences were observed between non-progressors and women who developed > CIN2 during follow-up. For validation by qRT-PCR differentially expressed genes were selected by statistical significance and by the extent of deregulation. Differences could be confirmed by qRT-PCR for 5 cellular genes between HPV-negative and HPV16-persisters and for 3 cellular genes between non-progressors and progressors to >CIN2 that have not been described before. Currently, expression of viral transcript amounts and of p16/INK4A is analysed and will be presented.

Conclusions: We could identify putative novel persistence markers and novel markers predictive of subsequent progression to CIN2 or worse among women with persistent HPV16 infection.

O-31.04

KALLICREIN-7 AND SUPEROXIDE DISMUTASE-2 AS PROGRESSION MARKERS OF CERVICAL DISEASE

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Background: The screening of differentially expressed proteins between normal cervical tissue, precursors lesions and cervical cancer, could contribute to the identification of lesions with higher probability to progress to malignancy. In the present study, we have evaluated hK7 (kallikrein 7) and SOD2 (superoxide dismutase 2) protein levels in cervix histological samples. Kallikrein 7 mediates the proteolytic degradation of cohesive intracellular structures associated with epithelial differentiation. Recent data also suggest that this protein may be causally involved in carcinogenesis, particularly in tumor metastasis and invasion, and, thus, may represent an attractive drug target to consider for therapeutic intervention. The superoxide dismutase 2 (SOD2) belongs to a family of enzymes involved in the conversion of superoxide radicals in molecular oxygen. Numerous in vivo studies have shown that the superoxide dismutases can be highly expressed in aggressive human solid tumors.

Methods: 367 cervical samples including 35 cases of benign cervix, 31 low-grade cervical intraepithelial neoplasia (CIN; CIN1), 51 high-grade CIN (CIN 2/3), 197 squamous cervical carcinomas (SCC) and 53 cervical adenocarcinomas, were analyzed by immunohistochemistry (IHC).

Results: Samples were grouped depending on the percentage of stained cells as weak or moderate (<50% of stained cells) and intense (>50% of stained cells). We observed an increase in the number of cells hK7 and SOD2 positive as a function of disease progression. While 15.2% and 23.1% of inflammatory/low-grade samples presented intense staining for these proteins, 92.5% and 65.4% of adenocarcinomas presented this staining pattern for hK7 and SOD2, respectively. Moreover, 68% and 39.6% of squamous cell carcinomas samples, exhibited more than 50% of positive cell staining for hK7 and SOD2, respectively.

Conclusions: Differences in expression patterns of these proteins could potentially be used in the characterization of lesions with higher risk to progress to malignancy.

O-31.05

TRIAGE OF ASC-US AND LSIL CYTOLOGY CASES BY P16 IMMUNOCYTOCHEMISTRY

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Background: Detection of over-expression of cell-cycle regulator protein p16 in cervical cytology specimens has been suggested as a surrogate marker for transforming HPV infections.

Objectives: A large retrospective pan-European study was performed to assess the performance characteristics of p16 immuno-staining in cervical cytology specimens categorized as ASC-US or LSIL to identify women with underlying CIN2+.

Methods: 810 LBC samples categorized as ASC-US (n=385) or LSIL (n=425) were retrieved from the archives of 5 cytopathology laboratories in Europe. Availability of histology follow-up data and access to corresponding tissue blocks was a selection criterion for study cases. Leftover material was used for p16-staining (CINtec Cytology Kit, mtm) and independently reviewed by two cytopathologists and a team of cytotechnologists. Presence of cells immuno-reactive for p16 and showing morphologic abnormalities generated a positive test result. HPV testing with Hybrid Capture 2 (Qiagen) was performed on all specimens. Consensus diagnoses of expert gynecopathologists on histology follow-up specimens served as Gold standard.

Results: For p16 immuno-cytochemistry, sensitivity of adjudicated cytopathologists results for the identification of women with underlying CIN2+ (n=222) reached 95% for ASC-US and 96% for LSIL cases. Specificity of cytopathologists slide reviews ranged from 66 to 71% for the ASC-US group, and from 47 to 53% for LSIL cases. For independent cytotechnologists ' slide reviews, sensitivity for CIN2+ was found at similar rates (ASC-US: 93%; LSIL: 92%), and specificity was 63% for ASC-US and 37% for LSIL cases, respectively. HPV testing resulted in sensitivity of 90% (ASC-US) and 96% (LSIL), and specificity of 38% (ASC-US) and 19% (LSIL).

Conclusions: Interpretation of p16 cytology slides has the potential to provide a comparable sensitivity, but significantly higher specificity for the identification of high-grade CIN as HPV testing in the triage of women with Pap cytology results categorized as ASC-US or LSIL.

O-31.06

THE USE OF AN RNA-BASED HPV-TEST IN HPV TRIAGING

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Objectives. Tests for detection of human papillomavirus (HPV) are used as confirmatory tests in the Norwegian Cervical Cancer Screening Programme in cases where cytology is uncertain or show low grade changes. We present our findings from the University Hospital of Northern Norway (UNN).

Methods. Our study comprises samples from 1295 women which cytology examinations including HPV-RNA tests were performed from 2006 and 2007. The tests were done according to the guidelines of the Cancer Registry of Norway. We detected HPV-mRNA with a test that detects 5 high-risk genotypes (16, 18, 31, 33 and 45). The results of the HPV-mRNA tests were compared to cytology and later biopsy findings up to April 2008.

Results. Forty nine percent (49%) of the women with a positive HPV-mRNA test had CIN2+ (CIN2, CIN3 or carcinoma). The sensitivity to detect CIN2+ was 86%. The negative predictive value was 0.98. In our material CIN2+ was detected in biopsies from 2% of the HPV-RNA negative cases.

Conclusions. Due to higher specificity HPV-mRNA test is more suitable than HPV-DNA test as a confirmatory test in uncertain and low grade cytology changes.

O-31.07 RESULTS OF THE FRENCH APTIMA HPV SCREENING EVALUATION (FASE STUDY)

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Background: The APTIMA HPV assay (AHPV, Gen-Probe Incorporated) detects E6/E7 mRNA from 14 high-risk types. The FASE study evaluated the use of the AHPV assay in France undergoing screening for cervical cancer.

Objectives: To assess the performance of the AHPV assay in comparison with the HC2 assay (Qiagen) for detection of HR HPV and detection of high-grade CIN in conjunction with liquid-based cytology (LBC).

Methods: This was a regional, cross-sectional cervical cancer screening study of 5000 women (age 20-65 years) for detection of CIN. Women cytologically abnormal or positive for either HPV test were sent for colposcopic evaluation. Biopsy specimens were taken from women with abnormal colposcopy or normal colposcopy following a positive screening test.

Results: Fewer specimens tested positive with the AHPV assay compared with the HC2 especially in women with less than CIN1 where there were almost twice as many HC2 positives compared to AHPV. Clinical performance results from 1528 women to date show that in a disease positive population (CIN2+), the sensitivity of the AHPV assay was 87.4% compared to HC2 sensitivity of 95.8%. The clinical specificity of the AHPV assay was significantly higher than the HC2 assay at 72.4% compared to 52.4% in the HC2 assay. Sensitivity in a CIN3 population was 100% for both assays, specificity was 69.1% for AHPV and 49.7% for HC2.

Conclusions: The results to date show that the AHPV assay had statistically equivalent sensitivity, but higher specificity than the HC2 assay for detection of CIN2+. The AHPV assay may provide improved clinical utility in comparison to HPV DNA testing.

O-31.08

A NOVEL BROAD-SPECTRUM PCR-LUMINEX ASSAY FOR IDENTIFICATION OF WART HPV

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Background A large number of HPV types, distributed over five papillomavirus genera, infect the skin. HPV types belonging to three of those (alpha, gamma and mu genus) have frequently been detected in cutaneous warts. A state-of-the-art HPV genotyping assay for these cutaneous wart-associated HPV types however does not exist, although warts constitue a highly prevalent skin condition especially in children (20-50%) and organ transplant recipients (44-49%).

Objectives To develop and evaluate a reverse hybridization genotyping assay, thereby extending the coverage of skin HPV typing systems with all known wart-related HPV types.

Methods A PCR was used to amplify a 76-84 bp fragment from the L1 ORF. Subsequent identification was carried out in a Luminex based genotyping assay.

Results and Conclusions The PCR was shown to efficiently amplify DNA of all known wart-associated HPV types from the alpha- (HPV2, 3, 7, 10, 27, 28, 29, 40, 43, 57, 77, 91 and 94), gamma- (4, 65, 95, 48, 50, 60 and 88), mu- (HPV1 and 63) and nu-genus (HPV41). Specific probes were designed for these HPV types and evaluated on plasmid HPV DNA, as well as on a panel of wart biopsies previously characterised by PCR and sequencing. The skin wart HPV Luminex based typing system identified all known wart HPV types with a high analytical specificity. Results of testing a series of 40 materials derived from patients suffering from (multiple) warts revealed the presence of HPV27 (35%), HPV1 (22.5%), HPV2 (20%), HPV57 (12.5%), HPV7 and HPV3 (both 2.5%). Two samples tested HPV negative.

In conclusion this Luminex based genotyping system amplifies all known wart HPV types including related types, is highly specific and suitable for large scale epidemiological studies.

O-31.09

HPV MRNA AND P16INK4A AS TRIAGE MARKERS IN CERVICAL CYTOLOGY

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Background: Cervical cancer screening is hampered by the low sensitivity of Pap cytology for the detection of cervical precancer. While HR-HPV DNA detection has a higher sensitivity to detect precancer, it cannot discriminate between transient and transforming HPV infections and therefore has limited specificity. Therefore, research on novel biomarkers is warranted to improve current screening algorithms.

Objectives: We evaluated two novel biomarkers for transforming HPV infections, HPV E6/E7 mRNA detection and p16 immunocytology in patients with abnormal cervical cytology.

Methods: 274 liquid based cytology specimens (PreservCyt, Cytyc) were collected from patients referred to colposcopy due to abnormal cytology and from patients during follow-up after previous treatment for cervical dysplasia. The APTIMA HPV E6/E7 mRNA test (Gen-Probe) was performed and Thinprep slides were stained with the CINtec p16 cytology test (mtm Laboratories). p16 staining was assessed according to a previously described nuclear scoring algorithm. In addition HR-HPV DNA was detected by the Hybrid Capture 2 assay (HC2) (Qiagen) from the same PreservCyt vial. 36.1% of the patients had CIN3 or worse in a punch biopsy or cone tissue, 24.4% had CIN2, 12.8% CIN1, and the remaining were disease negative. We compared assay performance to detect histology proven CIN2 or greater (CIN2+) and CIN3 or greater (CIN3+).

Results: All assays yielded a comparable sensitivity for the detection of CIN3+ (96.0% for APTIMA, 96.7% for CINtec, 95.9% for HC2). Clinical specificity for CIN3+ was 51.4% for APTIMA, 57.4% for CINtec and 41.0% for HC2. Sensitivity/ specificity for the detection of CIN2+ was 89.2%/70.4% for APTIMA, 85.1%/74.2% for CINtec and 93.0%/58.1% for HC2.

Conclusions: These results confirm the substantially improved specificity / sensitivity profile of CINtec p16 cytology test in comparison to the HC2 test, whereas detection of E6/E7 mRNA with the APTIMA test displayed an intermediate specificity / sensitivity profile.

POSTER ABSTRACTS SESSION 31

POSTER SESSION V THURSDAY 10.00

FEASIBILITY OF THE ABBOTT HRHPV ASSAY ON THREE LBC MEDIA

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Background: High risk (HR) HPV DNA testing is recommended for triage after an ASC-US cytology result. Objective: To evaluate the feasibility of the Abbott RealTime HRHPV assay (HRHPV) for detection of HPV16, HPV18 and other 12 HR HPV in cervical samples taken on three liquid-based cytology (LBC) media commonly used in France. Methods: A total of 146 cervical samples with an ASC-US cytology result, previously tested with Hybrid-Capture 2 (HC2) (Qiagen), were tested with HRHPV assay; 50 EasyFix (Labonord), 50 CytoScreen (Seroa) and 46 SurePath (Tripath). Sample preparation for HC2 was performed as described previously. HRHPV was performed using the automated Abbott m2000sp instrument by loading samples directly. Discrepant samples were tested with the Linear Array (LA) using extracted DNA from Abbott m2000sp.

Results: All samples gave a positive result for internal control. Sixty-nine samples were positive with HRHPV; 9 with HPV16, 11 with HPV16+other, 1 with HPV18+other, 48 with other. Overall correlation between HRHPV and HC2 was 87%. None of the 58 HC2 negative samples tested positive with HRHPV. Nineteen samples (13%) were positive with HC2 and negative with HRHPV. Among these, 6 were negative with the LA assay, 9 contained low risk and/or undetermined risk HPV, 4 were positive for HR HPV DNA by LA. Three of these 4 were amplified with HRHPV but beyond cutoff.

Conclusion: The HRHPV assay demonstrated its feasibility on CytoScreen, EasyFix and Surepath media. The absence of detection of low risk HPV DNA detected by HC2 may indicate a better analytical specificity of HRHPV for detecting HR HPV DNA. Further correlations studies are needed on unselected ASC-US samples taken on various LBC media to evaluate the performances of the HRHPV versus HC2 and other tests for predicting CIN2 +.

P-31.11

HPV16 VARIABILITY IN CROATIA ASSESSED BY HIGH RESOLUTION MELTING (HRM)

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Background: Viral genome variants, diverging by 2% within a viral type, contribute to the carcinogenic potential of HPV types presumably altering its transforming properties and/or immunogenicity. Most of the studies on HPV16 variability were concerned with E6 and E7 regions that encode for well known HPV oncogenes. However, studies of viral variants are very laborious and expensive as several genomic regions of each sample need to be sequenced to detect the few differing nucleotides that identify variants.

Objectives: To simplify and reduce the costs of variant identification we investigated the possible application of HRM analysis to determine HPV16 variants of the E6/E7 region.

Methods: The HRM analysis enables the distinction of PCR amplicons differing in even a sole nucleotide, and as such should be capable of easily indicate HPV variants. We have previously confirmed this assumption on a subset of samples. In this study, the performance of optimised HRM analysis was evaluated on 230 HPV16 positive cervical samples. The E6 region of HPV16 was amplified by a nested PCR and then melted. Samples giving an ambiguous result were additionally remelted in the presence of wild type HPV16 DNA to confirm the melting result. To evaluate the HRM method performance, a subset of samples was sequenced.

Results: In about half of Croatian women, the European German variant as indicated by the presence of E6-350G mutation was found. In addition, it appears that no other variants are common.

Conclusions: Our results indicate that HRM approach can indeed be informative, while saving time and significant cost of sequencing of each sample and it is interesting in low resource setting or where cost efficiency is the issue.

P-31.12 MOLECULAR VARIANTS OF HPV TYPE 16 E6 AMONG HONDURAN WOMEN

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BACKGROUND: Previous studies have suggested that variants of HPV-16 may show varying degrees of association with cervical neoplasia. Although these variants differ in prevalence, biological and biochemical properties, their implication in the etiology of cervical cancer is still uncertain.

OBJECTIVE: To investigate the prevalence of HPV 16 variants in Honduran women with normal cytology and with dysplasia or cervical cancer.

METHODS: As part of a larger longitudinal study, we analyzed genomic sequences of HPV 16 among 106 HPV 16 positive women, by analysis of the E6/E7 region by reverse hybridization.

RESULTS: The E-350G variant was the most prevalent variant in all different disease stages being present in 18% of cervical cancer, 13% of cervical intraepithelial neoplasia III, 5% of cervical intraepithelial neoplasias II, 5% of cervical intraepithelial neoplasia grade I, and 20% of control samples.

The highest number of cases was seen in women less than 30 years with 32 cases of E-350G variant.

CONCLUSIONS: We found that most infections in all clinical groups belong to the European variants, suggesting that HPV 16 non European variants do not represent an additional factor associated with increased occurrence of high grade cervical lesions in the studied population, as European and non-European variants appeared in equal frequencies among the histological types of lesions.

This study shows the diversity of HPV-16 variants in cervical samples of Honduran women.

P-31.14

HPV L1-CAPSID PROTEIN DETECTION AND PROGRESSION OF ANAL SQUAMOUS NEOPLASIA

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BACKGROUND: In the development of cervical intraepithelial lesions to invasive cancer, there is a reduction in HPV L1-capsid antigen (L1) expression, which may serve as a predictor of progression. Similar to cervical neoplasia, HPVassociated oncogenesis has been implicated in the development of anal squamous cell cancers (SCC). OBJECTIVES: We have sought to investigate the expression of HPV L1 across the spectrum of anal squamous neoplasia including normal mucosa (NM), SCC-in-situ (SCC-IS), SCC, local recurrences (LR) and metastases. METHODS: Analyzed tissues included 11 NM, 26 SCC-IS, 18 SCC, 7 LR and 6 metastases (4 lymph node, 2 distant) derived from 36 patients. HPV L1 expression was identified by immunohistochemistry using a monoclonal antibody (Thermo) on formalin-fixed paraffin-embedded sections. Statistical comparisons were performed using the Fisher Exact Test. RESULTS: Our population was comprised of 17 men and 19 women with a median age of 48 years (range 26-81). Eight patients were immunocompromised (5 HIVpositive, 3 transplants). HPV L1-positivity is characterized by nuclear staining and was identified in 38% (10/26) of SCC-IS, however there was no detection in NM, SCC, LR and metastases. Cytoplasmic staining was identified in 9% NM, 4% SCC-IS, 22% SCC, 29% LR and 17% metastases. Of those SCC-IS associated with a concomitant invasive SCC, only 15% (2/13) demonstrated nuclear L1 expression as compared to 62% (8/13) of isolated SCC-IS (p=0.02). CONCLUSIONS: The expression of L1-capsid antigen appears to be an important early marker in the development of anal squamous neoplasia. As in cervical cancer, nuclear expression of L1 protein is lost in the progression from SCC-IS to SCC, which may be an indicator of viral integration. A detailed examination of HPV genotyping and integration status will be presented. Loss of L1 antigen expression in SCC-IS may represent a possible prognostic marker of enhanced malignant potential.

P-31.15 EVALUATION OF THE APTIMA CERVICAL SPECIMEN COLLECTION AND TRANSPORT KIT

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Background: We have developed a Cervical Specimen Collection and Transport (CSCT) Kit for use with the APTIMA HPV Assay (APTIMA) as an alternative to using the PreservCyt liquid-based cytology (LBC) specimens. The APTIMA Assay is a CE marked test that detects E6/E7 mRNA of 14 high risk HPV types.

Objective: Determine the clinical performance of the APTIMA CSCT kit.

Methods: The CSCT kit consists of a cervical cleaning swab, a collection brush and a specimen collection tube. A total of 267 cervical specimens were collected from women (19 years of age or older) referred for colposcopy. For each subject enrolled in the study, a CSCT sample was collected followed by collection of a LBC sample. The LBC sample was processed for cytology and an aliquot of the residual LBC solution was diluted into an APTIMA Specimen Transport tube. A single replicate of both the CSCT and diluted LBC sample were tested with the APTIMA Assay according to Package Insert instructions. Concordance between the CSCT and LBC results was determined overall and by cytology category.

Results: The overall, positive and negative agreement between the CSCT and LBC samples was 94%, 98.2%, and 86.4%, respectively. For patients with a cytology diagnosis of ³ ASC, the percent positive agreement for the paired CSCT and LBC samples was 100% and the percent negative agreement was 81%. For patients with cytology diagnosis of LSIL and HSIL, there was 100% positive and negative agreement between the paired CSCT and LBC samples.

Conclusion: APTIMA Assay results showed good agreement between cervical specimens collected with the CSCT kit and LBC indicating the CSCT kit is suitable for E6/E7 mRNA testing of direct cervical samples. The use of the CSCT kit provides a cost effective alternative for molecular detection of HPV.

P-31.16

HPV MRNA GENOTYPE DISTRIBUTION IN CERVICAL CANCER IN SOUTH AFRICA

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Background: Slight variations in HPV genotype prevalence between continents and countries have been observed. The mapping of genotype prevalence in different geographical areas is important when designing HPV tests.

Objectives: To determine HPV genotype distribution in cervical cancer in South Africa and to compare HPV DNA and E6/E7 mRNA analysis for the detection of cervical cancer

Methods: 88 women with cervical carcinoma from Pretoria Academic Hospital. Cervical specimens were collected in PreTect TM (NorChip) together with biopsies prior to treatment. HPV DNA was detected with G5+/6+ PCR followed by Reverse Line Blot analysis. E6/E7 mRNA from the five HPV types 16,18,31,33 and 45 was detected with the NASBA based PreTect HPV-Proofer (NorChip). In addition E6/E7 mRNA from the HPV types 35,52 and 58 was detected using NASBA.

Results: HPV was detected in 83/88(94%) of the cases. Type 16 was the most common (42%) followed by 18(18,2%) and 35(13,6%). Other types included high-risk types 45(8%), 33(4,5%), 52(3,4%) and 31(2,3%), and the types 69(2,3%), 73(1,4%) and 30(1,4%). In seven cases a multiple infection was found. Of the HPV positive cases, 95% was detected with the mRNA testing, targeting eight HPV types and 97,5% was detected with DNA testing targeting 39 HPV types.

Conclusion: In South Africa HPV type 35 seems to be relatively frequent compared to other geographical regions. This highlights the importance of mapping the prevalence of the different HPV types in the various countries when designing HPV tests. Single infections with the "non-defined risk types" 30, 69 and 73 may suggest a role for these types in carcinogenesis, however, other non-virus related aberrations may be present. E6/E7 mRNA detection with eight HPV types had a sensitivity equal to that of a DNA test covering 39 types; a high sensitivity can be maintained while achieving a high specificity with mRNA testing

P-31.17 RESULTS FROM STRIPSCAN, GENOTYPING SOFTWARE FOR HPV LINEAR ARRAY

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HPV Linear Array and its predecessor, HPV Line Blot assay, have been used in more than 80 studies of cervical disease and cancer. Although technically simple, the HPV Linear Array assay involves multiple manual steps. For laboratories genotyping large numbers of samples this can prove challenging. Roche StripScan is internal research software for acquiring and processing images of strips using an office type scanner. The software measures the intensities of the probe regions on the strips, and makes initial probe call determinations that are reviewed and accepted by expert users. The results and associated plate data are exportable for downstream processing.

To describe the implementation and testing of HPV StripScan software for capture and interpretation of HPV Linear Array results.

We compared genotype results read manually and adjudicated by a second manual read to genotype results from StripScan. Two commercial scanners (Epson and HP) were used to record images of the strips. Discordant positive and negative results were analyzed for both scanners to establish optimal threshold for a positive result and determine if scanner adjustments changed the results.

1080 HPV Linear Array results from clinical samples were assayed manually and with StripScan. The rate of discordance from the manual reads (both negative and positive)was calculated and an optimized threshold determined to minimize discrepancies. Using the optimized threshold the rate of false negatives with StripScan was 1.3% for HP,1.5% for Epson. There were two samples that had incorrect manual interpretation because of mis-entry of sample number or similar. This software may provide a useful tool for interpretation of HPV Linear Array results and could significantly reduce workload for high-throughput labs.

P-31.18 HPV L1 IMMUNOCYTOCHEMISTRY STAINING IN LSIL AND ASCUS CYTOLOGY

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Background: The immunostaining of HPV L1 capsid protein could be used as indictor of HPV integration and prognosis factor for cervical disease.

Objective: The purpose of this study is to evaluate the prediction ability of human papillomavirus (HPV) L1 capsid protein in low-grade squamous intraepithelial lesion (LSIL) and atypical squamous cells of undetermined significance (ASCUS) cytology with HPV 16 infection in Korean women.

Method: From 2006 to 2007, Pap smears from 69 women in whom LSIL and ASCUS with HPV 16 infection had been diagnosed by cytology and HPV DNA chip. The slides of Pap smear at first visit were immunocytochemically stained for the HPV L1 protein. The study objects had undergone at least 2 times Pap smear tests with interval of 4~6 months or been followed longer than 1 year without treatment. We had examined Pap smear at every visit and assessed progression or regression of Pap smear during follow up. We compared the results of immunocytochemistry staining with a series of Pap smear.

Results: HPV L1-positive women showed regression in 59.1% of cases and HPV L1-negative women showed progression in 21.3% of cases.

Conclusion: HPV L1-positivity and negativity could be used to predict the progression or regression of LSIL and ASCUS of cervix.

EPIGENETIC MODIFICATIONS IN DAPK AND TIMP-3 GENE PROMOTERS IN HPV-SILS

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Background. Gene promoter hypermethylation is one of the mechanisms responsible for loss of expression in critical genes and the most common epigenetic mechanism leading to carcinogenesis.

Objectives. The goal of this study was to examine the methylation status of CpG islands in TIMP-3 and DAPK gene promoters in HPV infected cervical intraepithelial neoplastic (CIN) lesions. Most of the samples examined were HPV 16, a high-risk type or HPV53/66, two types with debatable neoplastic potential. Another goal was to correlate disease and methylation status with MTHFR genotyping (for C677T mutation).

Methods. DNA from 67 cervicovaginal samples with precancerous lesions and HPV and 20 samples negative for HPV and precancerous lesions was extracted (QIAGEN, Germany) and further subjected to sodium bisulfite conversion (Zymo Research, USA). Methylation-specific PCR (MSP) was employed to detect DAPK and TIMP-3 gene promoter methylation. Finally, PCR-RFLP was performed for the MTHFR genotyping.

Results-Conclusions. According to our results, TIMP-3 promoter was methylated in 21/67 (31.3%) of HPV samples and DAPK promoter was methylated in 42/67 (63%). In the negative samples, no aberrant methylation was detected. MTHFR genotyping showed 12 homozygotes, 24 heterogygotes and 31 wild-type patients. No statistical significant correlation was found between DAPK and TIMP-3 promoter methylation and age, HPV type (low or high-risk) and severity of precancerous lesions. A negative correlation (p=0.006) found between TIMP-3 methylation and HPV types 53 and 66 is a strong evidence for these two being low-risk. MTHFR homozygosity showed correlation with precancerous lesions and HPV, since higher numbers of homozygotes were found in patients with precancerous lesions and HPV than in healthy unselected population (17.9% compared to 8.1%, p=0.015, OR=2.47, CI=1.06-5.74).

P-31.20

CROSS-REACTIVE MONOCLONAL ANTIBODIES TO HPV-E6 PROTEINS BY CONSENSUS PEPTIDE APPROACH.

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Background: Human Papillomavirus (HPV) has been identified as a causative agent of cervical cancer, a disease that is associated with 233,000 deaths annually. The E6 protein expressed by oncogenic HPV subtypes has been shown to target the tumor suppressor protein p53 for ubiquitin mediated degradation. Loss of p53 mediated apoptosis can lead to dysregulated cell growth and subsequent tumor formation. Direct detection of multiple oncogenic E6 proteins may facilitate identification of patients at high-risk for cervical cancer progression with more specificity than nucleic acid tests.

Objectives: To establish and characterize monoclonal antibodies cross-reactive to multiple oncogenic HPV E6 subtypes using synthetic peptides corresponding to regions of high amino acid homology.

Methods: Alignment of oncogenic HPV E6 amino acid sequences was used to identify a region of high homology from which two peptides were chosen for synthesis. Consensus peptides were immunized into mice and hybridomas were established using a rapid RIMMS (repetitive immunizations multiple sites) protocol. Cultures positive in ELISA were cloned by limiting dilution and the antibodies were purified by protein-A chromatography. Monoclonal antibodies were further characterized by Western blot, sandwich ELISA, and PDZ binding assay using recombinant proteins and cell lysates.

Results: Five monoclonal antibodies were characterized that displayed a wide variety of cross-reactivity profiles when tested in ELISA and Western blot. One antibody strongly bound 6 different oncogenic subtypes including 16 and 18 in direct ELISA, but did not bind non-oncogenic 6b or 11 E6. Antibodies were also able to bind multiple E6 subtypes captured by a PDZ binding protein in ELISA. HPV 16E6 was detected from SiHa cell lysates using consensus peptide capture antibodies with HPV 16E6 specific detector antibodies in sandwich ELISA.

Conclusions: Using consensus peptides as immunogens resulted in novel monoclonal antibodies capable of binding a variety of E6 subtypes in diagnostically relevant formats.

ANTIBODIES AGAINST E4 AS MARKERS OF LOW-GRADE CERVICAL LESION.

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Background. Cervical persistent infection by oncogenic Human Papillomaviruses (HPV) may cause lesions and Cervical Cancer (CC). Pap smear and colposcopy detect late stages of the disease and there is a need to develop tests for early diagnosis. Viral DNA shows infection, but not viral activity. Though, detection of antibodies to early viral proteins sequentially expressed during the viral cycle may be useful to identify exposure, course of the infection and disease progression. Also, a specific immunoglobulin-type profile may be detected in each stage. Antibodies against E4 have been considered as markers of LSIL while E7 antibodies as markers of CIN-III/ CC, although, the antibody-type profile is unknown. OBJECTIVE. Identify the HPV16 E4 antibody profiles associated to persistent infection and LSIL in Mexican women. METHODOLOGY. Heterosexual women from general population with stable sexual partner answered a standardized questionnaire, donated blood for the antibody test, and cervical cells for cytology and HPV genotyping (Reverse-line blot). Data was analyzed by logistic regression. RESULTS. From 499 women, 14% were HPV positive, from which 74% had oncogenic types and 72% simple infection. HPV59 (22%) and HPV16 (14%) were the most frequent types. The statistical analysis showed that having more than 2 sexual partners during the last year (OR=2.7) and smoking (OR=1.9) were risk factors for HPV infection. Anti-E4 was found in 51% of the women, from which 11% were HPV positive. Age influenced the presence of HPV (high-risk and low-risk) and of anti-E4. Antibodies were associated to CIN-I (OR=5.0). IgM-anti-E4 was detected in HPV negative women with CIN-I, while the profile IgG+IgA-anti-E4 was detected only in HPV positive women with CIN-I. CONCLUSION. Life-style influences exposure (high anti-E4 prevalence) and infection by high-risk HPV types in young women. Anti-E4 seems a good marker for CIN-I and for HPV activity depending on the immunoglobulin-type detected.

P-31.22

RELATING CERVICAL PATHOLOGY TO PATTERNS OF VIRAL BIOMARKER EXPRESSION.

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There is considerable interest at present in using molecular biomarkers to improve the accuracy of cervical diagnosis, as diagnosis on the basis of pathology alone can be subjective and suffer from inter-lab variation. To overcome this, several types of biomarker have been proposed that can distinguish between key life cycle events that change during neoplastic progression. Important amongst these are surrogate markers of E6/E7, such as PCNA, MCM and p16 that identify cells that are 'in cycle'; E4, which marks cells supporting viral genome amplification; and L1, which marks cells containing virus particles. As HPV gene expression determines cellular phenotype, it is now important to accurately link viral gene activity to pathology.

Here we have made use of digital imaging to link the expression patterns of these three key classes of biomarker to cervical pathology in the same tissue section, by overlaying multi-colour immunostains onto the Haemotoxylin and Eosin stained tissue image. To do this cervical lesions of diverse pathology (>50) were first identified as being caused by HPV16 using a combination of HPV DNA typing and E4 detection using the monoclonal antibody TVG405. Tissue sections were subsequently double or triple-stained to detect MCM (red), E4 (green) and L1 (infra-red) and DNA using DAPI (blue). Following digital imaging of the fluorescence images, tissue sections were subject to conventional H+E staining and analysis by light microscopy. Immunofluorescence is lost during H+E staining, allowing routine pathology examination prior to correlation of captured expression patterns with cellular changes. Details of the approach and the status of ongoing experiments will be presented.

The linking of changes in HPV gene expression with the associated pathology phenotype in the same lesion, provides a rational basis for determining how molecular diagnosis using biomarkers may be implemented.

QPCR OF P16INK4A AND SURVIVIN EXPRESSION IN A CERVICAL LBC-SETTING.

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Background: The main mediators of cervical carcinogenesis, HPV E6 and E7 oncoproteins, interact with host proteins. Furthermore, HPV infection leads to changes in the expression levels of host genes. Improved insights in this molecular cascade led to the discovery of clinically useful biomarkers. As measurable indicators of cellular changes, they can facilitate prediction of clinical outcome and improve the objectivity, reproducibility and reliability of cervical cancer screening. Efficient implementation of biomarkers in routine clinical practice could be supported by the introduction of molecular biological techniques, such as qPCR.

Objective: This study assessed whether p16INK4a and/or survivin expression measured by qPCR, could predict cytological outcome or HPV16-positivity in a routine liquid-based cytology (LBC) setting.

Methods: Sixty PreservCyt-fixed cervical samples, divided in three groups based on cytological diagnosis, were subjected to HPV typing and analysis of p16INK4a and survivin expression by qPCR.

Results: Statistical analyses showed that p16INK4a and survivin expression was not significantly different between the cytological groups. Moreover, neither p16INK4a nor survivin expression enabled discrimination between HPV16-positive and –negative samples.

Conclusions: Our results indicate that qPCR is practically feasible for PreservCyt-fixed LBC- samples, but inappropriate to assess biomarker expression. This could be explained by the heterogeneous nature of the starting material. The majority of fixed cells are normal, non-dysplastic cells, which show a basal biomarker expression. Therefore, the total isolated RNA mainly comprises p16INK4a and survivin transcripts from normal cells, which dilutes the increased biomarker expression of dysplastic cells. Spiking experiments based on HaCaT and HeLa cells confirmed this hypothesis.

P-31.24

P16INK4A IDENTIFIES LOW GRADE SQUAMOUS INTRAEPITHELIAL LESIONS IN LIQUID CYTOLOGY

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BACKGROUND. Infection by high risk human papillomavirus (HR-HPV) is considered to represent an important risk factor for the development and progression of cervical invasive carcinoma. The protein p16INK4a is overexpressed in premalignant and malignant lesions in cervical tissue samples and this overexpression is related to the oncoprotein E7 of the HR-HPV, which can be a useful marker in squamous intraepithelial lesions (SIL). OBJECTIVE. Determine the p16INK4a expression in liquid based cytological samples and correlate it to the HR-HPV type and low grade SIL (LSIL). METHODS. One-hundred and five women in which HR-HPV presence was determined by PCR-RFLP were included in the study. Liquid based cytology (LiquiPREP) by the Papanicolaou technique and immunocytochemistry to detect p16INK4a (clone 16PO4/JC2) was carried out. RESULTS. Thirty-four normal cytologies were diagnosed (32.7%) and 71 (67.3%) LSIL, of the latter, 68 presented koilocytic changes from infection by HPV and 3 koilocytes plus mild displasia. Nine different HR-HPVs were typed (16, 18, 31, 33, 39, 45, 52, 58 y 59). Immunocytochemistry showed 60.9% (60) of the total number of samples analyzed were positive for p16INK4a expression. Among these 60 samples, we found that 13 (38.2%) cases with normal cytology, 48 (70.6%) cases with LSIL with koilocytes and 3 (100%) cases with LSIL plus mild displasia, overexpressed p16INK4a. CONCLUSIONS. p16INK4a expression is related to the presence of HR-HPV infection and can be utilized as an alternative to improve early detection of cervical invasive carcinoma, through the liquid based cytology method.

SERUM GROWTH FACTORS LEVEL IN GENITAL HPV INFECTION

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Background: Persistent genital infections with HPV are major cause of uterin cervix cancer, the second most prevalent type of cancer. Some studies indicate that bFGF is able to inhibit apoptosis, plasma level of this growth factor beeing associated with the evolution point of cancer. Also, receptors for growth factors play a central role in growth stimulation of cells, including tumor cells. As a consequence, EGFr is investigated as a potential target of anticancer therapy.

Objective: Investigation of plasma bFGF and EGFr levels in patients with HPV infected cervical lesions with one or more HPV strains.

Methods: We collected blood samples from 20 female patients with HPV infected cervical lesions, with ages between 23 and 60 years old (33,8±9,24). We further divided these patients into subgroup 1 including patients infected only with one HPV strain, and subgroup 2 including patients infected with multiple HPV strains. We used ELISA standardized kits for plasma bFGF and EGFr. For statistical analysis of our data we used GraphPad INSTAT.

Results: There is no statistically significat differences of plasma bFGF concentration between subgroup 1 and subgroup 2 of patients. There is a significantly higher plasma EGFr concentration in the subgroup 2 (p=0,03). Also, we found a negative correlation between the plasma bFGF and EGFr concentration values for the entire group. This is in agreement with data from other studies.

Conclusions: Our study indicates that the presence of multiple HPV strains in infected cervical lesions is associated with an increase of plasma EGFr concentration relative to patients infected with only one HPV strain. It is desirable to further investigate plasma level of other growth factors (i.e., VEGF).

P-31.26

DNA DAMAGE INDUCED BY PERSISTENT AND HIGH RISK HPV INFECTION

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BACKGROUND: The persistent HPV cervical infection with high risk genotypes is the cause of cervical cancer and of its precursor lesions. HR-HPV might have an effect on the DNA integrity in cervical cells and contribute to the development of cervical cancer. OBJECTIVES: To evaluate the DNA damage in persistent HPV infections, as well as in intermittent reinfections, in women that developed LSIL and in women whose cytology is still normal. METHODS: 20 women of the southern Mexican state, Guerrero, were studied. They were monitored between two and nine years for the detection of infection by HPV. DNA damage was assayed by alkaline comet assay in cervical cells according to standard protocol and DNA migration on cells was measured by image analysis software. Olive tail moment (OTM) was used as the DNA damage parameter. HPV detection was done by PCR (MY09/11 or GP5+/6+ primers) and were typed by RFLPs or sequencing. RESULTS: The OTM mean in 15 women with one year persistence or more of any HPV was 21.5, in five women with the persistence of HR-HPV was 23.5 and in 11 women that evolved to LSIL was 21.7. In three women with LR-HPV the OTM was 16.6, in five women with intermittent reinfections was 16.2 and in women without infection by HPV was 1.5. If the OTM is 0-20 DNA is considered not to be damaged. However, if OTM is 21-60 DNA is considered to have a minor damage. CONCLUSIONS: The persistence of infection for more than a year by any HPV type and the infection with HR-HPV cause DNA damage, which is an early event in cervical carcinogenesis.

PROGNOSTIC VALUE OF HPV MRNA TEST IN HSIL-/HC2+ PATIENTS

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Background: HPV tests increase screening sensitivity, but decrease the specificity. Therefore there is a need for additional tests to guide the management of HPV-positive women.

Objectives: To evaluate the capability of mRNA test to predict the onset of histological high grade lesions during a two-year follow up of HPV-positive patients with a negative Pap-test or with mild cytologic abnormalities.

Methods: We analyzed cervicovaginal samples of a series of 92 HSIL-/HC2+ patients by liquid-based cytology (Cytyc, Italy), HPV DNA-based Hybrid Capture 2 test (Qiagen, Italy) and HPV mRNA-based PreTect HPV Proofer assay (Norchip, Italy). The 92 patients were followed up for a minimum of 6 months and a maximum of 2 years (mean 11.8 \pm SD 4.6; median 12 months) with histology endpoint. 35 out of 92 women had a histological diagnosis at baseline classified as less severe lesion than CIN2.

Results: Only 3 of the 92 patients were diagnosed as CIN2+ during the follow-up, two after 6 months and one after 13 months. All the three CIN2+ patients presented a positive mRNA assay at baseline: two CIN2+ patients had a RNA transcript from HPV genotype 16 and one from HPV genotype 18. Among the other 89 CIN2- patients, only 16 (18%) had a positive mRNA test at baseline and 73 (82%) tested mRNA negative. On the basis of these results in our series of patients, unconditional logistic regression model evidenced that a HSIL-/HC2+ woman, with positive mRNA test at baseline, was 31.2 times more likely to be diagnosed as histological CIN2+ within 2 years compared to a mRNA test-negative woman. Conclusions: Our data suggest a prognostic significance of this biomarker. In fact, the mRNA test positivity, in patients

Conclusions: Our data suggest a prognostic significance of this biomarker. In fact, the mRNA test positivity, in patients HSIL-/HC2+, should become mandatory for a strict follow-up, the patients being more prone to develop a CIN2+ during the subsequent two years.

P-31.28

NM23 AND P16 AS BIOMARKERS OF HR-HPV AND CIN2+ DETECTION

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Background: The nucleoside diphosphate kinase nm23 has been shown to be involved in development and differentiation control and to have antimetastatic potential in human carcinomas. Preliminary studies have suggested that the E7 protein from high risk (HR) HPV types, interacts with nm23 protein affecting its stability and function thus favouring the HPV tumorigenic potentiality.

Objectives: to verify whether lack of nm23 expression may be a biomarker useful in identifying HR-HPV infection and/or high grade cervical lesions or carcinomas (CIN2+), comparing nm23 findings with p16 overexpression, which has been reported in HR-HPV infected preneoplastic and neoplastic lesions of the cervix.

Methods: We evaluated nm23 (monoclonal antibody 37.6) in parallel with p16 (monoclonal antibody E6H4) immunohistochemical staining in 143 cervical biopsies including negative cases, low- and high-grade lesions, and squamous carcinomas (SC). We also performed HR-HPV testing by Hybrid Capture 2, on the corrispondent cervico-vaginal samples.

Results: HR-HPV infection was found in 76% of the samples. Nm23 was expressed in 53% of normal tissues, and, unexpectedly, in 70% of the CIN1, 74% of the CIN2, 75% of the CIN3, and 100% of the SC ($p\chi^2$ trend <0.0001). p16 immunoreactivity was detected in 19% of the normal tissues, 43% of the CIN1, 100% of the CIN2, 95% of the CIN 3 and 94% of the SC ($p\chi^2$ trend <0.0001). Regarding CIN2+ detection, p16 showed the best sensitivity and specificity, the differences with nm23 being statistically significant. Moreover, we found that p16 specificity in identyfing HR-HPV infection was significantly higher than nm23, although nm23 and p16 sensitivity was comparable.

Conclusions: These findings indicate that nm23 immunostaining does not appear to be a better biomarker than p16 in identyfing high grade or worse diagnosis, or HR-HPV infected samples.

P-31.29 ROCHE LINEAR ARRAY AND PROTOTYPE RESULTS COMPARED IN SCREENING SAMPLES

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Background: The Roche Research Use Only Linear Array (LA) for HPV DNA Typing is a refinement of their prototype line blot assay (Prototype). Published comparisons of these two platforms have uniformly found results to be comparable, but with LA showing increased sensitivity and detection of more types than the Prototype assay. These comparisons have been largely conducted in women with cervical abnormalities. This study was conducted to evaluate whether the increased sensitivity of the LA would have a greater impact on samples from a screening population.

Methods: Residual DNA extracts from 3032 samples previously evaluated by the Prototype assay were retested with the RUO Roche Linear Array. Overall concordance of the assays was determined as well as type-specific concordance for HPV 16.

Results: Of 3032 samples, 1658 were negative and 721 were positive in both assays, 13 were positive only in Prototype and 640 were positive only in LA (kappa = 0.54 + - 0.02). For HPV 16 detection and typing, 59 samples were positive in both assays, 4 were positive only in prototype and 77 positive only in LA (kappa = 0.58 + - 0.04).

Conclusions: The LA assay detected nearly twice as many HPV infections in this sample compared with the Prototype results (1361 versus 734). Similar increases were noted in type-specific detection of HPV 16. The difference in assay performance in this sample set is greater than previously reported. We hypothesize that a higher proportion of women in screening populations may have lower viral loads accounting for the striking difference between the assays with known differences in analytic sensitivity. Comparisons between assay platforms must also consider the clinical-epidemiologic context of sample collection.

P-31.30

A NOVEL HPV-GENOTYPING MICROARRAY SYSTEM WITH TYPE-SPECIFIC PRIMERS AND PROBES.

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HPV-genotyping microarray system using type-specific PCR primers and probes designed in E6, E2, E7, L1, and L2 genes was developed. With this microarray system, designated as GeneSQUARE, 24 specimens can be genotyped at the same time for 23 HPV types (14 high-risk and 9 low-risk types) in 5.5 hours. The cross reaction among these 23 HPV genotypes was hardly observed. This HPV-genotyping system was evaluated for use in clinical diagnosis with 147 cervical swabs collected from female commercial sex workers in the Philippines. The result was compared with those of PCR using the original and modified GP5+/6+ primers designed in L1 gene and sequencing cloned PCR products (sequencing method). Of the 147 samples 85 were positive and 35 were negative for HPV DNA by both the assays. In all the 85 HPV-positive samples equal and more HPV types were detected by GeneSQUARE than the sequencing method, when disregarding HPV types which probe is not included in GeneSQUARE. In 26 samples HPV DNA was detected only by GeneSQUARE, and their positive results were confirmed by type-specific PCR and sequencing. In the remaining one sample HPV DNA was detected only by the sequencing method and the detected HPV type was 67, which probe is not included in GeneSQUARE. Therefore, the specificity and sensitivity of GeneSQUARE were 99.1%, and 97.2%, respectively. These finding suggest that GeneSQUARE is useful for rapid detection of the 23 HPV types from clinical specimens with high sensitivity and specificity. This system can be used for the epidemiology investigation of HPV infection as well as monitoring the efficacy of HPV vaccines.

A REFLEX GENOTYPING TEST BASED ON HYBRID CAPTURE TECHNOLOGY

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Background: The digene High-Risk HPV HC2 DNA Test (HC2) generates a qualitative result for the detection of high-risk human papillomavirus (hrHPV) in cervical specimens. It has been shown that HPV16 and HPV18 positive women have an increased risk of high-grade cervical intraepithelial neoplasia compared with women positive for other hrHPV types. In addition, HPV18 and HPV45 have been closely linked to aggressive and difficult to detect adenocarcinomas.

Objective: To develop a reflex test based on the Hybrid Capture[®] technology capable of specifically detecting the most important carcinogenic HPV types; 16, 18, and 45. This proposed HPV genotyping assay does not require target amplification and is compatible with core HC2 reagents.1

Methods: The assay is based on the Hybrid Capture[®] technology and utilizes hybrid-specific antibodies for the detection of DNA targets. For the HC2 screening test, the detection of 13 hrHPV types uses a mixture of long in vitro transcribed RNA probes. However, due to the high homology of HPV types, long probes lack the specificity that is required for accurate detection of individual HPV types. Replacement of long RNA probes with a mixture of short type-specific oligonucleotides and other reagent modifications using a combined hybridization and capture methodology provides the specificity required for HPV genotyping. The assays for HPV 16, 18 and 45 are performed in separate wells.

Results and Conclusions: The proposed genotyping assay specifically detects HPV 16, 18, and 45 with 5000 copies/assay analytical sensitivity. The assay is highly specific and does not detect other high-risk or low-risk types at 108 copies per reaction. Utility of the genotyping test was demonstrated on STM samples and confirmed by PCR.

P-31.32 PROEXC AND HPV MRNA FOR HSIL DETECTION IN SUREPATH SPECIMENS

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Background: Liquid-based cytology (LBC) provides excellent sensitivity for the detection of high grade squamous intraepithelial lesions (HSIL), however, specificity is low. Assays that can be performed from LBC samples with a high positive predictive value for HSIL detection would decrease the number of unnecessary colposcopies.

Objective: This study evaluated the performance of LBC cytology with ProExC immunocytochemistry and the PreTect HPV-Proofer assay for the detection of biopsy confirmed high grade cervical disease (CIN2+).

Materials and methods: Cervical cytology specimens collected into SurePath preservative fluid and found to be ASC-US+ on cytology were included in the study (n=85). An additional LBC slide was prepared for immunocytochemical staining using ProExC (BD Diagnostics) containing antibodies to MCM2 and TOP2A which detect aberrant S-phase induction. RNA was isolated from the residual enriched cell pellet using the RecoverAll Total Nucleic Acid Isolation Kit (Ambion) and utilized in the PreTect HPV-Proofer assay (NorChip) designed to detect E6/E7 mRNA from HPV types 16, 18, 31, 33 and 45. Biospy results were available on 73 patients.

Results: Positivity for the ProEx C and Proofer assays by cytology category was 50% and 30% (ASC-US), 36.8% and 63.2% (LSIL), 100% and.100% (ASC-H) and 90.9% and 72.7% (HSIL), respectively. ProEx C was found to have a sensitivity of 84.8%, specificity of 66.7%, PPV of 81.3% and NPV of 72% for detection of CIN2+. Among the subset of 23 patients for which both ProEx C and Proofer results were available, the sensitivity for each assay was 92.3% and 84.6%, specificity 60% and 50%, PPV 75% and 68.8% and NPV 85.7% and 71.4%, respectively.

Conclusion: Both ProEx C and the PreTect HPV-Proofer assay increase PPV for the detection of CIN2+ disease over cytology alone, with ProEx C appearing to have better clinical performance in this pilot study.

HPV DETECTION AND TYPING IN THIN PREPS COMPARING THREE METHODS

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Introduction: Multiple assays for HPV detection/genotyping are available. No gold standard currently exists. Objective: We compared three HPV detection methods in thin prep supernates: Digene Hybrid Capture II (HC), Roche Linear Array HPV Genotyping (LA) and Kurabo GeneSquare Microarray (GS). Our goals were 1) correlate cytology with HPV detection and 2) determine agreement between assay pairs. Interpretation of HPV 52 (LA)-positive tests is confounded by cross-reactivity with the 52/33/35/58 probe. We performed type-specific (TS)-PCR (33, 35, 52, and 58)in such specimens.

Methods: Thin prep Pap smears were performed (n=207), and supernates tested by HC. Extracted DNA from supernates was tested using LA and GS. LA uses primer pools and tests for 37 types. GS uses TS multiplex PCR for 23 types. For specimens reacting with the HPV 52/33/35/58 probe and HPV 33, 35, or 58, then TS-PCR was performed for HPV 52, 33, 35, or 58. Binomial proportions and Kappa coefficients were calculated for agreement.

Results: Cytology results and supernatant were available for 202 subjects. By cytology, HPV-positivity increased with worsening abnormality in all assays. For all cytologic groups, LA and GS detected more HPV (all and oncogenic). However, for shared types, differences in HPV detection between assays were less pronounced. Overall, the highest agreement was between LA and GS (89.1%, K 0.77). Using TS-PCR, HPV 52 was amplified from five of 14 specimens originally LAnegative using the algorithm provided (Roche). All five specimens were GS-positive for HPV 52, thus improving agreement between assays. Conclusions: 1. HPV detection increased with worsening cytologic abnormality for all assays.

2. The best correlation for HPV detection/genotyping was between LA and GS.

3. Using TS-PCR, five additional HPV 52 infections were found.

P-31.34

ANALYTICAL COMPARISON OF THE COBAS® 4800 HPV TEST TO HC2

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Background. Many studies have demonstrated that high risk (HR) HPV testing is more sensitive than cytology in the detection of CIN2+ lesions. In this study we have evaluated the analytical detection of 14 HR-HPV genotypes using the prototype cobas 4800 HPV test and Hybrid Capture 2 (HC2). The prototype cobas* 4800 is a highly automated system that performs, sample preparation, real-time HR-HPV amplification and simultaneous detection of 12 HR-HPV genotypes in a single pool, with separate detection of HPV16, HPV18, as well as the human beta globin gene, all in single tube.

Methods. Aliquots of cervical specimens from 430 consecutive patients for whom a HC2 test was requested in the followup of an abnormal Pap were stored for analysis with the prototype cobas* 4800 HPV test and the Linear Array (LA) HPV genotyping test. HC2 and cobas® 4800 HPV results were available for 399 patients, with valid LA results on 391/399 patients. Cytology and/or histology results were available from all patients.

Results. Among the 399 patients, 367/399 (92%) had concordant hc2 and prototype cobas 4800 HPV results (Kappa = 0.84) Cobas 4800 Positive, hc2 Negative discordants results were observed with 5 patients. Analysis by LA found 2 HPV negative, 2 had low-risk HPV, and one had a high-risk HPV. Cobas 4800 negative, hc2 positive discordant results were observed in 27 patients.- LA analysis found 3 HPV negative, 7 positive for HR-HPV , and 17 positive only for low-risk HPV genotypes, including 5 samples with HPV53.

Conclusion. This evaluation of the prototype cobas® 4800 HPV test indicates that the system is reliable in the detection of HR-HPV genotypes, is easy to use, and shows similar sensitivity, specificity, PPV and NPP compared to the widely used HC2 in the detection of CIN2+ lesions.

P-31.35 QUANTITATIVE ANALYSIS OF CELLULAR PROTEINS AFFECTED BY HIGH-RISK HPV ONCOPROTEINS

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Background: Human papillomaviruses (HPVs) are involved in the pathogenesis of different types of human cancers, including cancers of the cervix and oropharynx. The E6 and E7 oncoproteins interact with many cellular proteins to immortalize cells.

Objectives: To identify cellular proteins altered by the presence of these oncoproteins.

Methods: Differences in protein expression in E6- and E7- expressing cells were measured via liquid chromatographytandem mass spectrometry (LC-MS/MS). Samples were analyzed on the LTQ (linear trap quadrupole) Orbitrap Hybrid Mass Spectrometer from Thermo Scientific. The LTQ Orbitrap allows for quantitative analysis of proteins between samples. We performed gene expression analyses using Gene Chip[®] Exon ST Arrays (Affymetrix) arrays to correlate RNA and protein data.

Results: For U2OS-E6 cells compared to U2OS-NEO, a total of 140 proteins, identified by at least 4 unique peptides, were found to be differentially expressed by 1.5-fold or greater. Of those, 66 proteins were down-regulated, such as UV excision repair protein RAD23 homolog A (RAD23A). Conversely, 74 proteins were up-regulated, including the double-stranded RNA-specific adenosine deaminase (ADAR1), an RNA-editing enzyme important for miRNAs. For U2OS-E7 cells compared to U2OS-NEO, 108 proteins were identified using the same parameters. Ninety-four proteins were down-regulated, such as fragile X mental retardation syndrome-related protein 1 (FXR1), a component of the RNA-induced silencing (RISC) complex. Also, 14 proteins were up-regulated, including Ras-related protein Rab-13 (RAB13), tyrosine-protein phosphatase non-receptor type 1 (JKTBP), and Ras GTPase-activating-like protein (IQGAP).

Conclusions: These studies will identify novel markers that may be useful in the diagnosis of HPV-associated cancers. We are also employing these techniques to identify targets of miRNAs that may be important for cervical and oropharyngeal cancers.

P-31.36 COMPARISON OF THREE COMMERCIAL ASSAYS FOR HPV GENOTYPING IN LBCSAMPLES

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Persistent infection with high-risk (HR) human papilloma virus (HPV) is strongly associated with risk of cervical cancer. HR HPV genotyping in LBC samples by molecular methods may be useful in combination with cytology for cervical cancer screening. This study was performed to compare the performance of three commercially available HPV genotyping assays, namely Linear array (LA, Roche), Inno-Lipa genotyping extra (IL, Innogenetics) and Papillocheck DNA chip (PC, Greiner). These assays detect 21 HPV genotypes in common including detection of 17 HR HPV (HPV16, -18, -31, -33, -35, -39, -45, -51, -52, -53, -56, -58, -59, -66 -68, -73, -82).

HPV DNA was extracted from a panel of clinical samples and EQC samples from UKNEQAS. Cervical cells collected from 1 mL Papspin (Thermo) liquid cytology were pre-treated with proteinaseK and subsequently extracted using the Generic protocol of the Nuclisens EasyMag system. Next, isolated DNA was used in parallel to perform the Amplicor HR HPV pooled-probe detection assay (Roche) and genotyping assays (LA, IL and PC) according to the manufacturer's instructions.

A random panel of 46 cervical samples was tested for the presence of HR HPV genotypes using the Amplicor HPV detection assay, which showed that 59% (n=27) of the specimens were HR HPV positive and 41% (n=19) were negative. Subsequently, these HR HPV positive samples were further genotyped in parallel by LA, IL and PC genotyping assays, which confirmed the presence of one (70%, n=19) or multiple (30%, n=8) HR HPV genotypes in each specimen, although minor differences in HR HPV genotype patterns were observed in the majority of the HR HPV co-infected samples.

In conclusion, this preliminary data suggests that the performance of the HPV genotyping assays for detection of HR HPV genotypes in cervical specimens is comparable. Currently, these assays are further investigated using a larger cohort of samples

LUMINEX®-BASED ASSAY FOR MULTIPLEX GENOTYPING OF 45 MUCOSAL HPV TYPES

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Background: The introduction of type-specific HPV vaccines requires type-specific post-vaccine surveillance to monitor the effectiveness of the vaccines and detection of possible type-replacement or escape variants. Many commercial and home brewed genotyping methods have been described, with a wide range of methodological approaches, performance characteristics, type coverage and cost.

Objective: We developed a Luminex-based assay that can type 45 mucosal HPVs. The assay was designed to combine, as much as possible, maximum sensitivity, broadest coverage of HPV types, ability to detect multiple infections, higher throughput and lower cost.

Methods: Amplified single stranded HPV DNA carrying a biotin tag was generated using PGMY and GP5+/GP6+ primers in a nested PCR reaction. A set of 45 Luminex microspheres coupled with 45 unique HPV probes was used for detection and typing. This method was compared to the Roche LinearArray using 149 cervical samples.

Results and Conclusions: The Luminex method identified 45 mucosal HPV types without cross hybridization. In the comparison with the LinearArray, the Luminex method showed a slightly higher sensitivity, i.e.,85 vs 73 positive samples, and 171 vs 164 total HPV types detected, with 47 multiple infections detected with both methods. LinearArray showed slightly better sensitivity for detection of multiple infections with 3 or more types (26 vs.19). The overall distribution of HPV types is similar between the 2 methods, with the exception of HPV 52, which is less frequently detected by the Luminex method (8 vs 18). However, all but one sample, in which HPV 52 was detected only by LinearArray, also contained one of more of the HPV types that cross react with the LinearArray HPV 52 probe. In conclusion, we have developed a HPV typing method which is comparable to a commercial kit and which offers flexibility, lower cost and less hands-on time.

P-31.38

VALIDATION OF A NON-COMMERCIAL REVERSE HYBRIDIZATION ASSAY FOR HPV GENOTYPING

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Background. Human papillomavirus (HPV) infection with oncogenic types is a prerequisite for cervical cancer development. Different tests for human papillomavirus (HPV) genotyping are commercially available. However, they are usually expensive, which results in a difficult availability in areas with low economical resources. So, it is necessary to develop not expensive typing procedures for HPV, with sensitivity and specificity comparable to those reported by commercial assays.

Objectives. The purpose of this study is to compare a non-commercial HPV genotyping assay (NCA), using GP5+/GP6+ PCR followed by a non-radiactive reverse line blotting, with a commercial method for HPV genotyping, Linear Arrays HPV (LA) genotyping test (Roche).

Methods. A total of 99 HPV DNA-positive cervical samples by hybrid capture method were genotyped. Non-commercial reverse hybridization assay was done with previous GP5+/GP6+ PCR amplification. Poly-(d)T tail probes were designed to detect HPV genotypes. Linear Array HPV Assay (Roche Diagnostics) was used as method validation. Comparison analysis was limited to the HPV genotypes common to both assays (6, 11, 16, 18, 31, 33, 39, 45, 51, 52, 59).

Results. There were concordant results (absolute agreement between assays) in 57 (57.57%) samples and compatible results (correspondence for some but not all genotypes) were found in 22 (22.22%) samples. Twenty samples were considered as discordant (did not show any similarity between the tests); of them, twelve were negative by NCA and positive by LA and, in the other 8 samples, HPV16 and/or HPV31 were detected by NCA and not by LA, or HPV39, HPV51 and/or HPV52 were detected by LA and not by NCA.

Conclusions. Preliminary results show that the new genotyping method could be suitable for clinical and epidemiological studies, although further studies are necessary with more samples.

HETEROGENICITY IN CERVICAL CANCER REGARDING HPV TYPES AND E6/E7 EXPRESSION

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Background: In cervical cancer samples, more than one HPV type may be present, while E6/E7 gene expression of more than one type is less frequently observed. Based on the hypothesis that cervical cancer is a monoclonal disease, acquisition of "passenger" HPV types during the carcinogenic process, which may be detected in different locations of the tumor, is possible.

Objectives: To investigate the extent of heterogenicity in terms of HPV presence and oncogenic activity in cervical tumors.

Methods: 88 women with histologically confirmed cervical cancer have been recruited from Pretoria Academic Hospital. Cervical specimens have been collected with tampons and cytobrush in PreTect TM (NorChip AS) together with biopsies at 3 o'clock and 9 o'clock positions prior to treatment. HPV DNA was detected with G5+/6+ PCR and Reverse Line Blot analysis, detecting 39 HPV types. Detection and typing of E6/E7 mRNA from the five high-risk HPV types 16, 18, 31, 33 and 45 were performed with PreTect HPV-Proofer (NorChip AS). In addition, E6/E7 mRNA from the three HPV types 35, 52 and 58 was detected with NASBA.

Results: In 19 (22%) of the 88 cases, diversity in HPV types between cervical samples and biopsies or between the biopsy pairs was found. Also, differences in terms of the presence of HPV DNA and E6/E7 expression were seen. Details will be presented.

Conclusion: This study shows a relatively high degree of heterogenicity within cervical cancer tumors, both in terms of different HPV types present and E6/E7 mRNA expression. This suggests that "passenger" HPV types not initially contributing to the carcinogenic process are likely to be present also in malignant tissue and may falsely be regarded as 'high-risk' HPV types. This highlights the need for additional basic research studies of, among others, the characteristics of the E6 and E7 proteins of different types.

P-31.40

GENERATION OF HPV-16, 18 AND 58 SPECIFIC ANTIBODIES AS BIOMARKERS

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Persistent infection by high-risk HPV types can lead to the development of cervical cancer. HPV DNA is found in nearly all cases of cervical cancer (>99.7%), and is a useful biomarker when combined with cytology or pathology-diagnosis. When used alone, HPV typing in lesions cannot distinguish active infections from those that are latent, and cannot conclusively identify the causative HPV type in lesions harboring multiple infections. To overcome this problem, recent work has focused on markers of active infection such as viral mRNA and proteins, and cellular proteins that can be used as surrogate markers of viral gene activity. P16 and multicopy maintenance protein are surrogates of E6/E7 expression and identify cells expressing the HPV oncogenes, while the viral E4 protein, which is a distinct class of marker, identifies cells supporting genome amplification and virus assembly. E4 is highly abundant, and is expressed in differentiated epithelial cells according to lesion grade, making it a good candidate as a molecular biomarker of active HPV infection. To examine the suitability of E4 detection as a diagnostic tool, type specific anti-E4 Abs were generated against E4 proteins of HPV-16, HPV-18 and HPV-58. Rabbits and mice were injected with HPV type-specific immunogens, and polyclonal Abs were evaluated for HPV type-specificity by ELISA and Western blots. In addition, HPV-16, HPV-18 and HPV-58 transfected rafts were used for further testing of the specificity of the Abs. An HPV-16 E4 specific monoclonal Ab was also produced. Qualification experiments have shown that these HPV type-specific Abs did not cross react with any other high risk HPV E1^E4 fusion proteins tested, nor with other types of raft tissues. Specificity and sensitivity of these Abs were also confirmed by immunohistochemistry on CIN1, CIN2 and a subset of CIN3 formalin-fixed paraffin-embedded lesions associated with HPV-16, 18 and 58 types.

EVALUATION OF HPV-GENOTYPING TEST IN CERVICAL INTRAEPITHELIAL LESIONS AND CANCER.

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Background: Detection of HPV types for using multiple assays are now available or being under development. Currently, the Hybrid Capture assay-2 (HC-II) is the most popular tool for cervical cancer screening but has limitations for following up of HPV-infection and/or CIN. In this study, we evaluated three methods from cytological specimens such as Qiagen HC-II, Roche LINEAR ARRAY HPV Genotyping Test(LA) and Kurabo GeneSQUARE HPV Genotyping Microarray(GS).

Method: Cytological pap smears were performed from the patients who visited to the out-patient clinic of Kanazawa University Hospital from 2006-2008 for receiving colposcopy examination and/or follow-up of CIN (n=136). All the subjects were randomly selected, and their clinical stages were diagnosed by pathological evaluation on colposcopy-guided biopsy samples. Pap smears were tested by HC-II. DNA was extracted from specimens, and was tested using GS and LA. The GS uses multiplex PCR for 23 types of HPV including high-risk, low-risk or risk-unknown types. LA provides typing for 37types of HPV using universal (pooling) primers for PCR.

Result: The positive rates of high-risk types infection were 68%, 76% and 85% by HC-2, GS and LA, respectively. Concordance of HPV genotypes between GS and LN was 97% in single infection, while 41% in multiple infection. However, concordances in some types in multiplex infection were observed in 97%. In normal and CIN1, a perfect match was found in 66% and 55%, while a concordance in some types was 89% and 86%. In contrast, a perfect concordance was observed in 92% and a concordance in some types was 97% in CIN2 and more malignant lesions.

Conclusions: GS and LN appear to be more sensitive than HC-2 in detection of high-risk HPV types. Genotyping can be useful for detecting HPV in higher-grade CIN and cancer.

P-31.43

AGREEMENT FOR THE DETECCION OF HPV GENOTYPES BETWEEN MOLECULAR TECHNIQUES

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The HPV DNA assay permit to know the HPV genotype and the multiple-type infections. Detection of high-risk human Papillomavirus (HPV) types infection is an important tool in the screening of cervical cancer. The different techniques for detection of this cancer need to be contrasted and validated. OBJECTIVE. Knowing the agreement for the detection of the different HPV genotypes by means of three molecular techniques; PCR and subsequent typing by means of digestion with restriction enzymes (rePCR) (PVHfast[®], Genomica), Inno-LiPA HPV genotyping (Innogenetics), Clinical Arrays Papillomavirus® (Genomica) in gynaecological samples, received in the Service of microbiology of the Hospital Clínico Universitario de Valladolid (Spain). METHODS: analyzed the agreement between the three assays, for the detection of HPV genotypes (Kappa value). RESULTS: Upon analyzing the agreement between LiPA and Microarrays for the HPV genotypes, could be observed that the agreement was excellent to detect HPV 42, very good for detect HPV 16, 18 and 53 and HPV 6 and 31. Between LiPA and rePCR, the agreement was quite worse. Only was acceptable, for the HPV 16, 31, 53 and 58. Finally, the agreement between Microarrays and rePCR only was acceptable, for HPV 16, 31 53 and 58. Upon being compared the three techniques, the agreement among them was excellent to detect the genotypes 16, 18 and 53, and acceptable for HPV 11, 31 and 58. CONCLUSION: The interest in the design of molecular assays of detection can be influenced by the existing prior literature, because probably they prioritize the capacities of identification and detection of some genotypes set against other types. The best agreement was between the LiPA assay and Microarrays, since for some genotypes (HPV 42, 16, 18 and 53) the Kappa value > 0, 8.

ROCHE AMPLICOR GENOTYPING OF AGE-MATCHED CONTROL STUDIES IN GENITAL WARTS

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Introduction: The relationship between high-risk and low-risk HPV in genital wart lesions is not clearly understood, and there are limited age-matched controlled studies comparing HPV genotype distribution in HIV negative and positive men with anal warts with and without intraepithelial neoplasia (IN).

Methods: Since the establishment of a surgical database of perianal/anal warts in 1996, a significant collection of paraffin embedded material has been stored. This has enabled us to perform the following age and anatomical site matched control studies in HIV negative men with perianal/anal warts and no intraepithelial neoplasia, and compare them to the following groups of men with perianal/anal warts:

(i) HIV positive men with high-grade IN in the excised lesions

- (ii) HIV positive men with no IN
- (iii) HIV negative men with high-grade IN

The studies were performed using Roche Amplicor genotyping.

Results: The genotyping results will be presented. For the first study 20 men age range 24.9 – 49.3 are being compared to 20 men age range 24.9 - 53.9 (11 anal site alone, 6 both perianal and anal, 3 by perianal site). In the second study, 12 men age range 33.9 to 49.3 are compared to men age range 28.8 – 47.3 (5 anal site alone, 5 perianal and anal sites, 2 perianal). In the third study 28 men (21 anal, 4 perianal and anal, and 3 perianal) sites are compared.

Conclusions: Based on the data the following will be discussed:

Whether being HIV positive makes any difference to the genotype distribution in men with genital warts with high-grade IN or genital warts without IN; whether the HPV types are the same in perianal disease as for the anal canal in the same person; and whether the distribution of high and low-risk types is the same in all scenarios.

P-31.45

EVALUATION OF HUMAN PAPILLOMAVIRUS TESTING IN CERVICAL CANCER SCREENING

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Objective: To compare the performance of human papillomavirus(HPV) assays plus pap cytology with Pap cytology alone for cervical cancer screening in Japan.

Methods: Between August and April in 2007, 1519 women who had pap smears alone for cancer cervical cancer screening. HPV testing plus Pap cytology was performed on 1570 women April to August in 2008.

HPV testing was performed in women who desired it with informed concent.

Pap smears were evaluated at Tomishiro central hospital and diagnosed using the Bethesda system.

HPV testing was performed using the Hybrid capture II HPV DNA Assay.

HPV DNA typing was performed using direct sequencing of PCR products.

Results: Pap smear abnormalities were observed in 1.1% (17/1519) in 2007, 0.3%(5/1570) in 2008. HPV DNA was detected in 7.4%(19/243) in 2008.

The prevalence rates of HPV in the group aged 20-29, 30-39, 40-49, 50 years and upwards were 14.3%, 10.3%, 4.2%, 7.7% , respectively.

The women with HPV positive-but normal cytology were detected in 18 cases and 12 cases of them were able to be examined for coloposcopy and biopsy.

Three cases of them were diagnosed as cervical intraepithelial neoplasia (CIN1:1), CIN2:2) finally.

Conclusion: Although HPV testing of women having Pap smears has clinical usefulness, there are a few reports of it in Japan.

It is very important that we found CIN cases in HPV positive-normal cytology cases.

Our study suggests that HPV testing could be an effective way to improve the performance of cervical cancer screening. Thus, cervical cancer is more than ever a virtually preventable disease.

GENETICS CAFÉ: A WEBSITE CONSORTIUM OF HPV INFORMATION

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Background: The utilization of HPV testing and correlative results varies according to lab and platform. There currently is no central database to compare HPV prevalence, persistence, and pertinent medical history. Access Genetics is an innovative business that provides expertise in molecular diagnostic operations and provides test interpretation through an integrated web portal. Advances in this web portal include tools to abstract and correlate analytic results of HPV detection, genotype distribution, medical demographics, sensitivity, and specificity.

Objective: To facilitate a centralized, real-time consortium of HPV testing information for review by researchers and industry.

Methods: Access Genetics has built an interactive website using a widget (an abstraction of medical or demographic data) to compare information from one lab to all others within the system. Widgets allow comparison of HPV results with test platform, time, morphology, and demographics. The comprehensive website, Genetics Café, permits data retrieval as well as the ability to upload new data in an attempt to create a consortium of HPV information. The largest data element is based on cumulative results of HPV PCR testing with RFLP from 50 distinct laboratories.

Results: The overall database contains 275,953 HPV test results with a 28.8% HPV positivity rate (n=79,391). HPV-16 is the most prevalent type, followed by -53, -52 and -31. This is a sampling of results further illustrated in the web-based widgets.

Conclusions: HPV test results, genotype distribution, and corresponding demographics contribute to a growing body of HPV DNA information. We created Genetics Cafe with embedded HPV tools in an effort to improve quality of HPV testing in the United States. These advantages include inter-lab, inter-platform, morphological and molecular correlations. This model will add value to those who are developing vaccines, appreciate the health economics of HPV, and seek to make inter-lab comparisons.

P-31.47 QUALITY CONTROL ON SPECIFICITY OF HPV TYPING IN PARAFFIN BLOCKS

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on behalf of the RIS HPV TT study group

Background: HPV 16, 18, 31, 33 and 45 account for nearly 80% of the invasive cervical cancers worldwide and some cross protection effects are being described in the HPV vaccination trials. The cervical cancer attributable fraction to each HPV type is relying on the specificity of the testing systems.

Objective: As part of the quality control procedures in the RIS HPV TT study on cervical cancer this study evaluates the specificity of the typing procedures between closely related HPV types using different methodologies.

Methods: Specimens of cervical cancer preserved in paraffin blocks and pathology confirmed were examined by the SPF10 and LiPA 25 genotyping strip (version1) for HPV type. A sample of specimens found to contain HPV 16, 18, 31, 33 or 45 as single infections were further examined using GP5+/6+, PYGMY and sequencing. Sequencing results will be compared to the BLAST HPV data base sequences.

Results: Detailed comparisons on type specific detection and concordance between HPV typing methods will be presented.

PREVALENCE OF SPECIFIC HPV TYPES IN WOMEN WITH CERVICAL LESIONS.

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Background: Over 100 different types of HPV constituting different taxonomically related groups have been formally described. Objective: The objective of this study was to detect the most prevalent human papillomavirus (HPV) types in women with histological lesions of the cervical epithelium. Methodology: In this analysis, 193 samples from women attending the cervical pathology department of the Santa Casa de Misericórdia in Goiás, Brazil because of an abnormal cervical smear were investigated. HPV DNA was detected by polymerase chain reaction using PGMY09/PGMY11 primers, and genotyping was performed by reverse dot blot hybridization. Results: The overall prevalence of HPV in women with an abnormal cervical smear was 85.7% (168/196), of which 82.1% (161/196) consisted of cases of high-risk oncogenic types. Of the total of HPV-positive women, 45.2% (76/168) were infected with HPV 16 as a single or multiple infections. HPV 31 and 35 were, respectively, the second and third most prevalent types. These viral types belong to group 9. The prevalence of HPV 16 in the women with a histological diagnosis of cervical intraepithelial neoplasia (CIN) 1 was 32.7% (18/55). Considering the most severe lesions, this prevalence was 60% (21/35) in cases of CIN 2, 47.5% (19/40) in CIN 3 and 87.5% (7/8) in cases of invasive carcinoma. Type 31 was detected in 10.9% (6/55) of cases of CIN 1, 8.5 % (3/35) of cases of CIN 2 and 12.5 % (5/40) of cases of CIN 3. Conclusion: The HPV types most frequently detected in women with cervical lesions belong to species 9 of the remotely related taxonomic groups.

P-31.49

CXCR4 AND CXCL12 POLYMORPHISMS ASSOCIATED WITH CERVICAL CANCER SURVIVAL

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Mutations in the chemokine receptor CXCR4 have been linked to inherited impaired immune response to HPV. Its ligand, CXCL12 (SDF1), provides a chemotactic signal that directs leukocyte migration. We tested the hypothesis that cervical cancer survival is associated with common genetic variation in these genes. Two of four CXCR4 tagSNPs and 9 of 12 CXCL12 tagSNPs were assayed in 379 women diagnosed with FIGO stage 1B or higher cervical cancer in a population-based study conducted in the Seattle area between 1986 and 2004. Hazard ratios (HR) derived from Cox proportional hazards models were used to estimate the risk of death due to cervical cancer. All estimates were adjusted for age and race. Fifty-two of the 379 cases (13.7%) had died of cervical cancer through November 2008. There was a 2-fold increased hazard associated with a variant allele in the 3' UTR of CXCL12, rs1801157 (HR 2.0, 95% CI 1.0-3.8, p<0.05), and a 2-fold decreased hazard associated with a variant allele in the 5' UTR of CXCR4, rs2680880 (HR 0.5, 95% CI 0.2-1.0, p<0.05). Further assessment of these relationships in other populations is warranted.

IDENTIFICATION OF METHYLATION MARKERS FOR CERVICAL ADENOCARCINOMAS AND PRECURSOR LESIONS

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Background: Currently, cervical screening programs based on cytology have resulted in a decrease in the incidence of squamous cell carcinomas (SCC), while the incidence of adenocarcinoma (AdCA) has remained the same or even increased. This indicates that particularly cervical AdCA and its glandular precursor lesion, i.e. adenocarcinoma in situ (ACIS) are frequently missed in screening programs. Although women harbouring hrHPV, particularly HPV 18, 16 and 45, have an increased risk of ACIS/AdCA, only a fraction will have or develop ACIS/AdCA. Therefore, novel biomarkers are needed that allow prediction of ACIS/AdCA in hrHPV positive women. Based on methylation profiling and expression analysis we identified 12 known and novel candidate markers specific for AdCA

Objectives: To assess the value of promoter methylation analysis of 12 genes to detect and distinguish women with ACIS/ AdCA.

Methods: In a first pilot all 12 candidate markers (including SPARC, NPTX-1, IRF8, TP73, RASSF1a, MGMT and APC) were evaluated using methylation specific PCR (MSP). The 4 most promising markers (SPARC, NPTX-1 and 2 novel ones) were evaluated further using quantitative MSP on cervical biopsies representing AdCA, ACIS and SCC. All samples were positive for hrHPV. Normal cervical biopsies were tested as controls.

Results: In AdCAs methylation rates of 75%, 83%, 60% and 41% were found for the 4 most promising markers. Lower methylation rates in ACIS were found (33%, 38%, 33% and 33%, respectively). In SCC methylation rates were 89%, 78%, 44% and 11%, respectively. The 4 genes were not or rarely (5-11%) methylated in normal controls. When combining the 4 markers 98% of AdCAs, 89% of SCC, 60% of ACIS, and 14% of normal controls tested positive.

Conclusions: Two methylation markers, including SPARC, detected both AdCa and SCC at a high frequency, while two other markers, including NPTX-1, were more specific for AdCA.

P-31.51

ABERRANT DNA METHYLATION IN HPV-POSITIVE CERVICAL SQUAMOUS INTRAEPITHELIAL LESIONS

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Background: Aberrant methylation of tumor suppressor genes (TSG) has been increasingly recognized as an important mechanism in HPV-associated oncogenesis. Direct interactions between HPV and cellular proteins involved in DNA methylation have been demonstrated. Methylation of TSGs in cervical cancers has been examined, however there are relatively few studies examining methylation in pre-neoplastic lesions by HPV status.

Objectives: The purpose of this study was to determine the prevalence of aberrant TSG methylation and its association with HPV infection in low (LSIL) and high-grade (HSIL) cervical squamous intraepithelial lesions.

Methods: We examined 95 residual liquid-based cytology specimens (63 LSIL and 32 HSIL). HPV genotyping was conducted using the Roche Linear Array HPV Genotyping Test. Methylation of p16INK4a (p16), retinoic acid receptorb (RARb), death-associated protein kinase (DAPK), E-cadherin (CDH1), adenomatosis polyposis coli (APC) and tissue inhibitor of metalloproteinase-3 (TIMP3) was assessed using quantitative real-time PCR.

Results: The mean age among women was 32 (SD=13) years old with an overall HPV prevalence of 86%. Among HPV-positive women, methylation rate was highest for APC (44%), followed by CDH1 (26%) and DAPK (14%). The prevalence of methylation for RARb, TIMP3 and p16 was less than 5%. DAPK methylation was significantly higher in HSIL lesions as compared to LSIL (24% vs. 0%; p=0.007). HSILs had marginally more methylated genes than LSILs (p=0.09).

Conclusions: Methylation of CDH1, APC and DAPK was observed among HPV-positive SILs with DAPK methylation occurring exclusively in HSIL. Additional patient follow-up will confirm whether methylation of CDH1 and APC in LSIL is associated with progression to HSIL. The assessment of a panel of epigenetic biomarkers in readily available liquid-based cytological samples is feasible and may serve as a predictive methodology for determining the potential for histologic progression in HPV-positive SIL.

DEVELOPMENT OF PCR-HIBRYDIZATION ASSAY FOR DETECTION OF HPV MUCOSAL GENOTYPES

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Background. Different tests for human papillomavirus (HPV) screening are commercially available, detecting high-risk oncogenic HPV types with a pool of genotype-specific probes. However, HPV typing is required in the management of pre-cancerous lesions, epidemiological studies and vaccination trials. Objectives. Commercial assays for genotyping are usually expensive and cannot be used in some areas with high prevalence of cervical cancer, as Cuba. We have developed a simple and not expensive typing procedure for 11 mucosal HPV types using a nonradiactive reverse line blotting procedure by general primers (GP5+/6+) PCR.

Methods. Genotype-specific probes for HPV genotypes 6, 11, 16, 18, 31, 33, 39, 45, 51, 52 and 59 were selected. These probes were enzymatically provided with a 25-mer poly(dT) tail. Subsequently, probes were immobilized as parallel lines on nitrocellulose membrane strips, with a positive control biotinylated DNA in the top line. Different probes concentrations were used to optimize the test. Plasmids containing full genome of HPV corresponding to genotypes assayed were amplified by GP5+ and GP6+ biotinylated primers. Briefly, reverse hybridization was performed with PCR product, denatured by adding NaOH solution, followed by hybridization at 50°C for 1 h, incubation with alkaline phosphatase-streptavidin conjugate and addition of substrate (5-bromo-4-chloro-3-indolylphosphate and nitroblue tetrazolium). Optimal dilution for conjugate was 1:2500 and for probes was determined in 2.5 pmol/uL.

Results. PCR sensitivity was determined in serial dilutions of plasmid containing complete genome of HPV16, HPV 18 and HPV 45. The limits of detection were 20 fg for HPV 16, 2 fg for HPV 18 and 0.2 fg for HPV 45. Specificity of the method was evaluated with GP5+/GP6+ PCR products for the panel of HPV types and non-crossreactivity was observed.

Conclusions. We have obtained a simple and not very expensive method with good sensitivity that can be used for extensive epidemiological studies.

P-31.53

COMPARATIVE ANALYSIS OF CBG HPV ASSAY AND HYBRID CAPTURE ASSAY

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Background: Detection of high-risk HPV has proved its usefulness in complement of abnormal cervical cytology. Objectives: To compare the accuracy of the CBG HPV DNA Assay(CBG, Catch By Gene Co.) and Hybrid Capture 2 (HC2, QIAGEN) assay in cervical samples.

Methods: We investigated 355 cervical samples classified into six groups according to cytological results [benign 202(56.9%); ASCUS 59(16.6%); LSIL 73(20.5%); HSIL 16(4.5%); SCC 2(0.6%); adenocarcinoma(0.8%)] and CBG and HC2 were tested. In CBG, amplified DNA was detected by colorimetry, and chemiluminometry was used in HC2. Then DNA chip assay was performed on 148 samples including 43 samples showing discordant results between the 2 tests and 105 samples showing concordant results.

Results: The positive results were detected in 63.4% of samples by CBG and 56.9% by HC2. The concordant rate between CBG and HC2 was 90.1%. The concordant rate for the presence or absence of high risk (HR)-HPV between CBG and DNA chip assay was 89.2%, but was 77% between HC2 and DNA chip assay. For the Overall analysis, CBG demonstrated 80 true positive, 10 false positive, 52 true negative, 6 false negative, 93.0% of sensitivity, 83.9% of specificity, 88.8% of PPV and 86.9% NPV. HC2 demonstrated 59 true positive, 7 false positive, 55 true negative, 27 false negative, 71.4% of sensitivity, 88.3% of specificity, 89.3% of PPV and 71.4% of NPV.

Conclusions: The result of CBG showed higher positive rate than HC2 in ASCUS, L-SIL and H-SIL. Degree of concordance between CBG and DNA chip assay was higher than between HC2 and DNA chip assay. CBG HPV assay would be effective to screen more common High risk HPV. CBG can test larger number of samples than HC2 per unit time because CBG can use automatic ELISA system.

E6/E7 EXPRESSIONS IN INFECTED AND IN TRANSFORMED CERVICAL EPITHELIAL CELLS

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Background: Persistent human papillomavirus (HPV) infection is essential for the development of cervical intraepithelial neoplasia (CIN) and cervical cancer. E6 and E7 oncoproteins appear in the very early phase of HPV infection. Increased expression of E6 and E7 is observed in cells with HPV DNA integration, resulted in an increased proliferation rate and genomic instability of the epithelial cell. This phenomenon highly correlates with the malignant transformation of cells infected by high-risk HPV types and is the key mechanism of developing high-grade cervical intraepithelial neoplasia (CIN) and invasive cancer.

Objectives: We attempt to allocate the E6 and E7 oncoproteins in clinical samples of HPV infected cervical epithelial cells and in malignant transformed cells by high-risk HPV infection.

Methods: Efficient monoclonal antibodies, having developed by our groups for staining of histological and cytological specimens, against E6 and E7 proteins were applied. Expression and localization of theE6 and E7 oncoproteins in the histological and in the cytological specimens was investigated.

Results: We discovered that these oncoproteins are present only in the nucleus of HPV infected cells, but are present in both the nucleus and the cytoplasm of invasive cervical cancer. A level increase of E7 expression in cytoplasm correlates with the severity of disease, noted as ~0% in CIN 1, 38% in CIN 2, 52% in CIN 3 and 95% in invasive cervical cancer.

Conclusions: The clinical utility in detecting HPV oncoproteins in various manifestations of HPV related lesions, either in histological or in cytological specimens, can be further investigated.

P-31.55

WILL DIAGNOSTIC MARKERS HELP TO PREDICT PROGRESSION OF CERVICAL DYSPLASIA?

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Background: Several biomarkers have been evaluated in order to improve histological diagnosis of cervical intraepithelial neoplasia (CIN). Ki 67, p16 and expression of E6/E7 mRNA have been proposed also as biomarkers for progression. However, the CIN showing progression/regression/persistence is difficult to study in detail. We have established a model for studying progression by selecting a patient material in relation to pregnancy.

Objectives: The aims of the study was to compare whether positivity to HPV mRNA, p16, Ki67 or HPV type DNA was related to stage of CIN and/or progression.

Methods: Among all pregnant women in 1996-7 in Norway, those with diagnosed CINI-III during pregnancy were identified from the Cancer Registry of Norway. 155 women with minimum of two serial histology specimens were included into the study. The archival paraffin embedded tissue blocks were identified and histology diagnoses were confirmed by two independent pathologists (BH, WR). Real-time NASBA (NorChip AS, Klokkarstua, Norway) was used for E6/E7 mRNA from HPV16,18,31,33,45. For HPV DNA GP5+/6+ was used, followed by reverse line-blot for typing of 22 different types of HPV. P16/Ki67 were detected immunohistochemically (Cell Marque, p16, 16P04 and Assey, Filled Disp., CONFIRM Anti-KI76 K-2, Ventana). Prevalence of biomarkers by dysplasia grade (normal, CINI-III) was estimated. Time dependent Cox model was used to investigate role of biomarkers for progression and patients with an observation-time less than 6 months were excluded.

Results: The patients were divided into 4 groups, depending on the development of the CIN lesion: persistence (N=59), progression (N=28), regression (N=11) and point prevalence (52).

We found a statistically significant correlation between severity of CIN grade and presence of biomarkers studied. Presence of biomarkers, however, was not predictive for progression in our study.

As a conclusion, p16 and Ki 67, although being very good diagnostic markers, did not indicate progression.

QUANTITATIVE CONSENSUS MULTIPLEX REALTIME HPV TYPING AND DETECTION

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We have developed a quantitative L1 consensus realtime multiplex PCR test for oncogenic HPV types 16, 18, 31, 33, 35, 39, 45, 51, 56, 58, 59 and 66. A 200 bp fragment is amplified using consensus primers. Detection and typing occurs by the 3' nuclease (TaqMan) assay using TaqMan MGB or LNA (locked nucleic acid) probes. The test is adapted for the ABI prism 7300 and uses all four colour channels (FAM, VIC, NED and ROX). Full typing and detection uses two 4-plex and two 3-plex reactions, one of which includes a competitive internal control (HPV6). Analytical sensitivity is 5000 copies or better in a background of 100ng/microliter human DNA. We will report the results of comparison with HCII and sequencing in a set of 200 cervical samples.

P-31.58

PAPTYPE™ VERSUS HC2: A BLINDED 100 PATIENT PANEL STUDY

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PapType[™] HPV Detection and Typing System (Genera Biosystems) was utilized to detect high-risk HPV DNA in a cohort of 100 women undergoing treatment for abnormal cervical cytology. All patients were enrolled at the Royal Women's Hospital, Melbourne, Australia and had histology, Hybrid Capture[®] 2 High-Risk HPV DNA Test[™] (HC2) and Roche Linear Array results available for comparison. PapType testing was performed blinded to other results. PapType[™] returned positive results for all fourteen high risk types the test is designed to detect, including fourteen HPV16, eight HPV18, nine HPV31, five HPV33, three HPV35, eight HPV39, six HPV45, four HPV51, seven HPV52, six HPV56, four HPV58, four HPV59, nine HPV66 and six HPV68 infections. Of all specimens returning high risk HPV-positive results by PapType[™], three returned negative results for the fourteen PapType[™] high risk types by Roche Linear Array. In the first case, a CIN3 histology specimen was called HPV16 positive by PapType[™] but only low risk HPV6 by Roche Linear Array. In the second case, a normal histology specimen was called HPV68 positive by PapType[™] but HPV70 positive by Roche Linear Array. In the third case, a CIN3 histology specimen was called HPV68 positive by PapType[™] but HPV70 positive by Roche Linear Array. PapType[™] exhibited a higher clinical sensitivity for detection of CIN2+ than HC2 in the population studied. When compared to CIN2+ histology results, PapType[™] exhibited clinical sensitivity and specificity values of 84.8% and 50%, respectively, compared with the respective clinical sensitivity and specificity values for HC2 of 47.1% and 51.0%. Evaluation of a larger cohort is currently underway.

PREVALENCE AND HPV GENOTYPE DISTRIBUTION IN GREENLAND USING MOLECULAR HPV-DIAGNOSTICS

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Introduction: Greenland, with its population of 55000 people, has a cervical cancer incidence of 37/100000 women, thereby rating Greenland as on of the highest cervical cancer incidence areas in the world.

Methods: HPV-DNA microarray analysis were performed on 212 individual, clinical samples from women undergoing routine cervical cancer screening on the west coast of Greenland using a low-density HPV MA system (Genomica S.A.U.) containing specific probes for 35 mucosal HPV types.

Results: 212 women, age 18-68 years, participating in the regular screening programme on Greenland were in addition to pap-smaer testing, tested using our MA system to determine the prevalence of HPV infection in this unselected population. 51 of 212 (24%) women were postive for HPV infection, with 21 of 52 (40%) being positive for multiple HPV genotypes. Data from HPV postive samples have resulted in a newly ranked list of HPV genotypes. Among the HPV postive women, most commonly found was Genotype70 (9%), followed by Genotype16 (8%), Genotype51 (8%), Genotype53 (7%), and Genotype58 (6%), respectively. However, in this unselected study population, it was of interest to note that 67% of all HPV genotype findings belonged to the group of High Risk HPV genotypes, whereas genotypes associated with condylomas were relatively rare, genotypes 6 and 11 only seem in 5% and 2% of the HPV postive samples, respectively. An analysis of HPV co-infection patterns was subsequently performed and most commonly seen was Genotype 51 present in 23% of the women with a multiple HPV infection, whereas the high risk genotypes 16 and 18 were only present in 14% of the co-infected.

Conclusion: Use of HPV-DNA MA technology greatly improves the diagnostic power with regard to de-termin-ing single as well as multiple HPV infections associated with atypical cervical cell findings.

P-31.60

MODELING RT-PCR EFFICIENCY AND FLUORESCENT SIGNAL INTENSITY FOR GENE QUANTIFICATION

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Background: Several methods are available to quantify a viral load in a sample processed by real-time PCR. However, the construction of a standard curve and the amplification efficiency hypothesis are still required.

Objectives: An innovative mathematical method, based on the amplification rate model of the PCR reaction is evaluated. This technique estimates the initial fluorescence value of a sample, which is theoretically proportional to its DNA content, making thus possible the absolute quantitation of a given sample with a single standard.

Methods: Serial dilutions of high-risk HPV plasmids were co-amplified with genomic human DNA on a real time thermocycler (CFX96[™] Bio-Rad).

Results: Simulations showed that HPV concentrations estimated by the patented mathematical algorithm were similar to those acquired with the most currently used methods requiring a standard curve. The loss in precision and reproducibility due to the use of a single standard are insignificant. The accuracy is reliable for most applications.

Conclusions: This new Bio-Rad algorithm to analyze Q-PCR data is fully automated and requires only one standard point for precise and consistent target quantitation. The independent estimate of the amplification efficiency for each sample, useful to detect a potential inhibition within the reaction, is one of the advantages. Furthermore, it is less time consuming and cost effective in clinical applications.

P-31.61 FAILURE TO DETECT HPV16/18 IN SERUM OF COLPOSCOPY CLINIC PATIENTS

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Background. The causal role of HPV in cervical cancer establishes a place for high-risk HPV testing in clinical practice. Objective. To investigate the feasibility of using HPV DNA in the circulation as a cervical cancer screening tool.

Methods. Serum was analyzed from a subset of colposcopy clinic patients enrolled in a cross-sectional study of biomarkers correlating with cervical disease through the NCI Early Detection Research Network. The subset included women with cervical HPV16/18 (with/without other HPV types), or high-risk HPV negative. Cervical HPV testing was based on Roche prototype L1 consensus PCR and line probe hybridization. HPV testing on DNA extracted from 1mL serum was conducted without knowledge of cervical HPV status, using a 3-plex PCR and mass spectrometry-based (PCR-MS) assay with HPV16, HPV18 and beta-globin (detection threshold: 250 copies HPV genome/mL serum).

Results. By PCR-MS, of 116 (85.3%) beta-globin positive serum samples, all (100%) were negative for HPV16 and HPV18. Most participants were Black (73.3%) and non-Hispanic (89.6%), with median age of 27 years. Over half (51.7%) had cervical HPV16 or HPV18 infection (43.3% single and 56.7% multiple infections). Degree of cervical intraepithelial neoplasia (CIN) was 31.1%, 10.3%, and 50.9%, respectively, for high grade (CIN II/III), low grade (CIN I) and no disease. Regarding HPV cofactors, 28.5% ever regularly smoked cigarettes, 70.7% had early onset of sexual intercourse (\leq 17 years), and 75% ever used oral contraceptives.

Conclusions. PCR-MS did not detect HPV16/18 DNA at a level of 250 copies/mL in serum of women with representative distribution of cervical HPV infection, clinical disease, and HPV cofactors. Our finding is consistent with the lack of a viremic state for HPV infection and suggests that serum HPV DNA detection may have limited application as a cervical cancer screening tool.

P-31.62

HPV GENOTYPING IN CERVICAL SAMPLES COMPARING TWO HPV-DNA DETECTION ASSAYS

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Implementation of DNA testing for HPV has increased the sensitivity and cost effectiveness of cervical cancer screening programs by detecting "high risk" lesions. Currently there are many different molecular techniques which allow the detection of multiple HPV types and are highly specific.

This study describes the comparison of two commercially available systems for HPV detection and typing.

DNA was extracted from 45 cervical samples following each manufacturer s' instructions. The first method was based on filter columns and the second one on manifold filtration. In each methodology, the highly conserved L1 region of the virus was amplified by PCR. The biotynilated PCR products were then hybridisized to specific probes with streptavidin. During the hybridisation process, the first method involved attached probes on a microarray surface while the second method involved probes attached on a line strip. The number of HPV genotypes detected by each assay was 35 and 37 respectively.

The majority of the samples (75%) showed concordance for the genotype result in both methods. The inter-assay disagreement in 11% of the samples involved samples with multiple infections. A subset of five samples (11%) were found negative by the first assay, but were typed successfully by the second one. All samples contained both low and high risk types which were detected by both assays.

These results suggest that other parameters such as the viral load may provide information regarding the different sensitivity of the HPV genotype detection in both methods described above. Despite the fact that both methods are highly comparable, type specific reproducibility and robustness of commercial HPV genotyping tests is always under evaluation. The accurate monitoring of HPV prevalence will greatly support future vaccination programmes.

ASSESSING HPV-RELATED DISEASE THROUGH DETECTION OF HPV MRNA RATIOS

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Background. Screening for HPV DNA is proven to be beneficial for diagnosing cervical cancer. However, only a small proportion of individuals with a HPV-positive screening result will develop cancer. Thus, a confirmatory test with a higher positive-predictive value may help reduce the number of colposcopies and ensuing health-care costs. A potential assay biomarker is the ratio of E6/7 mRNA over other HPV mRNAs, which hypothetically changes during disease progression (patent, US6355424B1).

Objectives: The objectives were to develop assays to detect HPV mRNA, determine RNA stability and correlate HPV expression with progression of HPV disease in specimens of exfoliated, cervical epithelia.

Methods. HPV RNA was isolated using QIAGEN Rneasy Plus, FastLane and other technology. RNA detection was by hybrid-capture technology without target amplification. RT-PCR was used to validate the assay and determine RNA stability.

Results. The mRNA of SiHa was stable up to 24 days in LBC medium. The limit of detection of SiHa mRNA in LBC was approximately 800 cells per assay. Probes detecting early (E6/7) and late HPV 16 mRNAs were designed and then used to determine the ratio of early over late mRNA. The early:late mRNA ratio was high, approximately 25 for SiHa cells in LBC media, and in pools of HPV-positive clinical specimens (a pre-cancer model). The mRNA ratios were detected in clinical specimens. RT-PCR assay results correlated with the results from non-target amplification assays.

Conclusions. Assays for HPV 16 mRNAs were developed and applied in the detection of mRNA ratios of SiHa samples and clinical specimens. Assays for other HPV types are in progress. These assays of HPV mRNA ratio may be useful to help predict disease progression, and clinical research in this area is ongoing. This assay is under development and is not a product and not commercially available in the US or EU.

P-31.65 HPV-L1 CAPSID PROTEIN: A PROMISING RISK ASSESSMENT BIOMARKER FOR CIN2+

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Less than 10% of HPV-infected women will eventually develop CIN2+ lesions. Identifying women with high risk of progressing to CIN2+ is clnically important. HPV L1 expressed at the HPV episomal stage and is gradually lost during viral integration into the host genome. We assessed whether HPV L1 can be used as a risk assessment biomarker for progression to CIN2+. Immunocytochemical analysis of HPV L1(Cytoimmun, Germany) was performed on 130 HPV-positive women on ThinPrep Pap tests. The cytologic diagnoses consisted of ASC(40), LSIL(38), ASC-H(27), and HSIL(25). Histologic follow-up was available for all patients 2-18 months after initial cytologic diagnosis. Histologic examination revealed 47 benign, 43 CIN 1 and 40 CIN 2+. Overall 32%(42/130) of cases expressed HPV L1 which included 25 (58%) of CIN 1, 13 (33%) of benign cervix and 4(10%) of CIN 2+. Among patients with CIN2+ lesions, 90% (36/40) of patients had HPV L1 negativity on their previous PAP smears, compared to 10% (4/40) with HPV L1 positivity (p<0.001). In correlation of L1 negativity to cytologic evaluation, the risk of developing CIN2+ lesions with loss of HPV L1 was approximately 85% for HSIL, 36% for ASC-H, 29% for LSIL, and 15% for ASC. Among 78 cytologic ASC/LSIL cases, none of L1-positive ASC/LSIL had CIN 2-3 on follow up. However, 22%% of ASC/LSIL cases with L1 negativity developed CIN 2-3 lesions (p<0.01). Our study demonstrates that expression of HPV L1 capsid protein is associated with transient HPV infection and spontaneous regression. In contrast, loss of HPV L1 in HPV-pos women with abnormal cytology identifies a subset of HPV-positive women with high risk in progressing to CIN2+ lesions. Therefore, detection of L1 capsid protein in PAP tests can be a useful molecular biomarker in risk assessment and prognostic prediction.

EXPRESSION OF KI-67 IN CERVICAL CONES WITH NEGATIVE MARGINS

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BACKGROUND. The nuclear antigen Ki-67 is related to normal cell cycle, and its presence due to cells with high proliferative activity are associated with the appearance of premalignant and malignant lesions, therefore its evaluation is important. OBJECTIVE. Determine the expression pattern of Ki-67 in biopsies with high grade NIC in cervical cones with negative margins. METHODS. A transversal observational study was carried out, we used 30 samples of cervical tissue from women with a histopathologic diagnosis of high grade NIC, 20 of which corresponded to NIC III (moderate and/or advanced displasia), and 10 cases of carcinoma in situ. All samples were analyzed for Ki-67 (clone MIB-1) by immunohistochemistry. RESULTS. Ki-67 expression was found in the nucleus (ochre color). Positive immunostaining for Ki-67 was found in two thirds of the epithelium in 17 cases (57%), and in 3 cases (10%) in the first third of the epithelium, meanwhile in the carcinoma in situ the positive reaction was found in the entire tissue (33%). All the negative margins were negative for Ki-67. In the normal tissue close to the high grade NIC, Ki-67 expression was found in deep basal or parabasal cells. CONCLUSIONS. Immunohistochemical determination of proliferative activity against antigens related to the cell cycle, such as Ki-67, allows us to establish an exact diagnosis and constitutes an excellent tool to help the histopathological study to differentiate the proliferative state of high grade NIC.

P-31.67

HIGH-RISK HPV E7 ONCOPROTEIN DETECTION IN CERVIAL ADENOCARCINOMA

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Background: Persistent infections by high-risk human papillomavirus (HPV) types are the main etiologic factors for cervical cancer. These viruses can infect epithelial squamous and glandular cells in the cervical mucosa. At least 15 high-risk HPVs associated with intraepithelial lesions with a high potential for progression to invasive cancer are described. Adenocarcinomas develop in glandular cells of the cervical mucosa and account for 10 to 20% of all cervical cancer cases. The vast majority of the adenocarcinomas are positive for HPV-16 or HPV-18.

Objective: The objective of this study was to evaluate whether HPV-16 and HPV-18 E7 oncoproteins are adequate as a marker for the detection of cervical adenocarcinoma.

Method: HPV typing was conducted in biopsies of 39 cervical adenocarcinomas and 22 normal cervical glandular epithelia. The HPV-16 E7 and HPV-18 E7 oncoprotein levels were monitored by immunohistochemistry using rabbit anti-HPV-16 E7 and HPV-18 E7 antibodies.

Results: High HPV E7 oncoprotein levels were detected in all cervical adenocarcinomas analyzed.

Conclusion: The results suggest that rabbit antibodies against the HPV-16 E7 and HPV-18 E7 oncoproteins are potential tools to detect the vast majority of cervical adenocarcinomas.

TAXONOMY AND HPV DATABASES

SESSION 32

PROGRAMME

THE 25TH INTERNATIONAL PAPILLOMAVIRUS CONFERENCE MAY 8-14 2009, MALMÖ, SWEDEN

TIME NUMBER TITLE

2009-05-14

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K1-3

ROOM

O-32.01 PAPILLOMAVIRUS TAXONOMY: REPORT OF THE ICTV STUDY GROUP.

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Papillomaviruses are described as "types", and the terms "serotype" and "subtype" should be avoided. A new PV type is described as a completely cloned PV genome whose L1 nucleotide sequence differs >10% from previously designated PV types, and designated by letters indicating the English or scientific name of its host (e.g. CRPV, cottontail rabbit papillomavirus). A new HPV type is established, when a complete genome and sequence is submitted to the Reference Center for Papillomaviruses, Heidelberg, for confirmation and assignment of a number, a system not yet adopted for non-human PVs.

Classification of PV "types" is regulated among PV researchers, while the official classification on the taxonomic levels of "species", "genus", and "family" is conferred by the International Committee on Taxonomy of Viruses (ICTV), which nominated the authors of this abstract as members of a study group to provide an interface between PV experts and the ICTV. Past achievements of this interaction were the acceptance of the family Papillomaviridae, separate from the Polyomaviridae, a consensus that "PV types" are not "PV species" in spite of significant sequence diversity and evolutionary age even between related PV types, grouping of PV types into numerically distinguished species based on relationship and biology, and of PV species into genera designated by Greek letters. For example, HPV-16, 31 and five other HPV types form species 9 in the genus Alpha-papillomavirus. (Details published in de Villiers et al., Virology 324, 17, (2004)).

Pending taxonomic concerns address the exhaustion of the Greek alphabet due to the finding of numerous new animal PVs, the classification of new HPV types with genomic idiosynchrasies setting them apart from established HPV genera (alpha, beta, gamma, mu and nu HPVs.), and a nomenclature for variants of HPV types.

O-32.02

PAPILLOMAVIRUS EPISTEME (PAVE): A COMPREHENSIVE PAPILLOMAVIRIDAE DATABASE AND ANALYSIS RESOURCE

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The PapillomaVirus Episteme (PaVE) has been established to provide information and analysis resources to the scientific community for research on the Papillomaviridae family of viruses. The overarching goal of the PaVE is to provide the core components that will accelerate scientific progress and ultimately our understanding, detection, diagnosis, and treatment of diseases caused by papillomaviruses. The PaVE consists of a relational database and web applications that support the storage, annotation, analysis, and exchange of information. As much as possible, the PaVE adopts an Open Source software approach and emphasizes integration and reuse of existing tools. The planned first release will consist of over 70 complete genomic sequences of various genera, species and strains from the Papillomaviridae family. Viral sequences, protein structures, and annotations have been extracted from publicly available databases. The PaVE also provides analytical tools that add value to the data and aid in discovery. These include tools for the comparative analysis of different viral isolates as well as visualization tools to better display the results of various analyses. In this presentation we debut the initial development of what will become a comprehensive resource that facilitates the process of extracting knowledge from the distributed Papillomaviridae data. The seamless integration of the data and the analytical tools will assist in the identification of potential targets for papillomavirus vaccines and the development of therapeutics and diagnostics.

O-32.03

EVOLUTION OF PRIMATE ALPHA PAPILLOMAVIRUSES

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Non-human primate PVs have been detected from a wide range of apes and monkeys. All characterized primate PVs cluster into distantly separate genera- alpha (genital/mucosal), beta- and gamma-PVs (cutaneous). This suggests that divergence of a common ancient PV into different host ecosystems (e.g., tissue tropisms-genital/mucosal vs cutaneous) must have existed prior to the primate host speciation events. The observation that primate PVs infecting different hosts do not form distinct monophyletic groups challenges the notion of strict virus-host co-evolution. Among the alpha papillomaviruses, over 20 types have been detected in the genital/mucosal epithelia of non-human primates; all of which cluster into 5 clades related to, yet separate from HPVs. Using a Bayesian Markov chain Monte Carlo method and the previously published evolutionary nucleotide change rates of feline PVs, we calculated the approximate divergence times of primate alpha PVs from their most recent common ancestors (MRCAs). Results indicate that the common ancestor(s) of primate PVs successfully evolved to colonize distinct host ecosystems around 64 million years ago (Mya), the period of time shortly after the divergence of primates and rodents (70 Mya). Approximately 24-37 Mya, a variety of primate alpha PV common ancestors may have existed, evolving to eventually colonize more specific host ecosystem within primates (e.g., external genital tissue). The divergence times of genital macaque PVs and chimpanzee PVs from the common ancestors of human PVs correspond to the time periods encompassing the evolution of their hosts. This molecular clock data strongly supports the ancient virus-host co-evolution of primate PVs and their hosts within the alpha PV lineage. By applying host species divergence times, we have predicted the evolutionary rates for each alpha PV ORF, which is consistent with those extrapolated from feline PVs. A timescale model of alpha PV evolution is proposed encompassing genera, species and variant PV genome emergence.

O-32.04 MODELING CARCINOGENIC HPV GENITAL INFECTIONS AND DISEASE WITH RHPV1

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Rhesus papillomavirus type 1 (RhPV1) causes sexually transmitted infections (STIs) in rhesus macaques with disease similar to carcinogenic HPVs in human genital tracts. RhPV1 E6, E7, and L1 share high homology with the same proteins in HPV16. RhPV1 E5, E6, and E7 expression transforms cells in vitro. We find RhPV1 genomes are replication competent in rhesus genital epithelial cells (RGECs). The RhPV1 early promoter and RNA structures are conserved with aspects unique and functionally important to carcinogenic genital HPVs. High-titer RhPV1 virion stocks made via the 293TT system are infectious in RGECs. The genital tracts of five RhPV DNA and Ab negative female macaques were exposed to RhPV1 virions (1e9 vge/animal). Sampling was every 8 weeks up to 6 months, 10 and 12 months. ELISA shows 4/5 animals sero-converted by 2 months post RhPV1 exposure, and 5/5 sero-converted by 4 months, similar to anogenital HPV incident infections. Pap analysis was normal for all animals at 0 and 2 months. Thereafter, one animal was diagnosed with ASC-US and two different animals with LSIL indicative of RhPV1 infection. One animal with an LSIL resolved at the next sampling. One animal demonstrated persistent LSIL in three consecutive samplings spanning 6 months. Initial PCR data detecting RhPV1 viral genomes correlate well with the Pap results. In summary, our data are consistent with RhPV1 exposure, clinical viral infection (potential persistence), and clinical pathogenesis analogous to carcinogenic HPV STIs in humans. The rhesus model provides advantages over current animal models: natural genital infections with an HPV homologue; genetic homology of macaques to humans (i.e., anatomical, physiological/hormonal, and immunological responses). Lastly, the model provides for PV infection assessment in an AIDS-related immunosuppressive context conferred by SIV infection.

O-32.05

NOVEL HPV SEQUENCES IN CERVICAL CARCINOMA CONTAINING SINGLE HPV GENOTYPES

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Background: The identification of HPV genotypes in cervical carcinoma specimens is crucial to elucidate the role of HPV in cervical carcinoma development. HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82 have already been reported to be present as a single infection in cervical carcinoma specimens. However, the carcinogenic potential of other HPV types is still unclear.

Objectives: The aim of this study was to assess the presence of 1) novel HPV sequence variants of known (oncogenic) types, and 2) reported HPV types not (yet) classified as oncogenic or as non-oncogenic, as a single genotype in cervical carcinoma.

Methods: The established SPF10PCR/DEIA/LiPA25 system (version 1) was used to analyse 11,248 cervical carcinoma specimens. Based on the algorithm SPF10PCR/DEIA-positivity (general HPV positivity) and LiPA25-negativity (for HPV6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68/73, 70, and 74), novel HPV sequence variants or low prevalent HPV types were selected for sequence analyses in the L1 region.

Results: 96 SPF10PCR/DEIA-positive cervical carcinoma specimens remained untypeable by the algorithm. BLAST database comparison revealed 28 unique sequences that did not show a complete match with any reported (oncogenic) HPV sequence. In addition, 68 sequences were identical to previously reported HPV types which have not (yet) been classified as oncogenic (i.e., HPV20, HPV26, HPV30, HPV34, HPV61, HPV67 and HPV69). The LiPA25 test also identified non-oncogenic HPV types (i.e., HPV70, HPV6, HPV11, HPV42, HPV44, and HPV74) present as a single HPV genotype.

Conclusions: The SPF10PCR/DEIA/LiPA25 system combined with sequence analyses proved to be an excellent algorithm in cervical carcinoma specimens to identify the presence of single HPV sequences that are novel, not previously classified as oncogenic or non-oncogenic.

O-32.06

ALPHA AND BETA PAPILLOMAVIRUSES DIFFER IN SYNONYMOUS CODON USAGE

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BACKGROUND: Papillomaviruses use rare codons with respect to the host. This is the basis for the commonly used technique of "codon optimization" to allow improved expression in in vitro systems. The success of that work led us to become interested in codon manipulation as a tool to improve immunogenicity of the Cottontail Rabbit Papillomavirus (CRPV) in vivo. Our observations that synonymous codon changes in the CRPV genome can result in phenotypic changes led us to become interested in synonymous codon usage of the alpha and beta papillomaviruses.

OBJECTIVES: We wished to determine if alpha and beta papillomaviruses utilize the same codons; we hypothesized that differential codon usage might help to explain tissue and species specificities.

METHODS: Using Chi Square analysis on sequence data available from GenBank, we compared codon usage between different genes of the same virus and between the same genes of different viruses. We chose to focus on the E1, E2, L1 and L2 genes, as they are large enough to allow for good statistical analysis.

RESULTS: We found that certain amino acids code differently in different genes of the same virus. For example, in the alpha viruses, serine codes the same in E1 and E2 and in L1 and L2 but the codings used for the E1/E2 and L1/L2 pairs are different from each other. On the other hand, in the beta viruses, serine codes differently in E2 relative to the codings in the other three proteins.

CONCLUSIONS: Alpha and beta viruses can be separated by their differential codon usage for serine. Other differences between the two groups were also found. We hypothesize that these differences may hold clues to tissue specificities.

O-32.07

CHARACTERIZATIONS OF NINE MACACA FASCICULARIS PAPILLOMAVIRUS (MFPV) COMPLETE GENOMES

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INTRODUCTION: Related alpha-PVs have been identified in Old World primates; however, few studies have systematically characterized genital PVs from non-human primate populations.

METHODS: Through sequencing of the MY region within the L1 ORF, we identified 9 distinct types of PVs from exfoliated cervicovaginal cells of female cynomolgus macaques (Macaca fascicularis). These included 5 novel genotypes and 4 previously identified genotypes found in rhesus macaques (Macaca mulatta) (RhPV-d, RhPV-a, and RhPV-1) and cynomolgus macaques (MfPV-a). Type-specific primers were designed to amplify the complete genoms of these novel types using a overlapping PCR method.

RESULTS: Four MfPVs were associated with cervical intraepithelial neoplasia (CIN). The most prevalent virus type was MfPV-3 (formerly RhPV-d), which was identified in 60% of animals with CIN. In addition, the complete genomes of variants of MfPV-3 and RhPV-1 were characterized. These variants are 97.1% and 97.7% similar across the L1 nucleotide sequences with the prototype genomes, respectively. Sequence comparisons and phylogenetic analyses using multiple algorithms indicate that these novel MfPVs cluster together within the α 12 PVs (> 75% similarity across the L1 nucleotide sequences). The closest HPVs to the α 12 species are the α 9 (e.g., HPV16) and α 11 species (e.g., HPV34), indicating these types may share a most recent common ancestor (MRCA).

CONCLUSION: Our data expand the molecular diversity of the non-human primate PVs and sugget a novel aspect of primate PV evolution.

O-32.08

TOWARDS A TIME SCALE FOR THE EVOLUTION OF PAPILLOMAVIRUSES

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O Bininda - Emonds, Carl von Ossietzky Universität Oldenburg, Oldenburg, Germany
M Göker, Universität Tübingen, Tübingen, Germany
I Nindl, German Cancer Research Center-Charite, Heidelberg-Berlin, Germany
M Gottschling, Ludwig-Maximilians-Universität München, Munich, Germany

Background A number of evolutionary mechanisms drive papillomavirus (PV) diversification, including co-divergence with their hosts, colonisation of a new host species by horizontal transmission, establishment of new ecological niches within the same host (adaptive radiation), and recombination. However, a comprehensive evolutionary scenario of PV has not been provided so far. Objectives To bring PV evolution into a temporal context. Methods We compared the phylogenetic relationships of PV to those of the corresponding hosts they infect, using different topology-based and distance-based algorithms. PV phylogeny was reconstructed using maximum likelihood methods (RAxML) and Bayesian inference (BEAST). Results Global co-divergence was statistically supported, but topological consistencies between the phylogenies of the hosts and of the virus-host associations. Translating this information into temporal references, we inferred a time scale for the evolution of the PV. The last common ancestor of PV infecting mammals was dated to some 70-110 million years ago (Mya) and the appearance of the four major PV supertaxa to 50-70 Mya. Conclusion The early evolution within PV superclades appears strongly influenced by evolution within the mammalian hosts. However, a more systematic sampling of viral diversity, and a more thorough analysis of host specificity, are still wanting and will provide key information about PV evolution.

POSTER ABSTRACTS SESSION 32

POSTER SESSION V THURSDAY 10.00

P-32.09

THREE NOVEL CANINE PAPILLOMAVIRUSES SUPPORT TAXONOMIC CLADE FORMATION

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More than hundred human papillomaviruses (PVs) are identified and sequenced on their whole genome. Most of these human PVs can be classified into three distinct genera, the alpha- beta-, and gamma-PVs. Noteworthy, only one or a few PVs have been identified for each individual animal species. Yet, four canine papillomaviruses (COPV, CPV2, CPV3, and CPV4) are described and their entire genomic sequences published. Based on their sequence similarities, they belong to three distinct genera.

DNA was isolated from one case of pigmented plaques, one case of inverted papilloma and one case of in situ squamous cell carcinoma. Circular DNA was amplified from the lesional skin by rolling circle amplification, and PV genomic sequences were determined. Analysis of the sequences revealed all the conserved sequence elements of known PVs as homologues of three not yet described viruses. Therefore, we designated the new viruses as CPV5, CPV6, and CPV7.

The genomes of all seven known CPVs were analysed and compared in order to identify genetic characteristics of these viruses and their proposed genera. Interestingly, phylogenetic analyses revealed that CPV5 grouped well with the unclassified CPV3 and CPV4, CPV6 with COPV (lambda-PV), and CPV7 with the yet unclassified CPV2. Pairwise sequence alignments of the corresponding major capsid protein gene further support these classifications. CPV5 shares 71.1% and 63.5% identity to CPV3 and CPV4, respectively. CPV6 shares 65.9% to COPV and CPV7 66.4% to CPV2. An allocation of CPVs into so far three distinct genera could therefore be supported. Thus similarly to human PVs, it might be that the known and yet unknown canine PVs are related and form just a few clades or genera.

P-32.10

PHYLOGENETIC CHARACTERIZATION OF ISOLATED NOVEL CHIMERIC CETACEAN PAPILLOMAVIRUSES

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Background Cetacean PV (Omicronpapillomavirus) are closely related to Alphapapillomavirus in early genes analysis and share similar properties such as a common viral tissue tropism. In late genes analysis, Omicronpapillomavirus and Xipapillomavirus are closely related that can be explained by co-divergence in the Cetartiodactyla with their hosts. Incongruent tree topologies between early and late PV genes phylogenies have been explained by recombination events in the past. Objectives If recombination has taken place in cetacean PV, non-recombinant viruses closely related either to Alphapapillomavirus in late genes phylogenetic analysis, or to Xipapillomavirus in early genes analysis, are to be expected.

Methods We isolated and sequenced the complete genomes of five novel PV types from cetacean genital and esophageal lesions using the rolling circle amplification technique. Phylogenetic analyses were performed using Maximum Likelihood and Bayesian approaches.

Results Complete genomes were isolated from Delphinus delphis, Lagenorhynchus acutus, and Phocoena phocoena, designated DdPV, LaPV, PpPV-1, PpPV-2, and PpPV-4, respectively. As inferred from early genes analyses, Omicronpapillomavirus including the five novel types constituted the sistergroup of Alphapapillomavirus. Analyzing late genes, Omicronpapillomavirus (with the exception of PpPV-4) were the closest relatives of Xipapillomavirus. PpPV-4 was the only Omicronpapillomavirus type that was closely related to Alphapapillomavirus also in late genes analysis.

Conclusions With the only exception of PpPV-4, the known cetacean PV appear as chimeric viruses, consisting of genome elements either of close relatives of mucosotropic Alphapapillomavirus (i.e., early genes) and of cutaneotropic Xipapillomavirus (i.e., late genes), respectively. PpPV-4 appears as the only known non-recombinant cetacean PV-type and a putative relative of a donor, which has contributed the early genes to such chimeric viruses.

P-32.11

MACACA FASCICULARIS PAPILLOMAVIRUS TYPE 1(MFPV-1): A BETA-GENUS NON-HUMAN PRIMATE PV

Sj Ghim, James Graham Brown Cancer Center, University of Louisville, Louisville, KY, USA K Hopper, Mannheimer Foundation, Homestead, FL, USA K Van Doorslaer, The Albert Einstein College of Medicine and The Albert Einstein Cancer Center, Bronx, NY, USA J Sundberg, Jackson Laboratory, Bar Harbor, ME, USA J Joh, James Graham Brown Cancer Center, Louisville, KY, USA Ab Jenson, James Graham Brown Cancer Center, Louisville, KY, USA

Papillomaviruses (PVs) are a group of small, non-enveloped DNA viruses that cause mucosal or cutaneous neoplasia in a variety of animals. While most papillomas will regress spontaneously, papillomas may persist or undergo malignant transformation. Aggressive, persistent, and extensive warts were observed on the hands and feet of a Cynomolgus macaque (Macaca fascicularis). The presence of PV in the wart biopsies was identified by immunohistochemistry and PCR amplification of PV DNA. The genomic DNA of this PV was cloned, sequenced, and designated as Macaca fascicularis papillomavirus type 1 (MfPV-1). Its genome was 7,588 base pairs (bps) in length and the organization of its putative open reading frames (ORFs) (E1, E2, E6, E7, L1, L2, and E4) was similar to other PVs. MfPV-1 has a short non-coding region (NCR) of 412 bps. Molecular analysis of MfPV-1 genomic DNA classified it into the beta-PV genus, to which all Epidermodysplasia verruciformis (EV)-type PVs belong. Diseases caused by PVs of the beta-genus are usually associated with natural or iatrogenic immunosuppression. The genomic characterization performed in this study shows that MfPV-1 clusters within the beta-PV genus and also contains EV-type specific motifs in its NCR. Further characterization of this virus and its host interactions may allow us to develop a non-human primate model for beta-genus HPVs, a genus populated by HPV types causing EV.

P-32.12 THE HPV16 GENOME EVOLUTIONARY STATUS AND AMINO ACID VARIANTS

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Background: Nucleic acid sequencing data show that many natural HPV variants exists, and some of these are associated with amino acid changes in functional and antigenic domains and are liable to introduce unique biological and immunogenic properties, conferring differences in evolutionary status and an unexpected divergence.

Objectives: The study analyzed the HPV16 genome polymorphisms and evaluated the evolution status among cervical cancers in China.

Methods: Genetic diversity of HPV16 genome was estimated by calculating pi using DnaSP 4.004. Tajima's test was performed to determine factors influencing diversity of HPV. Haplotype network was constructed using Network 4.109. Neighbor joining trees were constructed using the Kimura's two-parameter method.

Results: Median joining network analysis of HPV16 in cervical cancer group showed that these coding regions had low diversity (E2: pi=0.00752, Tajima's D=-0.67519, p>0.01; E5: pi=0.00605, Tajima's D=-1.51622, p>0.01; E6: pi=0.00758, Tajima's D=-1.17258, p>0.01). The common variant among E2 coding region was P219S (nt3410C \rightarrow A and or T), and median network analysis showed that the haplotype with 3410T and haplotype which possessed C3410T, T3384C, G3449A, T3524C were more frequent and maybe had the strongest fitness. I39L and I60V were observed frequently in E5 coding region. Phylogenetic analysis showed that the two mutants made the evolution cluster into a clade, which accounted for 89.9% of E5 haplotypes. Among E6 haplotypes, the most prevalent variants were Asian variant (nt178 T \rightarrow G, 64.4%; nt178A, 2.97%) and European variant (nt350T, 22.8%; nt350G, 6.9%), accounting for 68% and 30%, respectively, whereas African variant only accounting for 2%.

Conclusion: E5 and E6 code regions may be subjected to purifying selection (E5: Ka/Ks=0.485; E6: Ka/Ks=0.436), whereas E2 code region may be evolved neutrally (Ka/Ks=1.08). The two mutants I39L and I60V of E5 ORF may make the HPV16 have more advantages to escape from immunological surveillance to survive and spread.

P-32.13

L1 POLYMORPHISM OF HPV TYPES RELATED TO HPV-16.

G Cornut, CHUM, Montreal, Canada S Gagnon, CHUM, Montreal, Canada C Hankins, McGill, Montreal, Canada D Money, Un of British Columbia, Vancouver, Canada K Pourreaux, , Montreal, Canada E Franco, McGill, Montreal, Canada F Coutlée, CHUM, Montreal, Canada

Background. The capsid of human papillomaviruses (HPV) is encoded by the L1 gene. Surface loops of L1 are exposed to the selective pressure from the host immune system. Objectives. The polymorphism of L1 genes from phylogenetically-related types 16, 31, 33, 35 and 52 is described and compared.

Methods. Cervicovaginal lavages collected from 1055 participants (732 HIV-seropositive, 323 HIV-seronegative) in the Canadian Women's HIV Study were screened initially for HPV DNA with consensus PCR. Cervical samples (n=351) positive for HPV types 16 (n=74), 31 (n=74), 33 (n=37), 35 (n=58) or 52 (n=108) were further analysed by amplifying and sequencing three overlapping regions of L1.

Results. A smaller number of variants in proportion to the number of isolates tested was obtained for HPV-33 (24.3%) compared to HPV-35 (60.3%, p=0.001, z statistic) or HPV-31 (56.4%, p=0.002), and between HPV-52 (32.1%) and HPV-31 (p=0.002) or HPV-35 (p=0.002), while the proportion of variants for HPV-16 was intermediate at 40.5%. Most of the non-synonymous variations were localised inside the putative hypervariable L1 loop regions. Synonymous variations were encountered in a mean of 1.7% (95% CI 1.1-2.3) of nucleotides in the loops and of 2.4% (95% CI 1.2-3.7) outside the loops. Non-synonymous variations were encountered in 1.8% (95% CI 1.1-2.5) of nucleotides within the loops and 0.2% (95% CI 0-0.4) of nucleotides outside the loops. The distance between HPV variants was greater in the five putative surface-exposed loops of L1 than outside the loops (p<0.01).

Conclusions. The frequency of non-synonymous variations was thus similar to that of synonymous variations within or outside the loops but was significantly smaller in extra-loop regions. Those findings suggest a greater genetic variability in loop sequences of L1, possibly because of the selective pressure of the immune system.

P-32.14

INTRATYPIC VARIANTS OF HPV TYPES 16/18/52/58 IN TAWAIN

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Human papillomavirus (HPV) 16 and 18 are most oncogenic HPV types, and HPV 52 and 58 are very prevalent in Asian populations. This study aims to examine the intratypic variants of HPV 16, 18, 52, and 58 in Taiwan. A total of 11,923 women were enrolled from seven townships in 1991-1993. The HPV DNA in their cervical cells was detected and typed by EasyChip HPV blot. A total of 807 participants were infected with one or more types of HPV 16, 18, 52 and 58 at enrollment. The intratypic variants of HPVs were determined in 548 participants who had high viral load in cervical cells by the polymerase chain reaction sequencing of the long control region (LCR) and E6 and E7 genes. We observed nucleotide exchanges in each of these four HPV types. LCR was more variable than E6 or E7. The Asian variant was the most prevalent variant (83.2%) of 161 HPV 16 samples; while the Asian-American variant was the most prevalent variant (81.3%) of 96 HPV18 samples. Non-prototype variants were detected in 243 (99.2%) of 245 HPV52 samples. These non-prototype variants of HPV 52 were found to cluster into two lineages based on the analysis of phylogenetic trees. The prototype-like variants of HPV58 were found only in 22 (20.0%) of 110 HPV 58 samples. It was concluded that frequency distributions of intratypic variants of HPV 16, 18, 52 and 58 were different from those reported in European and American populations.

P-32.15 ISOLATION AND GENOMIC CHARACTERIZATION OF THE FIRST INSECTIVORAN PAPILLOMAVIRUS

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Background Knowledge about biological diversity is the prerequisite to reliably reconstruct the evolution of pathogens such as papillomaviruses (PV). However, complete genomes of non-human PV have only been cloned and sequenced from 8 out of 18 orders within the Placentalia, although the host-specific variety of PV is considered much larger. Objectives To isolate and sequence the complete genome of the first insectivoran PV-type from hair follicle cells of the European hedgehog (Erinaceus europaeus), designated EHPV.

Methods We conducted phylogenetic analyses (Maximum Likelihood criterion and Bayesian inference) with the genomic information of a systematically representative set of 67 PV-types including EHPV. Results As inferred from amino acid sequence data of the separate genes E1, E2, and L1 as well as of the gene combination E6–E7–E1–E2–L1, EHPV clustered within the beta-gamma-pi-xi-PV supertaxon and constituted the closest relative of Betapapillomavirus infecting primates. Beside the typical organization of the PV genome, EHPV exhibited a 1,172 bp long, non-coding region between the E2 and the L2 open reading frames. Conclusions This trait has been previously described for the only distantly related Lambdapapillomavirus, but a common evolutionary origin of both non-coding regions is unlikely. Our results underscore the modular organization of the PV genome and the complex natural history of PV.

P-32.18

THE NEED FOR PROTECTING PAPILLOMAVIRUSES NOMENCLATURE AND TAXONOMY FROM COLLAPSE

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Recording the diversity of non-human papillomaviruses (PV) still remains in the fledgling stages. Viruses from not more than 50 different host species have been isolated and (partly) sequenced, but it is assumed that all of the 20,000 Amniota species potentially harbour a number of specific PV types.

The prospect for myriads of new PV types that will be discovered in near future due to improved sequencing techniques will challenge nomenclature and taxonomy of PV in several different ways: (1) Regarding nomenclature: (a) obligatory rules for naming PV-types are currently lacking (e.g., the names COPV, CfPV, and CPV have been designated to viruses isolated from the single host Canis familiaris); (b) changes of a host's scientific name lead to puzzling PV names of the same host (e.g., MnPV-1 and McPV-2 found in Mastomys coucha); (c) PV from different hosts but with identical initials have been likewise confused (e.g., there are three viruses with the name "CcPV" each isolated from Pan troglodytes, Capreolus capreolus, and Caretta caretta. (2) Regarding taxonomy, there are clearly more PV groups to be expected than Greek (and presumably even Chinese) letters are available to name them.

Simple solutions for these foreseeable problems are not in sight at present. As a consequence, we emphasise the need of an open discussion about PV nomenclature and taxonomy in order assure for the unambiguousness of scientific names in future. As it is the case for cellular organisms, a non-ambiguous naming system is the necessary prerequisite for reproducible, resuming, and experimental approaches, particularly in times of an exponential increase of our knowledge about viral diversity.

Declaration of conflicts of interest for speakers at the 25th International Papillomavirus Conference

The following speakers declare no conflict of interest:

Abigail I. Boster Adam Raff Ahti Anttila Alba Lucia Combita Alcina F Nicol Ali Rowhani-Rahbar Ameli Tropé Ana Paula Lepique Ann Roman Anna Manawapat Arne Stenlund Bakary S Sylla Berit Hammas Bettie M Steinberg Bladimiro Rincon Orozco Carolina Porras Carsten Lambert Cary Moody Charlotte Hellsten Cheng-Ming Chiang Christina Schellenbacher Christine Bergeron Cornelia Trimble D.C Rijkaart Deblina Datta Emma Ivansson Esther Roura Fatima Azerkan Felipe Andres Castro Florianne Henken Francesca Carozzi Francoise Thierry Gareth Maglennon Hans-Ulrich Bernard Harald zur Hausen Helena Faust Henry Kitchener Herbert Pfister Ibrahim I Daud Ignacio González Bravo Ilkka Kalliala Ingrid Hoffmann Ioanna Tsoumpou J Mooren J.P Klussmann

Jameela Khan Jasmin Ferley Jei-Hwa Yu Jia Ji Johannes Berkhof Johannes Bogaards John H Lee Jolanta Dickute Julia Gage Junona Moroianu Kai Schäfer Karen Smith-McCune Karin Hellner Kazunori Nagasaka Kristina M Michael Lara Termini Laura M Fahey Lauri Markowitz Leen J Blok Lena Silfverdal Loane Decrausaz Louise Kuhn Lysann Schädlich M Kocken M Saveria Campo M.N.C de Koning Maciej Lazarczyk Maria Kyrgiou Mario Schelhaas Martin J Sapp Melanie Liesenfeld Michael J Conway Mireia Diaz Sanchis Miriam Reuschenbach Mona Saraiya Murat Gök Nancy M Cladel Nicolas Wentzensen Nilam Sanjib Banerjee Patricia Martine Day Patrick Walker Paul F Lambert Peggy de Vos van Steenwijk Philip Davies Philip E Castle **R.D.M Steenbergen** Rachel Katzenellenbogen Rachel Schowalter/Christopher Buck Robert D Burk Rosita Accardi-Gheit

S.J Piersma Scott LaMontagne Silvia Franceschi Sophie Bellanger Stefanie Klug Sveinung Sørbye Takashi Yugawa Thomas Haugen Tilo Senger Tim Waterboer Tina Dalianis Ulrike Wieland Wenqiang Wei Yentram Huyen Yuzo Arima Zhi-Ming Zheng Zigui Chen Zizipho Mbulawa Alejandro M. Garcia Carranca Fang-hui Zhao Hang Yuan Beibei Lu Lisa G Johnson Björn Strander Anil Chaturvedi

ъреакет С	I ype of interest, eg. Fatent, shares, employment, association, payment (including details on any compound, work, etc.)	Name of commercial entity	Belongs to you, partner or unit?	Current interest? (or year ceased)	Is there anything else that could affect your objectivity of independence in the meeting or work, or the perception by others of your objectivity and independence?
Alan G Nyitray	My institution expects to receive research fundings in the future (with me as the principal investigator) to assess the incidence and persistence of anal HPV in men.	Merck & Co., Inc	Unit	Current	
Andreas M Kaufmann	Payment of congress fee and travel Speakers honoraria Advisory board honoraria	GSK, SPMSD GSK, SPMSD GenProbe	Myself Myself Myself	Current Current 2007/2008	
Ann Burchell	Supplementary and unconditional funding support for the HITCH Cohort Study was provided by Merck-Frosst Canada Ltd and Merck & Co Ltd. The funding organization had no role in the design and conduct of the study; the collection, analysis, and interpretation of the data; or the preparation, review, or approval of this conference presentation	Merck-Frosst Canada Ltd and Merck & Co Ltd	Unit	Yes	
Ann Nielsen	Free provision of LiPA kits	Innogenetics	Unit	2005	
Anna Giuliano	Consultant fees Grant Speaker's Bureau Consultant	Merck Merck Merck GSK		Current Current Current Ended 2007	
Anna-Barbara Moscicki	Advisory Board honorarium	Merck	Myself	+	
Anne Szarewski	Advisory boards/conference hospitality/lecture fees	SPMSD, GSK, Hologic	Me	Ongoing	

The following speakers declare conflict of interest:

Speaker	Type of interest, eg. Patent, shares, employment, association, payment (including details on any compound, work, etc.)	Name of commercial entity	Belongs to you, partner or unit?	Current interest? (or year ceased)	Is there anything else that could affect your objectivity of independence in the meeting or work, or the perception by others of your objectivity and independence?
	Funding for research in department	Qiagen, Hologic, GenProbe, Genomica, mtm Laboratories, Norchip, Roche Diagnostics	Unit	Ongoing	
	Investigator in HPV vaccine trials	GSK	Me/unit	Ongoing	
Carina Eklund	Research grants to the group (but not for this study)	Merck	Unit	Current interest	
Charles JN Lacey	I was a principal investigator at Imperial College, London, on the Merck/SPMSD Future	Merck/SPMSD	CJNL	Ceased 2008	
	II phase III trial of a quadrivalent HPV vaccine. I have received one-off consultancy fees from	SPMSD	CJNL		
	SPMSD in the past			The last was in 2006	
Christopher Fairley	Own shares in CSL Biotherapies the manufacturer for Gardasil	Superannuation fund	Both	Current	
	Have received honoraria from CSL Biotherapies	Self	Self	Last year	
	Research funding from CSL Biotherapies	University of New South Wales	Universityof NSW	Current	
Cornelis J Melief	Employment as Chief Scientific officer 75% of	Immune System		75% of	
	time	Activation		salary	
D.T. Geraets	Funding	Qiagen	N.A.	Yes	

Speaker	Type of interest, eg. Patent, shares, employment, association, payment (including details on any compound, work, etc.)	Name of commercial entity	Belongs to you, partner or unit?	Current interest? (or year ceased)	Is there anything else that could affect your objectivity of independence in the meeting or work, or the perception by others of your objectivity and independence?
Douglas R Lowy	An inventor of the technology that underlies the L1-based prophylactic virus-like particle (VLP) HPV vaccine and technology that underlies an L2-based prophylactic HPV vaccine. The NIH has licensed the technology for the L1 VLP vaccine to Merck and to GSK. NIH receives royalties from both pharmaceutical companies, and US Federal law entitles me to a limited share of the royalties. The L2-based technology is the subject of a cooperative research and development agreement between the NCI, Johns Hopkins University, and Shantha				
Elliot J. Androphy	Shares	Elagen Inc	Self	Yes	
Elmar Joura	Research funding (through institution), lecture fees	Merck, SPMSD, GSK			
Geoffrey P Garnett	Consultancy Consultancy Consultancy	GSK Merck SPMSD	Me Me Me	Yes 2007 2007	
Guglielmo Ronco	Minor payment for participation to two internal scientific advisory workshops	GenProbe	Me	Ceased	
Gypsyamber D'Souza	Consultant Research Grant	Merck Co Merck Co	Self Self	Current Current	
Helen Trottier	Paid consultant for and has received travel assistance from GSK	GSK	To me	Travel assistance	

Speaker	Type of interest, eg. Patent, shares, employment, association, payment (including details on any compound, work, etc.)	Name of commercial entity	Belongs to you, partner or unit?	Current interest? (or year ceased)	Is there anything else that could affect your objectivity of independence in the meeting or work, or the perception by others of your objectivity and independence?
				2007. Consultancy 2005	
lan Frazer	Holder of several patents relevant to HPV vaccines and HPV immunotherapy	Merck, GSK, Uniquest, The	Me	Current	
	Derive royalties from the sale of HPV vaccines	university of Queensland,			
	Consult for Merck and GSK and give paid talks	Implicit Bioscience.			
	-	Coridon			
	Hold research funding (in kind vaccine contributions from Merck and GSK)				
Ivonne Rubio	Patent	DKFZ	No	Yes	
Jack Cuzick	Advisory Board/Speaker Bureau Personal support (all but Merck)	Qiagen Roche	Self	Current	
		GenProbe Abbott			
		Merck			
Jacques Archambault	Member of the scientific advisory board (SAB) of Anaconda Pharma (Paris, France)	Anaconda Pharma	No	Current	
Jessica Kahn	Investigator on a government-funded grant, for which Merck provides vaccine and immunogenicity testing	Merck	Myself	Yes	
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Speaker	Type of interest, eg. Patent, shares, employment, association, payment (including details on any compound, work, etc.)	Name of commercial entity	Belongs to you, partner or unit?	Current interest? (or year ceased)	Is there anything else that could affect your objectivity of independence in the meeting or work, or the perception by others of your objectivity and independence?
Jie Ting	Denise T Kruzikas is an employee of GSK	GSK	D.T Kruzikas	Current	
	Jennifer Smith has received research grants, or contracts or consulting fees or honoraria for the last four years from GSK and Merck	GSK Merck & Co Inc	J.S Smith		
Jo Waller	In the past four years received honoraria for lectures and funding to attend meetings from GSK and SPMSD	GSK/Cervarix SPMSD/ Gardasil			
Joakim Dillner	Research grants Advisor/Consultant & Lecturer	Merck/SPMSD Merck/SPMSD	Unit Me	Current 2008	
	Grant reviewer	GSK	Me	2008	
Joel Palefsky	Research grant support	Merck and Co	My unit	Yes	
John T Schiller	Inventor of U.S. government owned patents and patent applications covering the VLP, L2, and pseudovirus-based vaccines and entitled to limited royalties from these patents as specified by U.S. law				
José Jeronimo					PATH has projects on VIA, HPV vaccination and HPV-DNA testing in several countries
Joseph Monsonego	Member Advisory Board	GenProbe Merck Roche Abbott Qiagen			Current

Speaker	Type of interest, eg. Patent, shares, employment, association, payment (including details on any compound, work, etc.)	Name of commercial entity	Belongs to you, partner or unit?	Current interest? (or year ceased)	Is there anything else that could affect your objectivity of independence in the meeting or work, or the perception by others of your objectivity and independence?
Julia Brotherton	Investigator on study which received unrestricted grant funding for HPV serology component of study performed at Merck for national Australian serosurvey. Study independently designed and analysed.	CSL/Merck	Me	2007	
	Investigator on WHINURS study that received unrestricted and equal grant funding from HPV vaccine manufacturers.	CSL and GSK	Me	2007	
Karen Canfell	Employed to September 2005 by a company developing cervical screening equipment. Since then, no association or employment with them and do not hold any shares. This entity does not have a direct interest in the work reported.	Polartechnics Limited	Myself	Ceased 2005	
Kathleen Irwin	Served on three expert advisory panels as paid expert on HPV	Merck Co		No. Ceased in 2006.	
Kristin Andersson	Research grants to the group (but not for this study)	Merck	Unit	Current	
Loretta Brabin	Research Grant Honoraria for presentations/meetings	GSK GSK	Unit Self	Jan 2009 2005-2008	
	Conference fees Honoraria for presentations/travel	GSK MSD	Self Self	2007 2006, 2008	
Louise T Chow	Two research applications pending Honoraria and travel expenses	Merck Merck	Self T Broker	Current 2008	
	Technical consultant	Kurabo Industries	T Broker	Future	
M.J.P Welters	Vaccine production and immunomonitoring of	ISA	1	Yes	

Speaker	Type of interest, eg. Patent, shares, employment, association, payment (including details on any compound, work, etc.)	Name of commercial entity	Belongs to you, partner or unit?	Current interest? (or year ceased)	Is there anything else that could affect your objectivity of independence in the meeting or work, or the perception by others of your objectivity and independence?
	clinical trial is partially financed	Pharmaceuticals			
Marc Arbyn	Travel funding for participation at conferences	SPMSD, GSK		2008	
Mario Sideri	Consultancy	GSK, Sanofi Pasteur, Quiagen/Digene , Innogenetics, MTM Lab	Unit (IEO)	Current	
Mark H Einstein	Unit has received grant funding for clinical trials that I have initiated or been the Montefiore PI from GSK, Merck, Third Wave Technologies (Hologic), Nventa, and Tigris. Advised consulted, or participated as a speaker, but do not receive any honorarium, from the following companies: GSK, Merck, Roche, Tigris, Nventa, PDS Biotechnology, Digene (Qiagen), and Cytyc (Hologic)				
Mark Schiffman	Employed by NIH/NCI and declare no personal financial conflict of interest. For organization: Costa Rican HPV Vaccine Trial is a long-standing collaboration between investigators in Costa Rica and the NCI. I am the NCI co-Project Officer and co-Medical Monitor. The trial is sponsored and funded by the NCI, the National Institutes of Health Office for Research on Women's Health, and the Ministry of Health of Costa Rica. Vaccine was provided	Glaxo Smith Kline	NCI	Current	

Speaker	Type of interest, eg. Patent, shares, employment, association, payment (including details on any compound, work, etc.)	Name of commercial entity	Belongs to you, partner or unit?	Current interest? (or year ceased)	Is there anything else that could affect your objectivity of independence in the meeting or work, or the perception by others of your objectivity and independence?
	by GSK. GSK also provides support for aspects of the trial associated with regulatory submission needs of the company. US government-owned HPV vaccine patents are licensed to GSK and Merck and result in royalties to NIH as specified by federal law. NCI investigators publish data from trial independently, with a separate set of monitoring authorities.				
Mark Stoler	Consultant Consultant	Merck Ventana Medical Systems, MTM laboratories	No No	Yes Yes	
Markus Schmitt	L.G received consulting fees from Qiagen and Abbott, V.D and C.C received consulting fees from Genprobe. M.S., M.P. and L.G. have submitted a patent application: Diagnostic transcript and splice patterns of HPV16 in different cervical lesions. Europe patent application EP08168608.1-222.				
Martin Kast	Payment Contract Research Research Collaboration	Wyeth Nventa Alphavax	Me Me Me	2007 2008 2009	
Martin Müller Mayumi	Patent Patent application on HPV E6 protein T cell	DKFZ NA	No UAMS	Yes No	

Speaker	Type of interest, eg. Patent, shares, employment, association, payment (including details on any compound, work, etc.)	Name of commercial entity	Belongs to you, partner or unit?	Current interest? (or year ceased)	Is there anything else that could affect your objectivity of independence in the meeting or work, or the perception by others of your objectivity and independence?
Nakagawa	epitopes and uses thereof				
Mel Krajden	Contract Research	Roche	N/A	Current	
	Contract Research	Qiagen	N/A	Current	
Merilyn Hibma	Member of GSK Advisory Committee, New Zeeland	GSK	No	2007- current	
Michelle A	US Patents: "Methods for detecting, titering,	PennState Univ			
Ozbun	and determining susceptibility to				
	"RhPV as a model HPV-induced cancers"	Univ Mexico			
		Science &			
		Technology			
Ole-Erik Iversen	Received honorarium through institution for	GSK and Merck	No	Yes	
	clinical vaccine trials, and teaching fee				
	Scientific Advisory Committee	Merck	No	Yes	
Patrick S Moore	MCV-related patents pending	Univ of Pittsburgh			
Pekka Nieminen	Member in External Endpoint committee of	GSK		Current	
	Chief colnosconist (Finland) in two HPV	GSK and MSD		Current	
	vaccine studies				
Peter L Stern	Independent Advisory Board Member	GSK	Self	Current	
Rhonda Christine Kines	Patent Filed	N/A	Me	Yes	
Richard Roden	L2 Patent royalties	Shantha	Me	Υ	Co-inventor of technology
		Biotechnics Acambis	Me	Y	for pseudovirion
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Speaker	Type of interest, eg. Patent, shares, employment, association, payment (including details on any compound, work, etc.)	Name of commercial entity	Belongs to you, partner or unit?	Current interest? (or year ceased)	Is there anything else that could affect your objectivity of independence in the meeting or work, or the perception by others of your objectivity and independence?
		PaxVax	Me	Y	licensed for internal use to Inviragen, GSK, Delsite
	Consultant	Merck	Me	2008	Biothechnologies, Dynavax Technologies,
	Educational Grant Funding	GSK	To my fellow	Y	Merck & Co, Large Scale Biology Corporation,
	Licensing fees	See below	Me	Y	Indian immunologicals, LG Life Sciences.
					Medimmune, Biotrin, Viropan, Ribovax
					(terminated), German
					Cancer Research Center (DKFZ), Arbor Vita
Robert G Pretorius	Grant for support of research	Merck Inc	Unit	Current	No financial support for any entity except SCPMG
i					(my employer)
Sam Ratnam	Research Grant	Merck Frosst Canada	Me	Yes	
S-E Olsson	Performed clinical studies Giving lectures	Merck SPMSD	No No	Current Current	
Sholom Wacholder	Presenting results of a pooled analysis of safety of Cervarix. Presentation includes data from PATRICIA, a study sponsored by GSK and from Costa Rica HPV Vaccine Trial (CVT). CVT is fully supported by the National Cancer				

Speaker	Type of interest, eg. Patent, shares, employment, association, payment (including details on any compound, work, etc.)	Name of commercial entity	Belongs to you, partner or unit?	Current interest? (or year ceased)	Is there anything else that could affect your objectivity of independence in the meeting or work, or the perception by others of your objectivity and independence?
	Institute (NCI), except that GSK provided the vaccine and paid for regulatory submission and				
	commercial licensure needs. CVT is being conducted autonomously from other trials of				
	Cervarix financed by GSK. NCI and Costa Rica				
	investigators retain the right to independently analyze and publish data from the trial. CVT is				
	the only publicly funded trial of an HPV				
	vaccine. USK provided data on pregnancy for this miscarriage analysis from their trial				
	PATRICIA. Following interim analysis,				
	including safety review, in December 2006 the				
	Data and Safety Monitoring Board (DSMB) of the NCI trial requested an assessment of				
	possible effects of the vaccine on miscarriages				
	performing a pooled post-hoc analysis of data from the two narallel trials Roth hoards				
	recommended that NCI prepare a manuscript				
	describing the results for publication in the				
	scientific literature. GSK scientists provided background information and data from				
	PATRICIA, and provided suggestions on the				
	methods, analysis and interpretation. CVT				
	investigators from NCI and Costa Rica prepared				
	this manuscript with input from the expert and consultants. GSK scientists commented on draft				

Speaker	Type of interest, eg. Patent, shares, employment, association, payment (including details on any compound, work, etc.)	Name of commercial entity	Belongs to you, partner or unit?	Current interest? (or year ceased)	Is there anything else that could affect your objectivity of independence in the meeting or work, or the perception by others of your objectivity and independence?
	manuscripts, but CVT investigators from NCI and Costa Rica made the final decisions about its contents. Presentation based on material in this manuscript.				
Shreva Kanodia	Therapeutic reagent (VRPs) and funding support was provided to Dr W.M Kast	Wyeth	Wyeth	Ceased in 2007	
Simon Dobson	Member of Advisory Board	Merck	Self	2008	Member of the National Advisory Committee on Immunization (Canada) 2000-2008. Member of the BC Immunization Sub-Committee 2006- present
Sjoerd H. van der Burg	Co-inventor on patents on synthetic long peptide vaccines Received support covering a period within the past 4 years for running clinical trials with synthetic long peptides as well as for immunomonitoring of these trials. There will also be support for this in the future, for new trials. Non-paid member of the strategy and steering committees of ISA Pharmaceuticals	ISA Pharmaceuticals ISA Pharmaceuticals ISA Pharmaceuticals	LUMC LUMC, Depts. of IHB, Gyne- Cology, Clinical Oncology Me	Yes Yes Yes	
Susanne Krüger Kjaer	Unrestricted grant Unrestricted grant	SPMSD Merck	Unit Unit	2008 2007	

Deaker	Type of interest, eg. Patent, shares, employment, association, payment (including details on any compound, work, etc.)	Name of commercial entity	Belongs to you, partner or unit?	Current interest? (or year ceased)	Is there anything else that could affect your objectivity of independence in the meeting or work, or the perception by others of your objectivity and independence?
	Unrestricted grant	SPMSD	Unit	2007	
Thomas R. Broker	Honoraria and some travel expenses for participating in four workshops or seminars 2006 (twice) and 2008 (twice)	Merck Vaccines Division	Self	No (2008)	Not likely. Any relations have been based on education and stimulating
	Technical consultant	Kuraho	Self	Future	research and develonment almost all
		Industries			were performed gratis, even paying my own travel
Toos Daemen	Minority share	ViciniVax	No	Yes	
	Part-time director pre-clinical research	ViciniVax	No	Yes	
W.G.V Quint	Funding	GSK	Me	Yes	
Jorma Paavonen	Research grants through the University Hospital Research Institute	GSK, MSD	Unit		
	Travel grants Consultation fees	GSK, MSD	JP		
Ana Cecilia Rodriquez	Vaccine was provided for our trial by GSK Biologicals, under a Clinical Trial's Agreement with the National Cancer Institute. GlaxoSmithKline also provides support for aspects of the trial associated with regulatory submission needs of the company under grant FDA-IND7920. The NCI and Costa Rica Investigators make final editorial decisions on this abstract; GSK has the right to review and comment				

Speaker	Type of interest, eg. Patent, shares, employment, association, payment (including details on any compound, work, etc.)	Name of commercial entity	Belongs to you, partner or unit?	Current interest? (or year ceased)	Is there anything else that could affect your objectivity of independence in the meeting or work, or the perception by others of your objectivity and independence?
Rachel Skinner	Principal investigator on sponsored clinical trial - budget provides to collect data (no salary) Advisory Board membership	GSK	Myself	Current	
	Travel grants	GSK, CSL	Myself	Current and past	
	Research grants	GSK, CSL CSL	Myself Myself	Past Current	
John Doorbar	Consultancy Collaborative Research	SPMSD GSK	To JD To UK MRC	Current Current	
Eduardo L Franco	Advisory Committee service Advisory Committee service Advisory Committee service Advisory Committee service Advisory Committee service Advisory Committee service Unconditional grant to supplement funding for research work conceived, initiated, and conducted by the speaker and his team (research that is not related to the commercial entity's products)	Merck GSK Roche Qiagen Gen-Probe Innovus Merck	Self Self Self Self Self Self	Current Current Current Current Current Current	My entire research program has been funded by Canadian and US governmental agencies and charities that use peer review to evaluate merit. I have received the following salary awards: Distinguished Scientist award from the MRC- Canada, National Scholar award from FRSQ- Quebec, and James McGill Professorship from McGill University.

Speaker	Type of interest, eg. Patent, shares, employment, association, payment (including details on any compound, work, etc.)	Name of commercial entity	Belongs to you, partner or unit?	Current interest? (or year ceased)	Is there anything else that could affect your objectivity of independence in the meeting or work, or the perception by others of your objectivity and independence?
Nicolas Van de Velde	Unrestricted grant	Merck Frosst Canada	Unit	Current	
Yurii B Shvetsov	Yurii B Shvetsov Pending research funding	Merck	Partner: B.Y. Hernandez	Yes	
Marc T Goodman	Pending research funding	Merck	Partner: B.Y. Hernandez	Yes	

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	Α
Abdul Hamid, N	
Abdullah, A	P-10.38, P-29.60
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Ackermann, M.	
Acs, N	
Actis, P	
Adadevoh, S	
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Allaire, G	P-16.10

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Ankur, K	P-18.09
Anson, JR	O-14.04, P-18.07
Anthony, GB	O-19.07
Antonio - Véjar, V	P-22.29
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Aoki, F	
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Arai, YP-04.34	Baladron,
Arbiza, JP-06.67	Balakhon
Arbyn, M O-19.01, O-21.07, O-26.08, P-19.10, P-19.20	Baldauf, J
Archambault, JO-24.06	Baldomer
Ardeleanu, CP-04.30	Baldwin,
Ardila, J	Baleja, J
Arduino, JMP-06.38	Ball, S
Arima, Y	Ballestero
Arnheim Dahlström, LO-22.04, P-10.07, P-22.09	Balogh, K
Arnold, M	Baltazar,
Arnold, S	Bandaru,
Arreola - Castro, YPP-31.24	Banerjee,
Arslan, A	Banister,
Arun Pokhrel, A	Banken, J
Asakawa, SP-03.35	Banks, L
Ashman, SP-19.13	Banura, C
Astudillo, AP-14.14	Baranosk
Atanasiu, VP-31.25	Barley - N
Atkins, K	Barnabas
Attner, PO-17.04, P-17.15	Barnett, J
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Aubin, F	Barros, N
Austin, NP-19.09	Barrow -
Auvinen, E	Bartellas,
Auvinen, P	Bartlett, S
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Ayuso, M	Barton - I
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Bachran, C	
Backes, D.M.	P-27.12
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Bae, J-H	
Bae, JH	P-03.46, P-06.42, P-31.18
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Baggio, ML	
Bahena - Román, M	
Bahtz, R	O-18.04
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Bailey, AS	
Bailey, R.C.	
Baker, J	P-18.32, P-31.20
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Baladron, I	
Balakhontseva, O.S.	P-18.33
Baldauf, J-J	P-29.31
Baldomero, J.	P-07.19
Baldwin, A	O-09.05
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Ballesteros, J	P-26.114
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Bininda - Emonds, O	O-32.08

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Blettner, M	
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Boardman, L	
Bobst, M	
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Bockstall, K	
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Boer, J. M.	
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Boissel, JP	
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Bollands, A	
Boller, K	
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Bonagura, V	
Bonde, J	
Bonde, R.K.	
Bonilla Saus, S.	
Bonsdorff, S	
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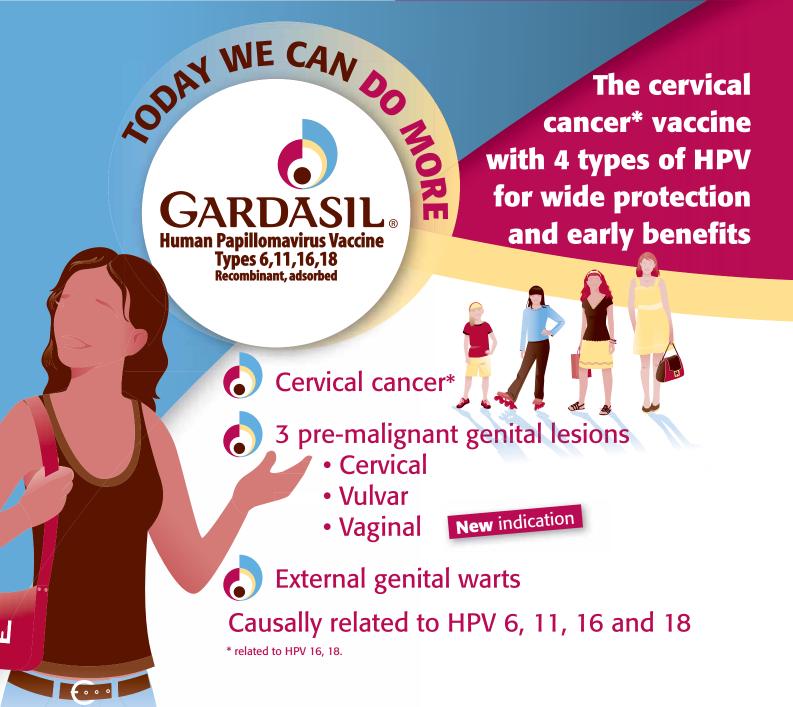
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Abridged Prescribing Information for use in the European Area. GARDASIL* (Human Papillomavirus Vaccine [Types 6, 11, 16, 18] (Recombinant, adsorbed)). *Refer to Summary of Product Characteristics for full product information.* **Presentation:** Gardasil is supplied as a single dose pre-filled syringe containing 0.5 ml of suspension. **Active ingredients:** Each dose contains L1 protein of HPV type 6 (20 µg), type 11 (40 µg), type 10 (40 µg) and type 18 (20 µg), adsorbed on amorphous aluminium hydroxyphosphate sulphate adjuvant. **Indications:** Gardasil is a vaccine for the prevention of premalignant genital lesions (cervical, vulvar and vaginal), cervical cancer and external genital warts (condyloma acuminata) causally related to Human Papillomavirus (HPV) types 6, 11, 16 and 18. The indication is based on the demonstration of efficacy of Gardasil in adult females 16 to 26 years of age and on the demonstration of immunogenicity of Gardasil in 9- to 15-year old children and adolescents. Protective efficacy has not been evaluated in males. The use of Gardasil should be in accordance with official recommendations. **Dosage and administration:** The primary vaccination series consists of 3 separate 0.5ml doses administered according to the following schedule: 0, 2, 6 months. If an alternate schedule is necessary the second dose should be administered to be administered by intramuscular injection. It is recommended that subjects who receive a first dose of Gardasil complete the 3-dose vaccination course with Gardasil. **Contraindications:** Hypersensitivity to any component of the vaccine. Hypersensitivity after previous administration of Gardasil. Acute severe febrile illnes. **Warnings and precautions:** As with all vaccines, appropriate medical treatment should always be available in case of rare anaphylactic reactions. Syncope (fainting) may follow any vaccination, especially in adolescents and young adults. Syncope, sometimes associated with falling, has occurred after vaccination with Gardasil. Therefore, vaccineees sh





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