28th International Papillomavirus Conference & Clinical and Public Health Workshops

Nov 30 - Dec 6, 2012 • San Juan, Puerto Rico

Abstracts of research presented at the conference selected by Edson Natal Fedrizzi (Universidade Federal de Santa Catarina) e Mauro Romero Leal Passos (Universidade Federal Fluminense)

We believe that this activity is a way to socialize and spread the knowledge to those who have not participated in important international scientific event.

Resumos de pesquisas apresentadas no congresso selecionadas por Edison Natal Fedrizzi (Universidade Federal de Santa Catarina) e Mauro Romero Leal Passos (Universidade Federal Fluminense)

Consideramos que esta atividade é uma forma de disseminar e socializar o conhecimento para aqueles que não participaram de importante evento científico internacional.

Basic Science Basics of prophylactic vaccines

Cross-Reactivity to VLP HPV 31, 45 and 58 in a Group of Women Vaccinated with the Quadrivalent Vaccine in Bogotá, Colombia

Presenter: Alba Lucia Combita, PhD

Investigators/Collaborators: Combita AL, Molano M, Duarte D, Rodríguez J, Martínez L, Trujillo L, Gonzales M, Luna J, Ortiz N, Touzé A, Corsaget P

Country: Colombia

Objectives: To characterize the humoral immune response (HIR) to HPV16, 18, 31, 45 and 58 HPV capsids in women who has been vaccinated with the quadrivalent vaccine. **Method:** 82 women aged 18-28 years old attending HPV clinic at the Instituto Nacional Cancerología were enrolled and vaccinated according to the vaccination squeme. Follow-up visits were scheduled at 1, 6, 18 months after last vaccination dose. Before and after vaccination, samples for Papanicolaou tests, HPV DNA typing, and immunological assays were collected. IgG Ab were measured by ELISA using HPV16, 18, 31, 45 and 58 VLPs. HPV-DNA detection was done by GP5+/GP6+PCR-ELISA and typing of 37 types was performed using a reverse line-blot assay. **Results:** Before vaccination, anti-VLP HPV16, 18, 31, 45 and 58 prevalence was 39%, 31.7%, 15.9%, 31.7% and 23.2% respectively. This prevalence increased to 98.8%, 97.5% and 98.7% for types 16, 18 and 58 respectively one month after vaccination. For HPV31 and 45 the prevalence was 88.8% and 86.4% respectively. Six months after vaccination, prevalence of anti-VLP HPV16, 18 and 58 remained high 98.6%, 87.7% and 89.0% respectively. a decrease was observed for HPV31 and 45 types: 65.7% and 56.2%, respectively. after 18 months, prevalence of anti-VLP HPV16 was 95.8%, while for HPV18 was 76%. For HPV31, 45 and 58 the prevalence was reduced to 46.5%, 38% and 53.5% for respectively. the HPV DNA prevalence before vaccination was 39%, which slightly increased after one month of vaccination (46.9%), but decreased to 26% and 23% after six and 18 months. HPV16 and 18 prevalence was 6.1% for both types before vaccination and remained similar one month after (7.4%). Six months after vaccination this prevalence decreased to 2.7% for HPV16, while HPV18 not infections were detected (0%). 18 months post vaccination no HPV16 and 18 infections were observed. Before and one month after vaccination the prevalence of types related to HPV16 was 13.4%, and decreased six months after vaccination (5.5%). However, it increased to 9.8% after 18 months of vaccination. For types related to HPV18, the prevalence was low before and after vaccination (2.4%). For other HR-HPV types unrelated, we observed an increase in prevalence after vaccination (15.5%) compared to before vaccination (9.8%). **Implications and Impact:** It was observed an increase in HIR to VLP16 and 18 after vaccination. For VLP16 this response was maintained during all follow up, while to VLP18 this response decrease slightly. For other HPV VLPs types like HPV31, 45 and 58 also was observed an increase in the HIR. This response decrease about 50% after 18 months of follow up. These results could suggest a possible cross-reactivity to VLP HPV 31, 45 and 58.

Basic Science Basics of prophylactic vaccines

HPV16 L1 Virus-Like Particle Expressed in Lactobacillus casei Induces Mucosal and Systemic Immune Responses in Vivo

Presenter: Ji-Na Won, Student

Investigators/Collaborators: Won JN[1,2], Sung MH[3,4], Lee IH[4], Poo H[1,2]

1 Viral Infectious Disease Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejon, Korea.
2 University of Science and Technology, Daejeon, Korea
3 Department of Bio and Nanochemistry, Kookmin University, Seoul, Korea
4 BioLeaders Corporation, Daejeon, Korea
Country: Republic of Korea

Objectives: Human papillomaviruses (HPVs) are non-enveloped DNA viruses causing warts on skin or genital track, and malignant cancer as well. among more than 100 different strains or types, HPV type 16 (HPV16) has been regarded as one of the most causative agents for cervical cancer. L1, the major capsid protein of HPV, self-assembles into virus like particles (VLPs), and is currently used as a HPV vaccine component because of its strong immunogenicity in vivo. **Method:** We have constructed a lactose-inducible system using lactose operon promoter of Lactobacillus casei, lacT, (LacT), and have expressed the L1 major capsid proteins of HPV16. For immunization, BALB/c mice were administrated with equal amount of the L. casei-HPV16L1 by the oral routes. Anti-VLP immunoglobulin a (IgA) and IgG were also detected in serum, vagina. **Results:** We generated the HPV16 L1 VLPs in Lactobacillus casei (L. casei), which is a potential vaccine vector for the induction of both mucosal and systemic immune responses. Oral administration of L. casei/HPV16 L1 induced strong systemic IgG and mucosal IgA antibody responses in Balb/c mice, which is comparable to the immune responses of conventional HPV16 L1 VLP-injected group. Also, oral immunization of L. casei/HPV16 L1 resulted in higher neutralizing activity against HPV16 pseudovirus infection to 293TT cells in vitro. More importantly, L. casei/HPV16 L1 conferred significant protection against pseudovirus challenge through the genital tract, a major infection route of HPV. **Implications and Impact:** Our results show that L. casei/HPV16 L1 could be an efficacious prophylactic vaccine which induces strong neutralizing immune responses in mucosa with the safety and ease of administration.

Basic Science Basics of prophylactic vaccines

Multivalent HPV L1 DNA Vaccination Utilizing Electroporation

Presenter: Kihyuck Kwak, Ph.D. candidate
Investigators/Collaborators: Kwak K[1], Jiang R[1], Jagu S[1], Wang J[1], Christensen ND[2], Roden R[1]
1 Johns Hopkins University, Baltimore, MD USA
2 Penn State University, Hershey, PA USA
Country: United States

Objectives: The two licensed HPV vaccines require a cold chain and remain expensive to produce. Naked DNA vaccines can be manufactured at low cost and are stable at ambient temperature, but require a novel delivery technology. in vivo electroporation is an emerging approach to deliver naked DNA, and here we explore its potential for multivalent HPV L1 and L2 DNA vaccination. **Method:** Balb/c mice were vaccinated three times at two week intervals with L2 N-termini multimer protein or its DNA expression vector, DNA constructs expressing L1 only or L1+L2 of a single HPV type, or as a mixture of several high-risk HPV types utilizing electroporation, i.m. injection or gene gun. Serum was collected two weeks and 3 months after the last vaccination. Sera from immunized mice were tested for in-vitro neutralization titer, and protective efficacy upon passive transfer to naive mice and vaginal HPV challenge. To explore co-assembly, 293TT cells were transfected with DNA constructs, HPV6, HPV16, and HPV18 L1 together, and HPV18 L1 immunoprecipitated with monoclonal antibody, H18. F8. Chimeric VLP was detected by western blotting of immunoprecipitates with anti-HPV6, 16, 18 L1 antibody. **Results:** Electroporation with L2 multimer DNA did not elicit detectable neutralizing antibody titer, whereas L1 DNA induced robust neutralizing antibody titers, approaching those induced by Gardasil. L1+L2 DNA vaccination induced similar levels of type restricted neutralizing antibodies as L1 only DNA vaccine. Reduced neutralizing antibody titers were observed when vaccinating with a mixture of L1 (or L1+L2) vectors of multiple HPV types, likely resulting from co-assembly of mixed particles observed in coimmunoprecipitation studies. High titers were restored by vaccinating with individual constructs at different sites. **Implications and Impact:** Delivery of HPV L1 DNA via in vivo electroporation produces a stronger antibody response compared to i.m. injection or i.d. ballistic delivery via gene gun, and was not augmented by co-expression of L2. Electroporation with L2 multimer DNA failed to induce a neutralizing response. When L1 DNA vaccines derived from multiple HPV types are mixed prior to administration, this leads to a reduction in neutralizing antibody titer, likely reflecting the formation of mixed type VLPs. This issue could be resolved by spacial separation of individual type L1 DNA vaccines at different sites, but not by co-expressing the cognate L2 proteins.

Basic Science Basics of therapeutic vaccines & antivirals

Effect of Local Tumor Irradiation on Homing of Tumor-Specific CTLS

Presenter: Oana Draghiciu, M.Sc.
Investigators/Collaborators: Walczak M[1], Hoogeboom BN[1], Meijerhof T[1], Nijman HW[2], Daemen T[1]
1 Department of Medical Microbiology, Molecular Virology Section, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands
2 Department of Gynecology, University Medical Center Groningen, University of Groningen,Groningen, the Netherlands
Country: Netherlands

Objectives: Current therapeutic approaches of cervical cancer involve aggressive surgery and/or radiotherapy combined with chemotherapy, depending on the stage of disease. Non-invasive therapies, like immunotherapy, might optimize treatment outcome of these patients. Therapeutic immunization protocols aim at inducing antigen-specific cytotoxic T lymphocytes (CTLs), capable of specific recognition and

eradiation of established tumors. To achieve optimal benefit from immunization protocols, strategies need to be developed that support optimal migration and activity of CTLs into the tumor microenvironment. Evidence is accumulating that local irradiation therapy can induce chemokines involved in CTLs recruitment to the tumor site. Therefore, combination of immunization, to induce antigen-specific CTLs, with local irradiation therapy could increase the antitumor effect. **Method:** We developed a method allowing us to assess the trafficking of tumor-induced, adoptively transferred as well as vaccine-induced antigen-specific CTLs into tumors. To obtain antigen-specific CTLs, donor mice were immunized with recombinant Semliki Forest virus (rSFV) encoding human papilloma virus (HPV)-E6,7 tumor antigens. in vitro restimulated E6,7-specific CTLs were CFSElabeled and adoptively transferred into TC-1 (HPV-transfected) tumor-bearing recipient mice four days after local tumor irradiation. Homing of both tumor-induced effector T cells and adoptively transferred E6,7-specific CTLs to TC-1 tumors was analyzed one day after transfer. **Results:** Local tumor irradiation induced a significant increase in intratumoral levels of adoptively transferred E6,7-specific CTLs. a similar effect was observed in the infiltration of tumor-induced effector T cells and MDSCs (myeloid derived suppressor cells), when compared with non-irradiated tumors. To assess the effect of irradiation on tumor trafficking of vaccine-induced antigen-specific CTLs, TC-1 tumor bearing mice were locally irradiated and one day later intramuscularly vaccinated. Local tumor irradiation caused a drastic increase in intratumoral levels of both tumor- and vaccine-induced specific CTLs. Intratumoral levels of vaccineinduced specific CTLs were 5-fold higher than intratumoral levels of adoptively transferred E6,7-specific CTLs. **Implications and Impact:** In summary, we demonstrated that vaccine-induced CTLs home into tumors and that local tumor irradiation increases the tumor homing efficacy of antigen-specific CTLs. This study indicates that rSFV-based immunotherapy combined with tumor radiotherapy could improve treatment outcome.

Basic Science Basics of therapeutic vaccines & antivirals

HPV VLPs Can Directly Prevent HPV Tumoral Effect

Presenter: William Bonnez, MD

Investigators/Collaborators: William Bonnez[1] and Carrie DaRin[1]

1 University of Rochester School of Medicine and Dentistry, Rochester, NY 14642 (USA)

Country: United States

Objectives: HPV VLPs and infectious HPV particles share the same general conformation, thus may interact with the same host molecules and compete with one another. This simple assumption creates the possibility of interfering with the viral cycle by the prior exposure of the target tissue to HPV VLPs. We are presenting a proof of this concept. **Method:** Human neonatal foreskin fragments were exposed to different dilutions (1:1, 1:10, 1:100, and diluent alone) of a suspension (8 μg) of HPV VLPs prior to being exposed to a suspension of infectious HPV-6 virions (5x10^8 DNA copies). These grafts were singly implanted subcutaneously on the flank of a SCID mouse. For each replicate experiment 2 foreskin donors were used, one for each side of the mouse. Three mice were used for each HPV VLP dilution, and each experiment was done in quadruplicate, requiring a total of 48 mice. the animals were sacrificed 12 weeks later, and the planned primary endpoint was graft size, measured as the composite geometric mean diameter (cGMD) of the two grafts born by each mouse. the secondary endpoints were graft histology for the presence of HPV and average copy number of HPV-6 cDNA in the mouse grafts. Separate experiments were conducted with L1 VLPs of HPV types 6, 16, and 18 (supplied by Merck USA). **Results:** HPV VLPs regardless of the dilutions tested or genotype strongly inhibited HPV-6-induced graft proliferation. the mouse graft sizes (mean±SD cGMD in mm) for the HPV-6 VLP dilutions of 1.1, 1:10, 1:100, and control (diluent), were respectively 2.0 ± 0.5, 2.0 ± 0.5, 2.0 ± 0.5, and 3.1 ± 0.6 (p = 10-7). with HPV-16 VLPs they were 1.5 ± 0.5, 1.7 ± 0.4, 1.8 ± 0.3, and 2.6 ± 0.3 (p = 10-6), and with HPV-18 VLPs, 2.1 ± 0.3, 2.0 ± 0.2, 2.0 ± 0.2, and 2.8 ± 0.4 (p = 5 x 10-7). in contrast, HPV VLPs did not prevent HPV-6 infection of the graft as measured by histology or HPV-6 cDNA copy numbers (data not shown). **Implication and Impact:** HPV VLPs, regardless of genotype, can directly prevent HPV-6 induced tumor proliferation when applied prior to virus challenge. Our inability to reach a limiting dilution of the HPV VLPs and the use of a highly supra-physiologic virus challenge dose is very encouraging for a potential clinical use of this effect, as it is likely to persist under less extreme conditions of challenge. Although we anticipated a mechanism of action by specific blockade of virus entry, the occurrence of this effect in the presence of active viral expression suggests at least a different, but undefined antiviral mechanism of action. From a practical and clinical standpoint, the inhibition of the pathogenesis of any HPV genotype irrespective of the genotype of the blocking VLPs is an attractive feature.

Basic Science, Basics of therapeutic vaccines & antivirals

The Development of a Novel Curcumin-Based Vaginal Cream Vacurin Which Selectively Eliminates Cervical Cancer Cells

Presenter: Mario Castellanos, MD

Investigators/Collaborators: Priya Ranjan Debata, Ph.D., Mario Castellanos, M.D., Jimmie Fata, Ph.D., Sara Baggett, Sritha Rajupet, M.D., Anita Szerszen, D.O., Sultana Begum, Anita Mata, Lynne M.Opitz, M.D., Probal Banerjee, Ph.D.

Country: United States

Objective: Globally, human papillomavirus (HPV) infections remain a leading cause of morbidity and mortality. Despite significant progress, an effective antiviral treatment remains elusive. Curcumin, a component of the culinary spice turmeric, has potent anticancer and antiviral properties. However, its use is limited by low plasma solubility, rapid clearance and overall poor oral bioavailability. To overcome these limitations, we develop and test a curcumin-based vaginal cream as new approach to treat cervical lesions associated with HPV. **Method:**

First, we show that curcumin eliminates HPV (+) cervical cancer cell lines. HeLa, ME-180, SiHa, SW756 and normal fibroblast cells were treated with different doses of curcumin (96 hours). WST-1 assays performed and IC50 values obtained. Cells were also treated with curcumin (50 μM) for 8 h. Western blotting of cell lysates done, HPV E6 and E7, EGFR, Rb and p53 levels measured. Next, intravaginal formulation containing 2%, 5%, 10% and 20% (w/w) curcumin were developed, named Vacurin-2, Vacurin-5, Vacurin-10, and Vacurin-20, respectively. Uniformity of these colloid mixtures were examined by measuring curcumin fluorescence on thin spreads. Vacurin was then tested for curcumin release and effectiveness. HeLa cells were cultured in a 12-well cluster plates and different concentrations of Vacurin were placed on a porous membrane in a tissue culture insert suspended 1mm above the cultured cells for 72 hours. Juxtaposed and peripheral cell death measured. Toxicity of Vacurin was determined by examining daily intravaginal infusions of either PBS, vehicle alone or Vacurin-20 for 2-3 weeks in mice. Animals euthanized and histopathology of lower reproductive tract done. **Results:** Curcumin selectively eliminates HeLa, Me180, SiHa, and SW756 cells. It suppresses HPV E6, dramatically inhibits the expression of EGFR and concomitantly induces p53. We show that Vacurin is a uniform colloidal solution of curcumin in a clinically used amphipathic cream. Vacurin eliminates juxtaposed HeLa cells in a culture system. in mice, daily intravaginal Vacurin-20 infusion produced no changes in body weight and when mice were scarified, the vaginal tract epithelium showed no mucosal injury or adverse effects. **Implications and Impact:** There is a worldwide need for an effective treatment against HPV, especially in developing countries with limited resources. Our compound is promising as it is derived from a low cost nutraceutical. Our curcumin-based vaginal cream effectively and preferentially eradicated HPV (+) cancer cells. Our preclinical data demonstrates a safe and novel therapy for the treatment of cervical lesions associated with HPV.

Basic Science Immunolgoy

Anal Condylomata as Potential Risk Factors for HIV-1 Acquisition

Presenter: Zoon Wangu, MD

Investigators/Collaborators: Zoon Wangu, MD[1], Jeffrey Pudney, PhD[2], Joseph Politch, PhD[2], Lori Panther, MD, MPH[3], Antonio de las Morenas, MD[4], Deborah Anderson, PhD[5]

1 Fellow, Department of Pediatric Infectious Diseases, Boston Medical Center, Boston, Massachusetts

2 Research Associate Professor, Department of Obstetrics & Gynecology, Boston University School of Medicine, Boston, Massachusetts

3 Assistant Professor, Harvard Medical School & Department of Infectious Diseases, Beth Israel Deaconess Medical Center, Boston, Massachusetts

4 Professor, Department of Pathology and Laboratory Medicine, Boston University School of Medicine, Boston, Massachusetts

5 Professor, Departments of Obstetrics & Gynecology, Microbiology and Medicine, Boston University School of Medicine, Boston, Massachusetts

Country: United States

Objectives: Anal HPV infections in young homosexual men have been associated with increased risk of HIV acquisition and seroconversion independent of other sexually transmitted diseases; however, the specific mechanism is unclear. We hypothesize that HPVinduced low grade condylomata enhance HIV transmission due to increased vascularity, friability and leukocytic infiltrates containing HIV host cells. Our objective was to compare presence and abundance of HIV target cells in anal condylomata vs. normal anal tissue. **Method:** Coded, archived samples of low-grade (up to AIN1) anal condylomata were obtained from the Boston Medical Center pathology department. Condylomata and normal anal tissue controls were sectioned and reacted with primary antibodies recognizing lymphocyte subsets (CD3+, CD4+, CD8+), antigen presenting & inflammatory cells (CD68+, CD15+, CD1a+) and HIV coreceptors (CCR5+, CXCR4+). Cells expressing these markers were visualized by an alkaline phosphatase detection system. Relative quantity & location of cell types were compared between samples and controls using a semi-quantitative scale. Differences in cell populations between samples and controls, and correlations between cell types within tissues, were assessed using the Spearman Rank Correlation Test. **Results:** Thirty condylomata samples were obtained from 24 patients (46% Caucasian, 46% male, median age 35 years). Forty-three percent of samples contained large numbers of epidermal CD1a+ dendritic cells, some with large focal dermal accumulations; 20% of samples contained large numbers of dermal CD68+ macrophages and CD4+ lymphocytes. in contrast, normal tissue contained few such dermal or epidermal cells. CD3+, CD15+ and CD8+ cells were present in varying numbers in most samples but were more numerous in condylomatous tissue compared to controls. All samples contained CCR5+ cells, whereas few (30%) contained CXCR4+ cells. in the condylomata epidermis, CD8+ lymphocyte infiltrates were associated with increased numbers of CD15+ granulocytes and CD1a+ dendritic cells. **Implications and Impact:** Our results indicate that low-grade condylomatous anal tissue often contains large accumulations of HIV target cells in contrast to normal anal epithelium. These data provide evidence that condylomata may enable HIV entry and infection, especially in the setting of typical lesion vascularity and friability. If so, treatment and/or prevention of anal condylomata may decrease HIV acquisition. This provides additional impetus for HPV vaccination both in populations with high HPV prevalence and in the general population especially before the age of sexual debut.

Basic Science Novel Diagnostics

Bead-Based Detection of Sexual Transmitted Infections and their Association with Cervical Cancer

Presenter: Markus Schmitt, PhD

Investigators/Collaborators: Christophe Depuydt, Michel Stalpaert and Michael Pawlita

Country: Germany

Objectives: Data on prevalence of sexual transmitted infections (STIs) and bacteria of the normal genital flora are scarce. in addition, it remains controversially discussed whether STIs may act as co-factors in the development of cervical cancer. **Method:** We report the development and validation of a novel multiplex genital pathogen assay (MGPA) that detects different genital pathogens in cervical swabs using a single multiplex PCR followed by Luminex bead-based target-specific hybridisation. MPGA was used to reanalyse HPV-characterised

samples from a Mongolian populations- and a cancer-based study. **Results:** MGPA specifically detects Chlamydia trachomatis, HSV1, HSV2, Treponema pallidum, Trichomonas vaginalis, Neisseria gonorrhoeae, Mycoplasma genitalium, M. hominis, M. pneumonia, Ureaplasma urealyticum and U. parvum, and quantifies BVassociated Atopobium vaginae and Gardnerella vaginalis as well as the normal genital flora Lactobacillus iners, L. crispaticus and L. jensenii. As little as 10 to 100 target copies per PCR could be detected for all pathogens. Quantification of A. vaginae and G. vaginalis was possible over 4 to 5 orders of magnitude. Additional validation, prevalence and association data will be presented. **Implications and Impact:** In conclusion, the novel MGPA assay is a powerful high-throughput tool in assessing sexual transmitted infections.

Basic Science Transformation and carcinogenesis

Association of Chlamydia trachomatis Infection in HPV Positive Women with Severity of Cervical Neoplasia

Presenter: Luiz Carlos Zeferino, PhD

Investigators/Collaborators: Juçara Maria de Castro-Sobrinho, Silvia Helena Rabelo-Santos, Rosane Ribeiro Figueiredo Alves, Sophie Françoise Mauricette Derchain, Luis Otávio Zanatta Sarian, Denise Rocha Pitta de Moraes, Elisabete Aparecida Campos, Luísa Lina Villa, Luiz Carlos Zeferino

Country: Brazil

Objectives: Human Papillomavirus (HPV) is widely accepted as the central cause of cervical cancer. Cofactors might affect the risk of the progression from HPV infection to cervical precursor lesions and invasive cancer. Bacterial co-infection by Chlamydia trachomatis (CT) in women with a history of HPV infection has been studied as a potential factor that contributes to the development of cervical intraepithelial neoplasia and cervical cancer. This study was designed to analyze the association of co-infection HPV and CT with the severity of cervical neoplasia. **Method:** This was a cross sectional study that included women who were subjected of LLETZ or conization due to cervical intraepithelial neoplasia (CIN) 2 and CIN 3 by biopsy; suspicious image penetrating the cervical canal and those in whom colposcopy was unsatisfactory and second cervical smear was abnormal. From 290 consecutive women, 251 were infected by high risk HPV (86.6%) and they were included in the study. the average age was 34.2 years and the median age was 32 years, ranging from 17 to 75 years. HPV- DNA was amplified using PGMY09/11 HPV specific primers and HPV-DNA genotyping was performed using a reverse line blot hybridization assay. the detection of CT was done by PCR to amplify a sequence in the cryptic plasmid generating a fragment of about 512 base pairs. **Results:** The prevalence of CT in HPV positive women was 15.1% (38/251). Significant association was observed between women with 30 years or older and CIN 2 or worse diagnosis for those CT negative (OR 2.11; 1.13-3.95), but this association was not observed for those CT positive (OR 2.03; 0.5-8.23). HPV 16 and/or HPV 18 were detected in 50% of the women under 29 years with CIN 2 or worse who were negative CT, and in 19.5% for those women with CIN 1 or cervicitis. in these women the association between HPV 16 and/or 18 and CIN 2 or worse revealed OR of 5.83 (2.19-15.57), but this association also was not observed considering the group CT positive (OR = 0.28; 0.04-1.98). **Implications and Impact:** This study did not show any association between CT infection and CIN2 or worse diagnosis among women with high risk HPV, specifically considering the types HPV 16 and or HPV 18.

Basic Science, Transformation and carcinogenesis

The Implication of HPV Infection in Human Reproduction

Presenter: Franco Borruto, PhD

Investigators/Collaborators: Ciro Comparetto[1], Valérie Giordanengo[2], Alain Treisser[3], Franco Borruto[4]

1 Division of Obstetrics and Gynecology, City Hospital, Azienda USL 4, Prato, Italy

2 PU-PH, Chef de Service, Laboratoire de Virologie, Centre Hospitalier Universitaire de Nice, Hôpital de l'Archet II, Nice (France)

3 Division of Obstetrics and Gynecology, Centre Hospitalier Princesse Grace, Monaco

4 Professor of Obstetrics and Gynecology, Centre Hospitalier Princesse Grace, Monaco

Country: Monaco

Objectives: Beside other mucosal targets, HPV-16 was reported to infect the placenta and to replicate in trophoblastic cells. Since these cells share invasive properties of tumoral cells, they represent an ideal model to investigate several oncogenic processes. in the present work, we analyzed the impacts of HPV-16 E5, E6, and E7 oncoproteins on the trophoblastic model. We sought to determine if HPV infection of extravillous trophoblast cells reduces cell invasion and if placental infection is associated with adverse reproductive outcomes attributed to placental dysfunction. **Method:** We conducted apoptosis and invasion assays using extravillous trophoblast cells that were transfected with a plasmid containing the entire HPV-16 genome. in order to associate HPV infection with reproductive outcomes, we conducted a case-control study to detect HPV DNA in the extravillous trophoblast region of placentas from cases of spontaneous preterm delivery, severe preeclampsia requiring delivery at < 37 weeks and controls who delivered at term. **Results:** Our results showed that E5 impaired the viability of trophoblastic and cervical cell lines but E6 and E7, favoring cell growth, neutralized the E5 cytotoxic effect. in addition, E5 decreased the adhesiveness of trophoblastic cells to the tissue culture plastic and to endometrial cells similarly as described previously for E6 and E7. E5 and E6 plus E7 increased also their migration and their invasive properties. Cells expressing HPV-16 early proteins under the control of the long control region endogenous promoter displayed growth advantage and were also more motile and invasive compared with control cells. Rates of apoptosis were 2- to 4- fold greater in transfected cells than in non-transfected cells or cells transfected with an empty plasmid. Invasion of transfected cells through extracellular matrices was 35-75% lower than that of the controls. HPV was detected more frequently

in placentas from spontaneous preterm deliveries than in placentas from controls (p = 0.05). Identification of HPV in placentas from cases of pre-eclampsia was not significantly different to controls. **Implications and Impact:** HPV-16 early proteins enhance trophoblastic growth and intensify the malignant phenotype by impairing cell adhesion leading to increased cellular motile and invasive properties. HPV infection of extravillous trophoblast induces cell death and may reduce placental invasion into the uterine wall. Thus, HPV infection may cause placental dysfunction and is associated with adverse pregnancy outcomes, including spontaneous preterm delivery.

Clinical Science Biomarkers: New and improved for cancer prevention/management of disease

Overexpression of ANXA1 in Penile Carcinomas Positive for High Risk HPVs

Presenter: Paula Rahal

Investigators/Collaborators: Rahal P[1]; Calmon MF[1]; Mota MTO[1]; Candido NM[1]; Girol AP[1]; Mendiburu CF[2]; Thomé JA[2]; Rosa BM[3]; Soares FA[4]; Oliani SM[1]; Villa LL[5]; Vassallo J[4]

1 São Paulo State University; S. J. do Rio Preto-SP, Brazil

2 IAPC. S. J. do Rio Preto-SP, Brazil

3 College of Medicine of São José do Rio Preto. S. J. do Rio Preto-SP, Brazil

4 Hospital A.C. Camargo. São Paulo-SP, Brazil

5 Department of Radiology and Oncology, School of Medicine, University of São Paulo, Brazil

Country: Brazil

Background: Penile squamous cell carcinoma (PSCC) is associated with a number of established risk factors and associated diseases including phimosis with chronic inflammation, Human Papillomavirus (HPV) infection, poor hygiene and smoking. in penile carcinomas the most common HPV types are HPV 16 and HPV 18 being that HPV 16 is most prevalent in North America, Europe, South America and India. **Objectives:** The objective of this study was to identify genes related to penile carcinoma. **Methods:** Forty-seven patients diagnosed with PSCC were enrolled in this study. HPV detection was done by PCR with generic primers GP5+/GP6+ and HPV typing was done by INNO-LIPA kit. RaSH methodology was performed in PSCC positive for high-risk HPV and normal penile tissues to identify differential expression in PSCC. the genes selected were validated in fresh tumour samples by qPCR and their proteins expressions were analyzed by immunohistochemistry. **Results:** HPV DNA was detected in 48.9% of PSCC cases. High-risk HPV were present in 42.5% of cases and low-risk HPV were detected in 6.4% of PSCC. the RaSH approach identified differential expression of Annexin A1 (ANXA1), p16, RPL6, PBEF1 and KIAA1033 in high-risk HPV positive penile carcinoma; ANXA1 and p16 were overexpressed in tumoral cells by qPCR. ANXA1 and p16 proteins were significantly more expressed in the cells from HPV-positive penile carcinoma as compared to HPV-negative tumors ($p < 0.001$) by immunohistochemistry. **Conclusion:** Overexpression of ANXA1, which has anti-inflammatory, antipyretic and anti-hyperalgesic activities and is associated with various physiological processes including cellular differentiation, cell proliferation and signal transduction, was demonstrated in penile squamous cell carcinoma samples and its protein expression is strongly associated with high risk HPV infection. We suggested the p16 could be a marker for penile carcinoma, confirming the diagnosis of malignant penile lesions infected with high risk HPVs.

Clinical Science, Biomarkers: New and improved for cancer prevention/management of disease

HPV 6 in One Case of Invasive Cervical Cancer: Analysis of Biomarkers

Presenter: Alcina F Nicol, PhD

Investigators/Collaborators: Amaro Filho SM[1]; Golub JE[2]; Levi JE[3]; Andrade CV[4]; Russomano F[4];Tristão A[4]; Pires A[5]; Nuovo GJ[6]; Nicol AF[1]

1 Laboratory of Interdisciplinary Medical Research (LIPMED), IOC – FIOCRUZ, RJ, Brazil

2 Johns Hopkins University

3 Instituto de Medicina Tropical Laboratório de Virologia – USP, São Paulo, SP

4 Instituto Fernandes Figueira Institute (IFF)

5 Fonte Medicina Diagnostica Laboratory, Niterói, RJ

6 Department of Pathology, University Hospitals, Columbus, OH, USA. e-mail: sergio@ioc.fiocruz.br

Country: Brazil

Objectives: The goal of this study is to report an unusual case where a single infection with low-risk HPV (type 6) is observed in an invasive cervical cancer sample and to analyze the expression of p16, Ki-67, MCM-2 and p53 in this rare case. **Method:** One invasive cancer specimen was analyzed by means of immunohistochemistry for p16, Ki-67, MCM-2 and p53. HPV DNA was detected by PCR following the genotyping by sequencing. **Results:** The single HPV 6 infection was confirmed by the three techniques: automatic sequencer, INNO-LiPA and PapilloCheck. the markers related to proliferation, Ki-67 and MCM-2, were overexpressed with a mean of 65% and 35% positive cells per field, respectively. the literature has reported high p16 positivity but low-level expression of p53 in ICC. Curiously, in this case p16 was reported negative, while p53 was overexpressed showing a mean greater than 90% of positive cells per field. **Implications and Impact:** Consideration should be given to alternate pathways leading to virally induced carcinogenesis. Other factors such as polymorphic or epigenetic events may play a role in an association with cervical cancer. Introduction: Single low-risk HPV infections in highgrade lesions are rare and p53 has often been associated with low expression in ICC. Objective: the goal of this study is to report an unusual case where a single infection with low-risk HPV (type 6) is observed in an invasive cervical cancer sample and to analyze the expression of p16, Ki-67, MCM-2 and p53 in this rare case. Methodology: One invasive cancer specimen was analyzed by means of immunohistochemistry for p16,

Ki-67, MCM-2 and p53. HPV DNA was detected by PCR following the genotyping by sequencing. Additionally, INNO-LiPA and Papillo-Check Kit were used in order to confirm the single HPV type infection. **Results and Discussion:** The single HPV 6 infection was confirmed by the three techniques: automatic sequencer, INNO-LiPA and PapilloCheck. the markers related to proliferation, Ki-67 and MCM-2, were overexpressed with a mean of 65% and 35% positive cells per field, respectively. the literature has reported high p16 positivity but low-level expression of p53 in ICC. Curiously, in this case p16 was reported negative, while p53 was overexpressed showing a mean greater than 90% of positive cells per field. **Conclusions:** Consideration should be given to alternate pathways leading to virally induced carcinogenesis. Other factors such as polymorphic or epigenetic events may play a role in an association with cervical cancer.

Clinical Science, Biomarkers: New and improved for cancer prevention/management of disease

Effect of Condom Use after CIN Treatment on HPV Positivity and Other Biomarkers: a Randomised Controlled Trial

Presenter: George Valasoulis, MD

Investigators/Collaborators: George Valasoulis[1], Maria Kyrgiou[2], Marc Arbyn[3], Sofia Melina Stasinou[1], Pierre Martin-Hirsch[4], Aristotelis Loufopoulos[5], George Koliopoulos[1], Petros Karakitsos[6], Evangelos Paraskevaidis[1]

1 Department of Obstetrics & Gynaecology, Ioannina University Hospital, Ioannina, Greece

2 West London Gynaecological Cancer Center, Department of Obstetrics & Gynaecology, Queen Charlotte's and Chelsea Hospital, Hammersmith Hospital, London, UK

3 Unit of Cancer Epidemiology, Scientific Institute of Public Health, Brussels, Belgium and European Cooperation on Development and Implementation of Cancer Screening and Prevention Guidelines, IARC, Lyon, France

4 Department of Gynaecologic Oncology - Obstetrics & Gynaecology, Royal Preston Hospital, Central Lancashire Teaching Hospitals, Preston, UK

5 Second Department of Obstetrics & Gynecology, Aristotle University of Thessaloniki, Hippokration Hospital, Thessaloniki, Greece

6 Department of Cytopathology, Attikon Hospital, University of Athens, Athens, Greece

Country: Greece

Objectives: To investigate whether consistent condom use after treatment of CIN reduces HPV positivity post-operatively and possibly as a result, the risk of treatment failure. **Method:** Design: Single-blinded randomised controlled trial. Period: From May 2009 to February 2012 Setting: University Hospital of Ioannina Inclusion criteria: Women planned to undergo conservative treatment for CIN. Intervention: Women randomly allocated to Group a were given recommendation for condom use, whilst women in Group B received routine post-treatment information. An LBC sample was tested for HPV DNA and typing, E6 & E7 mRNA (NASBA technique), E6 & E7 mRNA by flow cytometry, p16INK4a and microspectroscopy at 0 (pre-treatment), 6 and 12 months. a questionnaire to assess compliance was also completed. Outcomes: HPV and other biomarkers status at 6, 12, 18, 24 months, treatment failures at 24 months and compliance rates. Analysis: the relative risk (RR) and absolute RR (ARR) were calculated in an intention-to-treat analysis. the number needed to treat (NNT) and compliance to condom use recommendation, were also assessed. **Results:** A total of 204 women were recruited. All of them have completed the 6 and 176 the-12 month follow-up. the positivity for all the tested markers at follow-up was significantly reduced in Group A. in particular, 29.8% of women tested positive for HPV in Group a in comparison to 69.2% in Group B [RR:0.569(95%CI:0.376-0.702), ARR:0.394(95%CI:0.244-0.518) at the 6month visit. the NNT was 2. So far, we had 12 treatment failure cases with significantly higher proportion in control arm. Analysis of HPV positivity in relation to the excision margins, treatment failures and compliance rates as well as histological data for both groups will be presented. **Implications and Impact:** Post-treatment condom use significantly reduces HPV positivity. It remains to be confirmed whether this will also result in decreased number of treatment failures.

Clinical Science Biomarkers: New and improved for cancer prevention/management of disease

Susceptibility To Human Papillomavirus Infection and Association with Other Microbial Pathogens

Presenter: Maria Clara Bicho, MD PhD

Investigators/Collaborators: Andreia Matos[1]; M. Carreira[3]; Carina Farinha[2]; Marcia Veiga[2]; Hermínia Pereira[3]; Carlos Cardoso[3]

1 Laboratory of Genetics, Medical School, University of Lisbon, Portugal

2 Gynecology/Oncology ambulatory of Santiago Hospital, Lisbon, Portugal

3 Clinical Analysis Laboratory of Dr. Joaquim Chaves

Country: Portugal

Objectives: To analyze the possible relation with microbial gynecology and increase risk for severe dysplasia. **Method:** We studied 193 patients mean age 36, 96 ± 11, 36 (range: 17 – 66 years old) from the Gynecology/Oncology ambulatory of Santiago Hospital. the cervical samples were obtained for cytology, HPV, *Ureaplasma parvum*, *Chlamydia trachomatis* and *Mycoplasma hominis* detection. the method used for HPV detection and genotyping determination was Polymerase Chain Reaction followed by hybridization. Chlamydia trachomatis was detected by using a Real-Time Polimerase Chain Reaction (qPCR). the statistical methods used were Chi-square (Primer version 5) and ANOVA (SPSS version 20). the statistical significance level was established for $p < 0.005$. **Results:** Among 193 patients, there were 53 (32.3 %) patients with positive HPV DNA among women with normal and abnormal cytology. HPV DNA positive exhibited 16 (30.2 %) with normal and 37 (69.8 %) with negative results cytology. in 55 patients with HPV the incidence was highest in women aged 20 – 40 years old (58, 2 %). We identified 18 HPV types, which HPV 16 was the most predominated (10 (14.49 %) followed by HPV types 31, 52 and 53 (7 (10, 14 %)), type 58 (6 (8.70 %)) and types 66 and 33 (5 (7, 25%)). According to HPV results, we aggregated it in low (4 (2.4 %)), high (34 (20.7 %)), 2 or more high risk types (15 (9.1 %) and unknown (3 (1.8%). Molecular diagnostic tests and among HPV positive women, we found that 16 (33.33 %) with normal cytology, 6 (12.5 %) with High - grade squamous intraepithelial lesions (HSIL), 11 (22.92 %) with

Low-grade squamous intraepithelial lesions (LSIL) and 15 (31.25 %) with atypical squamous cells of undetermined significance (ASCUS). When we associated HPV results between inflammation and ASCUS, we found a significant prevalence of ASCUS with HPV positive (15 (56 %), ($\chi 2 = 4.297$, $p = 0{,}038$). We found a trend to association with molecular diagnostic tests for genital microorganisms and HPV DNA positive (39%, $p = 0.079$). When considered the HPV stratification, 2 or more high risks, was significantly associated with a positive molecular diagnostic tests (Positive: 66.7% versus negative: 33.3%, $p = 0.040$). Ureaplasma parvum was more prevalent particularly in high risk (22.2%) and 2 or more high risks (33.3%) of detected HPV ($p = 0.038$). **Implications and Impact:** Although the study population was small, we found a significant association between the presence of *Ureaplasma parvum* and HPV positive women, particularly in high risk and 2 or more high-risks HPV. We propose that the screen for the presence of different microorganisms could be important in prevention of severe dysplasias.

Clinical Science Biomarkers: New and improved for cancer prevention/management of disease

Detection of Anal Neoplasia Using Anal Cytology (PAP Smear) - Can We Do Better?

Presenter: John Thornhill, MD

Investigators/Collaborators: Dr Naveena Singh, Ms Damilola Awosika, Dr Michael Sheaff, Dr Mayura Nathan

Country: United Kingdom

Objectives: To assess the performance of anal cytology against High Resolution Anoscopy (HRA) guided biopsy for the detection of high grade anal intraepthelial neoplasia. **Method:** We retrospectively compared anal cytology results with histological diagnosis of anal HPV disease/neoplasia. We included all patients referred to our tertiary referral service in London, UK. Episodes with concurrent anal cytology and histopathology results (from HRA directed biopsy) were included. Liquid based anal cytology and HRA was performed (any area suspicious of disease was biopsied). Data pertaining to cytology, histopathology, number of affected disease quadrants (by area), and HIV status were recorded. of 3,520 episodes, 522 had concurrent anal cytology and histotology and therefore included. Anal cytology was deemed "positive" if any grade of abnormality was detected. Disease was considered "high grade" if the histopathology was AIN 2/3. Sensitivity and specifcity analyses of anal cytology were performed with respect to "high grade" disease and for "any disease". **Results:** 522 matched samples were included. 58.6% were HIV+ and 35.1% were HIV-. the sensitivity of anal cytology for detecting high grade disease was 77.5% (95% CI 67-82) while the specificity was 39.7% (95% CI 34-44). the sensitivity and specificity for high grade disease in HIV- individuals was 75% and 49.3%, while in the HIV+ group it was 75.2% and 29.9%. the no. of patients who were HIV+ with 2+ quadrants involved was 110. the sensitivity and specificity for high grade disease in this group was 87.9% and 15.6%. in the HIV- group with 2+ quadrant disease it was 92.9% and 41.7%. of those with negative smears and high grade disease (n = 36), the majority (80.5%) had zero to 1 quadrant disease (29/36) on HRA, 5/36 had two or more quadrants of disease. 10 of those with negative smears had AIN 3, the remainder had AIN 2. **Implications and Impact:** The sensitivity of anal cytology for detection of high grade AIN disease was 77.5%. This was comparable in HIV+ and HIV- groups (75% and 75.2% respectively). the sensitivity of anal cytology improved when 2+ quadrant disease was present on HRA; this was true in both the HIV+ and HIV- groups (87.2% and 92.9%, respectively) Anal cytology detects the majority of "large" lesions in HIV+ and HIV- individuals, however there may be scope for it's use with other biomarkers to improve performance.

Clinical Science, Biomarkers: New and improved for cancer prevention/management of disease

Comparison of Carcinogenic HPV Detection by Urine, Vulvar, and Cervical Sampling among Women Attending a Colposcopy Clinic

Presenter: Vikrant Sahasrabuddhe, MBBS, MPH, DrPH

Investigators/Collaborators: Vikrant V. Sahasrabuddhe, Patti E. Gravitt, S. Terence Dunn, David Brown, Richard A. Allen, Katie Smith, Rosemary E. Zuna, Michael A. Gold, Mark Schiffman, Philip E. Castle, Joan L. Walker, Nicolas Wentzensen

Country: United States

Objectives: Non-invasive, urine-based sampling for carcinogenic HPV testing offers the potential for improving anogenital cancer screening in hard-to-reach populations. We sought to compare HPV detection through urine-based sampling versus cellular sampling of thecervix and vulva, and correlate results with cervical disease status in a controlled clinical setting. **Method:** In a cross-sectional study at a colposcopy clinic, 72 participants provided a urine sample prior to a clinician collecting a vulvar (external genital) sample using a Dacron swab and cervical sample using a cytobrush. Cervical disease status was defined by combining colposcopy/histology and cervical cytology results. HPV genotyping of urine (after centrifugation), vulvar, and cervical samples was conducted using the Linear Array HPV Genotyping Test. **Results:** Carcinogenic HPV genotypes (HPV16/18/31/33/35/39/45/51/52/56/58/59/68) were present in 42/72 (58.3%) urine samples, 49/68 (72.1%) vulvar samples, and 53/72 (73.6%) cervical samples. Carcinogenic HPV detection in urine samples showed a 'moderate' agreement with HPV detection in cervical samples (Kappa: 0.55) and a 'substantial' agreement with vulvar samples (Kappa: 0.62). the agreement between carcinogenic HPV detection in cervical and vulvar samples was also 'substantial' (Kappa: 0.70). Highgrade cervical lesions (CIN2+ histology or HSIL cytology) were present in 25/66 (37.9%) women. Urine-based detection of carcinogenic HPV had a clinical sensitivity of 76.0% and a clinical specificity of 56.1% for diagnosing high-grade cervical lesions. the corresponding sensitivity and specificity values for vulvar sampling were 88.0% and 39.5%, and those for cervical sampling, 92.0% and 39.0% respectively. HPV16 was the most common carcinogenic genotype, detectable in 25.0% of urine, 33.8% of vulvar, and 31.9% cervical samples. **Implications and Impact:** HPV detection based on sampling from non-cervical sites may offer a simple approach for anogenital cancer screening in medically underserved areas. Population-based research studies are needed to evaluate the operational challenges in implementation of such sampling approaches.

It will be important to evaluate the acceptable levels of the decrement in sensitivity (as compared to cervical HPV) that may be offset by the benefits of non-invasive/non-obtrusive or home-based sampling.

Clinical Science, Clinical immunology

Immunogenicity of Quadrivalent HPV Vaccine among Girls Aged 11-13 Years Vaccinated Using Alternative Dosing Schedules: Results 32 Months after Third Dose

Presenter: D. Scott LaMontagne, PhD MPH, FRSPH
Investigators/Collaborators: LaMontagne DS[1]; VD Thiem[2]; VM Huong[1]; Y Tang[1]; Neuzil KM[1]
1 PATH, Seattle, USA and Hanoi, Vietnam
2 National Institute of Hygiene and Epidemiology, Hanoi, Vietnam
Country: United States

Objectives: To demonstrate that anti-HPV 16 and anti-HPV 18 immune responses more than 24 months post-dose 3 among 11-13 year old girls are non-inferior using three different alternative dosing schedules (0, 3, 9 months; 0, 6, 12 months; or 0, 12, 24 months) to those obtained when the vaccine is administered on the standard 3-dose schedule of 0, 2, 6 months. **Method:** We collected a single blood sample of 7 milliliters from girls enrolled in an original open-label, cluster-randomized trial of alternative dosing schedules that demonstrated non--inferior immunogenicity one-month post-dose 3 for two of three alternative dosing schedules compared to the standard schedule (Neuzil KM, et al. JAMA April 2011). Girls on the standard schedule were followed for 29 months and those on the alternative schedules for 32 months. An interim measure of 20 months post-dose 3 was performed for girls on the 0, 12, 24 month alternative schedule. the same type--specific competitive Luminex immunoassay was performed by Merck & Co. (USA) to quantify levels of neutralizing antibodies. Non--inferiority was defined as in the original trial: the lower bound of the 97.5% confidence interval for both the anti-HPV 16 and anti-HPV 18 GMT ratios between the alternative and standard schedule was > 0.50. Both parents and enrolled girls provided written informed consent to participate in this follow-up study. **Results:** Of the 809 girls aged 11-13 years who completed the original study, 741 were eligible for this follow-up study, and 518 had a valid blood sample at 29 or 32 months after dose three (which was 64% of the population who completed the original trial). Preliminary analyses indicate that the immunogenicity of HPV vaccine when delivered on either the 0, 3, 9 month or 0, 6, 12 month alternative dosing schedule was non-inferior for types 16 and 18 at 32 months post-dose 3 compared to girls at 29 months post-dose 3 who were vaccinated on the 0, 2, 6 month standard schedule. Results for girls vaccinated on an annual schedule (0, 12, 24 months) will be available at the time of the conference; however, an interim blood draw for this group showed that GMTs for types 16 and 18 remained high at 20 months post-dose 3. **Implications and Impact:** Young adolescent girls may have similar antibody responses for HPV 16 and 18 when vaccinated on a variety of dosing schedules. Provision of dosing flexibility without reduction in immunogenicity could facilitate more feasible delivery strategies in low resource settings and provide evidence for administration of missed doses up to two years after initiating the three-dose series.

Clinical Science, HPV testing: Prevention and management trials (RTC)

Overtreatment in See-and-Treat Management of Cervical Intraepithelial Lesions: Thirty Years of Experience in a Single Institution in the Netherlands

Presenter: Remko Bosgraaf, MD
Investigators/Collaborators: Remko P. Bosgraaf, MD, Peter-Paul Mast, BSc, Petronella H.T.H. Struik-van der Zanden, Johan Bulten, MD, PhD, Leon F.A.G Massuger, MD, PhD, Ruud L.M. Bekkers, MD, PhD.
Country: Netherlands

Objectives: The major cause of cervical intraepithelial neoplasia (CIN) is persistent infection of the cervix with high-risk types of human papillomavirus (hr-HPV). HPV appears to cause cervical dysplasia and may lead to the development of cervical cancer. Much controversy still exists on how to assess a patient referred for colposcopy after an abnormal cervical smear. a see-and-treat management is appealing because of low-costs, decreased patient anxiety and increased compliance. a major downside is the rate of overtreatment, with increased premature birthrate as major complication. the aim of this study is to determine the rate of overtreatment in see-and-treat management at colposcopy in relation to the cervical smear result, age, and colposcopic impression. **Method:** Out of a total of 4808 patients referred for an initial colposcopy to the Radboud University Nijmegen Medical Center over the last 30 years, 3192 (66%) patients underwent a see--and-treat protocol, and were analyzed in the current study. Overtreatment, defined as CIN 1 or less at final histopathological analysis, was investigated in relation to the age of the women, referred cervical smear result, colposcopic impression, and histopathology result. **Results:** The overall rate of overtreatment in see-and-treat management was 18.1%. the lowest overtreatment rate (4.5%) was seen in women with both a high-grade cervical smear, and a high-grade impression on colposcopy. the rate of overtreatment also showed a relation with age; women under forty were less likely to be overtreated than women above this age. **Implications and Impact:** This is the first time that age influences on the rate of overtreatment are studied in what is – as far as we know – the largest single institute case series. the overtreatment rate for patients in all age groups referred with a high-grade smear, and with a high-grade impression on colposcopy is low, and a see-and--treat approach in these patients is the preferred management strategy. in women with either a high-grade smear, or high-grade impression on colposcopy, see-and-treat may still be preferred but has higher overtreatment rates. Given the side effects of cervical surgery on pregnancy outcome, especially young women may benefit from a two-step approach if they have either a low-grade smear, or low-grade impression on colposcopy, while older women may prefer a see-and-treat policy.

Clinical Science New vaccine trials (Phase I-III; preventive and therapeutic trials)

Immunogenicity and Safety of the Bivalent HPV Vaccine in Female Juvenile Idiopathic Arthritis Patients versus Healthy Female Adolescents: a Prospective Controlled Observational Cohort Study

Presenter: Mirte Scherpenisse, PhD student

Investigators/Collaborators: Mirte Scherpenisse*[1,2], Marloes W. Heijstek*[3], Noortje de Groot[3], Carline Tacke[3], Anne-Marie Buisman, Guy A.M. Berbers[1], Nico M. Wulffraat*[3], Fiona R.M. van der Klis*[1]

1 Laboratory for Infectious Diseases and Screening, National Institute of Public Health and the Environment, Bilthoven, the Netherlands

2 Department of Pathology, VU University Medical Centre, Amsterdam, the Netherlands

3 Department of Pediatric Immunology, Wilhelmina Children's Hospital, University Medical Center, Utrecht, the Netherlands

* Contributed equally

Country: Netherlands

Objectives: In healthy young women the bivalent HPV vaccine is safe and immunogenic, and it induces a high degree of protection against HPV16/18 infection and their associated pre-cancer lesions. Children with chronic rheumatic diseases as juvenile idiopathic arthritis (JIA) are at increased risk of persistent HPV infections that may progress into HPV-associated malignancies. We conducted a prospective controlled clinical trial in order to assess the immunogenicity and safety of the HPV vaccine in female patients with JIA compared to healthy female adolescents. **Method:** Female patients (n = 68) and healthy female adolescents (n = 55) aged 12 to 18 years were recruited and vaccinated with Cervarix in a 2+1 vaccination schedule. Study visits with blood sampling occurred pre-vaccination and at 3, 7, and 12 months postvaccination. Serum samples were tested for HPV16/18-specific antibodies and antibody avidity using a multiplex immunoassay in which VLP16 and 18 were coupled to fluorescent microspheres. Memory B-cell responses were assessed by HPV16/18-specific ELIspot assays. Adverse events and the effect of vaccination on JIA disease were registered during the trial (ClinicalTrials.gov, registration number NCT00815282). **Results:** All participants were seropositive for HPV16 and 18 at 7 months. One patient turned seronegative at 12 months for both HPV16 and 18. No significant differences in HPV16/18-specific antibody concentrations were found between patients and controls but antibody concentrations were consistently lower in patients. No effect of anti-rheumatic drugs on HPV16/18 antibodies was detected (methotrexate, HPV16 p = 0.790, HPV18 p = 0.372). Patients using anti-TNFα were all seropositive after vaccination. Avidity Index of HPV16/18-specific antibodies at 12 months was similar in patients and controls for HPV16 and HPV18. the kinetics of HPV16/18 memory B-cell responses was comparable between patients and controls. However, the magnitude of memory B-cell responses at 7 and 12 months appeared lower in patients. No relevant differences in adverse events were found between both groups. No detrimental effect of the HPV vaccine on JIA disease activity was detected. **Implications and Impact:** The bivalent HPV16/18 vaccine is immunogenic, well tolerated and safe in JIA patients. However, HPV-specific antibody concentrations and the magnitude of B-cell responses were consistently lower in JIA patients compared to healthy controls during follow-up. It is possible that long-term protection against HPV infection is not guaranteed. Therefore, immunosurveillance in JIA patients seems warranted.

Clinical Science New vaccine trials (Phase I-III; preventive and therapeutic trials)

Cross-Protective Efficacy of HPV 16/18 AS04-Adjuvanted Vaccine: 4-Year End-Of-Study Analysis of Patricia Trial Utilising the Type Assignment Algorithm

Presenter: Barbara Romanowski, MD

Investigators/Collaborators: PATRICIA Study group

Country: Canada

Background: The human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine (Cervarix®, GlaxoSmithKline Vaccines) has shown high and sustained vaccine efficacy (VE) against infections and cervical intraepithelial neoplasia (CIN)2+ associated with HPV-16/18, and may provide a broader protection as shown by evidence of VE against some non-vaccine oncogenic types. **Objectives:** To present end-of-study results (Month 48) from the Phase III PATRICIA study with respect to cross-protective VE against infection and CIN2+, using HPV Type Assignment Algorithm (TAA). This is an alternative case definition assigning likely causality of CIN2+ lesions infected with multiple HPV types based on type-specific detection of HPV DNA in the lesion as well as in the preceding cytology samples. **Methods:** In this study (NCT00122681), women aged 15–25 years were randomised (1:1) to receive HPV-16/18 vaccine (N = 9319) or control (N = 9325) at Months 0, 1 and 6. Cervical samples were collected every 6 months for HPV DNA typing; gynaecological and cytopathological examinations were performed every 12 months. VE results are reported for the total vaccinated cohort (TVC)- naïve (women who received ≥ 1 vaccine dose, seronegative for HPV-16/18 and HPV DNA negative for 14 oncogenic HPV types, with normal cytology at baseline). **Results:** During the study period (overall mean follow-up for TVC-naïve was 44.3 months), VE (95% CI) against CIN2+ was 92.1% (68.1, 99.1) for HPV-31, 79.3% (44.6, 93.8) for HPV-33 and 100.0% (41.7, 100.0) for HPV-45. VE against CIN2+ associated with any non-vaccine oncogenic HPV type (HPV-31/33/35/39/45/51/52/56/58/59/66/68) was 55.3% (35.9, 69.3), and 69.8% (57.8, 78.8) against any oncogenic HPV type including vaccine types (HPV 16/18/31/33/35/39/45/51/52/56/58/59/66/68). VE against CIN3+ was 100% (15.5, 100) for HPV-31, 71.6% (-49.3, 97.1) for HPV-33, 100.0% (-429.7, 100.0) for HPV-45, 94.8% (79.8, 99.4) against any oncogenic HPV type including HPV 16/18, and 91.0% (63.2, 99.0) against any non-vaccine oncogenic HPV type. **Implications and Impact:** End-of-study (4-year follow-up) cross-protective VE results using TAA were similar to those using the primary case definition based on detection of HPV DNA in the lesion only (Wheeler CM, et al. Lancet Oncology 2012;13:100–10). These results strengthen the causal link between CIN2/3+ lesions and associated non-vaccine HPV types in the evaluation of cross-protective VE. Cervarix® is a registered trademark of the GlaxoSmithKline group of companies.

Clinical Science, New vaccine trials (Phase I-III; preventive and therapeutic trials)

Interchangeable Use of Gardasil and Cervarix: Preliminary Safety Data

Presenter: Vladimir Gilca, MD PhD

Investigators/Collaborators: Chantal Sauvageau MD, MSc; Geneviève Deceuninck MD, MSc; Nicole Boulianne RN, MSc; Gaston De Serres MD, MSc; Marc Dionne MD, MSc

Country: Canada

Background: Disruption in delivery of vaccines used in public immunization programs were previously reported in different countries. the availability of the same vaccine for the full course of vaccination should not be taken for granted. No data on interchangeable use of two HPV vaccines in humans are available. Evidence based data on the interchangeable use of two HPV vaccines is needed. **Objectives:** the main objective of this study was to assess the comparative immunogenicity and safety of Gardasil and Cervarix when administered to girls who previously received 2 doses of Gardasil. Here we present safety data. **Methods:** 416 12-14 year-old girls previously vaccinated with 2 doses of Gardasil at the age of 9-10 years according to a 0-6 months schedule were invited to participate in this blinded randomised (1:1) clinical trial. All reported adverse events were included in this analysis. **Results:** 366 girls accepted to participate and were randomised to receive Gardasil or Cervarix. after the administration of Gardasil or Cervarix, 81% and 91% reported a local ($p = 0.004$), and 58% and 59% a general adverse event ($p = 0.83$), respectively. after each of two vaccines similar proportions of vaccinees reported fatigue (34-35%), headache (26-29%), gastrointestinal symptoms (9-12%), rash (2-3%), myalgia (25-30%), artralgia (13-14%), urticaria (1-2%) and fever $\geq 37.5°C$ (0-1%) (all $p > 0.05$). Slightly higher proportion of vaccinees who received Cervarix reported redness (28% vs. 22%; $p = 0.15$) and swelling (26% vs. 18%; $p = 0.04$) than those who received Gardasil. Overall pain, and grade 3 pain at the injection site was more often reported after Cervarix when compared to Gardasil: 89% vs. 72% ($P < 0.001$) and 9% vs. 2% ($P = 0.003$), respectively. No vaccine related serious adverse event was reported. in the group who received 3 doses of Gardasil, a higher proportion of participants reported local (81% vs.62%) and general adverse events(58% vs. 42%) after the third dose when compared to first two doses of vaccine (all $p < 0.05$). **Implications and Impact:** The results of this study show that Cervarix has an acceptable tolerability profile when given to girls who previously received two doses of Gardasil. the higher proportion of vaccinees reporting adverse events after the third dose of Gardasil when compared to first 2 doses of the same vaccine is most probably related to the age when the vaccine was administered. the results from the ongoing immunogenicity tests will allow concluding on the possibility of interchangeable use of two HPV vaccines.

Clinical Science New vaccine trials (Phase I-III; preventive and therapeutic trials)

Efficacy and Safety of RO5217790 Treatment in Patients with High Grade Cervical Intraepithelial Neoplasia(CIN2/3)

Presenter: Pekka Nieminen, MD

Investigators/Collaborators: Pekka Nieminen[1], Diane Harper[2], Mark H. Einstein[3], Francisco Garcia[4], Gilbert Donders[5], Warner Huh[6], Thomas C. Wright[7], Mark Stoler[8], Alex Ferenczy[9], Olga Rutman[10], Anna Shikhman[10], Mimi Leung[10], Barry Clinch[11], Elizabeth Calleja[10]

1 Helsinki University Hospital, Helsinki, Finland
2 University of Missouri Kansas City School of Medicine, Kansas City, MO, USA
3 Montefiore Medical Center/Albert Einstein College of Medicine, Bronx, NY, USA
4 University of Arizona Health Science Ctr, Tuscon, AZ, USA
5 AZ Heilig Hart, Tienen, Belgium
6 University of Alabama at Birmingham, AL, USA
7 Columbia University Medical Center, New York, NY, USA
8 University of Virginia Health System, Charlottesville, VA, USA
9 McGill University and the Sir Mortimer B. Davis-Jewish General Hospital, Montreal, Quebec, Canada
10 Hoffmann La Roche, Inc, USA
11 Roche Prod Ltd, UK

Country: Finland

Objectives: NV25025 is a randomized Phase 2 study to determine the safety and efficacy of RO5217790 compared to placebo in CIN2/3 patients. RO5217790 is a targeted HPV immunotherapeutic comprised of Modified Vaccinia Ankara (MVA) with modified HPV16 E6 and E7 as well as the gene for human IL2. **Methods:** Patients with CIN2/3 were randomized 2:1 to receive subcutaneous injections of RO5217790 or placebo weekly 3 times within 60 days of initial biopsy diagnosis followed by conization at Month 6. Randomization was stratified by the presence of HPV16 monoinfection or other genotypes. Histology of biopsies obtained at baseline and from conization at Month 6 was centrally reviewed. HPV response was determined using the Roche linear array and defined as clearance of baseline HPV genotypes. Efficacy was assessed in the modified intent to treat population (mITT) defined as patients who received at least one study injection and had a CIN2/3 at entry by central pathology. **Results:** Overall, 206 patients were enrolled and dosed. the mITT population included 192 patients: 129 received RO5217790 and 63 received placebo. There were 85 patients with HPV16 mono-infection: 56 in the active arm and 29 in the placebo arm. the number of HPV16 mono-infected patients who achieved histologic resolution (no CIN) was 5 times greater in the active arm compared to the placebo (20% vs. 4%). a histologic response (< CIN2) was observed in this same group in 31% in the active arm vs. 22% in the placebo arm. the rates of histologic resolution and response in the mITT patients (all genotypes) were 15% higher in the RO5217790 arm than in the placebo arm (25% vs. 10% for resolution and 36% vs. 21% for response). At Month 6, patients treated with RO5217790 had a higher clearance of baseline HPV genotypes than those who received placebo: 38% vs. 9% in the HPV16 mono-infected group and 37%

vs. 14% in the mITT population. Treatment with RO5217790 was safe and well tolerated, with injection site reactions (ISRs)reported as the most frequent drug-related adverse events. the majority of the ISRs were mild to moderate in intensity. There were only 2 related serious adverse events: lymphadenopathy in the active arm and breast cancer in the placebo arm. There was one unrelated death in the placebo arm. **Implications and Impact:** RO5217790 showed higher activity when compared with placebo in both histologic resolution and response as well as viral clearance at 6 months in patients with CIN2/3. This targeted immunotherapy was safe and well tolerated.

Clinical Science New vaccine trials (Phase I-III; preventive and therapeutic trials)

Efficacy of an HPV16/18 Vaccine against Oral HPV Infections: a Randomized Clinical Trial

Presenter: Rolando Herrero, MD

Investigators/Collaborators: Rolando, Herrero, Wim Quint, Allan Hildesheim, Paula Gonzalez, Linda Struijk, Hormuzd a Katki, Carolina Porras, Mark Schiffman, Ana Cecilia Rodriguez, Diane Solomon, Silvia Jimenez, John T. Schiller, Douglas R. Lowy, Leen-Jan van Doorn, Sholom Wacholder, and Aimée R. Kreimer, for the CVT Vaccine Group.

Country: France

Objectives: Human papillomavirus (HPV) infection, particularly with type 16, causes a growing fraction of cancers of the oropharynx, whose incidence has significantly increased in recent decades, mainly in developed countries. We evaluated vaccine efficacy (VE) of an ASO4-adjuvanted HPV16/18 vaccine against oral HPV infections. **Method:** In a double-blind controlled trial, 7,466 women 18-25 years of age were randomized (1:1) to receive the HPV16/18 vaccine or hepatitis a vaccine as control. At the final blinded study visit, approximately four years after vaccination, 5 840 participants provided oral specimens (91.9% of eligible women) to evaluate VE against oral infections. Our primary analysis evaluated prevalent oral HPV infection at the four-year study visit in the cohort of all vaccinated women with oral and cervical HPV results. Corresponding VE against prevalent cervical HPV 16/18 infection was calculated for comparison. **Results:** Oral prevalence of identifiable mucosal HPV was low (1.9%) but comparable to previous reports. Approximately four years after vaccination, there were 15 prevalent HPV16/18 infections in the control group and one in the vaccine group, for a VE of 93.3% (95% CI = 63-100). Corresponding efficacy against prevalent cervical HPV16/18 infection for the same cohort at the same visit was 72.0% (95% CI = 63-79) (p versus oral VE = 0.04). There was no statistically significant evidence of protection against other oral HPV infections, though power was limited for these analyses. **Implications and Impact:** HPV prevalence four years after vaccination with the ASO4-adjuvanted HPV16/18 vaccine was much lower among women in the vaccine arm compared to those in the control arm, suggesting that the vaccine affords strong protection against oral HPV16/18 infection, with potentially important implications for prevention of increasingly common HPV-associated oropharyngeal cancer.

Clinical Science, New vaccine trials (Phase I-III; preventive and therapeutic trials)

Prevention of Anal Condyloma with Quadrivalent Human Papillomavirus Vaccination of Older Men who Have Sex with Men: a Nonconcurrent Cohort Study

Presenter: Stephen Goldstone, MD

Investigators/Collaborators: Kristin a Swedish, MD, MPH Stephen E. Goldstone, MD

Country: United States

Objectives: The quadrivalent human papillomavirus vaccine (qHPV) is FDA-approved for use in males 9 to 26 years old to prevent anogenital condyloma. QHPV has been shown to decrease anal and cervical high-grade dysplasia recurrence post treatment. the objective of this study is to determine if qHPV is effective at preventing anal condyloma among MSM 26 years of age and older. **Method:** This nonconcurrent cohort study evaluated HIV-negative MSM aged ≥ 26 years seen at a single site during 2007-2010. Patients either had no history of anal condyloma or had previously-treated anal condyloma recurrence-free for at least 12 months. We determined the recurrence rate of anal condyloma in vaccinated versus unvaccinated patients. **Results:** Of 308 eligible patients, 114 (37 %) patients had received the full 3-dose qHPV vaccine electively and 110 (35.7%) had history of anal condyloma. Vaccinated patients were significantly younger(vaccinated mean age 38.5 years with standard deviation [SD] 7.4, unvaccinated mean age 44.2 years with SD 10.4, p = 0.000) and were more likely to test positive for oncogenic HPV within 8 months prior to study entry (vaccinated 43.9%, unvaccinated 33.5%, p = 0.028). Groups were comparable in respect to race/ethnicity; insurance type; smoking status; history of anal condyloma; history of high-grade anal intraepithelial neoplasia; and history of gonorrhea, chlamydia, and syphilis. During 588.0 person-years follow-up, 8 (7.0%) vaccinated patients and 36 (18.6%) unvaccinated patients developed anal condyloma. Multivariable hazards ratio (HR) analysis at one year showed qHPV and testing negative for oncogenic HPV genotypes were significantly associated with decreased risk of anal condyloma (qHPV HR 0.34, 95% confidence interval [CI] 0.11-1.00, p = 0.049; negative HPV HR 0.17, 95% CI 0.04-0.76, p = 0.021). At two years, multivariable HR analysis showed testing negative for oncogenic HPV was associated with decreased risk of anal condyloma (HR 0.31, 95% CI 0.11-0.85, p = 0.024). qHPV approached significance for decreased risk of anal condyloma (HR 0.43, 95% CI 0.19-1.01, p = 0.052) and history of chlamydia approached significance for increased risk (HR 2.29, 95% CI 1.00-5.26, p = 0.051). Multivariable HR analysis at three years showed qHPV and testing negative for oncogenic HPV were significantly associated with decreased risk of anal condyloma (qHPV HR 0.43, 95% CI 0.19-1.00, p = 0.049; negative HPV HR 0.37, 95% CI 0.15-0.91, p = 0.030). **Implications and Impact:** Among MSM 26 years of age and older with and without history of anal condyloma, qHPV reduces the risk of anal condyloma development. a randomized controlled trial is needed to confirm these findings in this age group.

Clinical Science, Non-vaccine treatment for HPV associated disease

Treatment of Anal Intraepithelial Neoplasia in HIV+ MSM: A Triple Arm Randomized Clinical Trial of Imiquimod, Topical 5-Fluoruracil and Electrocautery

Presenter: Olivier Richel, MD
Investigators/Collaborators: Richel O, De Vries HJC, Van Noesel CM, Dijkgraaf M, Prins JM
Country: Netherlands

Objectives: Anal cancer is an increasing problem among HIV+ men-who-have-sex-with-men (MSM). Screening for its precursor lesion anal intraepithelial neoplasia (AIN) is subject of discussion. Current treatment options are suboptimal and have not been compared in a prospective trial. in this randomised clinical trial we compared efficacy and side effects of imiquimod, topical 5-fluoruracil (5-FU) and electrocautery (ECA) for the treatment of AIN. **Method:** 148 HIV+ MSM with histological confirmed AIN were randomised between 16 weeks of imiquimod (3 times a week), 5-FU (twice a week) or monthly ECA for 4 months. Participants were evaluated by high-resolution anoscopy with biopsies 4 weeks and 6 months after treatment. Response rates were compared by chi-square analysis. **Results:** 57% of patients had high grade (HG) AIN. in an intention to treat analysis imiquimod showed a response rate of 39% (95% CI 27-52), 5-FU of 29% (95% CI 18-43) and ECA of 48% (95% CI 34-62). Complete response was seen in 26% (95% CI 16-39), 17% (95% CI 8-30) and 41% (95% CI 28-56) respectively (p = 0.03), of which 25%, 57% and 17% recurred 6 months after treatment. in a multivariate logistic regression, HGAIN, peri-anal AIN and high plasma CD4 cell count were significantly associated with response to treatment, with odds ratios of 3.5 (p = 0.003), 31.9 (p = 0.003) and 1.003 (per cell/µl; p = 0.002) respectively. Severe side effects were seen in 43% (imiquimod), 27% (5-FU) and 18% (ECA) (p = 0.02). **Implications and Impact:** This study showed that regarding both efficacy and side effects electrocautery is superior to imiquimod and efudix in treatment of AIN, but recurrence rates are substantial.

Clinical Science, Non-vaccine treatment for HPV associated disease

Risk of Preterm Delivery after Treatment for Cervical Intraepithelial Neoplasia in England

Presenter: Alejandra Castanon, MD
Investigators/Collaborators: A Castanon, R Landy, P Brocklehurst, H Evans, N Singh, P Walker, J Patnick, D Peebles, P Sasieni, for the PaCT Study Group
Country: United Kingdom

Objectives: To estimate the association between treatment for cervical intraepithelial neoplasia and the risk of preterm birth in England. in particular, whether the amount of material excised modifies the risk. **Method:** We carried out a retrospective prospective cohort study (phase 1) with a nested case-control study (phase 2) using record linkage. We identified women with a histological sample taken at colposcopy from pathology and colposcopy records from 12 English hospitals between 1989 and 2011. These women were linked by HES (Hospital Episode Statistics) to hospital obstetric records between 1998 and 2009 for the whole of England to identify live births. the main outcome measure is the risk ratio of preterm births following excisional treatment (LLETZ/knife cone/laser cone) for cervical intraepithelial neoplasia. Using phase 2 data we also consider the amount of material excised. Analyses were adjusted by age at delivery, parity and the hospital where colposcopy was carried out. **Results:** Phase 1 included 18,441 singleton births: 4,176 before histology and 14,265 after. among first births subsequent to histology, the adjusted relative risk of a preterm birth associated with previous treatment was 1.19 (95% CI 1.01 to 1.41); among first births prior to histology the relative risk associated with subsequent treatment was 1.47 (95% CI 1.05 to 2.05). Combining these gives the relative risk associated with treatment adjusted for timing relative to histology of 0.91 (95% CI 0.66 to 1.26). Preliminary phase 2 data included 1842 births of which 1356 were after histology: about half of these births were preterm (by design). of those with a birth after histology 836 had a single treatment and 417 had a punch biopsy only. the height of the cone ranged from 1 to 30 mm with 20% being 14 mm or greater. the median height was 10 mm. About 8.5% of treated women had multiple treatments. We will have sufficient power to explore the risk of preterm delivery by treatment height in women with single and multiple treatments. **Implications and Impact:** The overall risk of preterm delivery in women treated by colposcopy in England was substantially less than that in many other studies, predominantly from Nordic countries. However only a small proportions of treated women had deep excision (only 10% were 15mm or greater) and it is possible that large excisions increase the risk of preterm birth.

Clinical Science Non-vaccine treatment for HPV associated disease

Effects of REBACIN in Treatment of Cervical Intraepithelial Neoplasia (CIN) Following LEEP Procedure

Presenter: Chunfa Zhang, PhD
Investigators/Collaborators: Li Cheng[1], G.Y. Wang[2], C.F. Zhang[3]
1 Dept. of Obstetrics and Gynecology, the 1st Hospital, Shanxi Medical University,Taiyuan, China
2 SR Biopharma Inc, Hainan, China
3 SR Life Sciences Institute Inc, MD, USA
Country: United States

Background: We have previously reported that REBACIN, a novel antiviral factor, is effective in high risk HPV infection clearance with notable virus negative conversion rate in a clinical study. REBACIN also exerts drastic suppression on the HPV E6/E7 oncogene expression, and largely reduces the growth of tumors induced by HPV16/18 infection in a mouse model. **Objective:** To evaluate the clinical effect of

REBACIN in the treatment of high grade cervical dysplasia following loop electrosurgical excision procedure (LEEP). **Methods:** 102 cases (patients) with CIN 2-3 and high risk HPV positive were LEEP treated and then were randomly divided into two groups. An experimental group of 53 cases received REBACIN treatment via vaginal administration for three months from the date LEEP was completed. a control group of 49 cases received no further treatment after LEEP. a 24-month follow up was conduced post the LEEP procedure, during which HPV-DNA test, colposcopy, and cervical biopsy were performed at 3-, 6-, 12- and 24-month intervals for both groups. the residual and recurrence disease rates were measured accordingly. **Results:** At both 12- and 24-month follow ups, 51 out of the 53 cases in the REBACIN-treated group were both CIN free and HPV negative. This 96% disease-free rate is significant higher than that of the control group, which is 73.5% accounting for 36 out of the 49 cases. 2 cases of the REBACIN group showed persistent HPV positive, and re-developed CIN again during the follow up, thus account for the disease recurrence rate of this group at 3.8%. the recurrence rate of the control group, on the other hand, is 26.5% for which 13 out of the 49 cases had CIN again in the course of the follow ups. **Conclusion:** These data has illustrated that REBACIN treatment in combination with LEEP procedure can effectively enhance the cure rate for high grade cervical intraepithelial neoplasia, and significantly reduce the disease recurrence rate compared with patients who were treated with LEEP only.

Epidemiology/Public Health, Biomarkers

Is HPV-16 Integration a Predictor Marker of Cervical Lesions?

Presenter: Rui Medeiros, PhD

Investigators/Collaborators: Joana Ribeiro[1,3,4], Dulce Teixeira[1,5], Joana Dias[1,6], Inês Baldaque[2], Rui Medeiros[1,4,7], Hugo Sousa[1,3,7]

1 Molecular Virology Laboratory of 2 Virology Service, Portuguese Institute of Oncology of Porto, Porto, Portugal

3 FMUP, Faculty of Medicine, University of Porto, Portugal

4 Portuguese League Against Cancer, Núcleo Regional do Norte, Portugal

5 ESTSP-IPP, School of Allied Sciences of the Polytechnic Institute of Oporto, Portugal

6 ESB-UC- Superior School of Biotechnology (Universidade Católica Portuguesa), Porto, Portugal

7 Molecular Oncology Group of Portuguese Institute of Oncology, Porto, Portugal

Country: Portugal

Objectives: The persistent infection with carcinogenic types of human papillomavirus (HPV) has been established as the main etiological factor for the development of squamous intraepithelial lesions of the cervix which may progress to high-grade dysplasia or invasive carcinoma. the integration of HPV genome into the host´s genome is considered the hallmark of HPV-associated carcinogenesis. However, the significance of HPV physical status detection remains unclear. the aim of this study was to characterize the physical status of HPV-16 in samples with different histological classifications. **Method:** We have selected 53 cervical specimens from women with different histological classification (7 normal, 15 atypical cells of undetermined significance (ASC-US), 12 low-grade squamous intraepithelial lesion (LSIL), 15 high-grade squamous intraepithelial lesions (HSIL) and 4 invasive cervical carcinoma (ICC)) that have been identified with HPV-16 infection (45 single infection and 8 co-infections). the physical status of HPV16 was analyzed using a multiplex Real-time PCR that allows simultaneous amplification of the E2 and E6 regions. HPV-16 status classification was based on the principle that, when integration occurs, the E2 gene is partially or totally disrupted while the E6 gene remains intact. **Results:** In this study, the prevalence of HPV16 integration was of 26.4% (14/53, 13 mixed forms and 1 integrated only). Results showed no significant association observed comparing HPV-16 integration with single vs co-infections with others HPVs ($p = 0.647$). Prevalence of HPV-16 integration among different cervical lesions was 28.6% (2/7) in samples without cytological lesion, 13.3% (2/15) in ASCUS, 33.3% (4/12) in LSIL, 33.3% (5/15) in HSIL and 25.0% (1/4) in ICC. Additionally, we no found statistical significant differences in HPV-16 integration distribution among the histological specimens ($p = 0.735$). **Implications and Impact:** Our study revealed that HPV 16 integration is not exclusive event of high-grade lesions/ICC. It was not possible to detect integrated forms in all cases of HSIL/ICC. This fact reveals the need to reconsider the role of viral genome integration in HPV associated carcinogenesis and suggests the requirement of further studies, preferably cohort studies, to follow-up normal, ASC-US and LSIL cases which present HPV integration and evaluate their progression.

Epidemiology/Public Health Epidemiology/Natural history of anogenital HPV in females

Microinvasive Adenocarcinoma of the Cervix in Young Woman Vaccinated against HPV: from Studies to Reality

Presenter: Julio Teixeira, PhD

Investigators/Collaborators: Julio Cesar Teixeira[1], Eliane R. Zambelli Oliveira[1], Círbia S. Campos Teixeira[1], Liliana A. L. A. Andrade[1], Carlos Eduardo Bacchi[2], Luiz Carlos Zeferino[1], Sophie Françoise Mauricette Derchain[1]

1 State University of Campinas - UNICAMP, Campinas, Brazil

2 Bacchi´s Laboratory Consultant Pathologist, Botucatu, Brazil

Country: Brazil

Objectives: Several factors are associated with an increased in the number of adenocarcinoma of the cervix. HPV vaccines and new screening technologies can counter this trend. It is expected a transition period with cases simulating limitations or flaws in the process vaccination-screening-treatment. the objective of this report is to illustrate with a real situation what may occur in the coming years. **Method:** A teenager 16 years old (she had a baby one year before), started in June 2005 the participation in the Phase III study (HPV-008/NCT00122681) to evaluate the efficacy of the HPV 16-18 AS04-adjuvanted vaccine (GSK). Received all three vaccine doses (zero, 30 and 180 days) and completed all procedures according to protocol: cervical sample collection every six months for PCR (SPF10 LiPA25) for identification

of 14 HR-HPV and 11 LR-HPV and yearly for cytology. Only cytology results, five negative exams, were available for clinical follow-up. Upon completion of the study and breakdown of blinding, this patient had received the HPV vaccine and had a HR-HPV test (+) at the final visit (May/2009). Due to HPV test (+), she was invited to continue the annual monitoring through an extension of the initial study, for up to four additional years. **Results:** In JAN/2011, remains the test HR-HPV (+) and cytology (−), with colposcopy (−). in JAN/2012, aged 23 years old, test HR-HPV (+) with an ASC-H cytology. the colposcopic evaluation had a focal area of aceto-white epithelium, slightly evident within a small ectropion, without further images. the biopsy showed an Adenocarcinoma in Situ in this area. We performed a LEEP cone with endocervical reinforce and the result was a Microinvasive adenocarcinoma of the cervix (invasion < 1 mm) with resections margins and canal reinforcement, negatives. She was instructed to keep strict follow-up. Due to HPV vaccination in 2005 and developments presented were obtained further information from the HPV-008 study: 7/8 of HPV testing performed in cervical samples from 2005 to 2009 detected a single infection with HPV-18, including the initial visit before the first dose. PCR and Immunohistochemical Study of material from the LEEP showed the presence of HPV-18 L1, p16 + and Ki-67 reagent. **Implications and Impact:** HPV-18 DNA was present in the cervix prior to vaccination. Persistent infection with HPV-18 can be considered an important factor in the development of lesions of the glandular epithelium. the glandular lesions are more difficult to be detected by cytology and colposcopy. It is evident the importance of vaccination before sexual debut and adequacy of screening in vaccinated.

Epidemiology/Public Health Epidemiology/Natural history of anogenital HPV in females

Molecular Detection of Chlamydia Trachomatis Infections in Screened Women for Cervical Cancer

Presenter: Cecilia Roteli Martins, PhD

Investigators/Collaborators: Renata RLM de Barros[1], Mariangela F Silveira[2], Dulce Stauffert[2], Marco Zonta[3], Priscila H de Oliveira[4], Ilana Di Fiore G Palermo[4], Solana Terrazas Martins[5], Cecilia M Roteli-Martins[6], Adhemar Longatto Filho[7]

1 Hospital – Secretaria da Saúde, São Paulo, Brazil
2 Universidade Federal de Pelotas (FAMED), Pelotas, RS, Brazil
3 Universidade Federal de São Paulo (Unifesp)/Lab in Cito, São Paulo, Brazil
4 Lab in Cito, São Paulo, Brazil
5 Departamento de Salud Pública, Pontificia Universidad Católica de Chile
6 L M de Barros Hospital – Secretaria da Saúde, São Paulo, Brazil
7 Universidade de São Paulo (LIM) Brazil, School of Health Sciences Universidade do Minho (ICVS), Braga, Portugal; Molecular Oncology Research Center, Hospital de Cancer Barretos, Brazil

Country: Brazil

Objectives: To verify the prevalence of CT infections in cervical samples obtained from women undergoing routine cytological screening for cervical cancer **Method:** Women aged 15 to 64 years were invited to routine cytological examination. Pap tests were collected and prepared with BD SurePath liquid-based. after cytological preparation residual samples were tested for CT using BD ProbeTec™ ET, an amplified DNA Assays, that use Strand Displacement Amplification (SDA) technology for the direct, qualitative detection of CT DNA **Results:** Women's mean age were 40.9 ± 11.2. the overall prevalence of CT was 15.7% (CI 95% 13.9%-17.6%). There was no statistical significant difference in CT prevalence within age groups(< 25 years: 16.4% (CI 95%: 9.6%-23.1%); 25 to 44 years: 16.9% (CI 95%: 14.3%-19.5%); 45 to 64 years: 14.0% (CI 95%: 11.2%-16.8%)). 15.0% (CI 95%10.5%-19.5%) of CT positive women had an abnormal cytology result (ASCUS or worse), with no statistical significant difference with the CT negative group (p = 0.689) **Implications and Impact:** Chlamydia trachomatis (CT) infection is one possible co-factor that lead to HPV persistence and cervical cancer. Basic epidemiological data about HPV and CT infection in Brazilian territory are still scarce. Our preliminary data indicate that the prevalence of CT infection in São Paulo, Brazil is high, but was not associated with abnormal Pap results. the present work could provide a framework for improving national strategies to control CT infection.

Epidemiology/Public Health, Epidemiology/Natural history of anogenital HPV in females

Human Papillomavirus (HPV) Perinatal Transmission and Risk of HPV Persistence among Children: a Cohort Study

Presenter: Helen Trottier, PhD

Investigators/Collaborators: Helen Trottier[1], Marie-Hélène Mayrand[2], Patricia Monnier[3], William Fraser[4], Ana-Maria Carceller[5], Diane Francoeur[4], François Coutlée[6]

1 Department of Social and Preventive Medicine, University of Montreal, Sainte-Justine Hospital,Montreal, Canada.
2 Dept. of Obstetrics and Gynecology, University of Montreal, St-Luc Hospital, Montreal, Canada
3 Dept. of Obstetrics and Gynecology, Mc Gill University, Royal Victoria Hospital; Montreal, Canada.
4 Dept. of Obstetrics and Gynecology, University of Montreal, Sainte-Justine Hospital, Montreal, Canada
5 Dept. of Pediatrics, University of Montreal, Sainte-Justine Hospital, Montreal, Canada.
6 Dept. of Microbiology, University of Montreal, Notre-Dame Hospital, Montreal, Canada

Country: Canada

Objectives: 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. **Objective:** To measure the probability of perinatal transmission of type-specific mucosal HPV (in conjunctival, laryngeal, buccal and genital mucosa of newborns) and its determinants; the risk of HPV persistence among children and its determinants; the presence of antibodies against HPV in mothers and children; and the association

between placental HPV infection and pregnancy outcome. **Method:** We are recruiting pregnant women 18-30 years of age during the first trimester of pregnancy in a prospective cohort study at three tertiary care centers in Montreal, Canada. Cervicovaginal swabs are taken at enrolment (1st trimester) and during the 3rd trimester for women positive at enrolment, and tested for 36 HPV genotypes by Linear Array. Children of positive mothers are followed until 2 years of age, at an interval of 3-6 months. At each visit, conjunctival, buccal, laryngeal and genital samples are collected for HPV testing. At each visit, mothers also provide information on sociodemographic, lifestyle, etc. Patient charts are reviewed to document pregnancy and outcome. Placenta specimens (biopsies and swabs) are also collected for HPV PCR testing. Blood samples from mothers and children are collected at different time points for HPV antibodies testing using HPV Virus-Like-Particle(VLP)-Based Enzyme Immunoassay for seroreactivity to HPV types 6/11/16/18 capsids. **Results:** Recruitment of pregnant women was completed at the end of June 2012 (N = 166). Follow-up is ongoing. PCR testing has been done for 160 women at enrolment (1st trimester). the prevalence of HPV was 44% (67% were positive for at least one HR-HPV) and 49% of them have multiple genotypes (range 2 to 11). 68 placentas were tested for HPV; 24% of the women with an HPV positive cervicovaginal sample during the first trimester had a positive placenta (8/33) whereas 3% of the women who were negative for HPV at enrolment had a positive placenta (1/35). 33 babies were born and tested for HPV at birth and all of them were HPV negative (conjunctival, buccal, laryngeal and genital samples). Final results (completed follow-up) will be available in 2015. **Implications and Impact:** We found a significant prevalence of HPV in pregnant women. Although HPV could be detected in the placenta, few newborns had HPV. This study will further our understanding of the perinatal transmission at different body sites in children born from HPV positive mother.

Epidemiology/Public Health, Epidemiology/Natural history of anogenital HPV in females

The Long-Term Study of GARDASIL™ in Previously Vaccinated Women: Absence of HPV Replacement Disease

Presenter: Joakim Dillner, MD

Investigators/Collaborators: Susanne Krüger Kjær, Bo Terning Hansen, Laufey Tryggvadóttir, Christian Munk, Lara Sigurdardottir, Maria Hortlund, Michael Ritter, Mari Nygård and Alfred Saah

Country: Sweden

Background: The GARDASIL™ long-term follow-up (LTFU) study is an ongoing extension of a pivotal randomized, placebo-controlled, doubleblind, 4-year study to investigate the safety, immunogenicity, and effectiveness of quadrivalent Human Papillomavirus vaccine (qHPV) on the incidence of HPV 16/18-related cervical intraepithelial neoplasia (CIN) 2 or worse in 16-to 23-year old women (Protocol 015). **Methods:** Follow-up of subjects is being accomplished in 2 ways: 1) registry-based follow-up for effectiveness data as well as safety data including but not limited to deaths, cancer, and hospitalizations; 2) active follow-up for blood collection for immunogenicity assessments. Effectiveness and safety data represent a mean follow-up of 8 years following the start of Protocol 015. Cohort 1 included approximately 2,700 subjects who received qHPV vaccine at the start of Protocol 015. Cohort 2 consists of approximately 2,100 subjects who received placebo at the start of Protocol 015 and qHPV vaccine prior to entry into the LTFU. Vaccine effectiveness against HPV 16/18-related CIN 2 or worse was estimated by calculating the expected incidence of CIN 2/3 or worse in an unvaccinated (placebo) cohort using historical registry data. the primary analysis approach was Generally HPV Naïve (GHN) for the HPV replacement analysis. **Results:** Previous data indicated no cases of CIN 2+ observed in the GHN population irrespective of HPV type. There were seven (7) cases of CIN 1 observed with follow-up time of 1,088.6 person-years regardless of HPV type in the GHN population. the incidence rate for this endpoint was 0.6 (95% CI: 0.3, 1.3) per 100 person-years at risk. the incidence rates for CIN 1 related to any of the 10 nonvaccine HPV types and not related to any of the 14 assay-identified HPV types were 0.4 (95% CI: 0.1, 0.9) and 0.2 (95% CI: 0.0, 0.7) per 100 person-years at risk, respectively. Data will be presented from 8 years of follow-up (not available at the time of abstract submission). Comparisons will be made to regionally obtained population-based rates of occurrence of certain HPV types prior to the introduction of the vaccine. **Implications and Impact:** HPV-type replacement did not occur at any appreciable level. HPV type replacement will continue to be assessed and further analyses will be performed at two-year intervals.

Epidemiology/Public Health, Epidemiology/Natural history of anogenital HPV in males

The Influence of Male Circumcision on the Incidence and Clearance of Genital Human Papillomavirus Infection in Men: Results from the HIM Study

Presenter: Ginesa Albero, PhD student

Investigators/Collaborators: Ginesa Albero[1,2], Luisa L Villa[3], Eduardo Lazcano-Ponce[4], William Fulp[5], Mary R Papenfuss[5], Xavier Castellsague[1,2], Alan G Nyitray[5], Beibei Lu[5], Martha Abrahamsen[5], F. Xavier Bosch[1], Anna R Giuliano[5]

1 Unit of Infections and Cancer (UNIC), Cancer Epidemiology Research Program (CERP), Catalan Institute of Oncology (ICO). L'Hospitalet de Llobregat (Barcelona), Spain

2 CIBER en Epidemiología y Salud Pública (CIBERESP). Spain

3 Ludwig Institute for Cancer Research, São Paulo, Brazil

4 Instituto Nacional de Salud Publica, Cuernavaca, Mexico

5 H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida, USA

Country: Spain

Objectives: To determine whether male circumcision (MC) affected the incidence and clearance of type-specific genital HPV infections in a multinational cohort study of men. **Method:** The HIM Study cohort enrolled healthy men, aged 18-70 years, from 2005 to 2009 in a study of genital HPV infections in Brazil, Mexico, and the USA. the current analysis included 4,033 men who were followed every 6 months for a

median of 17.5 months (interquartile range, 6.9 – 31.0 months). Saline-wetted Dacron swabs were used to collect exfoliated cell specimens from the coronal sulcus, glans penis, penile shaft, and scrotum at each study visit. Swabs were combined into one sample and genotyped for 37 HPV types using the Roche Linear Array. Circumcision status was determined by clinician exam. Cox proportional hazards models were used to evaluate the association between MC status and the incidence and clearance of HPV infections. Here we present models adjusted for age, country and sexual behavior variables. **Results:** The overall incidence of new genital HPV infection did not differ by MC status (adjusted hazard ratio (aHR) for circumcised men relative to uncircumcised men: 1.05 [95% confidence interval (CI): 0.98-1.13]). However, the incidence of HPV type 55 was significantly higher among circumcised men. Infection clearance was significantly decreased among circumcised men as compared to uncircumcised men (aHR: 0.86 [95% CI: 0.79-0.93]). Circumcised men were significantly less likely to clear HPV types 16, 51, 52, 58, and 82. in contrast, circumcised men were significantly more likely to clear HPV type 11. **Implications and Impact:** The data from this study show that incidence of new genital HPV infection is not associated with MC status. Clearance of HPV was reduced among circumcised men for certain HPV types but not for others. the use in our study of a combined sample from the coronal sulcus, glans, foreskin (if present), shaft, and scrotum likely limited our ability to identify a true effect at the distal penis. Additional prospective data on the effects of MC by specific anatomical sites are necessary to better assess the role of MC in the natural history of HPV infections in men.

Epidemiology/Public Health Epidemiology/Natural history of anogenital HPV in males

Alcohol Consumption and Inconsistent Condom Use Increase Risk for Acquisition of Oncogenic Genital HPV among Gay Men in South and North America: the HIM Study

Presenter: Alan Nyitray, PhD

Investigators/Collaborators: Authors: AG Nyitray[1], RJ Carvalho da Silva[2], ML Baggio[3], J Salmerón[4,5], M Quiterio[4], M Abrahamsen[1], M Papenfuss[1], LL Villa[3], E Lazcano-Ponce[4], AR Giuliano[1]

1 H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida, USA

2 Centro de Referência e Treinamento em DST/AIDS São Paulo, Brazil

3 Ludwig Institute for Cancer Research, São Paulo, Brazil

4 Instituto Nacional de Salud Pública, Cuernavaca, Mexico; 5Instituto Mexicano del Seguro Social, Cuernavaca, Mexico

Country: United States

Objectives: There are no reports of behavioral factors associated with acquisition of genital HPV among men having sex with men (MSM). Given that penile-anal sex increases risk for HPV infection in the anal canal, knowledge about modifiable risk factors may be particularly important for MSM, most of whom are over age 26 years and not eligible for HPV vaccination and among whom annual incidence of anal cancer in the United States may be up to 35/100,000 persons. the purpose of the current study was to assess modifiable factors that increase risk for genital HPV acquisition among MSM and men having sex with women and men (MSWM) recruited in São Paulo, Brazil, Cuernavaca, Mexico, and Tampa, USA. **Method:** Genotyping using the linear array method was conducted for genital specimens from men, ages 18-70, who attended semiannual visits for a 4–year prospective study. Eligibility included no history of genital warts or HIV. a total of 147 MSM and 259 MSWM provided evaluable specimens at greater than or equal to 2 visits. Specimens from the glans, penile shaft, and scrotum were combined for analysis. Twelve-month cumulative incidence was assessed among men negative for type-specific infection at the pre-enrollment visit. Baseline factors that may affect acquisition of genital HPV were assessed using Cox proportional hazard regression. **Results:** Median age of the MSM and MSWM was 29 and 33 years, respectively. the median follow-up time for MSM and MSWM was 19 and 18 months, respectively. During follow up, 68% of MSM and 59% of MSWM acquired one or more of 13 oncogenic HPV types. the 12-month cumulative incidence for any oncogenic type was 31.8% and 25.4% among MSM and MSWM, respectively, while 12- month cumulative incidence for HPV-16 was 9.9% and 5.0%. after controlling for confounders among MSM, modifiable behaviors that increased risk for oncogenic detection were alcohol consumption (compared to 0-30 drinks/month: Hazard ratio (HR), 2.30 [95% CI 1.10–4.83] for > 60 drinks/month] and inconsistent recent condom use for anal sex (compared to always using condoms, HR, 3.05 [95% CI 1.46–6.36]). among MSWM, former smokers were at decreased risk for detection of genital oncogenic HPV (compared to current smokers: HR, 0.16 [95% CI 0.05–0.51]). **Implications and Impact:** Acquisition of oncogenic HPV genotypes at the genitals was very common among MSM and MSWM. Decreased alcohol consumption and consistent condom use among MSM, and quitting smoking among MSWM are modifiable risk factors that may slow acquisition of genital HPV. Such knowledge may be important for gay and bisexual men not eligible for HPV vaccination.

Epidemiology/Public Health, Measuring the impact of HPV vaccination

Estimations of Herd Immunity Benefit Under Different Assumptions Regarding Natural Immunity: a Case-Study Based on the Australian National HPV Vaccination Program

Presenter: Igor Korostil, Mr

Investigators/Collaborators: Gareth Peters, School of Mathematics and Statistics, University of New South Wales, Sydney, Australia; Matthew Law, the Kirby Institute, University of New South Wales, Sydney, Australia; David Regan, the Kirby Institute, University of New South Wales, Sydney, Australia;

Country: Australia

Objectives: To investigate the differences in estimations of herd immunity benefit (when a vaccination benefits whose not vaccinated) in Australian setting, produced by mathematical models differing in assumptions regarding natural immunity to reinfection with the same HPV type. This is relevant in view of recent epidemiological findings suggesting that the assumption of life-long natural immunity, com-

monly accepted in health-economic evaluations of HPV vaccination programs using dynamic mathematical models, may be not plausible. Estimation of herd immunity benefit is an important component of such evaluations: the less significant the benefit is, the less effective is a vaccination program and the more likely is a need for public health decisions aimed at its improvement. **Method:** We develop three mathematical models to describe HPV-6/11/16/18 transmission in an Australian heterosexual population: an SIR type model (commonly used, assumes life-long natural immunity), an SIS model (less popular, no natural immunity) and a model representing a compromise between SIR and SIS (limited natural immunity). This model, which may provide a more accurate characterisation of natural immunity than SIR or SIS, is selected based on the recent literature and calibration of a variable structure general model to observational data. Calibration is performed using a statistically robust Bayesian approach based on an adaptive Markov chain Monte Carlo algorithm. **Results:** In our comparison herd immunity benefits, measured as relative reductions in HPV prevalence in unvaccinated female and male populations, estimated by the non--SIR models, are much more substantial than for the SIR model. in particular, for the unvaccinated female population, they are higher than for the SIR model by at least 27% (HPV-16), 45% (HPV-18) and 75% (HPV-6/11), depending on the assumed vaccine efficacy. Similar differences are observed for the unvaccinated male population. **Implications and Impact:** Studies evaluating vaccination programs which rely only on SIR models may significantly underestimate herd immunity benefits of these programs. As a possible solution, we suggest that several model structures should be considered in such studies, each assigned a "weight" derived from expert opinions or any other current information which can help improve our understanding of natural immunity.

Epidemiology/Public Health, Measuring the impact of HPV vaccination

Comparing the Cost-Effectiveness of the Bivalent, Quadrivalent and Nonavalent HPV Vaccines: a Model-Based Analysis

Presenter: Marc Brisson, PhD
Investigators/Collaborators: Marc Brisson, Nicolas Van de Velde, Jean-François Laprise, Mélanie Drolet, Marie-Claude Boily
Country: Canada

Objectives: Bivalent (HPV2) and quadrivalent (HPV4) HPV vaccines are now licensed in several countries, and a clinical trial is examining the efficacy of a nonavalent vaccine (HPV9). Our aim was to compare the cost-effectiveness of the HPV2, HPV4 and HPV9 to provide evidence for policy decisions. **Method:** We developed HPV-ADVISE, a multi-type individual-based transmission-dynamic model of HPV infection and disease (genital warts (GW), and cervical, anogenital and oropharyngeal cancers). We calibrated the model to epidemiologic data from Canada, and estimated Quality-Adjusted Life-Years (QALYs) lost and costs (2010$CAN) from the literature. We assumed vaccine-type efficacy is 95% for all vaccines, and cross-protective efficacy against HPV-31,-33,-45,-52,-58 is 77, 43, 79, 19,0% and 46, 29, 8, 18,6% for HPV2 and HPV4, respectively. in addition, we assumed duration of vaccine-type efficacy and cross-protection is 20 and 10 years, respectively. the analysis was performed from the healthcare provider perspective, and costs and benefits were discounted at 3%. Sensitivity analysis was performed varying vaccine efficacy, duration of protection, and burden of illness (QALY-lost and costs). Uncertainty in model predictions is presented using the median [10th-90th percentiles] of simulations. **Results:** Under base-case assumptions (vaccinating 10-year-old girls, 80% coverage, 95$/dose), using HPV2, HPV4 and HPV9 was estimated to cost $20,000[$16,000-$25,000], $14,000[$11,000-$19,000] and $12,000[$9,000-$17,000] per QALY-gained, respectively. At equal price, HPV9 remained more cost-effective than HPV2 and HPV4, even when assuming shorter duration of protection (HPV9 = 20 years vs. HPV2/HPV4 = lifelong) and lower vaccine-type efficacy (HPV9 = 85% vs. HPV2/HPV4 = 95%). HPV4 remained more costeffective than HPV2 under all scenarios investigated, except when simultaneously assuming longer HPV2 duration of protection (HPV2 = lifelong vs. HPV4 = 20 years), high cross-protection, and low burden of GW. Under base-case assumptions, the maximum additional cost/dose for HPV9 to be cost-effective compared to HPV2 and HPV4 was $67[57-74] and $12[3-39], respectively (using a $50,000/QALY-gained threshold). the maximum additional cost/dose of HPV4 for it to be cost-effective compared to HPV2 was $54[9-64]. Comparative cost-effectiveness results were most sensitive to differential durations of protection and GW burden of illness. **Implications and Impact:** Vaccinating pre-adolescent girls against HPV is predicted to be highly cost-effective. If equally priced, the most cost-effective vaccine is HPV9, followed by HPV4 and HPV2. However, ultimately, the most cost--effective HPV vaccine will be determined by the relative prices of the vaccines and durations of protection.

Epidemiology/Public Health Measuring the impact of HPV vaccination

HPV6/11/16/18 Vaccine Efficacy in Women 24 to 45: Follow-up Through 6.3 Years Post-Vaccination

Presenter: Joaquin Luna, MD
Investigators/Collaborators: on behalf of the Protocol 019 investigators
Country: Colombia

Objectives: The quadrivalent HPV (types 6, 11, 16 18) (qHPV) vaccine is highly effective in preventing HPV6/11/16/18-related persistent infection, CIN, EGL, and abnormal Pap smears in women aged 24 to 45 naïve to vaccine HPV types. Vaccine efficacy in the prevention of HPV6/11/16/18-related CIN or EGL was 95.7% (95% CI: (73.4, 99.9) through 3.8 years of follow-up. We present the results of an interim analysis of a long-term follow-up study of qHPV in women aged 24-45 designed to determine the long-term immunogenicity, effectiveness, and safety of the qHPV vaccine. This report summarizes data collected as of year 6 post-vaccination (relative to day 1 of the base study). Future analyses are planned at years 8 and 10 (end-of-study analysis). **Method:** This extension study was conducted in Colombia. Subjects who were vaccinated in the base study from Colombia are referred to as the "early vaccination group" (EVG) in this report. Subjects vaccinated after the completion of the base study (catch-up cohort) have not had sufficient follow-up as of this report. **Results:** Enrollment

into the base study was 1,610 in total (804 randomized to qHPV, 806 randomized to with placebo). a total of 1,360 Colombian subjects participated in this extension (84% of the subjects enrolled in base study in that country). No new cases of HPV 6/11/16/18-related genital warts/cervical dysplasia have been reported in the EVG (N = 684). Month 72 antibody titers against HPV 6/11/16/18 are comparable to those observed at Month 48 (end of base study), indicating no further diminution of titers between 4 years and 6 years post-vaccination. No serious adverse experiences have been reported in the EVG between years 4 and 6. **Implications and Impact:** In summary, data through 6.3 years after the start of the 019 base study shows that administration of the qHPV vaccine among women aged 24-45 is generally well tolerated. Anti-HPV 6, 11, 16, and 18 responses have persisted over the long-term, and no additional cases of disease related to vaccine HPV types 6/11/16/18 were observed.

Epidemiology/Public Health, Measuring the impact of HPV vaccination

Long-Term Effectiveness of GARDASIL™ in the Nordic Countries

Presenter: Susanne Krüger Kjær, MD

Investigators/Collaborators: Mari Nygård, Joakim Dillner, Brooke Marshall, Bo Terning Hansen, Lara G. Sigurdardottir, Maria Hortlund, Laufey Tryggvadóttir, Alfred Saah, Christian Munk

Country: Denmark

Objectives: The GARDASIL™ long-term follow-up (LTFU) study is an ongoing extension of a pivotal randomized, placebo-controlled, doubleblind, 4-year study to investigate the safety, immunogenicity, and effectiveness of quadrivalent Human Papillomavirus vaccine (qHPV) on the incidence of HPV 16/18-related cervical intraepithelial neoplasia (CIN) 2 or worse in 16-to 23-year old women (Protocol 015). the LTFU study is taking place in 4 Nordic countries (Denmark, Iceland, Norway, and Sweden). **Methods:** Every citizen in the Nordic countries is assigned a unique personal identification number (PIN) at birth, which is registered in the Civil Registration System in each country. Due to the existence of the PINs and the nationwide registers, it is possible to do follow up studies with virtually no loss to follow--up in the Nordic countries. All women in the trial are followed through different national registries for effectiveness data as well as safety data such as deaths, cancer, and hospitalizations. Effectiveness and safety analyses started approximately 2 years following completion of Protocol 015 and will occur approximately every 2 years thereafter for 10 years. Cohort 1 included approximately 2,700 subjects who received qHPV vaccine at the start of Protocol 015. Cohort 2 consists of approximately 2,100 subjects who received placebo at the start of Protocol 015 and qHPV vaccine prior to entry into the LTFU. Vaccine effectiveness against HPV 16/18-related CIN 2 or worse was estimated by calculating the expected incidence of CIN 2/3 or worse in an unvaccinated (placebo) cohort using historical registry data. the primary analysis approach was perprotocol. **Results:** In the initial analysis of effectiveness after the first 7 years, there were 1,080 subjects that contributed to the follow-up period out of a total of 2,195 eligible subjects in the per-protocol population in Cohort 1. in these subjects there were no cases of HPV 16/18-related CIN 2 or worse observed. There were also no cases of HPV 6/11/16/18-related CIN, vulvar cancer, and vaginal cancer observed. However, the follow-up time in person-years is insufficient to make a definitive statement about the effectiveness of the qHPV vaccine for the current time period. **Implications and Impact:** The qHPV vaccine shows a trend of continued protection in women, although there is as yet insufficient data to confirm that protection is maintained. the qHPV vaccine continues to be generally safe and well tolerated up to 7 years following vaccination.

Epidemiology/Public Health Measuring the impact of HPV vaccination

Decrease in Anogenital Warts Incidence after the Introduction of HPV Vaccination in Germany: Model Predictions and Real Data

Presenter: Andreas Kaufmann, PhD

Investigators/Collaborators: Rafael Mikolajczyk[1,2], Johannes Horn[1], Oliver Damm[3], Renate Schulze-Rath[4], Edeltraut Garbe[5], Andreas M Kaufmann[6]

1 Helmholtz Centre for Infection Research

2 Medical School Hannover, Hannover, Germany

3 Department of Health Economics and Health Care Management, School of Public Health, Bielefeld University, Bielefeld, Germany

4 Sanofi Pasteur MSD

5 Department of Clinical Epidemiology, BIPS - Institute for Epidemiology and Prevention Research, Bremen, Germany

6 Clinic for Gynecology, Charité - University Medicine, Berlin

Country: Germany

Objectives: Anogenital warts (AGW) are considered the best early indicator of HPV-vaccine effects on the population level, since in contrast to cervical intraepithelial neoplasia (CIN) anogenital warts become clinically apparent within months after the initial infection. in two recent independent projects, we addressed changes in the incidence of AGW. First, we developed a mathematical model to study long term effects of HPV vaccination. Second, we studied changes in the incidence of AGW at the population level using health insurance claims data. in this analysis, we aimed to derive model predictions regarding incidence of AGW and compare them with the empirical data. **Method:** A dynamic transmission model for HPV infection and disease progression was developed on the basis of previous models and calibrated to fit the HPV prevalence and incidence of HPV related lesions in Germany. with respect to AGW, the model utilised a multiplicative approach based on incident infections with HPV types 6 and 11 and was fitted to incidence of AGW derived from health insurance data for the years 2005 and 2006, i.e. before introduction of HPV vaccination in Germany. Assuming vaccination coverage of 50% among 17 years old girls with a market share of 90% for Gardasil and 100% effectiveness against anogenital warts, we used the model to predict the development

of AGW incidence in Germany after the introduction of HPV vaccination in 2007. in an independent analysis, changes in the incidence of AGW following the introduction of HPV vaccination up to the end of 2008 were studied based on the ICD-10 diagnoses A63.0 recorded in claims data from a large health insurance company including about six million insurants (8% of the population in Germany). **Results:** Empirical data demonstrated a decrease in the incidence of AGW among those 15 to 17 years old starting after the introduction of HPV vaccination and matching the vaccination coverage. the decline observed in real data was well reproduced in the modeling approach. Assuming that vaccination will continue at the same level, our model predicts an almost complete eradication of AGW in Germany 35 years after the introduction of the HPV vaccine. **Implications and Impact:** Changes in the incidence of AGW accompanying introduction of HPV vaccination in Germany are an early indicator of vaccine effects at the population level. Agreement between observed changes and model prediction verify the modelling approach. Eradication of AGW in Germany is possible, even at a relatively low vaccination coverage level of 50%.

Epidemiology/Public Health Measuring the impact of HPV vaccination

Equity in HPV Vaccination Uptake?

Presenter: Madelief Mollers, MSc

Investigators/Collaborators: Madelief Mollers, Karin Lubbers, Kor Spoelstra, Willibrord Weijmar, Schultz Toos, Daemen Tjalke, Westra Nynke, Koelma Marianne van der Sande, Hans Nijman Hester de Melker, Adriana Tami

Country: Netherlands

Objectives: In the Netherlands, the HPV vaccination (Cervarix) is part of a state funded national programme and should therefore be equally accessible for all girls invited for vaccination. the objective of this study was to investigate whether vaccinated girls are different from unvaccinated girls with regard to demographics, such as education and ethnicity (both associated with nonattendance in the Cervical Cancer Screening programme), sexual behaviour and knowledge of HPV and cervical cancer. **Method:** Online questionnaires were sent to approximately 20.000 randomly selected 16-17 year old girls, which were targeted in the catch-up vaccination campaign in 2010. Out of these girls, 2982 participated (65% vaccinated, 35% unvaccinated). Proportional differences between vaccinated and unvaccinated girls were tested by $\chi 2$. a knowledge scale composite score was calculated based on answers to 7 general knowledge questions (0-7) and 11 questions on HPV transmission knowledge (0-11). Mean scores were compared for vaccinated and unvaccinated girls by a t-test. **Results:** Vaccinated and unvaccinated girls were similar with regard to ethnicity, education level and knowledge of HPV transmission. However, vaccinated girls had slightly more general knowledge of HPV (2.0 vs. 1.9, $p < 0.001$), were from more urbanized areas (53% vs. 47%, $p < 0.01$) and were less likely to have a religious background (46% vs. 54%, $p < 0.0001$). Vaccinated girls were less aware of the Cervical Cancer Screening programme (48% vs. 57%, $p < 0.0001$), although they were more inclined to participate in the future (71% vs. 66%,$p < 0.03$). a higher percentage of vaccinated girls were sexually active (56% vs. 52%, $p < 0.04$) and amongst them, a higher number of lifetime partners was identified (2.2 vs. 1.9, $p < 0.01$). with regard to age of sexual debut, condom use and history of STIs, there were no differences amongst vaccinated and unvaccinated sexually active girls. Irrespective of vaccination status, 81% of the girls knew about the causal relationship between HPV and cervical cancer, but only 20% knew about the relationship between HPV and genital warts. **Implications and Impact:** Routine HPV vaccination in the Netherlands reduces the inequity of prevention of cervical cancer. For example, vaccination uptake is not associated with education and ethnicity. Vaccinated girls were slightly more sexually active indicating that the impact of vaccination is not overestimated in for example modelling studies.

Epidemiology/Public Health, Measuring the impact of HPV vaccination

HPV-Genotype Distribution in Urine Samples from 17-Year Old Girls in Norway in a Nonvaccinated Age-Cohort

Presenter: Christine Jonassen, PhD

Investigators/Collaborators: Christine M. Jonassen[1], Mona Hansen[1], Berit Feiring[2], Ellen Myrvang[1], Thu Nguyen[1], Roger Meisal[1], Elisabete Weiderpass[3], Lill Trogstad[2]

1 Department of Microbiology, Akershus University Hospital, Lørenskog, Norway

2 Department of Vaccines, Norwegian Institute of Public Health, Oslo, Norway

3 Cancer Registry of Norway, Oslo, Norway

Country: Norway

Objectives: The HPV vaccine was included in the Norwegian childhood immunisation programme from September 2009. the quadrivalent vaccine is offered to girls aged 12 years. No catch up vaccination for older girls is offered. As part of a national surveillance programme of the HPV-vaccination, HPV testing in urine was proposed as a surrogate sample for cervical infection in prescreening age cohorts to monitor the impact of the HPV immunisation programme on the occurrence and HPV type distribution in young women. Two age-cohorts were selected for surveillance; 17 and 22 year old girls. Cross-sectional HPV prevalence studies will be performed first in non-vaccinated cohorts, later in vaccinated cohorts as they reach 17 and 22 years of age. the objective of this study was to monitor HPV genotype distribution in urine samples from 17-year old girls in a non-vaccinated age cohort in Norway. **Method:** The whole cohort of girls born in 1994 (n = 25 400) was invited by mail to participate in the surveillance programme. Sampling materials were sent to the girls who had accepted to participate by returning the informed consent letter. Urine samples were analysed for HPV using a modified GP5+/6+ PCR protocol followed by Luminex-based genotype detection, a method genoyping 37 different HPV genotypes. Cell-content was tested for all samples through a beta-globin PCR. **Results:** A total of 5 433 of the 25 400 girls invited (21%) participated in the study by providing an urine sample. 3 179.

samples have been tested and validated to date. the results show an overall prevalence for HPV of 14%%, with the main HPV types detected being HPV 16 (18.3% of the HPV positive samples), followed by HPV 90 (14% of the HPV positive samples), HPV 42 (13.4% of the HPV positive samples), HPV 6 (13.1% of the HPV positive samples), HPV 18 (10.7% of the HPV positive samples) and HPV51 (10.2% of the HPV positive samples). HPV 11 was only detected in 1.5% of the positive samples, i.e in 0.2% of the screened population. Multiple infections were detected in more than 46% of the positive samples. **Implications and Impact:** In this population based study, HPV infection was highly prevalent among 17-year old girls. HPV 16 and 18, in addition to HPV 6, are among the most prevalent types, and the vaccine is therefore expected to significantly reduce the overall HPV prevalence in this population.

Epidemiology/Public Health Measuring the impact of HPV vaccination

Burden of Disease Due to Non-Cervical HPV–Related Cancers in Spain

Presenter: Javier Cortés, PhD MD, FIAC

Investigators/Collaborators: Xavier Castellsagué[1], Javier Cerdán[2], Javier Cortés[3], Pepi Hurtado[4], Carmen Morillo[5], Miquel Quer[6], Jesús Salinas[7]

1 Cancer Epidemiology Research Program, Institut Català d'Oncologia, L'Hospitalet de Llobregat, Spain
2 Department of Digestive Surgery, Hospital Clínico San Carlos. Madrid, Spain
3 Senior Consultant on Gynaecologic Oncology, Private Practice, Palma de Mallorca, Spain
4 IMS, Barcelona, Spain
5 Medical Department Sanofi Pasteur MSD, Spain
6 Department of Otolaryngology. Hospital de la Santa Creu i Sant Pau, Barcelona, Spain
7 Department of Urology, Hospital Clínico San Carlos, Madrid, Spain)

Country: Spain

Objectives: To estimate the burden of non-cervical HPV-related cancers in Spain in 2009 and the cost of treatment of these cancers from both the National Health System and social perspectives during five years' follow up period. **Method:** Data needs were identified by conducting a bibliographic search on management of pathologies, epidemiologic data and direct and indirect costs of oral cavity and pharyngeal, anal, vulvar, vaginal and penis cancer. Cases attributable to HPV-16 and HPV-18 were calculated by means of the fractions attributable to each type, using estimates with greater evidence in the medical literature and data obtained from interviews with scientists in the field. Cost of non-cervical HPV-related cancers included direct and indirect costs. Direct costs were added to the monetary value of diagnosis, hospitalization and surgery, ambulatory treatment, including chemo and radiotherapy, and follow up costs, together with recurrence treatment. Indirect costs included potential years of work life lost (PYWLL). **Results:** Between 30-75% of new annual cases can be directly attributable to HPV-16/18. Direct costs were higher in advanced stages of cancer, with the exception of anal cancer. Indirect costs were 55-75% of the total cost depending on the type of cancer. Total costs of non-cervical HPV–related cancers were estimated at 355,748,285€. the fraction attributable to HPV was 137,751,910€ and the fraction attributable to HPV-16/18 was 56,758,762€. There was the difficulty in the estimation of the underlying indirect costs as well as the intangible costs of these cancers concerning quality of life and psychological impact on patients and their families. **Implications and Impact:** These tumors could be of interest in Public Health and primary prevention strategies. Currently established vaccination programs for the prevention of cervical cancer may have an eventual impact in the reduction of the incidence and burden of non-cervical HPV-related cancers.

Epidemiology/Public Health, Measuring the impact of HPV vaccination

Impact of a 9-Valent Vaccine in HPV Related Cervical Disease

Presenter: Laia Alemany, MD MPH

Investigators/Collaborators: Serrano B[1], Alemany L[1,2], Tous S[1], Bruni L[1], Clifford GM[3], Weiss T[4], Bosch FX[1], de Sanjosé S[1,2]

1 Unit of Infections and Cancer. Catalan Institute of Oncology. Barcelona, Spain
2 CIBER Epidemiología y Salud Pública, CIBERESP
3 International Agency for Research on Cancer, Lyon, France
4 Global Health Outcomes, Merck & Co., Inc., West Point, PA

Country: Spain

Objectives: We estimated the relative contribution (RC) of the nine HPV types (HPV 6/11/16/18/31/33/45/52 and 58) included in a developmental HPV vaccine, in invasive cervical cancer (ICC) and precancerous cervical lesions. **Method:** Estimations on ICC were based on an international study of 8,977 HPV/DNA positive cases and estimations on precancerous cervical lesions were extracted from a published meta-analysis including 115,789 HPV/DNA positive women. Globocan 2008 and 2010 World Population Prospects were used to estimate current and future projections of new ICC cases. **Results:** RC of the 9 HPV types in ICC was 89.4% with 18.5% of cases positive for HPV 31/33/45/52 and 58. Regional variations were observed. RC varied by histology, ranging between 89.1% in squamous cell carcinomas and 95.5% in adenocarcinomas. HPV 16/18 and 45 were detected in 94.2% of adenocarcinomas. RC of the 9 types altogether decreased with age (trend test $p < 0.0001$), driven by the decrease in older ages of HPV 16/18 and 45. in contrast, HPV 31/33/52 and 58 were more frequent in older ages. Due to population growth alone, projected estimates of ICC cases attributable to the 9 HPV types are expected to rise from 493,770 new cases in 2012 to 560,887 in 2025. the addition of HPV 31/33/45/52 and 58 to HPV types included in current vaccines could prevent almost 90% of ICC cases worldwide. If the nine-valent vaccine achieves similar efficacy of existing HPV vaccines, world incidence rates could be reduced to 1.7 new cases per 100,000 women per year, driving cervical cancer from the 3rd to 19th most common female

cancer. Differences in the RC of each high risk HPV type in precancerous cervical lesions were large for most cytological and histological categories, with RC increasing in higher grades cervical lesions. **Implications and Impact:** Although HPV 16 is the most common type in all cervical cancer and pre-cancerous lesions, there is an under representation of HPV 18 and 45 in women with precancerous cervical lesions compared to the high contribution in ICC. At the meeting more detailed data regarding the impact of nine-valent vaccine (RC of 9 types) on pre-neoplastic cervical lesions will also be presented.

Epidemiology/Public Health Measuring the impact of HPV vaccination

Condyloma Protection of Quadrivalent HPV-Vaccine: Population Cohort Analysis of Dose Effectiveness

Presenter: Par Sparen, PhD Professor

Investigators/Collaborators: Amy Leval, RN[1]; Eva Herweijer, MSc[1]; Alexander Ploner, PhD[1]; Sandra Eloranta, MSc[1]; Julia Fridman Simard, ScD[2]; Professor Joakim Dillner, MD[1]; Eva Netterlid, PhD[3,4,5]; Professor Pär Sparén, PhD[1]; Lisen Arnheim-Dahlström, PhD[1]

1 Dept. of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden
2 Dept. of Medicine, Clinical Epidemiology Unit, Karolinska Institutet, Stockholm, Sweden
3 Swedish Institute for Communicable Disease Control, Stockholm, Sweden
4 Faculty of Medicine, Department of Clinical Sciences in Malmö, Lund University, Sweden
5 Department of Occupational and Environmental Dermatology, Skåne University Hospital, Malmö, Sweden

Country: Sweden

Objectives: The aim of this study is to examine quadrivalent HPV-vaccine effectiveness against condyloma per dose level. **Method:** To assess condyloma incidence in relation to vaccine dose level for females first vaccinated before age 20, an open cohort of all females living in Sweden between 2006-2010 (n = 1 045 083) were linked to numerous Swedish nationwide registers. Time-toevent analyses generating incidence rate ratios (IRR) of condyloma were estimated using Poisson regression with dose as a timedependent exposure, adjusting for attained age, parental education and stratifying on age-at-first-vaccination for two age groups (ages 10-16 and 17-19). **Results:** For girls vaccinated between ages 10-16, maximum effectiveness was seen with three doses 79% (95% CI 75-83). There was a significant difference between three versus two doses in this younger age group (p-value 0.007), with three doses offering 37% more effectiveness (95% CI 12-54). For individuals vaccinated between ages 17-19, maximum effectiveness (70%, 95% CI 63-76) was also seen with three-doses, offering 26% (95% CI 0-46) more effectiveness than two-doses (p-value 0.061), but with borderline significance. No differences in effectiveness were found for girls who received two-doses between ages 10-16 with that of individuals who received three-doses between ages 17-19 (p-value 0.633). **Implications and Impact:** Maximum protection against condyloma was found with three doses for younger girls and two doses are less effective than three.

Epidemiology/Public Health Measuring the impact of HPV vaccination

Quadrivalent HPV-Vaccine Effectiveness on Genital Warts: Population Cohort Study on over 2.2 Million Girls and Women

Presenter: Par Sparen, PhD Professor

Investigators/Collaborators: Amy Leval, RN[1]; Eva Herweijer, MSc[1]; Alexander Ploner, PhD[1]; Sandra Eloranta, MSc[1]; Julia Fridman Simard, ScD[2]; Professor Joakim Dillner, MD[1]; Cecilia Young, PhD[3], Eva Netterlid, PhD[4,5,6]; Professor Pär Sparén, PhD[1]; Lisen Arnheim--Dahlström, PhD[1]

1 Dept. of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden
2 Dept. of Medicine, Clinical Epidemiology Unit, Karolinska Institutet, Stockholm, Sweden
3 Sanofi Pasteur MSD, Solna, Sweden
4 Swedish Institute for Communicable Disease Control, Stockholm, Sweden
5 Faculty of Medicine, Department of Clinical Sciences in Malmö, Lund University, Sweden
6 Department of Occupational and Environmental Dermatology, Skåne University Hospital, Malmö, Sweden

Country: Sweden

Objectives: When assessing the effectiveness of human papillomavirus (HPV) vaccination strategies, genital warts (GW) incidence is the earliest disease outcome possible to measure. This study was conducted to assess GW incidence after vaccination with the qHPV vaccine in Sweden using individual-level data from the entire Swedish population. **Method:** An open cohort of the entire population of 10-44 year old women living in Sweden between 2006 and 2010 (n > 2.2 million) was linked to the national HPV Vaccination Register, the Patient Register, the Prescribed Drug Register, the Multi-generation Register and the Education Register to identify incident GW and parental education level in relation to HPV vaccination. Self-selection for vaccination in relation to GW risk was assessed by studying GW rates over time among the unvaccinated. Incidence rate ratios of GW were estimated using time-to-event analyses adjusting for attained age, parental education and stratifying on age-at-first vaccination. **Results:** 124000 women were vaccinated between 2006 and 2010, 90% of whom were in the subsidized target group (coverage 25-33%). High education-level of parents compared to low was a strong determinant of vaccination (OR: 15.3; 95% CI 14.5; 16.1). Above age 20, GW rates declined among unvaccinated, suggesting that HPV vaccines were preferentially used by women at high GW risk. Vaccination effectiveness for women below 20 was 76% and highest below age 14 (effectiveness = 93%; 95% CI 71-99). **Implications and Impact:** Above age 20, self-selection to women at high GW risk may have impaired effectiveness. Below age 20, there was considerable effectiveness. However, the on-demand vaccination strategy used during this time had substantial social inequity.

Epidemiology/Public Health Measuring the impact of HPV vaccination

A Registry-Based Long-Term Follow-up Study of the Safety of the Quadrivalent HPV (qHPV) Vaccine in Scandinavia

Presenter: Alfred Saah, MD

Investigators/Collaborators: Laufey Tryggvadóttir, Christian Munk, Mari Nygård, Bo Terning Hansen, Maria Hortlund, Lara G. Sigurdardottir, Jack Smith, Joakim Dillner and Susanne Krüger Kjaer

Country: United States

Background: The long-term follow-up (LTFU) study is an ongoing extension of a pivotal randomized, placebo-controlled, double-blind, 4-year study, FUTURE II, to investigate the safety, immunogenicity, and effectiveness of qHPV vaccine on the incidence of HPV 16/18-related CIN 2+ in young women. Long term assessment of safety is presented. **Methods:** Upon completion of FUTURE II, the National Research Study Centers of Denmark, Iceland, Norway and Sweden systematically searched their respective national registries for information on deaths, cancers, hospitalizations, and other outcomes for safety assessment. the full Intention to Treat (ITT) population (all subjects who received at least one dose of qHPV vaccine and consented to safety follow-up) was used for the analysis in this study. the second search of the hospital discharge registries covered the time period from the subject's last base protocol study visit through 01-Mar-2011 (approximately 48 months follow up after the end of the base study). **Results:** There were no signals or changes in the new medical conditions that would indicate any change in the safety profile of the qHPV vaccine. the number of subjects with cancer, conditions of potential autoimmune etiology, or who died was varied and small. Overall, there was no specific pattern of new medical conditions. Data will be presented from an extended follow-up time not available at the time of abstract submission. **Implications and Impact:** The qHPV vaccine continues to be generally safe and well tolerated in young women 8 years following vaccination.

Epidemiology/Public Health Measuring the impact of HPV vaccination

Catching up or Missing out? HPV Vaccine Acceptability among 18-26 Year Old Men who Have Sex with Men in a U.S. National Sample

Presenter: Nathan Stupiansky, PhD

Investigators/Collaborators: Nathan W. Stupiansky, PhD; Joshua G. Rosenberger, PhD; Gregory D. Zimet, PhD; David S. Novak, MSW Susan L. Rosenthal, PhD

Country: United States

Objectives: Men who have sex with men (MSM) have higher HPV infection rates than those in the general population and also face disproportionately high rates of anal cancer, yet HPV vaccine uptake among young MSM is minimal. the purpose of this study was to examine the factors associated with HPV vaccine acceptability among a U.S. national sample of young MSM. **Method:** All U.S. users between the ages of 18-26 years (M = 22.5, SD = 2.4) of an online social and sexual networking site for MSM were invited to complete an anonymous online survey of HPV vaccine history, attitudes, and acceptability. a total of 1,457 participants provided complete data including sociodemographics, HPV vaccine history, HPV vaccine acceptability, HPV-related health beliefs, and barriers to vaccination. among those not previously vaccinated, HPV vaccine acceptability was assessed from 0-100 at a cost condition of US $30 per dose to reflect the likely amount that would be paid out of pocket. **Results:** In this sample 6.7% (98/1457) reported receipt of one or more doses of HPV vaccine, and 2.7% (39/1457) reported series completion. For those who were unvaccinated (n = 1,359) there were several factors that related to HPV vaccine acceptability (M = 62.2, SD = 32). in multivariate models, safety concerns (B = -.137), cost (B = -.186), the feeling that one is not at risk for HPV (B = -.098), and the belief that the vaccine is for men who are promiscuous (B = -.084) were associated with decreased vaccine acceptability. Conversely, worry about getting infected with HPV (B = .230), agreeing to receive a shot in the future that a doctor would say is necessary (B = .10), and the belief that they may have already been exposed to the virus (B = 0.88) were associated with increased vaccine acceptability. Young men who had been tested in the past year for HIV (p = .01) or STDs (p = .01) and those who reported having a primary health care provider (p = .03) also rated the vaccine as more acceptable, as did those who had achieved higher levels of education (p = .01) and those who self-reported better health (p = .001). the majority of men who had not received vaccination had not been offered the vaccine by a doctor (96.3%). **Implications and Impact:** Overall, young MSM represent an at-risk group with generally high HPV vaccine acceptability. Men's reports of better overall health and sexual health behaviors (i.e. STD/HIV testing) were indicative of more favorable attitudes towards the HPV vaccine. Our findings suggest that structural interventions (e.g. offering vaccination in HIV testing venues), as well as interventions that address health beliefs may help to increase HPV vaccination rates among MSM.

Epidemiology/Public Health Measuring the impact of HPV vaccination

HPV Types Associated with High Grade Cervical Dysplasia: Impact of Vaccinationin California, 2008-2011

Presenter: Heidi Bauer, MD MPH

Investigators/Collaborators: Heidi M. Bauer[1], Ellen Luecke[1,2], Sharon McDonnell[1,2], Erin Whitney[1,2], Lauri Markowitz[3], Elizabeth R. Unger[4], Susan Hariri[3]

1 Sexually Transmitted Disease Control Branch, California Department of Public Health, Richmond, CA, USA

2 California Emerging Infections Program, Oakland, CA, USA

3 Division of STD Prevention, Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA

4 Division of High-Consequence Pathogens and Pathology, Centers for Disease Control and Prevention (CDC), Atlanta, GA USA
Country: United States

Objectives: HPV vaccination is expected to reduce disease related to vaccine types and change the distribution of HPV types in high grade cervical disease. the CDC-funded HPV-Impact project, comprising five sentinel sites in the U.S., initiated ongoing surveillance of histologically defined high-grade cervical dysplasia, specifically cervical intraepithelial neoplasia (CIN) grade 2 or 3 and adenocarcinoma in situ (AIS). Our objective was to examine trends in disease rates and distribution of HPV vaccine types 16/18. **Method:** The California catchment area includes over 317,000 adult women residents. Case reports are generated by laboratories and health care organizations. Representative cervical histology specimens from women age 18-39 years diagnosed in 2008-2011 underwent HPV typing using with L1 Consensus PCR. We compared HPV type distribution over time, by age, by high-grade diagnosis, and by vaccination status (defined as at least one dose). **Results:** From 2008-2011, 1,328 cases of CIN2/3/AIS were identified among women 18-39 years of age. the annual rate of disease declined 17% from 279 to 231 per 100,000 in that time period (P for trend = 0.04). Diagnoses included CIN2 (42%), CIN2/3 (22%), CIN3 (34%), and AIS (2%); these proportions were unchanged over time. of the 576 specimens tested, 553 (96%) were considered adequate and typed for HPV. of these, 96% were HPV-positive; 51% had types 16/18 and 20% had multiple types. the proportion of specimens with 16/18 did not vary by the year of diagnosis or the age of the patient. Higher grade lesions were more likely to have HPV 16/18: 46% of CIN2 and CIN2/3, 59% of CIN3, and 88% of AIS (P < .05). Only 27 (4.9%) specimens were from women who had received any HPV vaccine; notably 44% of these women received the vaccine after their diagnosis. the proportion of specimens with HPV 16/18 did not vary by vaccination status. **Implications and Impact:** The decline in incident lesions soon after vaccine introduction emphasizes the complexity of detecting the early endpoints of HPV vaccine impact and highlights the need for ongoing surveillance of HPV type distribution in cervical lesions. Ongoing measurement of vaccine coverage as well as cervical cancer clinical prevention practices will be critical for interpreting trends.

Epidemiology/Public Health, Measuring the impact of HPV vaccination

Long-Term Extension Study of GARDASIL in Adolescents; Results through Month 96

Presenter: Alfred Saah, MD
Investigators/Collaborators: on behalf of the Protocol 018 investigators
Country: United States

Objectives: Quadrivalent HPV vaccine has previously been shown to be safe and immunogenic in adolescents though 72 months after vaccination. We describe the first interim effectiveness data for a long-term immunogenicity, safety, and effectiveness study of GARDASIL™ among adolescents. **Method:** In the base study, 1781 sexually naïve boys and girls were assigned (2:1) to GARDASIL or saline placebo at day 1, months 2 and 6. At the end of the base study (month 30), the placebo group received GARDASIL™ following the same regimen. Those vaccinated with GARDASIL in the base study are the early vaccination group (EVG). Those vaccinated with GARDASIL during months 30-36 are the catch-up vaccination group (CVG). As this extension study does not have a placebo arm, effectiveness was assessed by calculating the incidence of the primary endpoints (HPV6/11/16/18 persistent infection or related disease) and comparing these rates with those from previous phase 3 studies in men and women aged 16-26. the median follow-up time for effectiveness was 6.8 years in EVG and 4.7 years in the CVG. **Results:** For each of HPV types 6, 11 and 16, the vaccination-induced anti-HPV response persisted long-term. Depending on HPV type, 88%-97% remained seropositive through Month 96. the lower vaccination-induced anti-HPV 18 response over time observed in V501-018-11 is consistent with the persistence profile observed in other studies in the GARDASIL™ program. No cases of HPV 6/11/16/18-related disease were observed. One serious adverse event was reported between months 72-96 (tonic-clonic movements) and was deemed not related to vaccine. **Implications and Impact:** Vaccine-type anti-HPV 6, 11, 16, and 18 responses generated through administration of GARDASIL™ among preadolescents and adolescents persist over the long-term, in accordance with expectations from previous GARDASIL™ studies. No breakthrough cases of disease related to vaccine HPV types 6, 11, 16, and 18 have been observed among preadolescents and adolescents vaccinated with GARDASIL™.

Epidemiology/Public Health the human element: Sociocultural and psychosocial factors related to cervical cancer prevention

Is the World Ready to Vaccinate Boys against HPV? Socio-Cultural and Psychosocial Lessons from the Implementation of the HPV Vaccine Programs for Girls

Presenter: Margaret Heffernan,OAM, PhD OAM: Order of Australia Medal
Authors: No conflict of interest
Investigators/Collaborators: Heffernan ME[1], Daley EM[2], Garland SM[3,4], Zimet GD[5]
1 RMIT University, Melbourne Australia (margaret.heffernan@rmit.edu.au)
2 College of Public Health, University of South Florida, Tampa USA
3 University of Melbourne
4 Royal Women's Hospital, Melbourne, Australia
5 Indiana University – Purdue University Indianapolis (IUPUI), USA
Country: Australia

Objectives: Sound arguments can be made for HPV vaccination of males, but much of the world is not prepared for male HPV vaccination programs. Female HPV vaccination programs globally overlooked critical social and system ramifications, and have proven to be complex and challenging. If crucial system, educative, psycho-social and cross-cultural factors are unaddressed, challenges will be posed for adoles-

cent male HPV vaccination across diverse nations. **Method:** (a) Results from an Australian cross-cultural qualitative study on parental and general medical practitioners (GPs) attitudes toward adolescent HPV vaccination. Participants for the qualitative semi-structured interviews were purposively selected: (i) parents of Anglo, Aboriginal, and Chinese descendency (n = 166) (ii) practicing GPs (n = 15). Recruitment was through hospital clinics and cultural networks; (b) a literature review of global HPV vaccination programs 2007-2012. **Results:** The study showed parental consent was based on passive acceptance and emotional vulnerability toward cancer, despite concerns about the vaccine's characteristics, relevance for young adolescents and psychosocial factors of 'shame' and 'stigma'. Public HPV education resources, derived from assumptive conclusions and ethnocentric discourses, ignored diverse socio-cultural paradigms and health practices. Dissatisfaction was held with protectionist discourses by the health sector which limited overall public awareness about HPV infection, and especially for males. Cultural and religious norms influenced parental and GPs' differing attitudes. the literature shows that the message framing of HPV vaccines and minimisation of HPV as an STI contributed to confusion about HPV infection and vaccination for males. Implementation strategies for female HPV vaccination programs, typically treated as different from other vaccines, failed to take advantage of findings from prior adolescent vaccination strategies and studies. **Implications and Impact:** The introduction of boys' HPV vaccination programs should not mimic that of girls and current implementation strategies require a system-change review. To aid support for male vaccination, an unmitigated education approach undertaken pre-vaccination should inform about male and female sexual acquisition and HPV diseases. Diverse resources, expanded time frames and public sexual discourses are required to change the social paradigms toward stigma and taboo in relation to HPV and other STIs. This comprehensive and socio-ecological approach to HPV-STI education programs and male vaccination considers conflicting values and will optimise delivery and uptake.

Epidemiology/Public Health the human element: Sociocultural and psychosocial factors related to cervical cancer prevention

Parents' Human Papillomavirus Vaccine Decision-Making for their Young Daughters: the Role of Vaccination Safety

Presenter: Andrea Krawczyk, PhD
Investigators/Collaborators: Bärbel Knäuper, Vladimir Gilca, Eve Dubé, Zeev Rosberger
Country: Canada

Objectives: Vaccination against the human papillomavirus (HPV) is an effective primary prevention measure for HPV-related cancers and associated diseases. For children and young adolescents, the uptake of the vaccine is contingent on parental vaccination consent. This study sought to identify key differences between parents who accept and parents who refuse the HPV vaccine for their daughters. **Method:** In the context of a free, universal, school-based HPV vaccination program, a random sample of 2,500 Québec parents of 9-10 year old girls were invited to participate in the study by mail. Participants completed a questionnaire based on the theoretical constructs of the health belief model (HBM) and additional relevant factors identified in the literature. **Results:** Of the 834 parents who completed the questionnaire (33% response rate), 88.2% reported accepting the HPV vaccine for their daughter. the HBM constructs distinguishing parents who have accepted and parents who have refused the HPV vaccine included: perceived susceptibility of daughters to HPV infection (OR = 1.12 CI = 1.06, 1.18), perceived benefits of the vaccine (OR = 1.27 CI = 1.20, 1.36), perceived barriers (OR = 0.92 CI = 0.90, 0.95), and cues to action (OR = 1.23 CI = 1.18, 1.28). in particular, parental perception of vaccine safety was the strongest factor associated with acceptance (OR = 2.30 CI = 1.96, 2.71). Further, perceived safety was a significant independent contributor beyond all other HBM constructs (OR = 1.73, CI = 1.36, 2.21). Other significant factors associated with parental vaccination acceptance were positive vaccination attitudes (OR = 1.13 CI = 1.08, 1.18), negative vaccination attitudes (OR = 0.89 CI = 0.86, 0.92), anticipated regret (OR = 0.61 CI = 0.54, 0.69), adherence to other routinely recommended vaccines (OR = 1.96 CI = 1.24, 3.10), social norms (OR = 1.65 CI = 1.43, 1.91), and media influence (OR = 1.64 CI = 1.43, 1.90). **Implications and Impact:** Parental perception of vaccine safety is a critical factor for HPV vaccination acceptance. the HBM provided a useful, but incomplete framework to identify the factors related to parental vaccination decision making. Comprehensive theories of vaccination decision making that include additional behavioural, social, and cognitive factors are warranted.

Epidemiology/Public Health, the human element: Sociocultural and psychosocial factors related to cervical cancer prevention

Effects of Condom Use on Genital Human Papillomavirus (HPV) Incidence and Clearance in Men: The HPV in Men Study

Presenter: Christine Pierce Campbell, PhD
Investigators/Collaborators: Christine M. Pierce Campbell[1], William Fulp[1], Hui-Yi Lin[1], Mary R. Papenfuss[1], Eduardo Lazcano-Ponc[e2], Luisa L. Villa[3], and Giuliano AR[1]
1 H Lee Moffitt Cancer Center, Center for Infection Research in Cancer, Tampa, FL, USA
2 Instituto Nacional de Salud Pública, Cuernavaca, México
3 Ludwig Institute for Research on Cancer, São Paulo, Brazil
Country: United States

Objectives: Male condoms offer substantial protection against many sexually transmitted infections; however, data supporting the efficacy of male condoms against HPV infection have been limited. the goal of this study was to determine if consistent condom use among certain groups of high-risk men would reduce the risk of acquiring genital HPV infections, or decrease the duration of HPV infections, compared with men who never use condoms. **Method:** A prospective analysis was conducted within the HPV in Men (HIM) Study, an ongoing co-

hort study of the natural history of HPV infections among men in the US, Brazil, and Mexico. the analytic cohort consisted of 3,323 men aged 18–70 years who reported recent sexual activity (3–6 months prior to baseline visit). All men completed risk factor questionnaires and underwent clinical examinations at baseline and subsequent follow-up visits. Genital HPV was assessed using the Roche Linear Array genotyping test. To assess under which circumstances condoms might impact HPV acquisition and clearance, men were categorized according to their risk potential for HPV infection (single, monogamous, or non-monogamous). Sex act- and partner-specific measures of condom use (always, sometimes, or never) were used to evaluate risk of HPV acquisition and median time to clearance. **Results:** Men (N = 3,323) were followed for a median of 17.3 months (range = 0.3–55.6). Risk of HPV acquisition and clearance varied significantly by risk group and condom use. the 12-month incidence of any HPV was higher among non-monogamous men (53.8%) than single (40.8%) or monogamous men (34.6%) (log-rank $p < 0.001$). among single men, those who always used condoms during vaginal sex had the lowest risk of acquiring any HPV within 12 months (32.2%) (sometimes [50.3%] and never [49.9%]; log-rank $p = 0.014$). the median time to clear an incident HPV infection was shorter among monogamous men (6.6 months) than single (7.2 months) or non-monogamous (7.6 months) men (log-rank $p = 0.008$). among non-monogamous men, those who always used condoms with non-steady partners cleared oncogenic HPV infections more quickly (median = 6.4 months) (sometimes [8.1 months] and never [11.1 months]; log-rank $p = 0.014$). No protective effects of condom use were observed among monogamous men. **Implications and Impact:** Consistent condom use may reduce the risk of acquiring some, but not all, grouped HPV infections and decrease the duration of these infections among high-risk men. Condom use is a cost-effective risk-reduction strategy that should be promoted in combination with population-based vaccination to prevent HPV infection, reduce HPV transmission, and decrease the burden of HPV-related diseases.

Epidemiology/Public Health the human element: Sociocultural and psychosocial factors related to cervical cancer prevention

Psychosocial Impact of Genital Warts and Other HPV-Related Diseases in South Korea

Presenter: Smita Kothari, PhD, MBA, RPh

Investigators/Collaborators: JuW[1], LeeTS[2], Yee KS[3], Kothari S[4], Lara N[5], Montse R[5], Giuliano A[6], Garland S[7]

1 Department of Obstetrics and Gynecology, Medical Research Center, School of Medicine, Ewha Women's Hospital, Seoul, Korea
2 Department of Obstetrics and Gynecology, Seoul Metropolitan Boramae Hospital, Seoul, Korea
3 Agile1, USA
4 Global Health Outcomes, Merck, USA
5 IMS Health Economics and Outcomes Research, Barcelona, Spain
6 Moffit Cancer Center, Florida, USA
7 Royal Women's Hospital, Victoria, Australia

Country: United States

Objectives: To assess the psychosocial impact of genital warts (GW) in males and select HPV-related diseases in females in South Korea. **Method:** During September 2011 through December 2011, male and female patients, 20-60 years of age from 4 different provinces (Seoul, Busan, Daegu and Daejeon) in South Korea were invited by their physicians to complete questionnaires to assess healthcare seeking and sexual activity behavior and the psychosocial impact of GW (males) and selected HPV-diseases (females). Patients diagnosed with GW or HPV-condition, without any significant comorbid condition or a concurrent/active sexually transmitted infection within the last 3 months of completing the questionnaire, were included in the study. Patients without diagnoses of GW or HPV-related diseases within the last 3 months were also included. Psychosocial impact was assessed using HPV Impact Profile (HIP), disease specific Questionnaire for Condylomata Acuminata (CECA) which was administered only to patients with GW, and EQ-5D in 250 females with (200) and without (50) HPV disease and 150 males with (75) and without GW (75). HIP and EQ-5D questionnaire scores were compared between male GW patients and females with HPV vs. those without. the CECA scores were compared between males and females with GW only. **Results:** The total HIP scores (range 1 (best) -100 (worst)), were statistically significantly higher in both males with GW ($p < 0.001$) and females with HPV ($p < 0.001$) than those without GW or HPV-related diseases, respectively. in males, the HIP scores were significantly higher than those without GW in all domains except "control-life impact." the HIP domain scores for females with HPV diseases was significantly higher in the worries/concerns ($p < 0.0001$), emotional impact ($p < 0.0001$), and partner issues/transmission ($p = 0.0001$) than the no HPV group. in GW patients, the CECA scores ((range 1 (worst) -100 (best)), for "emotional" ($p < 0.0001$) and "sexual activity" ($p < 0.0001$) domains were both statistically significantly higher in males than females. in females, EQ-5D anxiety/depression ($p = 0\text{-}0078$) domain was significant for the HPV disease group compared to no HPV group. **Implications and Impact:** In our study sample, there is a significant psychosocial impact on male patients with GW as compared to those without. in females, HPV disease including GW has high impact on worries/concerns, emotional and partner issues/transmission related issues than no HPV disease.

Received on: 28.12.2013
Approved on: 31.01.2013