WORLD HEALTH ORGANIZATION
International Agency for Research on Cancer

Primary End-points for Prophylactic HPV Vaccine Trials

IARC Working Group Report

Volume 7

IARC 2014

WORLD HEALTH ORGANIZATION

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

IARC

Working Group Reports

Volume 7

PRIMARY END-POINTS FOR PROPHYLACTIC HPV VACCINE TRIALS

This report represents the views and expert opinions of an
IARC Working Group that met in Lyon, France
23–24 September 2013

This Expert Meeting was jointly organized and supported by IARC and the
United States National Cancer Institute (NCI)

Published by the International Agency for Research on Cancer,
150 cours Albert Thomas, 69372 Lyon Cedex 08, France

Distributed by
WHO Press, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland
(tel: +41 22 791 3264; fax: +41 22 791 4857; email: bookorders@who.int).

IARC Library Cataloguing in Publication Data

Primary end-points for prophylactic HPV vaccine trials / IARC HPV Working Group (2013: Lyon, France)

(IARC Working Group Reports; 7)

1. Papillomavirus vaccines – therapeutic use 2. Papillomavirus vaccines – adverse effects
3. Papillomavirus infections – prevention and control 4. Uterine cervical neoplasms – virology 5. Endpoint determination – therapeutic use
I. IARC Working Group Report II. Series

ISBN 978-92-832-2451-8 (NLM Classification: W1)

WORKING GROUP MEMBERS

Dr David Collingridge
Editor, The Lancet Oncology
London, United Kingdom

Dr Jack Cuzick
Cancer Research UK
Wolfson Institute of Preventive Medicine
London, United Kingdom

Dr Silvia de Sanjosé Llongueras
Catalan Institute of Oncology
Barcelona, Spain

Dr Joakim Dillner
Professor in Infectious Epidemiology at the Department of Laboratory Medicine and the Department of Medical Epidemiology and Biostatistics
Karolinska Institute
Stockholm, Sweden

Dr Linda Eckert
Department of Obstetrics & Gynecology
University of Washington
Seattle, WA, USA

Dr Eduardo Franco
Division of Cancer Epidemiology
McGill University
Montreal, Canada

Dr Suzanne Garland
Department of Microbiology & Infectious Diseases
The Royal Women's Hospital
Parkville, Australia

Dr Maura Gillison
College of Medicine
The Ohio State University
Columbus, OH, USA

Dr Anna Giuliano
Chair, Department of Cancer Epidemiology
H. Lee Moffitt Cancer Center and Research Institute
Tampa, FL, USA

Dr Susanne Krüger-Kjaer
Head, Virus, Lifestyle and Genes Unit
Institute of Cancer Epidemiology
Danish Cancer Society
Copenhagen, Denmark

Dr Matti Lehtinen
School of Health Sciences
University of Tampere
Tampere, Finland

Dr Lauri Markowitz
Division of STD Prevention
Centers for Disease Control and Prevention
Atlanta, GA, USA

Dr Pieter Neels
Vice Chair of the Vaccine Group of the European Medicines Agency (EMA)
Belgium Medicine Commission
Brussels, Belgium

Dr Jorma Paavonen
Department of Obstetrics and Gynecology
University of Helsinki
Helsinki, Finland

Dr Joel Palefsky
Department of Medicine
UCSF School of Medicine
San Francisco, CA, USA

Dr Jeffrey Roberts
U.S. Food and Drug Administration
Center for Biologics Evaluation and Research
Office of Vaccines Research and Review
Silver Spring, MD, USA

Dr Margaret Stanley
Department of Pathology
University of Cambridge
Cambridge, United Kingdom

Dr Vivien Davis Tsu
Director, Cervical Cancer Prevention Project
Associate Professor, Reproductive Health, PATH
Seattle, WA, USA

Dr Elizabeth R. Unger
Chief, Chronic Viral Diseases Branch
National Center for Emerging and Zoonotic Infectious Diseases
Centers for Disease Control and Prevention
Atlanta, GA, USA

Dr Cosette Wheeler
Departments of Pathology and Obstetrics and Gynecology
Chief, MGM Translational Research
University of New Mexico
Albuquerque, NM, USA

NCI Participants

Dr Allan Hildesheim
Chief, Infections and Immunoepidemiology Branch
Division of Cancer Epidemiology & Genetics
National Cancer Institute (NCI)
Rockville, MD, USA

Dr Aimee Kreimer
Investigator, Infections and Immunoepidemiology Branch
Division of Cancer Epidemiology & Genetics
National Cancer Institute (NCI)
Rockville, MD, USA

Dr Douglas Lowy
Head, Signaling and Oncogenesis Section
Center for Cancer Research (CCR)
National Cancer Institute (NCI)
Bethesda, MD, USA

Dr Mahboobeh Safaeian
Infections and Immunoepidemiology Branch
Division of Cancer Epidemiology & Genetics
National Cancer Institute (NCI)
Bethesda, MD, USA

Dr Mark Schiffman
Clinical Genetics Branch
Division of Cancer Epidemiology & Genetics
National Cancer Institute (NCI)
Bethesda, MD, USA

Dr John Schiller
Center for Cancer Research
Head, Neoplastic Disease Section
Deputy Laboratory Chief
Center for Cancer Research (CCR)
National Cancer Institute (NCI)
Bethesda, MD, USA

Dr Sholom Wacholder
Biostatistics Branch
Division of Cancer Epidemiology & Genetics
National Cancer Institute (NCI)
Bethesda, MD, USA

WHO Participants

Dr Nathalie Broutet
Medical Officer
Department of Reproductive Health and Research
World Health Organization
Geneva, Switzerland

Dr Ulrich Fruth
Vaccine Development and Evaluation
World Health Organization
Geneva, Switzerland

Dr Andrea Vicari
Advisor, Immunization
World Health Organization
Regional Office for the Americas
Washington, DC, USA

Dr Susan Wang
Medical Officer, Expanded Programme on Immunization Plus
World Health Organization
Geneva, Switzerland

Dr Tiequn Zhou
Medical Officer, Essential Medicines & Pharmaceutical Policies
World Health Organization
Geneva, Switzerland

IARC Participants

IARC Participants

Dr Maribel Almonte
Prevention and Implementation Group
Section of Early Detection and Prevention
International Agency for Research on Cancer (IARC)
Lyon, France

Dr Gary Clifford
Infections and Cancer Epidemiology Group
Section of Infections
International Agency for Research on Cancer (IARC)
Lyon, France

Dr Hugo De Vuyst
Infections and Cancer Epidemiology Group
Section of Infections
International Agency for Research on Cancer (IARC)
Lyon, France

Dr Silvia Franceschi
Head, Infections and Cancer Epidemiology Group
Section of Infections
International Agency for Research on Cancer (IARC)
Lyon, France

Dr Paula Gonzalez
Prevention and Implementation Group
Section of Early Detection and Prevention
International Agency for Research on Cancer (IARC)
Lyon, France

Dr Rolando Herrero
Head, Prevention and Implementation Group
Section of Early Detection and Prevention
International Agency for Research on Cancer (IARC)
Lyon, France

Dr Martyn Plummer
Infections and Cancer Epidemiology Group
Section of Infections
International Agency for Research on Cancer (IARC)
Lyon, France

Dr Rengaswamy Sankaranarayanan
Head, Screening Group and Section of Early Detection and Prevention
International Agency for Research on Cancer (IARC)
Lyon, France

Dr Massimo Tommasino
Head, Infections and Cancer Biology Group and Section of Infections
International Agency for Research on Cancer (IARC)
Lyon, France

Dr Salvatore Vaccarella
Infections and Cancer Epidemiology Group
Section of Infections
International Agency for Research on Cancer (IARC)
Lyon, France

Dr Christopher P. Wild
Director
International Agency for Research on Cancer (IARC)
Lyon, France

Table of Contents

TABLES

Abbreviations

AIN	anal intraepithelial neoplasia
AIN1+	anal intraepithelial neoplasia of grade 1 or worse
ART	antiretroviral therapy
ASCUS	atypical squamous cells of undetermined significance
ASIL	anal squamous intraepithelial lesion
ATP	according-to-protocol
bHPV	bivalent HPV 16/18
CI	confidence interval
CIN2/3	cervical intraepithelial neoplasia of grade 2 or 3
CIN2+	cervical intraepithelial neoplasia of grade 2 or worse
cLIA	competitive Luminex immunoassay
COPV	canine oral papillomavirus
CSIL	cervical squamous intraepithelial lesion
CVT	Costa Rica Vaccine Trial
ELISA	enzyme-linked immunosorbent assay
ELISPOT	enzyme-linked immunosorbent spot
EMA	European Medicines Agency
FDA	United States Food and Drug Administration
GMT	geometric mean titre
HIV	human immunodeficiency virus
HNSCC	head and neck squamous cell cancer
HPV	human papillomavirus
HR	high-risk
HSIL	high-grade squamous intraepithelial lesion
IARC	International Agency for Research on Cancer
ICC	invasive cervical cancer
LEEP	loop electrosurgical excision procedure
LR	low-risk
LSIL	low-grade squamous intraepithelial lesion
MBC	memory B cell
MSM	men who have sex with men
MSW	men who have sex with women
NCI	National Cancer Institute
OR	odds ratio
PAF	population attributable fraction
PCR	polymerase chain reaction
PPV	positive predictive value
PSA	prostate-specific antigen
qHPV	quadrivalent HPV 6/11/16/18
RCT	randomized controlled trial
RR	relative risk
SEER	Surveillance, Epidemiology, and End Results
VAIN	vaginal intraepithelial neoplasia
VE	vaccine efficacy
VIN	vulvar intraepithelial neoplasia
VLP	virus-like particle
WHO	World Health Organization

Chapter 1. Summary of IARC/NCI Expert Meeting on Primary End-points for Prophylactic HPV Vaccine Trials

Douglas Lowy, Rolando Herrero, Allan Hildesheim, and John Schiller, for the HPV Working Group

1.1 Executive summary

The potential of the current licensed preventive virus-like particle (VLP) human papillomavirus (HPV) vaccines to have a major public health impact is leading to additional clinical trials. The goals of such trials may include reducing the number of doses of a current vaccine, evaluating a vaccine similar to a licensed vaccine, increasing the valency of a licensed vaccine by including VLPs for additional HPV types, or evaluating alternative prophylactic vaccines that also use a viral capsid protein or polypeptide to induce neutralizing antibodies.

The Working Group at the IARC/United States National Cancer Institute (NCI) Expert Meeting of September 2013 primarily reviewed the evidence as to whether it might be appropriate to use a virological end-point, rather than a disease end-point such as cervical intraepithelial neoplasia of grade 2 or worse (CIN2+), as the primary end-point for some future clinical efficacy trials, and the circumstances under which immunobridging trials might be sufficient for licensure. Virological end-points could accelerate vaccine evaluation and licensure. Several factors make virological end-points attractive when they represent valid surrogates for clinical premalignant disease end-points. They are more reproducible as end-points than disease end-points, they are the most definitive end-points when there is co-infection by more than one HPV type, and there are substantially more cases of infection by a given HPV type than cases of such disease. In the clinical trials that have been completed, efficacy against persistent infection has been found to be as stringent an outcome as CIN2+. Immunobridging trials have been used to extend the use of currently licensed vaccines to adolescents, an age group not evaluated in the efficacy trials. In these trials, the induction of non-inferior serum antibody titres after the same vaccine dosage schedule as in the efficacy trials has been used as the measure.

Based on a review of the scientific evidence, for most situations, such as infection of the cervix or anus of young adults (e.g. individuals aged 16–26 years), the Working Group recommended that persistent HPV infection of 6 months or longer be used as an appropriate end-point when protection is being evaluated, with reduction in disease being verified by post-licensure monitoring. If vulvar/vaginal protection is to be evaluated in a trial, it is recommended to use HPV 16/18-positive high-grade vulvar intraepithelial neoplasia/vaginal intraepithelial neoplasia (VIN/VAIN) as a disease end-point, as there is relatively little experience in using persistent HPV infection as a surrogate end-point for vulvar and vaginal disease. A persistent infection end-point could be considered at these two sites if subsequent studies validated the ability to detect persistent infection at these sites. A persistent HPV 16/18 infection end-point is recommended for evaluating oral/oropharyngeal infection because an oral/oropharyngeal disease end-point comparable to those associated with anogenital infection is not feasible with current technology. After a vaccine has been shown to be effective in one population group (e.g. individuals aged 16–26 years), immunobridging is sufficient for extending licensure to other population groups (e.g. individuals aged < 16 years). The Working Group recommended that immunological non-inferiority is an appropriate end-point in such situations, independent of the number of vaccine doses used to demonstrate such non-inferiority, with reduction in disease being verified by post-licensure monitoring. The need for standardization of virological and immunological assays was emphasized.

1.2 Background

1.2.1 HPV infection causes anogenital and oropharyngeal cancers

HPV infection causes several anogenital cancers as well as a subset of oropharyngeal cancers [1, 2]. Virtually all cases of cervical cancer are attributable to HPV infection [3]. HPV also causes > 90% of anal cancers and a smaller proportion of vulvar, vaginal, penile, and oropharyngeal cancers. The incidence of HPV-positive oropharyngeal cancer as well as that of anal cancer has been increasing in several industrialized countries; HPV-positive oropharyngeal cancers now account for about two thirds of the cancers at this site in the USA [4] and an even higher proportion in Sweden [5]. The incidence of HPV-positive oropharyngeal cancer is substantially lower in developing countries [1].

Cervical cancer accounts for > 90% of the HPV-positive cancers in developing countries, where it is the third most common cancer in women [1]. More than 95% of the HPV-associated cancers in developing countries occur in women. By contrast, in some industrialized countries, the incidence of the non-cervical cancers may be similar to that of cervical cancer, largely because cervical cancer screening has resulted in a substantial reduction in the incidence of this cancer and because the incidence of anal and oropharyngeal cancer has been increasing. About 40% of anal cancers and three quarters of HPV-positive oropharyngeal cancers occur in men, which means that about one quarter to one third of the HPV-positive cancers in industrialized countries arise in men. Thus, HPV-associated cancers in developing countries are dominated by cervical cancer, whereas HPV-positive cancers in at least some industrialized countries are divided more evenly among the different sites where HPV infection is widely accepted as potentially leading to malignant disease.

1.2.2 Pathogenesis of HPV-associated cancer

The stages leading to the development of cancer are best understood for cervical infection and cervical cancer [2, 6]. HPV infection of the cervix appears to be necessary for the development of cervical cancer, but it is not sufficient. The interval between infection and cancer is usually at least 20 years. The majority of infections are self-limited, and the likelihood of viral clearance is inversely related to the duration of infection. For those infections that lead to cancer, the infected cells continue to express viral RNA and protein, which is associated with progression first to premalignant disease (high-grade dysplasia) and then to invasive cancer [7]. Most HPV-positive cancers at other anogenital sites appear to arise by an analogous process.

The ability of cervical cancer screening to identify most high-grade dysplasias, which are then treated, has reduced the incidence of and mortality from this cancer [8, 9]. This success reinforces the conclusion that such dysplasias represent a necessary step in the development of cervical cancer.

1.2.3 Oncogenic HPV types

At least 13 HPV types are recognized as being able to cause cervical cancer [10–12]. HPV 16 is the most oncogenic and accounts for about 50–60% of cervical cancer. HPV 18 accounts for about 10–20% of cervical cancer. Infection by more than 10 other HPV types accounts for the 30% of cervical cancer not attributable to infection by HPV 16 or 18. For non-cervical cancers, HPV 16 accounts for 80–90%, with HPV 18 accounting for some additional cases [13].

1.2.4 Rationale for developing the current preventive HPV vaccines

The medical importance of cervical cancer provided the initial public health rationale for developing a preventive HPV vaccine [14]. The recognition that HPV also causes many cases of non-cervical cancer provided an additional incentive. Although cervical cancer screening is a validated secondary prevention approach in some countries for reducing the incidence of and mortality from this disease, it may be difficult to introduce and maintain, as it is costly and requires considerable infrastructure. Vaccination could provide primary prevention of these infections. In addition, a vaccine should be able to prevent the non-cervical cancers as well, and there are currently no validated public health screening procedures for the non-cervical cancers. The initial focus of vaccine development was on HPV 16 and 18 (HPV 16/18), given the overriding importance of these two types to the development of HPV-positive cancer.

1.2.5 Current HPV vaccines

Two commercial HPV vaccines have been licensed by the United States Food and Drug Administration (FDA), the European Medicines Agency (EMA), and many other regulatory authorities. Both are subunit vaccines composed of adjuvanted purified virus-like particles (VLPs) produced by expressing the L1 major structural viral protein in eukaryotic cells. One is a bivalent HPV 16/18 VLP vaccine manufactured by GlaxoSmithKline [15]. It is approved by WHO, and in many countries, for girls/young women aged 9–25 years. The other is a quadrivalent HPV 6/11/16/18 VLP vaccine manufactured by Merck [16]. HPV 6 and 11 together cause about 90% of genital warts. The quadrivalent vaccine is approved by WHO, and in many countries, for girls/young women aged 9–26 years; it is also approved for boys/young men in many industrialized countries [17, 18].

When given to unexposed women by the parenteral three-dose schedule approved by regulatory authorities, the vaccines are highly immunogenic and have demonstrated almost complete protection of several years' duration against infection and disease caused by the HPV types targeted by each vaccine (vaccine types). The vaccines also display some degree of cross-protection against phylogenetically related HPV types (non-vaccine types) [19, 20].

1.2.6 Post-licensure impact of HPV vaccination

Consistent with the efficacy results of the clinical trials, several countries have reported that vaccination with the quadrivalent vaccine has been associated with a substantial reduction in the incidence of genital warts in vaccinated girls [21–24]. For both vaccines, there has been an overall reduction in the prevalence of the HPV types targeted by each vaccine (HPV 16/18 for the bivalent vaccine and HPV 6/11/16/18 for the quadrivalent vaccine) among young women in the target age group for vaccination [25]. Evidence that herd immunity can extend to unvaccinated young men who have sex with women (MSW) in populations where most young women have been vaccinated (with the quadrivalent vaccine) has been seen in Australia, where the incidence of genital warts has decreased among MSW aged 25 years or younger [26]. There is also evidence that the incidence of high-grade cervical dysplasia is decreasing among vaccinated young women [27].

1.2.7 Rationale for additional clinical trials

There is considerable interest in conducting additional clinical trials of prophylactic HPV vaccines, given that three intramuscular doses are difficult to deliver, that almost one third of potentially oncogenic cervical infections are not specifically targeted by the current vaccines, and that the current vaccines are relatively expensive [28, 29]. Such trials may have various goals. For example, there may be trials to determine whether VLPs generated by additional manufacturers are equivalent to those in the licensed vaccines, whether reducing the number of doses of VLP vaccines may still induce durable high efficacy, or whether

increasing the valency of the vaccines, by adding more VLPs from additional HPV types, can broaden the strong protection to these additional HPV types. Other trials may use VLPs but test whether it would be advantageous to deliver them by alternative routes (such as via the skin or inhalation), or they may use viral capsomeres instead of VLPs to test whether such a formulation might have advantages. Trials to extend vaccination to infants and children are also feasible. It is also possible to test the clinical utility of vaccine vectors that encode L1 and are therefore designed to produce L1 in the vaccinee. Other trials may test an alternative vaccine approach, the use of peptides from the L2 minor capsid protein, because these peptides contain cross-neutralization epitopes shared by many HPV types. Such candidate L2 peptides may therefore serve as the basis for an inexpensive vaccine that could protect against infection and disease caused by a broad spectrum of HPV types.

1.2.8 Current primary end-points used for vaccine licensure

Until now, the pivotal vaccine efficacy trials have used protection against the development of premalignant disease as the primary end-point for determining efficacy [30]. The clinical lesions to be prevented have included cervical intraepithelial neoplasia of grade 2 or worse (CIN2+) and corresponding high-grade lesions in the vulva and vagina, and anal dysplasia of any degree of severity in men. The rationale for including moderate cervical dysplasia (CIN2) as part of the disease end-point was that the standard of care in many countries requires treatment of CIN2 lesions, although CIN2 is not considered premalignant by some investigators, and its diagnosis is less reproducible than severe dysplasia (CIN3). Protection against invasive cancer was not used as an end-point because the standard of care requires treatment of premalignant disease, which is recognized as being on the causal pathway to invasive cancer. Protection against genital warts by the quadrivalent vaccine has also been used as a primary end-point for men and women. Protection against incident and prevalent HPV infection during the trials has also been monitored and reported.

Large efficacy trials of both vaccines have been carried out in young women (aged 16–26 years), leading to licensure for this age group and sex [15, 16]. The quadrivalent vaccine has also been shown, in more limited studies in young men, to confer protection against genital warts and low-grade anal dysplasia or worse (anal intraepithelial neoplasia of grade 1 or worse [AIN1+]), resulting in licensure of this vaccine for young men in some countries [17, 18].

1.2.9 Licensure for young adolescents, the main target group for the vaccines

The principal target group for the vaccines is young adolescents who have not yet become sexually active, because the vaccines confer protection mainly by preventing incident infection and disease, and because individuals are at high risk of developing HPV infection soon after the onset of sexual activity. Regulatory authorities agreed that it was not feasible to conduct efficacy trials of reasonable duration in this young age group. Therefore, licensure for vaccination of young adolescents has been based on immunobridging trials of young adolescents that demonstrated that the serum immune responses to three vaccine doses in this group were not inferior, against the HPV types in the respective vaccine, to the immune responses induced in the older individuals in the respective efficacy trials. For both sexes, the immunobridging studies actually indicated that three vaccine doses in young adolescents induced stronger immune responses than those among the older individuals in whom the efficacy trials were conducted.

1.3 Rationale for the IARC/NCI Expert Meeting on Primary End-points for Prophylactic HPV Vaccine Trials

The main purpose of the Expert Meeting was to discuss whether it might be appropriate to consider using a virological end-point, rather than a disease end-point, as the primary end-point for at least some future clinical efficacy trials of prophylactic HPV vaccines. When valid,

virological end-points could accelerate vaccine development and evaluation, leading to faster approval and implementation of these potentially important public health measures. In addition, the cost and complexity of HPV vaccine trials are also greater when a disease end-point such as CIN2+ is used. Now that there are licensed prophylactic vaccines, it may be unethical for future trials to be placebo-controlled if a candidate vaccine is being tested in individuals aged 9–26 years. Instead, many future efficacy trials for people in that age range will be non-inferiority trials, where a new vaccine is compared with a licensed vaccine. For example, Merck, to evaluate its experimental nonavalent vaccine, which contains five oncogenic HPV types in addition to the four HPV types in its quadrivalent vaccine, conducted a trial that compared its efficacy against the five new types to that of the quadrivalent vaccine (http://www.mercknewsroom.com/news-release/research-and-development-news/mercks-investigational-9-valent-hpv-vaccine-v503-prevente). Given the high efficacy of the licensed vaccines against the vaccine HPV types in naive subjects, the use of a disease end-point such as CIN2+ in a non-inferiority efficacy trial requires a very large trial, which might discourage future vaccine development.

The principal questions to be considered and discussed at the Expert Meeting were: (i) whether there was now sufficient information about the performance of the preventive HPV vaccines that a change in primary end-point might be warranted for some clinical trials, and (ii) what clinical trials might still require the use of a disease end-point. Because immunobridging has also been used for vaccine licensure for young adolescents, it seemed desirable to discuss the circumstances under which immunobridging trials might be sufficient. The goal of the Expert Meeting would be to develop recommendations for clinical trial primary end-points that could also include post-trial follow-up recommendations.

The concept of holding an Expert Meeting to consider these issues was discussed and supported at a WHO scoping meeting held in Geneva in February 2013, focused on a future revision of the Technical Report Series on HPV Vaccines. At the February meeting, it was decided to hold a larger WHO meeting in Geneva in November 2013, to initiate the revision. To maximize the potential utility of the Expert Meeting on end-points for HPV vaccine trials, it was decided to hold it early enough to develop a draft of the report that would be made available to the participants at the November meeting.

1.4 Expert Meeting on Primary End-points for Prophylactic HPV Vaccine Trials

The Expert Meeting brought together a Working Group composed of experts from the non-profit public and academic sectors who possessed a wide range of expertise relevant to HPV vaccines. This included expertise in: basic and clinical HPV biology; HPV epidemiology; HPV vaccine research and development; the conduct of HPV vaccine trials, post-licensure surveillance, and cervical cancer screening trials; and HPV vaccine regulatory and implementation issues.

Conflicts of interest were fully disclosed, and relevant disclosures are included in this IARC Working Group Report.

The first day of the workshop was devoted to a discussion of the issues surrounding the use of disease versus virological end-points for different preventive HPV vaccines, for different anatomical sites of HPV infection, and for sex at anatomical sites where both sexes develop disease, as well as the role of immunobridging trials. The second day was devoted to developing recommendations.

There were five main sessions on the first day:

- Session 1: Trials of vaccine efficacy against cervical outcomes associated with HPV 16/18 infection

- Session 2: Trials of vaccine efficacy against cervical outcomes associated with other (non-HPV 16/18) types
- Session 3: Evaluation of durability of protection
- Session 4: Evaluation of alternative schedules and target ages
- Session 5: Trials of vaccine efficacy against non-cervical outcomes.

A background paper was prepared for each session; these papers in revised form, together with this Summary, comprise the current IARC Working Group Report.

It was agreed that virological end-points are attractive when they represent valid surrogates for clinical premalignant end-points. First, there are substantially more cases of infection by a given HPV type – whether the parameter used is a single time point or persistent infection of 6 months or longer – than there are of CIN2+ cases attributable to that HPV type, which implies that trials could be smaller and possibly of shorter duration. Second, co-infection by more than one HPV type occurs frequently; this fact can make it difficult to accurately attribute a CIN2+ lesion to a specific HPV type when more than one type is detected at the site of a lesion. By contrast, when a viral end-point is used and there is co-infection by more than one HPV type, infection by each type can be considered separately. Third, the diagnosis of CIN2+ is less reproducible than HPV positivity.

Session 1: Trials of vaccine efficacy against cervical outcomes associated with HPV 16/18 infection

It has been shown that viral infection with an oncogenic HPV type is part of the necessary pathway for the development of cervical cancer (see Section 1.2.2 above, "Pathogenesis of HPV-associated cancer"). Therefore, preventing viral infection should also prevent development of premalignant and malignant cervical disease, provided that the prevented infections were not skewed towards those infections that were not destined to result in premalignant disease. The Working Group recognized that the possibility of breakthrough infections progressing to disease at a higher rate has not yet formally been ruled out. However, a consensus view was developed that data from epidemiology and vaccine clinical trials indicate that this possibility is remote, and it should not prevent the use of persistent infection as a surrogate end-point. The Working Group members agreed that persistent infection of 6 months or longer is very likely to perform with high fidelity as a surrogate for advanced disease/cancer. For any trial, it is important that the method for sampling the epithelium and for HPV determination be standardized and reproducible.

The data from the efficacy trials that have been completed support the use of the above-mentioned viral end-points. Vaccine efficacy against vaccine HPV types was similar whether the end-point of persistent infection or CIN2+ was used. Vaccine efficacy for persistent infection was similar whether a 6-month end-point or a 12-month end-point was used. In addition to these instances of high vaccine efficacy against vaccine HPV types, vaccine efficacy against persistent infection was either similar to or lower than vaccine efficacy against CIN2+ for those non-vaccine HPV types in which partial cross-protection was observed. Thus, vaccine efficacy as determined by a persistent infection end-point has not been higher than for the CIN2+ end-point.

In future clinical trials of prophylactic non-VLP vaccines, whose immunogen is derived from capsid proteins and whose mechanism of action appears to be antibody-mediated, most Working Group members agreed that if the mechanism of protection induced by such immunogens was analogous to that of VLP vaccines, then it was acceptable for trials to use a virological end-point of persistent infection. However, a minority preferred the use of a clinical end-point until vaccine efficacy using a viral end-point was shown to be a valid disease surrogate for that non-VLP vaccine.

Immunogenicity end-points were deemed acceptable for bridging efficacy to younger age groups, provided that non-inferiority can be demonstrated. In immunobridging trials that use non-inferiority as the main end-point, the serological assay should be standardized and validated and the time frame defined and standardized. Validation should include establishing the correlation between the titres in the serological assay and titres in a standard in vitro neutralization assay.

Session 2: Trials of vaccine efficacy against cervical outcomes associated with other (non-HPV 16/18) types

End-point issues were considered for two distinct situations: (i) when adding new HPV types to a multivalent vaccine, and (ii) when evaluating cross-protection against non-vaccine HPV types.

When new HPV types are added to a multivalent vaccine (that presumably has high vaccine efficacy against HPV 16/18), most Working Group members agreed that it is not always feasible to use CIN2+ as an end-point, because the number of such cases, compared with the number of infections by those types, is substantially lower than for HPV 16/18 infection, and progression to CIN2+ may occur less frequently and more slowly compared with HPV 16/18 infection. Use of a composite end-point (e.g. all oncogenic HPV types) to evaluate efficacy against the new HPV types at the cervix could be a reasonable approach, given the low attack rate anticipated for many of the less common HPV types. The public health value related to the composite end-point was noted. The Working Group members also agreed that non-inferiority immunobridging, using a well-validated assay, could be used in two situations: (i) for HPV types shared by both the candidate vaccine and the licensed comparator vaccine, and (ii) for the new HPV types when studies are conducted to bridge efficacy to additional ages (typically < 16 years) after efficacy has been demonstrated for those types in another age group (typically 16–26 years).

For the purpose of evaluating cross-protection (i.e. protection against HPV types not included in the vaccine [non-vaccine types]), most Working Group members agreed that CIN2+ may not be feasible. It was agreed that persistent infection could be used for VLP vaccines, especially as cross-protection in the VLP trials already conducted had indicated that vaccine efficacy is not higher when persistent infection, rather than CIN2+, is used as the end-point. Protection should be demonstrated for individual HPV types. Given questions about the durability of cross-protective efficacy, special attention should be given to this issue in the design of the trial. Although duration of protection would not be a formal criterion for licensure, it is of public health importance. It should therefore be addressed in post-licensure surveillance studies.

Session 3: Evaluation of durability of protection

Demonstration of duration of protection, although important for public health, has not been a requirement for licensure of HPV vaccines or of other vaccines. It was therefore agreed that duration of protection in clinical trials should not be required for future licensure. However, evaluation of the durability of the immune response and of protective efficacy should be an important element of post-licensure monitoring. The design of pivotal trials should include plans for long-term follow-up; these plans, and the approach to updating the label by regulatory bodies, should be coordinated with them early in development of the clinical evaluation.

Session 4: Evaluation of alternative schedules and target ages

This session focused mainly on the immune response in different age groups and its relevance to extending vaccination by immunobridging to additional age groups (typically 9–15 years) once a vaccine had been shown to be effective in some age groups (typically 16–

26 years). There has been keen interest in this area because the immune response in young adolescents (individuals aged 9–14 years) of both sexes to three doses is stronger than that of individuals aged 16–26 years in the clinical efficacy trials; these findings have raised the question of whether it might be appropriate for immunobridging trials to be used for licensure of alternative schedules, including those that use fewer than three doses. The session also briefly discussed vaccination in immunosuppressed populations. It was agreed that trial end-points in immunosuppressed patients, such as HIV-positive individuals, were beyond the scope of this meeting, but that the risk–benefit ratio for subunit HPV vaccines, which are non-infectious, strongly favoured their evaluation or use in immunosuppressed populations.

There was unanimous agreement that immunobridging based on non-inferiority of the immune response was a useful approach, when a standardized validated immunological test whose results correlate with neutralizing activity was used. The key metric should be the level of the immune response, rather than the number of doses required or the dosage schedule for inducing the response. In principle, this approach is valid for alternative schedules for three doses or fewer than three doses, and is not inherently age-specific, in that it could also apply to groups older (or younger) than 9–15 years. However, in post-licensure studies, it would be useful to verify high efficacy and long duration of protection. In cases where immunological non-inferiority cannot be demonstrated – for example, as can be expected when only one dose is administered – efficacy against a viral end-point such as persistent infection would be required, as the minimum level of immunity required for protection has not been established.

Session 5: Trials of vaccine efficacy against non-cervical outcomes

The discussion focused on anal infection and disease and on oropharyngeal infection and disease. Vulvar and vaginal infection and disease are also important sites.

Anal infection and disease have much in common with cervical infection and disease, except that HPV infection and disease at the anus affect both men and women. The disease appears to be similar in men and women. More recent studies strongly suggest that almost all cases of anal cancer are attributable to HPV infection. HPV 16 accounts for most cases, in both HIV-positive and HIV-negative individuals. In some studies of anal cancer, HPV types other than HPV 16/18 have been associated with up to 20% of the HPV-positive cases.

In women, anal infection is as common as cervical infection; however, the lower rate of anal cancer implies that infection at this site is less likely to progress to invasive cancer compared with cervical cancer. It was agreed that although the natural history of anal HPV infection is less well understood than that of cervical infection, end-points for anal disease prevention can be similar to cervical end-points for vaccine prevention trials.

Vulvar cancer is approximately one sixth as common as cervical cancer, and vaginal cancer is approximately one twentieth as common as cervical cancer. Although vulvar cancer usually occurs among elderly women, emerging evidence suggests that high-grade vulvar intraepithelial neoplasia/vaginal intraepithelial neoplasia (VIN/VAIN) is becoming increasingly common also among younger women. High-grade VIN/VAIN are surrogate markers for vulvar or vaginal cancers. Approximately half of vulvar cancer cases are caused by high-risk HPV types, primarily HPV 16/18. Hence, HPV 16/18 also dominate in high-grade VIN. The attributable proportion for HPV 16/18 is higher than that for high-grade CIN. The attributable proportion of other high-risk HPV types in high-grade VIN/VAIN has not been extensively studied. The end-points in the clinical trials so far have been disease end-points based on colposcopy biopsies. In such clinical trials, colposcopists must be trained to perform vulvar/vaginal colposcopy to obtain multiple biopsies from any lesions visible after acetic acid application. Not enough is known about using persistent HPV infection as an end-point.

A persistent infection end-point could be considered at these two sites if new studies validated the ability to detect persistent infection at them.

HPV infection of the oral cavity and oropharynx is less common than anogenital infection, and the natural history of infection at this non-genital site is less well understood than that of genital infection. Oral infection is more common in men than in women, and seems to persist longer in men than in women. Premalignant oropharyngeal lesions cannot be routinely identified. It is therefore not feasible to use a disease end-point at this site to evaluate vaccine efficacy. The only feasible vaccine end-point at the moment is prevention of oral infection. It was agreed that high vaccine efficacy as measured by this parameter would probably translate to protection against oropharyngeal cancer. However, it was also recognized that the relationship between that oral infection, as measured using current collection procedures, and HPV-positive oropharyngeal cancer is not as tightly linked as that for anogenital infection and premalignant disease.

1.5 Recommendations for vaccine end-points

1.5.1 General considerations

These recommendations are confined to prophylactic HPV vaccines that use immunogens derived from the L1 and/or L2 capsid proteins. The vaccines are divided into VLP vaccines and non-VLP vaccines. For all such vaccines, there is good experimental evidence that their main protective activity is antibody-mediated.

The Working Group considered vaccine end-points for three age groups: < 16 years, 16–26 years, and > 26 years. Although it is feasible to conduct trials in infants and children, the Working Group did not make recommendations for these age groups. For different scenarios, the main question addressed was whether it is appropriate to use a serological end-point (immunobridging), a viral end-point (e.g. persistent infection), or a disease end-point (e.g. CIN2+). Consensus was reached for almost all situations considered by the Working Group. In general, it was agreed that where protection was being measured, persistent infection was an appropriate end-point in most settings (for exceptions, see the next two paragraphs). However, it should be noted that the recommendation of an end-point upstream from a disease end-point does not mean that a more downstream end-point could not be used, but rather that it does not need to be used. For infections at sites that affect both sexes, no situation was identified where end-points should be sex-specific.

For individuals aged > 26 years, consensus for an appropriate protection end-point was not reached. Some Working Group members concluded that a persistent infection end-point was appropriate, by analogy to what is recommended for those aged 16–26 years in settings where protection should be evaluated. Others concluded that a disease end-point (CIN2+) was appropriate, given that the frequency with which incident infection acquired in this age group progresses to precancer is uncertain, as is the potential public health impact. For simplicity, this recommendation is given in the tables as both options. Another issue is that the vaccine is approved for this age group by the EMA but not by the FDA.

If vulvar/vaginal protection is to be evaluated in a trial, it is recommended to use the HPV 16/18-positive high-grade VIN/VAIN disease end-point, as there is relatively little experience in using persistent HPV infection as a surrogate end-point for vulvar and vaginal disease.

Because the natural history of oral infection is less well characterized, demonstration of efficacy on a disease end-point would, in principle, be preferable. However, the recommendation for a persistent infection end-point recognizes that with current technology, an oral disease end-point is not feasible. This recommendation is in contrast to those for the

anogenital end-point, where a disease end-point is technically feasible but is no longer necessary.

Many trials will be non-inferiority trials. The comparison group for establishing efficacy should be agreed upon in advance with regulatory authorities. Where vaccine efficacy is high, as occurs with protection against vaccine HPV types, the attack rate in vaccinees is anticipated to be very low, which means that demonstrating non-inferiority for an end-point within the trial may not be feasible. Such a situation may require exploration of alternative approaches for demonstrating efficacy, such as use of historical controls or use of concurrently collected prevalence data in the broader population in which the study is conducted.

1.5.2 End-points for placebo-controlled trial of a licensed HPV VLP vaccine: three doses

See Table 1.1. Given that currently available VLP vaccines have been licensed by many regulatory authorities, the Working Group members thought that such a placebo-controlled design might not be ethical in most situations for age groups in which the vaccine had already been widely licensed. However, they recognized that some countries may require such a trial for vaccine licensure in their country. Under those circumstances, the Working Group recommended use of persistent infection at the cervix, at the anus, or in the oral cavity (HPV 16/18) for individuals aged 16–26 years and an immunobridging non-inferiority trial for individuals aged < 16 years, and either a disease or viral end-point for individuals aged > 26 years.

1.5.3 End-points for development of a new HPV VLP vaccine similar to a licensed product or products: three doses

See Table 1.2. If immunological non-inferiority compared with the licensed product is demonstrated for an age group, that result would be sufficient for licensure for that age group. The immunological result should be followed by post-licensure confirmation of protection. In the case of a vaccine for which immunological non-inferiority is not demonstrated, protection against persistent infection should be the primary end-point for the trial. If that protection is demonstrated for a particular age group (such as 16–26 years), an immunobridging non-inferiority trial can be used for individuals aged < 16 years. Either a disease or viral end-point should be used for individuals aged > 26 years.

1.5.4 End-points for development of a new polyvalent VLP vaccine containing additional HPV types compared with a licensed product or products: three doses

See Table 1.3. To establish efficacy, the Working Group recommended using a composite viral end-point for the new HPV types, immunological non-inferiority for the common HPV types, and no obvious reduction in protection against the common HPV types at the cervix, with a similar end-point at the anus. After efficacy is established for the new types via a viral end-point in an exposed population (e.g. those aged 16–26 years), demonstrating immunological non-inferiority would be the appropriate approach to bridging efficacy to a younger population. The Working Group recommended either a disease or viral end-point for individuals aged > 26 years. The Working Group also recommended an HPV 16/18 viral end-point in the oral cavity.

1.5.5 End-points for one or two doses for an HPV VLP vaccine approved for three doses

1.5.5.1 If immunological non-inferiority can be demonstrated

See Table 1.4. Where feasible, the Working Group recommended an immunobridging non-inferiority trial for individuals aged < 16 years or 16–26 years, as the key metric is the level of the immune response, rather than the number of vaccine doses or dosage schedule. Post-

licensure evaluation of long-term efficacy against composite persistent infection and/or disease for vaccine HPV types would be especially important.

1.5.5.2 If immunological non-inferiority cannot be demonstrated

See Table 1.5. If non-inferiority was not successful or was not tried – for example, with the group aged 16–26 years for two doses or with any group for one dose – persistent infection at the cervix, at the anus, or in the oral cavity (HPV 16/18) should be used. The table indicates that a composite end-point should be used; however, the HPV 16/18 end-point should be evaluated separately from that of other HPV types. Post-licensure evaluation of long-term efficacy against composite persistent infection and/or disease for vaccine HPV types would be especially important. Either a disease or viral end-point should be used for individuals aged > 26 years.

1.5.6 End-points for cross-protection against non-vaccine types for any VLP vaccine

See Table 1.6. For individuals aged 16–26 years, the Working Group recommended showing statistically significant protection against an individual (or possibly composite) viral end-point. Long-term protection against viral and disease end-points would be evaluated post-licensure. For individuals aged < 16 years, an immunobridging non-inferiority trial is recommended. Long-term protection against viral and disease end-points should be evaluated post-licensure. Either a disease or viral end-point should be used for individuals aged > 26 years.

1.5.7 End-points for non-VLP vaccines

See Table 1.7. These vaccines may include a vector that expresses L1, purified L1 capsomeres, or L2-based vaccines. When the mechanism of protection is antibody-mediated, as in these examples, efficacy against persistent infection at the cervix, at the anus, or in the oral cavity (HPV 16/18) should be used. Demonstration of high efficacy is desirable. Long-term protection against viral and disease end-points would be evaluated post-licensure. Immunobridging non-inferiority trials could be conducted as for VLP vaccines.

References

1. de Martel C, Ferlay J, Franceschi S, Vignat J, Bray F, Forman D *et al.* (2012). Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. *Lancet Oncol*. 13(6):607–15. http://dx.doi.org/10.1016/S1470-2045(12)70137-7 PMID:22575588
2. Crosbie EJ, Einstein MH, Franceschi S, Kitchener HC (2013). Human papillomavirus and cervical cancer. *Lancet*. 382(9895):889–99. http://dx.doi.org/10.1016/S0140-6736(13)60022-7 PMID:23618600
3. Bosch FX, Lorincz A, Muñoz N, Meijer CJ, Shah KV (2002). The causal relation between human papillomavirus and cervical cancer. *J Clin Pathol*. 55(4):244–65. http://dx.doi.org/10.1136/jcp.55.4.244 PMID:11919208
4. Chaturvedi AK, Engels EA, Pfeiffer RM, Hernandez BY, Xiao W, Kim E *et al.* (2011). Human papillomavirus and rising oropharyngeal cancer incidence in the United States. *J Clin Oncol*. 29(32):4294–301. http://dx.doi.org/10.1200/JCO.2011.36.4596 PMID:21969503
5. Attner P, Du J, Näsman A, Hammarstedt L, Ramqvist T, Lindholm J *et al.* (2010). The role of human papillomavirus in the increased incidence of base of tongue cancer. *Int J Cancer*. 126(12):2879–84. http://dx.doi.org/10.1002/ijc.24994 PMID:19856308
6. Moscicki AB, Schiffman M, Burchell A, Albero G, Giuliano AR, Goodman MT *et al.* (2012). Updating the natural history of human papillomavirus and anogenital cancers. *Vaccine*. 30 Suppl 5:F24–33. http://dx.doi.org/10.1016/j.vaccine.2012.05.089 PMID:23199964

7. Howley PM, Schiller JT, Lowy DR (2013). Papillomaviruses. In: Knipe DM, Howley PM, editors. *Fields Virology*, 6th ed. Philadelphia: Wolters Kluwer/Lippincott Williams & Willkins, pp. 1662–703.
8. Schiffman M, Wentzensen N, Wacholder S, Kinney W, Gage JC, Castle PE (2011). Human papillomavirus testing in the prevention of cervical cancer. *J Natl Cancer Inst*. 103(5):368–83. http://dx.doi.org/10.1093/jnci/djq562 PMID:21282563
9. Vaccarella S, Lortet-Tieulent J, Plummer M, Franceschi S, Bray F (2013). Worldwide trends in cervical cancer incidence: impact of screening against changes in disease risk factors. *Eur J Cancer*. 49(15):3262–73. http://dx.doi.org/10.1016/j.ejca.2013.04.024 PMID:23751569
10. Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV *et al.*; International Agency for Research on Cancer Multicenter Cervical Cancer Study Group (2003). Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med*. 348(6):518–27. http://dx.doi.org/10.1056/NEJMoa021641 PMID:12571259
11. Smith JS, Lindsay L, Hoots B, Keys J, Franceschi S, Winer R *et al.* (2007). Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. *Int J Cancer*. 121(3):621–32. http://dx.doi.org/10.1002/ijc.22527 PMID:17405118
12. Guan P, Howell-Jones R, Li N, Bruni L, de Sanjosé S, Franceschi S *et al.* (2012). Human papillomavirus types in 115,789 HPV-positive women: a meta-analysis from cervical infection to cancer. *Int J Cancer*. 131(10):2349–59. http://dx.doi.org/10.1002/ijc.27485 PMID:22323075
13. Gillison ML, Chaturvedi AK, Lowy DR (2008). HPV prophylactic vaccines and the potential prevention of noncervical cancers in both men and women. *Cancer*. 113(10) Suppl:3036–46. http://dx.doi.org/10.1002/cncr.23764 PMID:18980286
14. zur Hausen H (2009). Papillomaviruses in the causation of human cancers - a brief historical account. *Virology*. 384(2):260–5. http://dx.doi.org/10.1016/j.virol.2008.11.046 PMID:19135222
15. Lehtinen M, Paavonen J, Wheeler CM, Jaisamrarn U, Garland SM, Castellsagué X *et al.*; HPV PATRICIA Study Group (2012). Overall efficacy of HPV-16/18 AS04-adjuvanted vaccine against grade 3 or greater cervical intraepithelial neoplasia: 4-year end-of-study analysis of the randomised, double-blind PATRICIA trial. *Lancet Oncol*. 13(1):89–99. http://dx.doi.org/10.1016/S1470-2045(11)70286-8 PMID:22075171
16. Muñoz N, Kjaer SK, Sigurdsson K, Iversen O-E, Hernandez-Avila M, Wheeler CM *et al.* (2010). Impact of human papillomavirus (HPV)-6/11/16/18 vaccine on all HPV-associated genital diseases in young women. *J Natl Cancer Inst*. 102(5):325–39. http://dx.doi.org/10.1093/jnci/djp534 PMID:20139221
17. Giuliano AR, Palefsky JM, Goldstone S, Moreira ED Jr, Penny ME, Aranda C *et al.* (2011). Efficacy of quadrivalent HPV vaccine against HPV infection and disease in males. *N Engl J Med*. 364(5):401–11. http://dx.doi.org/10.1056/NEJMoa0909537 PMID:21288094
18. Palefsky JM, Giuliano AR, Goldstone S, Moreira ED Jr, Aranda C, Jessen H *et al.* (2011). HPV vaccine against anal HPV infection and anal intraepithelial neoplasia. *N Engl J Med*. 365(17):1576–85. http://dx.doi.org/10.1056/NEJMoa1010971 PMID:22029979
19. Wheeler CM, Kjaer SK, Sigurdsson K, Iversen O-E, Hernandez-Avila M, Perez G *et al.* (2009). The impact of quadrivalent human papillomavirus (HPV; types 6, 11, 16, and 18) L1 virus-like particle vaccine on infection and disease due to oncogenic nonvaccine HPV types in sexually active women aged 16–26 years. *J Infect Dis*. 199(7):936–44. http://dx.doi.org/10.1086/597309 PMID:19236277
20. Wheeler CM, Castellsagué X, Garland SM, Szarewski A, Paavonen J, Naud P *et al.*; HPV PATRICIA Study Group (2012). Cross-protective efficacy of HPV-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by non-vaccine oncogenic HPV types: 4-year end-of-study analysis of the randomised, double-blind

PATRICIA trial. *Lancet Oncol*. 13(1):100–10. http://dx.doi.org/10.1016/S1470-2045(11)70287-X PMID:22075170
21. Read TR, Hocking JS, Chen MY, Donovan B, Bradshaw CS, Fairley CK (2011). The near disappearance of genital warts in young women 4 years after commencing a national human papillomavirus (HPV) vaccination programme. *Sex Transm Infect*. 87(7):544–7. http://dx.doi.org/10.1136/sextrans-2011-050234 PMID:21970896
22. Blomberg M, Dehlendorff C, Munk C, Kjaer SK (2013). Strongly decreased risk of genital warts after vaccination against human papillomavirus: nationwide follow-up of vaccinated and unvaccinated girls in Denmark. *Clin Infect Dis*. 57(7):929–34. http://dx.doi.org/10.1093/cid/cit436 PMID:23804192
23. Leval A, Herweijer E, Ploner A, Eloranta S, Fridman Simard J, Dillner J *et al.* (2013). Quadrivalent human papillomavirus vaccine effectiveness: a Swedish national cohort study. *J Natl Cancer Inst*. 105(7):469–74. http://dx.doi.org/10.1093/jnci/djt032 PMID:23486550
24. Markowitz LE, Hariri S, Lin C, Dunne EF, Steinau M, McQuillan G *et al.* (2013). Reduction in human papillomavirus (HPV) prevalence among young women following HPV vaccine introduction in the United States, National Health and Nutrition Examination Surveys, 2003–2010. *J Infect Dis*. 208(3):385–93. http://dx.doi.org/10.1093/infdis/jit192 PMID:23785124
25. Tabrizi SN, Brotherton JM, Kaldor JM, Skinner SR, Cummins E, Liu B *et al.* (2012). Fall in human papillomavirus prevalence following a national vaccination program. *J Infect Dis*. 206(11):1645–51. http://dx.doi.org/10.1093/infdis/jis590 PMID:23087430
26. Ali H, Guy RJ, Wand H, Read TRH, Regan DG, Grulich AE *et al.* (2013). Decline in in-patient treatments of genital warts among young Australians following the national HPV vaccination program. *BMC Infect Dis*. 13(1):140. http://dx.doi.org/10.1186/1471-2334-13-140 PMID:23506489
27. Gertig DM, Brotherton JM, Budd AC, Drennan K, Chappell G, Saville AM (2013). Impact of a population-based HPV vaccination program on cervical abnormalities: a data linkage study. *BMC Med*. 11(1):227. http://dx.doi.org/10.1186/1741-7015-11-227 PMID:24148310
28. Lowy DR, Schiller JT (2012). Reducing HPV-associated cancer globally. *Cancer Prev Res (Phila)*. 5(1):18–23. http://dx.doi.org/10.1158/1940-6207.CAPR-11-0542 PMID:22219162
29. Wang JW, Roden RB (2013). Virus-like particles for the prevention of human papillomavirus-associated malignancies. *Expert Rev Vaccines*. 12(2):129–41. http://dx.doi.org/10.1586/erv.12.151 PMID:23414405
30. Schiller JT, Castellsagué X, Garland SM (2012). A review of clinical trials of human papillomavirus prophylactic vaccines. *Vaccine*. 30 Suppl 5:F123–38. http://dx.doi.org/10.1016/j.vaccine.2012.04.108 PMID:23199956

Table 1.1. End-points for placebo-controlled trial of a licensed HPV VLP vaccine: three doses[a]

Age group	Immunobridging	Protection at anatomical site			
		Cervical	**Vulvar/vaginal**	**Anal**	**Oral**
< 16 years	Non-inferiority to the established dosing regimen in the population in whom efficacy was demonstrated (typically, individuals aged 16–26 years) for each vaccine HPV type	NA	NA	NA	NA
16–26 years	NA	Persistent infection with vaccine HPV types[b]	High-grade VIN/VAIN	Persistent infection with vaccine HPV types[b]	Persistent HPV 16/18 infection[b]
> 26 years	NA	Persistent infection with vaccine HPV types[b] or disease (CIN2+)	High-grade VIN/VAIN	Persistent infection with vaccine HPV types[b] or disease (AIN)	Persistent HPV 16/18 infection[b]

AIN, anal intraepithelial neoplasia; CIN2+, cervical intraepithelial neoplasia of grade 2 or worse; HPV, human papillomavirus; NA, not applicable; VIN/VAIN, vulvar intraepithelial neoplasia/vaginal intraepithelial neoplasia; VLP, virus-like particle.

[a] In most countries, it is probably not ethical to have a placebo group where regulatory authorities in many countries have already licensed the vaccine for the age groups in question. Recommended only if a national regulatory authority requires a placebo-controlled trial.

[b] 6 months or longer.

Table 1.2. End-points for development of a new HPV VLP vaccine similar to a licensed product or products: three doses

Age group	Immunobridging	Protection at anatomical site			
		Cervical	**Vulvar/vaginal**	**Anal**	**Oral**
< 16 years	Non-inferiority to immunity in the age group 16–26 years for each vaccine HPV type[a]	NA	NA	NA	NA
16–26 years	Non-inferiority for each vaccine HPV type compared with a licensed product[b]	Post-licensure: confirm efficacy with vaccine HPV types using virological and/or disease end-points	NA	NA	NA
> 26 years	NA	Persistent infection with vaccine HPV types[c] or disease (CIN2+)	High-grade VIN/VAIN	Persistent infection with vaccine HPV types[c] or disease (AIN)	Persistent HPV 16/18 infection[c]

AIN, anal intraepithelial neoplasia; CIN2+, cervical intraepithelial neoplasia of grade 2 or worse; HPV, human papillomavirus; NA, not applicable; VIN/VAIN, vulvar intraepithelial neoplasia/vaginal intraepithelial neoplasia; VLP, virus-like particle.

[a] Immunobridging can be done in the age group < 16 years if immunological non-inferiority for each vaccine HPV type has been demonstrated for the age group 16–26 years.

[b] A virological end-point would be required if non-inferiority could not be demonstrated.

[c] 6 months or longer.

Table 1.3. End-points for development of a new polyvalent VLP vaccine containing additional HPV types compared with a licensed product or products: three doses

Age group	Immunobridging	Protection at anatomical site			
		Cervical	**Vulvar/vaginal**	**Anal**	**Oral**
< 16 years	Non-inferiority to the established dosing regimen in the population in whom efficacy was demonstrated (typically, individuals aged 16–26 years) for each vaccine HPV type	NA	NA	NA	NA
16–26 years	Non-inferiority for each HPV type shared by both vaccines	Composite end-point of persistent infection with new HPV types[a,b]	High-grade HPV 16/18 VIN/VAIN	Composite end-point of persistent infection with new HPV types[a,b]	Persistent HPV 16/18 infection[b]
> 26 years	NA	Persistent infection with vaccine HPV types[a,b] or disease (CIN2+)	High-grade HPV 16/18 VIN/VAIN	Persistent infection with vaccine HPV types[a,b] or disease (AIN)	Persistent HPV 16/18 infection[b]

AIN, anal intraepithelial neoplasia; CIN2+, cervical intraepithelial neoplasia of grade 2 or worse; HPV, human papillomavirus; NA, not applicable; VIN/VAIN, vulvar intraepithelial neoplasia/vaginal intraepithelial neoplasia; VLP, virus-like particle.

[a] Monitor vaccine efficacy against each HPV type.

[b] 6 months or longer.

Table 1.4. End-points for one or two doses for an HPV VLP vaccine approved for three doses, for situations in which immunological non-inferiority can be demonstrated

Age group	Immunobridging	Protection at anatomical site			
		Cervical	**Vulvar/vaginal**	**Anal**	**Oral**
< 16 years	Non-inferiority to the standard three-dose schedule in the population in whom efficacy was demonstrated (typically, individuals aged 16–26 years) for each vaccine HPV type[a]	NA	NA	NA	NA
16–26 years	Non-inferiority to the standard three-dose schedule in the population in whom efficacy was demonstrated (typically, individuals aged 16–26 years) for each vaccine HPV type[a]	NA	NA	NA	NA
> 26 years	NA	Persistent infection with vaccine HPV types[a,b] or disease (CIN2+)	High-grade HPV 16/18 VIN/VAIN	Persistent infection with vaccine HPV types[a,b] or disease (AIN)	Persistent HPV 16/18 infection[b]

AIN, anal intraepithelial neoplasia; CIN2+, cervical intraepithelial neoplasia of grade 2 or worse; HPV, human papillomavirus; NA, not applicable; VIN/VAIN, vulvar intraepithelial neoplasia/vaginal intraepithelial neoplasia; VLP, virus-like particle.

[a] Post-licensure: confirm long-term efficacy against composite persistent infection and/or disease for vaccine HPV types. Monitor efficacy against individual vaccine HPV types.

[b] 6 months or longer.

Table 1.5. End-points for one or two doses for an HPV VLP vaccine approved for three doses: alternative approach for situations in which immunologic non-inferiority cannot be demonstrated

Age group	Immunobridging	Protection at anatomical site			
		Cervical	**Vulvar/vaginal**	**Anal**	**Oral**
< 16 years	Non-inferiority to the established dosing regimen in the population in whom efficacy was demonstrated (typically, individuals aged 16–26 years) for each vaccine HPV type	NA	NA	NA	NA
16–26 years	NA	Composite end-point of persistent infection with vaccine HPV types[a,b]	High-grade HPV 16/18 VIN/VAIN	Composite end-point of persistent infection with vaccine HPV types[a,b]	Persistent HPV 16/18 infection
> 26 years	NA	Composite persistent infection with vaccine HPV types[a,b] or disease (CIN2+)	High-grade HPV 16/18 VIN/VAIN	Composite persistent infection with vaccine HPV types[a,b] or disease (AIN)	Persistent HPV 16/18 infection

AIN, anal intraepithelial neoplasia; CIN2+, cervical intraepithelial neoplasia of grade 2 or worse; HPV, human papillomavirus; NA, not applicable; VIN/VAIN, vulvar intraepithelial neoplasia/vaginal intraepithelial neoplasia; VLP, virus-like particle.

[a] 6 months or longer; monitor vaccine efficacy against each HPV type. Post-licensure: confirm long-term high efficacy against composite persistent infection and/or disease for vaccine HPV types.

[b] The comparison group for establishing efficacy should be agreed upon in advance with regulatory authorities. Because the attack rate for persistent infection in, for example, both a two-dose and a three-dose group is anticipated to be very low, demonstrating non-inferiority for this end-point may not be feasible. Such a situation may require exploration of alternative approaches for demonstrating efficacy, such as use of historical controls or use of concurrently collected prevalence data in the broader population in whom the study is conducted. Post-licensure: confirm long-term efficacy against composite persistent infection and/or disease for vaccine HPV types.

Table 1.6. End-points for cross-protection against non-vaccine types for any VLP vaccine

Age group	Immunobridging	Protection at anatomical site			
		Cervical	**Vulvar/vaginal**	**Anal**	**Oral**
< 16 years	Non-inferiority to the established dosing regimen in the population in whom efficacy was demonstrated (typically, individuals aged 16–26 years) for each vaccine HPV type[a]	NA	NA	NA	NA
16–26 years	NA	Statistically significant efficacy against an individual (or possibly composite) persistent infection[a]	High-grade HPV 16/18 VIN/VAIN	Statistically significant efficacy against an individual (or possibly composite) persistent infection[a]	NA
> 26 years	NA	Statistically significant efficacy against persistent infection or disease for an individual HPV type (or possibly composite) persistent infection[a]	High-grade HPV 16/18 VIN/VAIN	Statistically significant efficacy against persistent infection or disease for an individual HPV type (or possibly composite) persistent infection[a]	NA

AIN, anal intraepithelial neoplasia; CIN2+, cervical intraepithelial neoplasia of grade 2 or worse; HPV, human papillomavirus; NA, not applicable; VIN/VAIN, vulvar intraepithelial neoplasia/vaginal intraepithelial neoplasia; VLP, virus-like particle.

[a] 6 months or longer. Post-licensure: confirm long-term efficacy for infection and/or disease end-points.

Table 1.7. End-points for non-VLP vaccines

Age group	Immunobridging	Protection at anatomical site			
		Cervical	**Vulvar/vaginal**	**Anal**	**Oral**
< 16 years	Non-inferiority to the established dosing regimen in the population in whom efficacy was demonstrated (typically, individuals aged 16–26 years) for each vaccine HPV type	NA	NA	NA	NA
16–26 years	NA	Persistent infection with vaccine HPV types[a,b]	High-grade HPV 16/18 VIN/VAIN	Persistent infection with vaccine HPV types[a]	Persistent HPV 16/18 infection
> 26 years	NA	Persistent infection with vaccine HPV types[a] or disease (CIN2+)[b]	High-grade HPV 16/18 VIN/VAIN	Persistent infection with vaccine HPV types[a] or disease (AIN)	Persistent HPV 16/18 infection

AIN, anal intraepithelial neoplasia; CIN2+, cervical intraepithelial neoplasia of grade 2 or worse; HPV, human papillomavirus; NA, not applicable; VIN/VAIN, vulvar intraepithelial neoplasia/vaginal intraepithelial neoplasia; VLP, virus-like particle.

[a] 6 months or longer; monitor vaccine efficacy against each HPV type.

[b] For non-VLP vaccine candidates, it may be acceptable to infer efficacy based on demonstration of immunological non-inferiority compared with a licensed VLP vaccine. The acceptability of this approach should be determined on a case-by-case basis, taking into account the product itself and whether preclinical data suggest that the mechanism of protection is expected to be similar to that for VLP-based vaccines. This table addresses situations in which demonstration of immunological non-inferiority compared with a licensed vaccine is either not possible or determined to be an unacceptable approach to demonstrating efficacy.

The comparison group for establishing efficacy should be agreed upon in advance with regulatory authorities. Because the attack rate for persistent infection in both the licensed vaccine and the candidate vaccine groups is anticipated to be very low, demonstrating non-inferiority for this end-point may not be feasible. Such a situation may require exploration of alternative approaches for demonstrating efficacy, such as use of historical controls or use of concurrently collected prevalence data in the broader population in whom the study is conducted.

Chapter 2. Next-generation HPV vaccines – potential for alternative end-points

Margaret Stanley

2.1 Introduction

Human papillomaviruses (HPVs) are a large family of small double-stranded DNA viruses that infect the squamous epithelium of the skin and internal mucosal surfaces of the anogenital and upper respiratory tracts. HPVs are classified as genotypes determined by sequences of the gene encoding the major capsid protein L1and numbered in the order in which they were isolated, i.e. HPV 1, HPV 2, and so on. At present, the DNA of more than 100 HPV types or strains has been isolated and cloned from clinical biopsies [1]. About 30–40 HPV types regularly or sporadically infect the squamous mucosal surfaces of the anogenital tract. These mucosatropic HPVs fall into two groups: low-risk (LR) viruses that cause genital warts, the most prevalent types of which are HPV 6 and 11 [2], and high-risk (HR) or oncogenic types associated with malignant disease, of which 12 types (HPV 16, 18, 45, 31, 33, 35, 52, 58, 51, 57, 39, and 59) are recognized as oncogenic [3].

Infection with one of the subset of HR HPVs is the cause of invasive cervical cancer in women. Globally, HPV 16 is the most common type to be detected, in 55% of cases, followed by HPV 18, in 15% of cases. Other oncogenic HPV types including 31, 33, 35, 45, 52, and 58 account for an additional 18% of all cases [4, 5]. Although cervical cancer represents the major burden of oncogenic HPV infection, a proportion of cases of cancer of the penis, vulva (40–50%), vagina (60%), and anus (> 80%) and of head and neck squamous cell cancer (HNSCC) (12–24%), particularly of the oropharynx, are attributed to HPV, with HPV 16 as the major type. In terms of absolute numbers, HNSCC, the sixth most common cancer globally, is second overall to cervical cancer for HPV-attributable cases. The most recent estimate is that HPV is the cause of 5.2% of all cancers [6].

The disease burden of the LR types HPV 6 and 11 is also very substantial. Genital warts are the most common viral sexually transmitted infection, with a lifetime risk of acquisition of 10% [7]. Prophylactic vaccination against HPV infections should prevent most HPV-associated cancers and related benign conditions and have a major public health impact.

This review addresses the following topics using data from etiological studies, screening trials, and vaccination trials:

- Can persistent infection be used as a surrogate end-point to express vaccine efficacy for HPV 16 and 18 and other oncogenic HPV types?
- Can persistent infection with the same HR type be used to replace cervical intraepithelial neoplasia of grade 2 or worse (CIN2+) as a primary efficacy end-point in clinical trials for non-vaccine HPV types?
- What are the uncertainties related to the use of an infection surrogate end-point?
- Immunogenicity and second-generation vaccines.

2.2 Licensed prophylactic HPV vaccines

The currently licensed prophylactic HPV vaccines are subunit vaccines comprising virus-like particles (VLPs) formed of only one protein, the major coat or capsid protein L1 [8]. HPV VLPs are made using sophisticated recombinant technologies in which the L1 gene of specific HPV types is recombined into the host genome of the yeast *Saccharomyces cerevisiae* or the insect virus baculovirus and the L1 protein is expressed via these recombinant vectors. The chemistry of the expressed protein is such that it spontaneously assembles into VLPs that are morphologically and antigenically similar to the wild-type virus

particle. However, VLPs lack DNA and are non-infectious and non-oncogenic. There are two licensed prophylactic HPV L1 VLP vaccines: Cervarix, a bivalent HPV 16/18 (bHPV) product from GlaxoSmithKline, and Gardasil (also known as Silgard), a quadrivalent HPV 6/11/16/18 (qHPV) product from Merck.

Both vaccines have undergone large randomized placebo-controlled double-blind phase III trials in young women aged 15–26 years. These trials were designed primarily to demonstrate efficacy in preventing high-grade intraepithelial neoplasms caused by infection related to the vaccine HPV types (both vaccines) or external genital warts (qHPV). Two phase III studies evaluated the qHPV vaccine (FUTURE I and II), and two evaluated the bHPV vaccine (PATRICIA and the Costa Rica Vaccine Trial [CVT]). The FUTURE I and II and PATRICIA trials were company-sponsored multicentre trials in Europe, the Americas, and the Asia–Pacific region. The CVT was a United States government-sponsored community-based trial in Guanacaste, Costa Rica. The primary end-point in the CVT was 12-month HPV 16/18 persistent infection.

End-of-study analyses of the pivotal phase III trials in young women have now been published [9–12]. For both vaccines, prophylactic efficacy against vaccine type-associated primary disease end-points is uniformly high in women in the according-to-protocol (ATP) cohort, i.e. women aged 15–26 years who were polymerase chain reaction (PCR)-negative and seronegative for vaccine HPV types at trial entry and after completion of the three-dose immunization regimen (reviewed in [13]). Efficacy against cervical disease caused by the HPV types in the vaccines is > 90% and is maintained for at least 8–9 years [14, 15]. Modelling studies suggest that duration of protection will extend over many decades and is probably lifelong [16, 17]. However, the vaccines have their limitations: they are, at present, expensive and type-restricted. There is cross-protection against HPV types not in the vaccine, but this is partial and the duration of such protection is questionable [18].

2.3 Next-generation vaccines

Next-generation vaccines that address either or both of these issues – cost and coverage of oncogenic HPVs in addition to HPV 16 and 18 – are in development. A nonavalent (HPV 6, 11, 16, 18, 31, 33, 45, 52, 58) HPV L1 VLP vaccine [19] developed by Merck is in phase III randomized controlled trials (RCTs). Interim data from these trials have been presented at medical meetings, with overwhelming efficacy (> 97%) for all vaccine types demonstrated in the per-protocol efficacy population [20]. Vaccines based on L1 pentameric subunits or capsomers produced in *Escherichia coli* [21] could address the issue of cost of production and potentially provide polyvalent vaccines. These products are immunogenic and show protection against virus challenge in animal models [22], but no immunogenicity or safety data for HPV capsomer vaccines are available as yet. Other alternatives for reducing manufacturing costs that are under investigation include the generation of L1 VLPs in alternative vectors such as the yeast *Pichia pastoris* [23] or in plants [24]. Live recombinant viral and bacterial vectors, including measles [25], adeno-associated virus [26], and *Salmonella typhi* [27], have been reported. Vaccines based on the minor coat protein L2 have been shown in in vitro systems and animal models to generate antibodies that are broadly cross-neutralizing and prevent infection with pseudovirions (surrogate HPV virus particles) of a range of genital and cutaneous HPV types [28]. However, apart from the nonavalent vaccine from Merck, none of the vaccine candidates described above are in clinical trials. Furthermore, all these vaccines, irrespective of the process by which they are generated, are new vaccines in regulatory terms and will face significant challenges in demonstrating efficacy if clinical disease remains the primary end-point.

2.4 Vaccine end-points

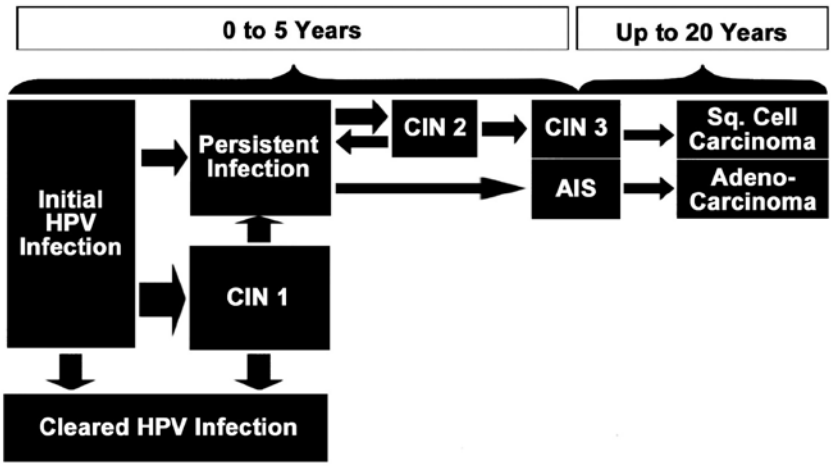
Traditionally, the measurable end-point to determine efficacy of primary intervention by prophylactic immunization has been disease incidence. However, the true disease end-point for HPV 16/18 vaccines is cancer. This disease end-point is (i) impractical, requiring huge and costly trials since cervical cancer is a rare outcome of infection with an oncogenic HPV and the interval between infection and disease is measured in decades, and (ii) unethical, since cervical cancer is a disease that can be prevented through detection of high-grade precancerous lesions by cervical screening and treatment.

The primary efficacy end-points accepted by regulatory authorities for the pivotal phase III studies of the licensed HPV vaccines were surrogate high-grade precancerous disease end-points, cervical intraepithelial neoplasia of grade 2 or 3 (CIN2/3), adenocarcinoma in situ, or cervical cancer associated with HPV 16/18, diagnosed by histopathology together with virus detection and typing [29]. This end-point was recommended by the Vaccines and Related Biological Products Advisory Committee, convened by the United States Food and Drug Administration (FDA) in 2001, and other national regulatory bodies, for a vaccine indication of prevention of cervical cancer. The rationale for a disease end-point of high-grade precancer is clear: high-grade intraepithelial neoplasms of the cervix, CIN3 and adenocarcinoma in situ, are the histopathological state that immediately precedes invasive cancer [30]. If left untreated, 30–40% of CIN3 progress to invasive cancer over a 30-year period [31]. Intervention strategies that remove CIN3 by ablative or excisional therapy lead to a reduction in incidence of cervical cancer [31]. CIN2 is a more heterogeneous group of lesions [32], but women with CIN2 are at an increased risk of cervical cancer [33].

2.5 Natural history of HPV infection in the genital tract

There is a wealth of epidemiological data to show that HPV is the cause of CIN of all grades and of cervical cancer [30, 34]. Epidemiological and natural history studies have shown that genital HPV infection in women is acquired soon after the onset of sexual activity, with a peak prevalence in the age group of 18–25 years, declining in the subsequent decades [35]. The evidence is that the majority of incident infections will clear with time, but a fraction (estimated as 10–20%) of these infections persist [36]. However, the longer a detectable oncogenic HPV infection lasts, the higher the risk that molecular “accidents” will occur during the repeat cycles of viral replication [37]. These accidents result in the deregulation of viral gene expression and the initiation of events leading to CIN3 and, hence, invasive cancer.

Fig. 2.1. Natural history of cervical HPV infection. Source: Pinto AP, Crum CP (2000). Natural history of cervical neoplasia: defining progression and its consequence. *Clin Obstet Gynecol*. 43(2):352–62. Adapted with permission from Lippincott Williams and Wilkins/Wolters Kluwer Health, copyright 2000.



HPV infection is determined by the detection of HPV DNA in swabs, smears, or biopsies taken mainly, but not exclusively, from the cervix. The term “clear” refers to the failure to detect HPV DNA in subsequent swabs or smears from the same anogenital sites. However, this issue is complicated by the persuasive evidence that HPV infections can become latent, with HPV genomes remaining in a population of basal epithelial cells, in which viral replication is controlled, it is speculated, by immune surveillance [37, 38]. Such latency requires biopsy rather than swabs or smears and is not easily detected even with biopsy. Further complexity comes from the variability in the sampling intervals and the length of follow-up, and the variability in assays used for HPV DNA detection and genotyping [39]. Studies detecting HPV infection with a specific type therefore reflect a complex picture: there may be new incident infections, reactivation of latent infections, or true persistent infections, and this is reflected in the patterns of infection [40, 41]. Thus, women may (i) be consistently HPV DNA-negative; (ii) be HPV DNA-positive and then consistently HPV DNA-negative; (iii) have fluctuating infections, HPV DNA-positive, -negative, and -positive – the infections usually clear in these women; or (iv) be consistently HPV DNA-positive for a specific HPV type – persistent infection. The consensus view is that the last category, persistent infection, constitutes the major risk for progression to high-grade CIN and, thus, to cervical cancer [33].

2.6 Persistent infection as a surrogate end-point for high-grade cervical disease

2.6.1 Persistent infection as a surrogate virological end-point

A central issue, however, is how to define a persistent infection. Probably the most commonly used definition is two or more positive HPV DNA tests 6 or 12 months apart. Various investigators have used duration of infection [42], the time to clearance [43], the proportion of HPV-positive visits, or any combination thereof [44–46]. These definitions have been further complicated by a lack of uniformity in the laboratory assays used for HPV DNA detection, in terms of sensitivity and specificity, of the detection of type-specific virus versus non-type-specific analysis, variation in the baseline HPV status, and clearance requirements.

2.6.2 Persistent infection as a predictor of high-grade disease

Two meta-analyses [47, 48] have attempted, by a systematic review of the literature, to detail the strength of the association between HPV persistence, high-grade CIN (CIN2/3), and invasive cervical cancer against this background of variation in study methodology and the HPV DNA detection assays. The primary aim of the meta-analysis and the review of the literature by Koshiol and colleagues [47] was to determine the strength of the association between persistent HPV DNA detection and high-grade precancer and invasive cancer of the cervix. In brief, the authors identified relevant studies via PubMed and other literature databases up to 30 January 2006. They identified 41 eligible studies covering > 22 000 women that analysed by different designs the association (relative risk [RR]) between HPV persistence and neoplasia. The authors abstracted RRs and 95% confidence limits (either as reported or as calculated by the authors) for the risk of cervical neoplasia (of low and high grade) among women with persistent HPV infections compared with other infection patterns, i.e. women who were consistently negative for the HPV types used to define persistence, women who had HPV infections that subsequently became undetectable, and women with mixed or fluctuating HPV infections. Cervical outcomes in this study included histologically confirmed invasive carcinoma, CIN2/3, cytologically determined high-grade squamous intraepithelial lesions (HSILs), low-grade squamous intraepithelial lesions, CIN1, atypical squamous cells of undetermined significance (ASCUS), or any combination thereof. Given the considerable variation in DNA detection methods, the lack of standardization in the diagnostic criteria for both histology and cytology, and the variability in the definition of persistence in these various studies, the consistency of the associations reported is important and impressive. In brief, the study findings were as follows. First, HPV persistence

was strongly and consistently associated with CIN2/3/HSIL, with variable RRs from 1.3 to 813.0. A total of 92% of RRs were > 3.0; the longer the duration of persistence (> 12 months) or the wider the testing interval (> 6 months or > 12 months), the stronger the associations. Second, the magnitude of the effect for HPV persistence in predicting CIN2/3/HSIL varied widely and was, to a large extent, dependent upon the referent group. Thus, as would be predicted, comparing persistent infections with the HPV-negative women gave the highest RRs since these HPV-negative women are at almost zero risk of developing high-grade disease within 5–10 years. A more recent study has shown that risk estimates for persistent infections in predicting progressive disease critically depend upon the referent group [49]. Third, the strength of the association between persistence and cervical neoplasia increased with increasing grade of intraepithelial disease.

In a second, more recent meta-analysis by some of these authors, the issue of what is a clinically relevant definition of HPV persistence has been addressed by a summary of the literature [48]. These authors performed a systematic review and meta-analysis to determine the influence of definitions of HPV persistence and study characteristics on estimated duration of infection and the proportion of women with persistently detectable HPV DNA over time. They reviewed and abstracted data from studies published up to 1 January 2010. Of the 4203 abstracts, 86 met the study inclusion criteria and reported non-duplicate results. Most studies were conducted in Europe (40%) and North America (29%), and the remainder were from Central or South America (20%), Asia (5%), Africa (3%), and Australia (1%). In more than half of the studies, women were aged 30 years or older. The majority of studies were in populations of women with normal cytology at baseline and were screening-based cohort studies using PCR-based detection methods. Definitions of persistence varied between studies, but the most common was two or more HPV DNA-positive samples taken 6–12 months apart (73%). Consecutive HPV-positive visits were usually required, but in 14% of studies intervening HPV-negative visits were permitted. The median duration for HPV detection of any HPV (both LR and HR types) was slightly less than 1 year in women with normal cytology. The proportion of women with infection persistent over 6 months varied across studies. Heterogeneity was largely a function of HPV type; the most persistent HR types in the α9 clade were HPV 16, 31, 33, and 52 and in the α7 clade were HPV 18, 39, 45, and 58. The least persistent types were HPV 35, 51, 66, and 68. The median duration of any HPV type was 10 months, with a median for HPV 16 of 12.4 months and for HPV 18 of 9.8 months. Very inconsistent results for the relationship of age to persistence were found, but the meta-analysis could not reliably compare persistence of prevalent versus incident infections.

The issue of age and incident/prevalent infection and persistence causes considerable confusion and should be considered in terms of the natural history of HPV infection. The peak acquisition of HPV occurs early in the years after the onset of sexual activity. HPV is endemic and easily transmitted [50]. The average age at the first sexual intercourse varies for different social and cultural mores but on average is in mid-to-late adolescence and early adulthood. Thus, in populations of young women, most infections are “new”, and in these age groups there will be little difference between incident and prevalent infections in terms of persistence and progression. In older women (aged > 30 years), persistent infections are more likely to be prevalent infection and progressive [51].

Other cohort studies have been published after 1 January 2010 [51–55], which, in essence, support the general conclusions from the meta-analyses described above. Kjaer and colleagues [56] described the results of a population-based cohort study in which 7679 cytologically normal women out of 8656 women from the general population in Denmark were examined twice, 2 years apart. At each examination the women underwent a gynaecological examination, cervical cytology, HPV detection by Hybrid Capture 2, and HPV type assignment by line probe assay. The women were then followed by the Danish

pathology databank for cervical neoplasia for up to 13.4 years. HPV 16, 18, 31, and 33 infection, but most specifically persistence of HPV 16 infection, was associated with high absolute risks of progression to CIN3. Thus, the estimated probability of developing CIN3 for those also positive at the second visit: with HPV 16 was 26.7% (95% confidence interval [CI], 21.1–31.8%), with HPV 18 was 19.1% (95% CI, 10.4–27.3%), with HPV 31 was 14.3% (95% CI, 9.1–19.4%), with HPV 33 was 14.9% (95% CI, 7.9–21.1%), and with HR types other than HPV 16, 18, 31, and 33 was 6.0% (95% CI, 3.8–8.3%). This study could not, because of its design, assess the duration of persistence but supports all previous studies in showing that persistence of oncogenic HPV types, particularly HPV 16, is associated with a high risk of progression to high-grade cervical lesions.

2.6.3 Evidence from randomized controlled trials of the licensed vaccines

The manufacturers of both licensed vaccines sponsored global clinical development programmes that included studies in which infection, both incident and persistent, alone or combined with a CIN2/3 disease end-point, was a primary end-point. In the CVT, the primary end-point was HPV 16/18 cervicovaginal infection that persisted for at least 12 months. Overall, the trials represent probably the largest systematic longitudinal collections of the natural history of HPV infection in women in the age group of 15–26 years by HPV type. End-of-study data from the manufacturer-sponsored trials for both vaccines and from the CVT are now available.

2.6.3.1 Cervarix bivalent HPV vaccine

In three manufacturer-sponsored phase II trials for the bHPV vaccine (trials 001, 007, and 023), the primary end-point was incident infection with HPV 16 or 18 and 12-month persistent infection. At trial entry, subjects were PCR-negative for the DNA of 14 HR HPV types and seronegative for HPV 16 and 18. Vaccine efficacy against persistent infection with HPV 16 and 18 was 100% for trial 007 at up to 7.3 years of follow-up [57] and for trial 023 (a subset of 007) was 100% at up to 8.4 years of follow-up [15]. In the pivotal RCT, 008 (PATRICIA), in the ATP cohort (i.e. women who received three doses of vaccine and were PCR-negative for 14 HR HPV types and seronegative for HPV 16 and 18 at enrolment and at month 6 after vaccination), vaccine efficacy against HPV 16/18-associated, HPV-positive CIN of grade 2 or worse (CIN2+) at the end of the study is shown in Table 2.1 [58]. Vaccine efficacy against the virological end-points 6-month and 12-month persistent infection with HPV 16/18 is shown Table 2.2. The correlation between persistent infection with HPV 16/18 and CIN2+ is very strong.

In the CVT, efficacy against the primary end-point of 12-month persistent HPV infection was 90.9% in the ATP cohort and 49% in the intention-to-treat cohort [59], comparable to efficacies seen in the PATRICIA trial [58].

Both licensed vaccines showed evidence of some efficacy against non-vaccine types, particularly HPV 31, 33, and 45 [12, 60, 61]. Analysis of efficacy of cross-protection against disease in these cases in the RCTs is complicated by multiple infections – situations in which more than one HPV type is detected in the diagnostic biopsy. The question then arises as to which of the HPV types detected is causal; this is particularly important when HPV 16 and/or 18 are co-infecting with other less oncogenic types since HPV 16 and 18 are more likely to be driving the neoplastic process. Virological end-points are more informative in such situations since the question is not which HPV caused the lesion but whether the vaccine has prevented infection with that specific HPV type in the vaccine group compared with the placebo [12].

Table 2.1. Vaccine efficacy against high-grade cervical lesions associated with HPV 16/18 (according-to-protocol [ATP] cohort) in the PATRICIA trial

HPV 16/18 end-point	ATP cohort[a]		
	End-of-study analysis[b]		
	Cervarix (*N* = 7338)	Control (*N* = 7305)	% Efficacy (95% CI)
	n[c]	*n*	
CIN2+	5	97	94.9% (87.7–98.4%)
CIN3+	2	24	91.7% (66.6–99.1%)

CI, confidence interval; CIN2+, cervical intraepithelial neoplasia of grade 2 or worse; HPV, human papillomavirus.

N = number of subjects included in each group

n = number of cases

[a] ATP cohort: includes women who received three doses of vaccine and were DNA-negative and seronegative at month 0 and DNA-negative at month 6 to the relevant HPV type (HPV 16 or 18).

[b] Mean follow-up of 40 months after dose 3.

[c] Including 4 cases of CIN2+ and 2 cases of CIN3+ in which another oncogenic HPV type was identified in the lesion concomitantly with HPV 16 or 18. These cases are excluded in the HPV type assignment analysis.

Source: Adapted with permission from: European Medicines Agency. Cervarix: EPAR - Production Information - Annex 1 Summary of Product Characteristics 2009; http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000721/WC500024632.pdf, March 2014.

Table 2.2. Vaccine efficacy against virological end-points associated with HPV 16/18 (according-to-protocol [ATP] cohort) in the PATRICIA trial

HPV 16/18 end-point	ATP cohort[a]		
	End-of-study analysis[b]		
	Cervarix (*N* = 7338)	Control (*N* = 7305)	% Efficacy (95% CI)
	n/*N*	*n*/*N*	
6-month persistent infection	35/7182	588/7137	94.3% (92.0–96.1%)
12-month persistent infection	26/7082	354/7038	92.9% (89.4–95.4%)

CI, confidence interval; CIN2+, cervical intraepithelial neoplasia of grade 2 or worse; HPV, human papillomavirus.

N = number of subjects included in each group

n = number of cases

[a] ATP cohort: includes women who received three doses of vaccine and were DNA-negative and seronegative at month 0 and DNA-negative at month 6 to the relevant HPV type (HPV 16 or 18).

[b] Mean follow-up of 40 months after dose 3.

Source: Adapted with permission from: European Medicines Agency. Cervarix: EPAR - Production Information - Annex 1 Summary of Product Characteristics 2009; http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000721/WC500024632.pdf, March 2014.

2.6.3.2 Gardasil quadrivalent HPV vaccine

In the global clinical development programme for the qHPV vaccine, a subset of studies 007, 012, and 019 had a composite end-point of persistent infection and disease (Table 2.3). In these studies, follow-up visits were scheduled every 6 months after completion of the immunization series. Swabs from several sites (cervical, vulval labial, perineal, and perianal) were taken for HPV DNA detection by a proprietary PCR-based assay.

Table 2.3. Studies of the quadrivalent HPV (qHPV) vaccine in women that assessed persistent infection

Protocol no. [ref.]	Study description	End-point assessed	qHPV *N*	Placebo *N*
007 [62]	Dose-ranging phase II study of qHPV versus placebo in young women aged 16–23 years	Persistent infection or disease related to HPV 6/11/16/18	276	275
012 [63]	Substudy of FUTURE I (phase III study) bridging to monovalent HPV 16 vaccine, in young women aged 16–26 years	Persistent infection related to HPV 16/18	1783	1788
019 [64]	Phase III study of qHPV versus placebo in women aged 24–45 years	Persistent infection or disease related to HPV 6/11/16/18	1910	1907
Total			3969	3970

HPV, human papillomavirus; no., number; ref., reference.

Source: Compiled from: Muñoz N, Manalastas R Jr, Pitisuttithum P, Tresukosol D, Monsonego J, Ault K *et al*. (2009). Safety, immunogenicity, and efficacy of quadrivalent human papillomavirus (types 6, 11, 16, 18) recombinant vaccine in women aged 24–45 years: a randomised, double-blind trial. *Lancet*. 373(9679):1949–57.

The definition of persistent infection in these studies was as follows. A subject was considered to have a case of HPV-related persistent infection if, after the day 1 visit, the subject could be classified into either of the following categories: (i) detected as HPV PCR-positive for the same vaccine HPV type in two or more consecutive swabs from the cervix, vagina, and external genital region or in biopsy samples obtained at least 6 months apart (within a ± 4 week window), or (ii) had a biopsy showing pathological evidence of HPV disease and was positive for HPV by PCR in an adjacent section from the same tissue block and was detected as PCR-positive for the same vaccine HPV type in the samples of the swabs or biopsy obtained immediately before or immediately after the biopsy showing HPV-associated disease.

Table 2.4 shows the number of cases of disease in each of the three studies analysed according to whether persistent infection with the same type had begun before the disease. Cases relating to both HPV 16 and 18 are counted, but infection and disease had to be related to the same HPV type.

A total of 39 individuals HPV-negative for HPV 16 and 18 at baseline developed HPV 16/18-related CIN2+. Of the 39, 38 had one HPV-positive swab before the diagnosis of disease. One subject with no prior HPV 16/18 positivity in swabs developed disease. The diagnostic biopsy was co-infected with HPV 16, 39, and 52. HPV 39 was detected in all previous swabs, but HPV 16 and 52 were detected only in the biopsy. This example reflects the problems relating to case assignment with multiple infection of the diagnostic biopsy.

Table 2.4. HPV 16/18-related CIN2 or worse by prior infection with the same HPV type (subjects naive to the relevant type receiving placebo)

Protocol no. [ref.]	Infection	No. of subjects	Disease	No disease
007 [62]	Yes	30	2	28
	No	221	0	221
	Total	251	2	249
012 [63]	Yes	254	29	225
	No	1407	0	1407
	Total	1661	29	1632
019 [64]	Yes	84	7	77
	No	1693	1	1692
	Total	1777	8	1769
Combined	Yes	368	38	330
	No	3321	1	3320
	Total	3689	39	3650

CIN2, cervical intraepithelial neoplasia of grade 2; HPV, human papillomavirus; no., number; ref., reference.

Source: Compiled from: Muñoz N, Manalastas R Jr, Pitisuttithum P, Tresukosol D, Monsonego J, Ault K *et al.* (2009). Safety, immunogenicity, and efficacy of quadrivalent human papillomavirus (types 6, 11, 16, 18) recombinant vaccine in women aged 24–45 years: a randomised, double-blind trial. *Lancet*. 373(9679):1949–57.

The data from the clinical trials establish a clear biological relationship between persistent infection with a HR HPV type and the subsequent development of CIN2+ in which that HPV type can be detected. However, virological end-points as surrogates compared with CIN3 are more distal to the true disease end-point of cervical cancer. Demonstrating a clear statistical (as well as a biological) relationship for persistent infection that satisfies the criteria for a surrogate statistical end-point [65] would reinforce the validity of persistent infection as an end-point.

2.6.4 Summary: persistent infection as a surrogate end-point for high-grade cervical disease

The studies reviewed above show that persistent infection with a HR HPV type is a prerequisite for the development of progressive cervical neoplastic disease. Both the biological and the statistical analyses in cross-sectional and cohort studies and the RCTs of the licensed vaccines strongly support the notion that persistent infection with a HR HPV type could be used to replace CIN2+ as a primary efficacy end-point in trials. Efficacy against 12-month persistent HPV 16/18 infection was the primary end-point in the CVT. However, regulatory authorities will be cautious about vaccine approval without robust evidence that efficacy against disease has been well established, and there are some issues that remain to be resolved.

The definition of persistent infection needs to be established and agreed upon. The cohort studies and the vaccine RCTs have shown that even short-term persistent infection for HPV 16 or 18 is predictive of disease. However, duration of infection is also a very important risk factor; the longer an HPV infection is detectable, the greater the risk of disease. It seems from the evidence that 6-month persistent infection as defined in the RCTs for both licensed vaccines, i.e. samples positive for the same HPV type on two or more consecutive visits at least 6 months apart, is de facto the accepted definition of persistent infection and is predictive of high-grade disease. There is robust evidence for this for HPV 16/18, but this

evidence is less strong for the other most prevalent carcinogenic types, HPV 31, 33, 35, 52, 58, and 45 [55, 56]. This is particularly important for HPV 45, which is the third most prevalent type in cervical carcinoma but a minor type for infection and CIN2+. The data from the large cohort studies [56, 66] show that persistent infection with types other than HPV 16/18 precedes precancerous lesions but that there is a slower progression to disease with these types – 12-month persistent infection may be a more robust predictor for these minor types. However, even if persistent infection is accepted as an end-point, regulatory authorities are conservative bodies and will most likely require that the clinical end-point of CIN2+ continue to be followed as a secondary end-point.

Very high vaccine efficacy against persistent infection with HPV 16 and 18 has been demonstrated in the pivotal RCTs for the licensed vaccines, and the correlation between persistent infection with HPV 16/18 and CIN2+ is very strong. Comparable efficacy against persistent infection with oncogenic types other than HPV 16 and 18 will have to be achieved in any trials with next-generation vaccines. If efficacy against persistent infection is moderate or low (≤ 70%), then it could be argued that those infections that are not prevented may be the ones that progress to cervical cancer.

Compared with histopathological end-points, virological end-points are more informative and robust in situations with multiple infections. In these cases, if histopathological end-points are used and more than one HPV type is detected in the diagnostic biopsy, the question arises as to which HPV type is causal. A virological end-point is unequivocal in such situations since either a persistent infection with a specific HPV type has been detected or it has not.

However, if a virological end-point is to be used as a primary end-point, the importance of internationally standardized, quality-controlled, and audited testing methodologies used for HPV detection and genotyping in trials cannot be overstated. Molecular testing and typing requires standardization of the sampling protocols and of the sites from which samples are taken in the anogenital tract, as well as standardization of the nucleic acid extraction protocols and of the amplification procedures. Since next-generation vaccines will target HPV types other than HPV 6, 11, 16, and 18, the sensitivity, specificity, and reproducibility of the detection and genotyping methods [67] will be crucial to comparability between trials.

2.7 Vaccine-induced immune responses

The current assumption is that antibody is the mechanism of protection afforded by HPV VLP vaccines. Rigorous evidence for this is based at present on preclinical studies in animals that showed that passive immunization of naive recipients with serum immunoglobulin purified from VLP immunized animals protected against high-dose viral challenge. Only intact VLPs could generate protective antibody, and these and other data provided evidence that conformational epitopes in L1 are required to generate neutralizing antibodies and that neutralizing antibody was required for protection (reviewed in [68]).

2.8 Monitoring vaccine-induced immunity – sero-assays

The measurement of specific immunoglobulin anti-L1 VLP antibodies is the parameter used to measure and monitor vaccine-induced immunity. The sero-assays used for the licensed vaccines differ. The qHPV vaccine used a high-throughput competitive Luminex immunoassay (cLIA). In this assay the VLPs are bound to microspheres and then incubated with a vaccine HPV type-specific neutralizing monoclonal antibody labelled with a dye, together with the serum sample [69, 70]. The assay measures only that one neutralizing antibody species that competes with the monoclonal antibody. The assay is therefore highly specific but relatively insensitive, since it measures only one of the several neutralizing antibodies that are generated and is an underestimate of the neutralizing antibody response

[71]. The bHPV vaccine used a conventional enzyme-linked immunosorbent assay (ELISA). This measures the polyclonal antibody response of both neutralizing and non-neutralizing species [72]. This assay is therefore highly sensitive but has a reduced specificity. A conventional neutralizing assay is not feasible for HPV since in vitro culture systems for the production of bulk amounts of infectious virus are not available. The most widely used neutralizing assay uses pseudovirions or surrogate virus particles. Pseudovirions are L1/L2 VLPs that have packaged a reporter plasmid that encodes an enzyme such as an alkaline phosphatase or a fluorescent protein. The expression of these after cell entry is a measure of how many pseudovirions have infected cells [73].

Careful studies have looked at the correlation between the data generated by each of these assays and have shown that the generation of neutralizing antibodies is highly correlated whichever assay is used [74]. However, as discussed above, the sensitivity and specificity of the assays differ. A key issue for future clinical studies using new-generation vaccines is the standardization and quality control of the protocols for the sero-assays, the reagents used in these assays, and the reporting of antibody concentrations using agreed International Units (IU).

2.9 Immunogenicity

In contrast to natural infections, in which the humoral immune response is slow and weak and only 50–70% of individuals seroconvert, systemic immunization with L1 VLP vaccines generates high serum antibody concentrations at least 50–1000 times those measured in natural infections [75] and virtually all vaccinees seroconvert [76–78]. After the three-dose immunization schedule, geometric mean titres (GMTs) for antibodies to the vaccine HPV types peak at month 7. GMTs then wane until month 18–24, when there is a plateau at about 10 times levels in natural infections [76, 78], which remains stable for at least 8–9 years after the primary immunization [14, 15]. This pattern of antibody response is consistent with the notion of the generation after the three-dose immunization schedule of a large population of antibody-secreting plasma cells with varying lifespans, some of which have the phenotype of long-lived plasma cells that migrate to the bone marrow and survive for life, maintaining a low but constant antibody production. Antigen challenge at 60 months after dose 1, with either vaccine, results in a rapid and robust anamnestic response with antibody concentrations rising within a week to levels greater than those achieved at peak (1 month after dose 3) in the initial immunization schedule, demonstrating the presence of reactive memory B cells [79, 80]. However, although the HPV VLP vaccines are highly efficacious and immunogenic, to date there is no immune correlate of protection against infection or disease. The minimum level of antibody needed for such protection and the role of B-cell memory if antibody wanes have yet to be established.

Experimental animal data using rodent cervicovaginal infection and challenge models may be informative. In these models, micro-wounds are induced at the cervical squamocolumnar junction in mice and macaques [81, 82]. The animals are then challenged vaginally with HPV pseudovirions, L1/L2 VLPs that have packaged a plasmid encoding a reporter molecule such as red fluorescent protein. Using sensitive longitudinal in vivo imaging technologies, the course of HPV pseudovirion infection and the effect of passive transfer of antibody to prevent infection can then be followed in living animals. Recent data using this model and passive immunization with sera from animals immunized with the commercially available vaccines show that very low concentrations of antibody are protective [83]. Such concentrations in vivo are up to 100-fold lower than those measured in vitro by the gold standard pseudovirion neutralizing sero-assay [84]. This suggests that very low levels of vaccine-generated antibody, below our capacity to measure at present, will be protective.

2.10 Immunogenicity and second-generation vaccines

The absence of an immune correlate raises challenges for second-generation vaccines, which it is assumed will include HPV 16 and 18 as immunogens. It is probable that the clinical trials evaluating such vaccines will be required, ethically, to use an active HPV vaccine comparator instead of a placebo control. In such a scenario the new vaccine will probably, as a regulatory requirement, need to show (i) seroconversion rates comparable to those of the current licensed products, and (ii) GMTs in young women that meet the statistical criterion for non-inferiority, i.e. that at the lower level of the confidence interval for the fold difference in GMTs between groups exclude a decrease of 2-fold or more for each HPV type.

This could be an issue for multivalent L1 VLP vaccines, since there is the potential for immune interference by the additional new L1 VLPs reducing the antibody response to HPV 16 and 18 compared with the comparator vaccine, and a problem for capsomer-based vaccines, since the immunogenicity of these is usually less than that of VLP vaccines [85]. Furthermore, because the prophylactic efficacy of both the current vaccines approaches 100% for both disease and infection and there have been no, or very few, cases of breakthrough infection, it has not been possible to define the minimum level of antibody that correlates with long-term protection against HPV infection and, increasingly, it looks unlikely that an immune correlate that is easily measurable will be defined. It is very unlikely, therefore, that the demonstration of immunogenicity alone for any new vaccine will be sufficient for regulatory authorities, and non-inferiority for persistent infection and/or CIN2+ will be required.

References

1. Bernard HU, Burk RD, Chen Z, van Doorslaer K, zur Hausen H, de Villiers EM (2010). Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. *Virology*. 401(1):70–9. http://dx.doi.org/10.1016/j.virol.2010.02.002 PMID:20206957
2. Lacey CJ, Lowndes CM, Shah KV (2006). Chapter 4: Burden and management of non-cancerous HPV-related conditions: HPV-6/11 disease. *Vaccine*. 24 Suppl 3:35–41. http://dx.doi.org/10.1016/j.vaccine.2006.06.015 PMID:16950016
3. Schiffman M, Clifford G, Buonaguro FM (2009). Classification of weakly carcinogenic human papillomavirus types: addressing the limits of epidemiology at the borderline. *Infect Agent Cancer*. 4(1):8. http://dx.doi.org/10.1186/1750-9378-4-8 PMID:19486508
4. de Sanjose S, Quint WG, Alemany L, Geraets DT, Klaustermeier JE, Lloveras B *et al.*; Retrospective International Survey and HPV Time Trends Study Group (2010). Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. *Lancet Oncol*. 11(11):1048–56. http://dx.doi.org/10.1016/S1470-2045(10)70230-8 PMID:20952254
5. Arbyn M, Castellsagué X, de Sanjosé S, Bruni L, Saraiya M, Bray F *et al.* (2011). Worldwide burden of cervical cancer in 2008. *Ann Oncol*. 22(12):2675–86. http://dx.doi.org/10.1093/annonc/mdr015 PMID:21471563
6. de Martel C, Ferlay J, Franceschi S, Vignat J, Bray F, Forman D *et al.* (2012). Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. *Lancet Oncol*. 13(6):607–15. http://dx.doi.org/10.1016/S1470-2045(12)70137-7 PMID:22575588
7. Kjaer SK, Tran TN, Sparen P, Tryggvadottir L, Munk C, Dasbach E *et al.* (2007). The burden of genital warts: a study of nearly 70,000 women from the general female population in the 4 Nordic countries. *J Infect Dis*. 196(10):1447–54. http://dx.doi.org/10.1086/522863 PMID:18008222

8. Stanley M, Lowy DR, Frazer I (2006). Chapter 12: Prophylactic HPV vaccines: underlying mechanisms. *Vaccine*. 24 Suppl 3:106–13. http://dx.doi.org/10.1016/j.vaccine.2006.05.110 PMID:16949996
9. Garland SM, Hernandez-Avila M, Wheeler CM, Perez G, Harper DM, Leodolter S *et al.*; Females United to Unilaterally Reduce Endo/Ectocervical Disease (FUTURE) I Investigators (2007). Quadrivalent vaccine against human papillomavirus to prevent anogenital diseases. *N Engl J Med*. 356(19):1928–43. http://dx.doi.org/10.1056/NEJMoa061760 PMID:17494926
10. FUTURE II Study Group (2007). Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. *N Engl J Med*. 356(19):1915–27. http://dx.doi.org/10.1056/NEJMoa061741 PMID:17494925
11. Lehtinen M, Paavonen J, Wheeler CM, Jaisamrarn U, Garland SM, Castellsagué X *et al.*; HPV PATRICIA Study Group (2012). Overall efficacy of HPV-16/18 AS04-adjuvanted vaccine against grade 3 or greater cervical intraepithelial neoplasia: 4-year end-of-study analysis of the randomised, double-blind PATRICIA trial. *Lancet Oncol*. 13(1):89–99. http://dx.doi.org/10.1016/S1470-2045(11)70286-8 PMID:22075171
12. Herrero R, Wacholder S, Rodríguez AC, Solomon D, González P, Kreimer AR *et al.*; Costa Rica Vaccine Trial Group (2011). Prevention of persistent human papillomavirus infection by an HPV16/18 vaccine: a community-based randomized clinical trial in Guanacaste, Costa Rica. *Cancer Discov*. 1(5):408–19. http://dx.doi.org/10.1158/2159-8290.CD-11-0131 PMID:22586631
13. Schiller JT, Castellsagué X, Garland SM (2012). A review of clinical trials of human papillomavirus prophylactic vaccines. *Vaccine*. 30 Suppl 5:F123–38. http://dx.doi.org/10.1016/j.vaccine.2012.04.108 PMID:23199956
14. Rowhani-Rahbar A, Mao C, Hughes JP, Alvarez FB, Bryan JT, Hawes SE *et al.* (2009). Longer term efficacy of a prophylactic monovalent human papillomavirus type 16 vaccine. *Vaccine*. 27(41):5612–9. http://dx.doi.org/10.1016/j.vaccine.2009.07.027 PMID:19647066
15. Roteli-Martins CM, Naud P, De Borba P, Teixeira JC, De Carvalho NS, Zahaf T *et al.* (2012). Sustained immunogenicity and efficacy of the HPV-16/18 AS04-adjuvanted vaccine: up to 8.4 years of follow-up. *Hum Vaccin Immunother*. 8(3):390–7. http://dx.doi.org/10.4161/hv.18865 PMID:22327492
16. Fraser C, Tomassini JE, Xi L, Golm G, Watson M, Giuliano AR *et al.* (2007). Modeling the long-term antibody response of a human papillomavirus (HPV) virus-like particle (VLP) type 16 prophylactic vaccine. *Vaccine*. 25(21):4324–33. http://dx.doi.org/10.1016/j.vaccine.2007.02.069 PMID:17445955
17. David MP, Van Herck K, Hardt K, Tibaldi F, Dubin G, Descamps D *et al.* (2009). Long-term persistence of anti-HPV-16 and -18 antibodies induced by vaccination with the AS04-adjuvanted cervical cancer vaccine: modeling of sustained antibody responses. *Gynecol Oncol*. 115(3) Suppl:S1–6. http://dx.doi.org/10.1016/j.ygyno.2009.01.011 PMID:19217149
18. Romanowski B, de Borba PC, Naud PS, Roteli-Martins CM, De Carvalho NS, Teixeira JC *et al.*; GlaxoSmithKline Vaccine HPV-007 Study Group (2009). Sustained efficacy and immunogenicity of the human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine: analysis of a randomised placebo-controlled trial up to 6.4 years. *Lancet*. 374(9706):1975–85. http://dx.doi.org/10.1016/S0140-6736(09)61567-1 PMID:19962185
19. Peres J (2011). For cancers caused by HPV, two vaccines were just the beginning. *J Natl Cancer Inst*. 103(5):360–2. http://dx.doi.org/10.1093/jnci/djr053 PMID:21357595
20. Joura E on behalf of the V503-001 study team (2013). Efficacy and immunogenicity of a novel 9-valent HPV L1 virus-like particle vaccine in 16- to 26-year-old women. EUROGIN Congress 2013, Florence, Italy. Abstract SS 8-4, p. 102.
21. Chen XS, Casini G, Harrison SC, Garcea RL (2001). Papillomavirus capsid protein expression in *Escherichia coli*: purification and assembly of HPV11 and HPV16 L1. *J Mol Biol*. 307(1):173–82. http://dx.doi.org/10.1006/jmbi.2000.4464 PMID:11243812

22. Yuan H, Estes PA, Chen Y, Newsome J, Olcese VA, Garcea RL *et al.* (2001). Immunization with a pentameric L1 fusion protein protects against papillomavirus infection. *J Virol*. 75(17):7848–53. http://dx.doi.org/10.1128/JVI.75.17.7848-7853.2001 PMID:11483728
23. Bazan SB, de Alencar Muniz Chaves A, Aires KA, Cianciarullo AM, Garcea RL, Ho PL (2009). Expression and characterization of HPV-16 L1 capsid protein in *Pichia pastoris*. *Arch Virol*. 154(10):1609–17. http://dx.doi.org/10.1007/s00705-009-0484-8 PMID:19756360
24. Rybicki EP, Williamson AL, Meyers A, Hitzeroth II (2011). Vaccine farming in Cape Town. *Hum Vaccin*. 7(3):339–48. http://dx.doi.org/10.4161/hv.7.3.14263 PMID:21358269
25. Cantarella G, Liniger M, Zuniga A, Schiller JT, Billeter M, Naim HY *et al.* (2009). Recombinant measles virus-HPV vaccine candidates for prevention of cervical carcinoma. *Vaccine*. 27(25-26):3385–90. http://dx.doi.org/10.1016/j.vaccine.2009.01.061 PMID:19200837
26. Kuck D, Lau T, Leuchs B, Kern A, Müller M, Gissmann L *et al.* (2006). Intranasal vaccination with recombinant adeno-associated virus type 5 against human papillomavirus type 16 L1. *J Virol*. 80(6):2621–30. http://dx.doi.org/10.1128/JVI.80.6.2621-2630.2006 PMID:16501072
27. Fraillery D, Baud D, Pang SY, Schiller J, Bobst M, Zosso N *et al.* (2007). *Salmonella enterica* serovar Typhi Ty21a expressing human papillomavirus type 16 L1 as a potential live vaccine against cervical cancer and typhoid fever. *Clin Vaccine Immunol*. 14(10):1285–95. http://dx.doi.org/10.1128/CVI.00164-07 PMID:17687110
28. Karanam B, Jagu S, Huh WK, Roden RB (2009). Developing vaccines against minor capsid antigen L2 to prevent papillomavirus infection. *Immunol Cell Biol*. 87(4):287–99. http://dx.doi.org/10.1038/icb.2009.13 PMID:19421199
29. Pagliusi SR, Teresa Aguado M (2004). Efficacy and other milestones for human papillomavirus vaccine introduction. *Vaccine*. 23(5):569–78. http://dx.doi.org/10.1016/j.vaccine.2004.07.046 PMID:15630792
30. Moscicki AB, Schiffman M, Burchell A, Albero G, Giuliano AR, Goodman MT *et al.* (2012). Updating the natural history of human papillomavirus and anogenital cancers. *Vaccine*. 30 Suppl 5:F24–33. http://dx.doi.org/10.1016/j.vaccine.2012.05.089 PMID:23199964
31. McCredie MR, Sharples KJ, Paul C, Baranyai J, Medley G, Jones RW *et al.* (2008). Natural history of cervical neoplasia and risk of invasive cancer in women with cervical intraepithelial neoplasia 3: a retrospective cohort study. *Lancet Oncol*. 9(5):425–34. http://dx.doi.org/10.1016/S1470-2045(08)70103-7 PMID:18407790
32. Moscicki AB, Ma Y, Wibbelsman C, Darragh TM, Powers A, Farhat S *et al.* (2010). Rate of and risks for regression of cervical intraepithelial neoplasia 2 in adolescents and young women. *Obstet Gynecol*. 116(6):1373–80. http://dx.doi.org/10.1097/AOG.0b013e3181fe777f PMID:21099605
33. Insinga RP, Dasbach EJ, Elbasha EH (2009). Epidemiologic natural history and clinical management of human papillomavirus (HPV) disease: a critical and systematic review of the literature in the development of an HPV dynamic transmission model. *BMC Infect Dis*. 9(1):119. http://dx.doi.org/10.1186/1471-2334-9-119 PMID:19640281
34. Muñoz N, Castellsagué X, de González AB, Gissmann L (2006). Chapter 1: HPV in the etiology of human cancer. *Vaccine*. 24 Suppl 3:1–10. http://dx.doi.org/10.1016/j.vaccine.2006.05.115 PMID:16949995
35. de Sanjosé S, Diaz M, Castellsagué X, Clifford G, Bruni L, Muñoz N *et al.* (2007). Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. *Lancet Infect Dis*. 7(7):453–9. http://dx.doi.org/10.1016/S1473-3099(07)70158-5 PMID:17597569
36. Rodríguez AC, Schiffman M, Herrero R, Wacholder S, Hildesheim A, Castle PE *et al.*; Proyecto Epidemiológico Guanacaste Group (2008). Rapid clearance of human papillomavirus and implications for clinical focus on persistent infections. *J Natl Cancer Inst*. 100(7):513–7. http://dx.doi.org/10.1093/jnci/djn044 PMID:18364507

37. Doorbar J, Quint W, Banks L, Bravo IG, Stoler M, Broker TR *et al.* (2012). The biology and life-cycle of human papillomaviruses. *Vaccine*. 30 Suppl 5:F55–70. http://dx.doi.org/10.1016/j.vaccine.2012.06.083 PMID:23199966
38. Abramson AL, Nouri M, Mullooly V, Fisch G, Steinberg BM (2004). Latent human papillomavirus infection is comparable in the larynx and trachea. *J Med Virol*. 72(3):473–7. http://dx.doi.org/10.1002/jmv.20013 PMID:14748072
39. Poljak M, Cuzick J, Kocjan BJ, Iftner T, Dillner J, Arbyn M (2012). Nucleic acid tests for the detection of alpha human papillomaviruses. *Vaccine*. 30 Suppl 5:F100–6. http://dx.doi.org/10.1016/j.vaccine.2012.04.105 PMID:23199952
40. Moscicki AB, Shiboski S, Broering J, Powell K, Clayton L, Jay N *et al.* (1998). The natural history of human papillomavirus infection as measured by repeated DNA testing in adolescent and young women. *J Pediatr*. 132(2):277–84. http://dx.doi.org/10.1016/S0022-3476(98)70445-7 PMID:9506641
41. Syrjänen K (2011). Persistent high-risk human papillomavirus (HPV) infections as surrogate endpoints of progressive cervical disease. Potential new endpoint for efficacy studies with new-generation (non-HPV 16/18) prophylactic HPV vaccines. *Eur J Gynaecol Oncol*. 32(1):17–33. PMID:21446320
42. Muñoz N, Hernandez-Suarez G, Méndez F, Molano M, Posso H, Moreno V *et al.*; Instituto Nacional de Cancerología HPV Study Group (2009). Persistence of HPV infection and risk of high-grade cervical intraepithelial neoplasia in a cohort of Colombian women. *Br J Cancer*. 100(7):1184–90. http://dx.doi.org/10.1038/sj.bjc.6604972 PMID:19293802
43. Moscicki AB, Ellenberg JH, Farhat S, Xu J (2004). Persistence of human papillomavirus infection in HIV-infected and -uninfected adolescent girls: risk factors and differences, by phylogenetic type. *J Infect Dis*. 190(1):37–45. http://dx.doi.org/10.1086/421467 PMID:15195241
44. Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD (1998). Natural history of cervicovaginal papillomavirus infection in young women. *N Engl J Med*. 338(7):423–8. http://dx.doi.org/10.1056/NEJM199802123380703 PMID:9459645
45. Wallin KL, Wiklund F, Angström T, Bergman F, Stendahl U, Wadell G *et al.* (1999). Type-specific persistence of human papillomavirus DNA before the development of invasive cervical cancer. *N Engl J Med*. 341(22):1633–8. http://dx.doi.org/10.1056/NEJM199911253412201 PMID:10572150
46. Schlecht NF, Kulaga S, Robitaille J, Ferreira S, Santos M, Miyamura RA *et al.* (2001). Persistent human papillomavirus infection as a predictor of cervical intraepithelial neoplasia. *JAMA*. 286(24):3106–14. http://dx.doi.org/10.1001/jama.286.24.3106 PMID:11754676
47. Koshiol J, Lindsay L, Pimenta JM, Poole C, Jenkins D, Smith JS (2008). Persistent human papillomavirus infection and cervical neoplasia: a systematic review and meta-analysis. *Am J Epidemiol*. 168(2):123–37. http://dx.doi.org/10.1093/aje/kwn036 PMID:18483125
48. Rositch AF, Koshiol J, Hudgens M, Razzaghi H, Backes DM, Pimenta JM *et al.* (2012). Patterns of persistent genital human papillomavirus infection among women worldwide: a literature review and meta-analysis. *Int J Cancer.* 133(6):1271–85. PMID:22961444
49. Syrjänen K, Shabalova I, Naud P, Kozachenko V, Derchain S, Zakharchenko S *et al.*; NIS and LAMS Study Research Groups (2011). Risk estimates for persistent high-risk human papillomavirus infections as surrogate endpoints of progressive cervical disease critically depend on reference category: analysis of the combined prospective cohort of the New Independent States of the Former Soviet Union and Latin American Screening studies. *Int J STD AIDS*. 22(6):315–23. http://dx.doi.org/10.1258/ijsa.2009.009365 PMID:21680666
50. Brown DR, Shew ML, Qadadri B, Neptune N, Vargas M, Tu W *et al.* (2005). A longitudinal study of genital human papillomavirus infection in a cohort of closely followed adolescent women. *J Infect Dis*. 191(2):182–92. http://dx.doi.org/10.1086/426867 PMID:15609227

51. Rodríguez AC, Schiffman M, Herrero R, Hildesheim A, Bratti C, Sherman ME *et al.* (2010). Longitudinal study of human papillomavirus persistence and cervical intraepithelial neoplasia grade 2/3: critical role of duration of infection. *J Natl Cancer Inst*. 102(5):315–24. http://dx.doi.org/10.1093/jnci/djq001 PMID:20157096
52. Schiffman M, Rodriguez AC, Chen Z, Wacholder S, Herrero R, Hildesheim A *et al.* (2010). A population-based prospective study of carcinogenic human papillomavirus variant lineages, viral persistence, and cervical neoplasia. *Cancer Res*. 70(8):3159–69. http://dx.doi.org/10.1158/0008-5472.CAN-09-4179 PMID:20354192
53. Nielsen A, Kjaer SK, Munk C, Osler M, Iftner T (2010). Persistence of high-risk human papillomavirus infection in a population-based cohort of Danish women. *J Med Virol*. 82(4):616–23. http://dx.doi.org/10.1002/jmv.21750 PMID:20166190
54. Schmeink CE, Melchers WJ, Siebers AG, Quint WG, Massuger LF, Bekkers RL (2011). Human papillomavirus persistence in young unscreened women, a prospective cohort study. *PLoS One*. 6(11):e27937. http://dx.doi.org/10.1371/journal.pone.0027937 PMID:22132173
55. Chen HC, Schiffman M, Lin CY, Pan MH, You SL, Chuang LC *et al.*; CBCSP-HPV Study Group (2011). Persistence of type-specific human papillomavirus infection and increased long-term risk of cervical cancer. *J Natl Cancer Inst*. 103(18):1387–96. http://dx.doi.org/10.1093/jnci/djr283 PMID:21900119
56. Kjaer SK, Frederiksen K, Munk C, Iftner T (2010). Long-term absolute risk of cervical intraepithelial neoplasia grade 3 or worse following human papillomavirus infection: role of persistence. *J Natl Cancer Inst*. 102(19):1478–88. http://dx.doi.org/10.1093/jnci/djq356 PMID:20841605
57. De Carvalho N, Teixeira J, Roteli-Martins CM, Naud P, De Borba P, Zahaf T *et al.* (2010). Sustained efficacy and immunogenicity of the HPV-16/18 AS04-adjuvanted vaccine up to 7.3 years in young adult women. *Vaccine*. 28(38):6247–55. http://dx.doi.org/10.1016/j.vaccine.2010.07.007 PMID:20643092
58. European Medicines Agency (2011). Cervarix: EPAR - Product Information. Available at http://www.ema.europa.eu/ema/index.jsp?curl=pages/includes/document/document_detail.jsp?webContentId=WC500024632&mid=WC0b01ac058009a3dc.
59. Herrero R, Wacholder S, Rodríguez AC, Solomon D, González P, Kreimer AR *et al.*; Costa Rica Vaccine Trial Group (2011). Prevention of persistent human papillomavirus infection by an HPV16/18 vaccine: a community-based randomized clinical trial in Guanacaste, Costa Rica. *Cancer Discov*. 1(5):408–19. http://dx.doi.org/10.1158/2159-8290.CD-11-0131 PMID:22586631
60. Brown DR, Kjaer SK, Sigurdsson K, Iversen OE, Hernandez-Avila M, Wheeler CM *et al.* (2009). The impact of quadrivalent human papillomavirus (HPV; types 6, 11, 16, and 18) L1 virus-like particle vaccine on infection and disease due to oncogenic nonvaccine HPV types in generally HPV-naive women aged 16–26 years. *J Infect Dis*. 199(7):926–35. http://dx.doi.org/10.1086/597307 PMID:19236279
61. Wheeler CM, Castellsagué X, Garland SM, Szarewski A, Paavonen J, Naud P *et al.*; HPV PATRICIA Study Group (2012). Cross-protective efficacy of HPV-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by non-vaccine oncogenic HPV types: 4-year end-of-study analysis of the randomised, double-blind PATRICIA trial. *Lancet Oncol*. 13(1):100–10. http://dx.doi.org/10.1016/S1470-2045(11)70287-X PMID:22075170
62. Villa LL, Costa RL, Petta CA, Andrade RP, Paavonen J, Iversen OE *et al.* (2006). High sustained efficacy of a prophylactic quadrivalent human papillomavirus types 6/11/16/18 L1 virus-like particle vaccine through 5 years of follow-up. *Br J Cancer*. 95(11):1459–66. http://dx.doi.org/10.1038/sj.bjc.6603469 PMID:17117182
63. Insinga RP, Perez G, Wheeler CM, Koutsky LA, Garland SM, Leodolter S *et al.*; FUTURE I Investigators (2010). Incidence, duration, and reappearance of type-specific cervical human papillomavirus infections in young women. *Cancer Epidemiol Biomarkers Prev*. 19(6):1585–94. http://dx.doi.org/10.1158/1055-9965.EPI-09-1235 PMID:20530494

64. Muñoz N, Manalastas R Jr, Pitisuttithum P, Tresukosol D, Monsonego J, Ault K *et al.* (2009). Safety, immunogenicity, and efficacy of quadrivalent human papillomavirus (types 6, 11, 16, 18) recombinant vaccine in women aged 24–45 years: a randomised, double-blind trial. *Lancet*. 373(9679):1949–57. http://dx.doi.org/10.1016/S0140-6736(09)60691-7 PMID:19493565
65. Prentice RL (1989). Surrogate endpoints in clinical trials: definition and operational criteria. *Stat Med*. 8(4):431–40. http://dx.doi.org/10.1002/sim.4780080407 PMID:2727467
66. Khan MJ, Castle PE, Lorincz AT, Wacholder S, Sherman M, Scott DR *et al.* (2005). The elevated 10-year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical practice. *J Natl Cancer Inst*. 97(14):1072–9. http://dx.doi.org/10.1093/jnci/dji187 PMID:16030305
67. Vaccarella S, Franceschi S, Snijders PJ, Herrero R, Meijer CJ, Plummer M; IARC HPV Prevalence Surveys Study Group (2010). Concurrent infection with multiple human papillomavirus types: pooled analysis of the IARC HPV Prevalence Surveys. *Cancer Epidemiol Biomarkers Prev*. 19(2):503–10. http://dx.doi.org/10.1158/1055-9965.EPI-09-0983 PMID:20142247
68. Stanley M, Pinto LA, Trimble C (2012). Human papillomavirus vaccines – immune responses. *Vaccine*. 30 Suppl 5:F83–7. http://dx.doi.org/10.1016/j.vaccine.2012.04.106 PMID:23199968
69. Opalka D, Lachman CE, MacMullen SA, Jansen KU, Smith JF, Chirmule N *et al.* (2003). Simultaneous quantitation of antibodies to neutralizing epitopes on virus-like particles for human papillomavirus types 6, 11, 16, and 18 by a multiplexed Luminex assay. *Clin Diagn Lab Immunol*. 10(1):108–15. http://dx.doi.org/10.1128/CDLI.10.1.108-115.2003 PMID:12522048
70. Dias D, Van Doren J, Schlottmann S, Kelly S, Puchalski D, Ruiz W *et al.* (2005). Optimization and validation of a multiplexed Luminex assay to quantify antibodies to neutralizing epitopes on human papillomaviruses 6, 11, 16, and 18. *Clin Diagn Lab Immunol*. 12(8):959–69. http://dx.doi.org/10.1128/CDLI.12.8.959-969.2005 PMID:16085914
71. Brown DR, Garland SM, Ferris DG, Joura E, Steben M, James M *et al.* (2011). The humoral response to Gardasil over four years as defined by total IgG and competitive Luminex immunoassay. *Hum Vaccin*. 7(2):230–8. http://dx.doi.org/10.4161/hv.7.2.13948 PMID:21307649
72. Kemp TJ, García-Piñeres A, Falk RT, Poncelet S, Dessy F, Giannini SL *et al.*; Costa Rica Vaccine Trial (CVT) Group (2008). Evaluation of systemic and mucosal anti-HPV16 and anti-HPV18 antibody responses from vaccinated women. *Vaccine*. 26(29–30):3608–16. http://dx.doi.org/10.1016/j.vaccine.2008.04.074 PMID:18541349
73. Buck CB, Pastrana DV, Lowy DR, Schiller JT (2005). Generation of HPV pseudovirions using transfection and their use in neutralization assays. *Methods Mol Med*. 119:445–62. PMID:16350417
74. Dessy FJ, Giannini SL, Bougelet CA, Kemp TJ, David MP, Poncelet SM *et al.* (2008). Correlation between direct ELISA, single epitope-based inhibition ELISA and pseudovirion-based neutralization assay for measuring anti-HPV-16 and anti-HPV-18 antibody response after vaccination with the AS04-adjuvanted HPV-16/18 cervical cancer vaccine. *Hum Vaccin*. 4(6):425–34. http://dx.doi.org/10.4161/hv.4.6.6912 PMID:18948732
75. Harro CD, Pang YY, Roden RB, Hildesheim A, Wang Z, Reynolds MJ *et al.* (2001). Safety and immunogenicity trial in adult volunteers of a human papillomavirus 16 L1 virus-like particle vaccine. *J Natl Cancer Inst*. 93(4):284–92. http://dx.doi.org/10.1093/jnci/93.4.284 PMID:11181775
76. Villa LL, Ault KA, Giuliano AR, Costa RL, Petta CA, Andrade RP *et al.* (2006). Immunologic responses following administration of a vaccine targeting human

papillomavirus types 6, 11, 16, and 18. *Vaccine*. 24(27–28):5571–83. http://dx.doi.org/10.1016/j.vaccine.2006.04.068 PMID:16753240
77. Harper DM, Franco EL, Wheeler C, Ferris DG, Jenkins D, Schuind A *et al.*; GlaxoSmithKline HPV Vaccine Study Group (2004). Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial. *Lancet*. 364(9447):1757–65. http://dx.doi.org/10.1016/S0140-6736(04)17398-4 PMID:15541448
78. Harper DM, Franco EL, Wheeler CM, Moscicki AB, Romanowski B, Roteli-Martins CM *et al.*; HPV Vaccine Study Group (2006). Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomised control trial. *Lancet*. 367(9518):1247–55. http://dx.doi.org/10.1016/S0140-6736(06)68439-0 PMID:16631880
79. Olsson SE, Villa LL, Costa RL, Petta CA, Andrade RP, Malm C *et al.* (2007). Induction of immune memory following administration of a prophylactic quadrivalent human papillomavirus (HPV) types 6/11/16/18 L1 virus-like particle (VLP) vaccine. *Vaccine*. 25(26):4931–9. http://dx.doi.org/10.1016/j.vaccine.2007.03.049 PMID:17499406
80. Moscicki AB, Wheeler CM, Romanowski B, Hedrick J, Gall S, Ferris D *et al.* (2012). Immune responses elicited by a fourth dose of the HPV-16/18 AS04-adjuvanted vaccine in previously vaccinated adult women. *Vaccine*. 31(1):234–41. http://dx.doi.org/10.1016/j.vaccine.2012.09.037 PMID:23063422
81. Roberts JN, Buck CB, Thompson CD, Kines R, Bernardo M, Choyke PL *et al.* (2007). Genital transmission of HPV in a mouse model is potentiated by nonoxynol-9 and inhibited by carrageenan. *Nat Med*. 13(7):857–61. http://dx.doi.org/10.1038/nm1598 PMID:17603495
82. Roberts JN, Kines RC, Katki HA, Lowy DR, Schiller JT (2011). Effect of Pap smear collection and carrageenan on cervicovaginal human papillomavirus-16 infection in a rhesus macaque model. *J Natl Cancer Inst*. 103(9):737–43. http://dx.doi.org/10.1093/jnci/djr061 PMID:21483020
83. Day PM, Kines RC, Thompson CD, Jagu S, Roden RB, Lowy DR *et al.* (2010). In vivo mechanisms of vaccine-induced protection against HPV infection. *Cell Host Microbe*. 8(3):260–70. http://dx.doi.org/10.1016/j.chom.2010.08.003 PMID:20833377
84. Longet S, Schiller JT, Bobst M, Jichlinski P, Nardelli-Haefliger D (2011). A murine genital-challenge model is a sensitive measure of protective antibodies against human papillomavirus infection. *J Virol*. 85(24):13253–9. http://dx.doi.org/10.1128/JVI.06093-11 PMID:21976653
85. Schadlich L, Senger T, Gerlach B, Mucke N, Klein C, Bravo IG *et al.* (2009). Analysis of modified HPV 16 L1 capsomeres: the ability to assemble into larger particles correlates with higher immunogenicity. *J Virol*. 83(15):7690–705. http://dx.doi.org/10.1128/JVI.02588-08 PMID:19457985

Chapter 3. Methodological issues for trials of vaccine efficacy against HPV types 16 and 18

Eduardo Franco, Mark Schiffman, Sholom Wacholder, and Margaret Stanley

3.1 Introduction

A key topic in cancer epidemiology is the validation of end-points for use in prevention, screening, and treatment trials. The relevant background literature has been summarized in Chapter 2. Essential to the points discussed in the present chapter is the accrued knowledge on the efficacy of first- and second-generation virus-like particle (VLP) vaccines and the natural history of human papillomavirus (HPV) infection and cervical cancer. Discussions about the advantages and disadvantages of lesion and virological end-points for future trials and about markers of immune response as possible end-points in trials are also found in Chapter 2 as well as in other chapters of this Report.

Specifically for the present chapter, we discuss the natural history and statistical basis for using virological outcomes in trials of new vaccines, whether based on VLPs constructed with the HPV L1 capsid protein only or both L1 and L2, as well as future vaccines based on L1 capsomers. The key premise in our line of reasoning is the expectation that successive candidate HPV vaccine formulations will increase the range of protection against multiple carcinogenic HPV types while increasing the vaccine efficacy (VE) or maintaining the same VE of previous-generation vaccines.

Although our goal is to cover cervical disease only, we recognize that decisions concerning trial design in relation to end-points for other anatomical sites will be different. Most of the knowledge concerning validity of end-points applies to the natural history of HPV infection in cervical cancer. We know relatively far less for other anatomical sites, such as the anus, vagina, vulva, penis, and oral cavity and pharynx.

As this chapter is intended to cover pragmatic questions to assist policy-makers and regulatory agencies, it provides only a limited number of references for the arguments advanced herein. Readers will find more detailed background information with comprehensive bibliographies elsewhere in this Report.

3.2 Validated and non-validated end-points

One of the most discussed topics in cancer epidemiology is the validation of end-points for use in prevention, screening, and treatment trials. We use the term “validation” to denote the acceptance of a particular end-point by a regulatory agency, such as the United States Food and Drug Administration (FDA) or the European Medicines Agency (EMA), as part of the pre-specified outcomes in a randomized controlled trial (RCT) proposed as primary objectives. The burden of proof that the proposed end-point is a validated one is typically left to the applicant (e.g. a pharmaceutical company) to the regulatory agency. In the specific case of HPV vaccination, regulatory agencies have indicated a priori that they will accept claims related to cervical cancer prevention only if based on results of RCTs with high-grade cervical intraepithelial neoplasia (CIN), i.e. CIN of grade 2 or 3 (CIN2/3), or worse lesions (cancer). Compared with earlier steps in the causal pathway based on infection, CIN2/3 is a “downstream” end-point in the natural history of cervical cancer. CIN3 is in fact considered an unequivocal precancerous state.

There are three advantages in using biological intermediates, all of which are of a pragmatic nature: (i) intermediate end-points are more common, which permits trials to be smaller while attaining adequate statistical power, (ii) they occur earlier, i.e. they are more “upstream” in

the natural history of cancer, which permits trials to be shorter in duration, and (iii) they do not require censoring because of treatment that is clinically indicated if a CIN2/3 lesion is present. To be suitable, intermediate end-points must fulfil statistical and biological criteria. Biological criteria relate to the plausibility that an intermediate end-point is truly a causal one and not a proxy or correlate of a true biological intermediate. For instance, serum prostate-specific antigen (PSA) levels are strongly predictive of whether a man has prostate cancer or will be diagnosed with prostate cancer in the future. An intervention that succeeded in preventing prostate cancer from occurring would also prevent PSA levels from rising. However, the converse is not true. A strategy to lower PSA levels will not necessarily prevent prostate cancer; it may do so only if it arrests the natural history of the disease. That being said, not all intermediate end-points, even if they are on the causal pathway, are good surrogate end-points, particularly if the disease in question has a broad multifactorial etiology and the intermediate end-point is upstream to only one of the causal factors. Wacholder's view [1] is that a suitable intermediate end-point ought to have a high population attributable fraction (PAF) for the cancer end-point and a high positive predictive value (PPV). In other words, a large proportion of the cancers would have to originate from the intermediate end-point and a large proportion of the intermediate end-points would eventually become cancer. The definition of "large" in the previous sentence implies how close the intermediate end-point is to being a necessary (PAF approaching 100%) and sufficient (PPV approaching 100%) step in the causal pathway to cancer.

The statistical criterion formulated by Prentice [2] indicated that an intermediate end-point would be valid only if it captures the entire statistical relation between the intervention and the final outcome. The extent with which an intervention prevents the intermediate end-point would be comparable to the effect in averting the final outcome; in the case of HPV vaccination, the VE represents such an effect. In practice, this means that the VE for intermediate end-points that do not progress to cancer equals the VEs for intermediates that do progress to cancer. However, as pointed out by Wacholder [1], this expectation is not verifiable in practice if one cannot fully observe a paradigmatic relation between the intervention and final outcome. Screening or HPV vaccination studies of cervical cancer prevention pose such a problem because of the ethical impossibility of conducting these studies with full completion, i.e. until cervical cancer has a chance to develop. In a research setting, investigators are expected to intervene and treat by ablation or excision any precancerous lesions, to avoid the risk of them progressing to cancer. Yet, currently accepted regulatory standards for end-points to be used in HPV vaccination trials state that CIN2/3 is universally accepted, even though no trial has had or will have the opportunity to demonstrate that Prentice's criterion is fulfilled in the setting of cervical cancer.

The reason for the high credibility of CIN2/3 as a validated end-point for cervical cancer prevention comes entirely from the time-tested consistency of the observation that the process of discovering and treating CIN2/3 via screening leads to a subsequent reduction in the incidence of and mortality from cervical cancer in different populations. The body of evidence is thus entirely ecological; there have never been – and likely never will be – any controlled trials to support this notion. The practical proof of validity will be the extent and timing of the reduction in cervical cancer in vaccinated women. Enhanced credibility also comes from abundant natural history and epidemiological evidence that virtually all cases of cervical cancer arise via a well-defined series of steps: sexual transmission of HPV infection, persistence of infection, development of cervical precancer, and invasion. The key assumption – which is accepted as a scientific canon – is that the PAF of cervical cancer for the pathway that involves CIN2/3 is 100%. Each step in the carcinogenic process is necessary, or has a PAF of 100%; preventing any single step prevents cancer development. Thus, in the absence of direct sexual contact, there is almost no cervical cancer (e.g. nuns have very low rates of cervical cancer). In the absence of HPV acquisition and persistence, there is practically no risk. Precancer (defined best as CIN3, as mentioned above) is a

logically necessary intermediate but proximal end-point. Therefore, for all practical purposes, regulatory agencies tacitly accept that trials of cervical cancer prevention can confidently rely on CIN2/3 as a validated end-point and that the magnitude of the impact (i.e. reduction in incidence) on CIN2/3 by an intervention such as HPV vaccination will be expected to result in a comparable impact on the incidence of cervical cancer in the future. If the VE for CIN2/3 is 100%, and the PAF of CIN2/3 for cancer is 100%, all cancer will be prevented.

This logical chain might break down in some theoretical settings. An assumption of equal VEs against an early and a late end-point is essentially equivalent to an assumption that the vaccine does not affect the transition or transitions between end-points. Especially when the VE is not high, a high PPV for cancer, given the intermediate end-point targeted by the vaccine, may be necessary to provide reassurance about the suitability of the end-point; a low VE against an intermediate end-point with a low PPV leaves open the disheartening possibility that the vaccine preferentially prevents end-points that do not go on to cancer. In principle, the greater the PPV for the chosen end-point, the more likely it is that vaccine effectiveness will translate into prevention of cancer.

It stands to reason that in a sequential causal pathway originating with a single necessary cause, such as HPV infection in cervical cancer, the impact (of the intervention) on a cancer end-point later in the natural history is closer to the impact on cancer, simply because fewer assumptions of no effect are required. Logically, reducing sexual activity by 50% would not necessarily prevent 50% of cervical cancer because of the heterogeneity in sexual transmission dynamics and the dependence on HPV prevalence in partners, conditions that lower the PPV from sexual activity. Our question is about steps later in the sequence than sexual behaviour, i.e. whether prevention of a certain percentage of virological intermediate end-points would lead to the same percentage of cervical cancer being averted. It is, nevertheless, reassuring that the incidence rates of cervical cancer in countries with sociocultural norms that are not conducive to transmission of HPV infection, for example Middle Eastern countries, are comparable to that in Finland, a country that has possibly one of the best organized cervical cancer screening programmes in the world. In other words, a downstream intervention (screening) that identifies and treats CIN2/3 exerts approximately the same impact on the final outcome (cervical cancer) as one exerted distally (risk behaviour avoidance), completely upstream in the causal pathway of this disease.

Despite the strong body of evidence concerning the validity of the linear causal pathway from sexual activity to HPV infection, then to CIN2/3, and finally to cervical cancer (the latter being observed only via ecological studies), regulatory agencies appropriately require a high burden of proof before a virological end-point, i.e. a measure of HPV infection, can be accepted for a trial to support a regulatory claim of preventive value against cervical cancer. They should note, however, that a virological end-point is important not only for scientific and public health reasons but also for pragmatic and economic reasons, to permit financially viable clinical development programmes by the private and public sectors.

Of course, not every virological end-point should be deemed adequate, even if cost-effective. Although a perfect measure of HPV acquisition might theoretically serve as an adequate surrogate end-point, one-time HPV positivity may represent simple deposition (i.e. detection of HPV DNA deposited in the cervical surface from very recent sexual activity and which may not necessarily become a productive infection) without true infection, the prevention of which would not lead to prevention of cervical cancer. Therefore, one must rely on some length of type-specific persistence that indicates a high probability of true infection (we are discounting the unlikely possibility of repeated deposition without infection).

Specifically, a question that has been highly debated and is central to the discussion of feasibility of end-points is whether a fixed period of HPV persistence (e.g. 6 months or

1 year) can serve as an adequate surrogate end-point for cervical cancer risk. For example, if a vaccine prevented 50% of persistent HPV 16 infection, would 50% of HPV 16-associated cervical cancer be prevented? As mentioned above, there is no direct evidence referring to cervical cancer itself. If we demand that level of evidence, there is no established surrogate, including histological precancer (which is generally taken as CIN2/3). For HPV 16 at least, we have direct evidence from vaccine trials that the magnitude of the reduction in incidence of persistent infection equals the reduction in incidence of CIN2/3. This is consistent with other observations showing that persistent HPV 16 infection and HPV 16-associated CIN2/3 are highly linked, with a demonstrated transition probability (i.e. a PPV) of nearly 50% [3].

An important assumption in the foregoing discussion about the validity of virological end-points is the quality of the measurement, i.e. accuracy, consistency, and reliability. There is abundant empirical proof that without properly validated assays our ability to measure the causal relation between HPV and cervical cancer is severely impaired [4]. Minimizing errors in the process that includes specimen collection, transportation, and testing for the presence of nucleic acids of specific HPV genotypes via validated laboratory assays is an imperative for the above-mentioned epidemiological arguments to hold. Fortunately, the advent of HPV-based prevention strategies (vaccination and screening) has led to international cooperation coordinated by WHO in ensuring quality control proficiency in laboratory testing for HPV DNA [5].

3.3 Are randomized controlled trials with CIN2/3 end-points feasible for the future generation of HPV vaccines?

Based on the above-mentioned premises of validated biological intermediates in cervical cancer prevention, the more upstream one focuses in the natural history, the more common the end-point. By the same token, the more downstream (i.e. proximal) in the causal pathway, the rarer the end-point and the longer it will take for it to be observed. The relationship between the frequency (or in sample size calculation parlance, the attack rate) of an end-point and the projected size of an RCT can be illustrated with data from the trial of HPV vaccination in Guanacaste, Costa Rica [6]. Table 3.1 shows that sample size requirements for a hypothetical HPV 16/18 vaccine decrease as one goes upstream in the type-specific natural history. Taking HPV 16 as an example, there were 671 cases of incident infection among women in the placebo arm of the Costa Rica Vaccine Trial. Among these, 238 persisted for 1 year and 94 for 2 years, and 18 of the infected women developed cervical intraepithelial neoplasia of grade 2 or worse (CIN2+).

The complexity of study management aside, the cost borne by the sponsor of an RCT is directly related to the final sample size requirements and the duration of the study. Reliance on a 1-year type-specific persistence for any given type rather than CIN2+ implies substantial savings for the study (Table 3.1). The sample size requirements for an end-point of CIN2 or worse lesion (the current paradigm) would be 14–16-fold greater than for a type-specific persistent infection measured at two time points, 1 year apart, after vaccination completion. Correspondingly, the savings consequent to the reduction in sample size requirements relative to those that would be necessary for upholding the standard of CIN2+ in an RCT would be > 90% for a definition of 1-year type-specific HPV infection. In the example chosen (50% protection), the ratio of sample size to the reciprocal of the relative frequency of the event is mostly > 100 and increases with severity. Overall, it can be seen that the sample size needed is inversely proportional to the attack rate.

Table 3.1. End-points based on HPV 16 and 18 virological and lesion frequencies in the placebo arm[a] of the Costa Rica Vaccine Trial and associated sample size requirements[b]

Statistic	HPV type	Composite end-point assuming conditionality[c]			
		Incident infection	1-year persistent infection	2-year persistent infection	CIN2+
Number of cases	HPV 16	671	238	94	18
	HPV 18	361	78	24	4
	HPV 16 or 18	1032	316	118	22
Proportion relative to entire cohort	HPV 16	19.33%	6.86%	2.71%	0.52%
	HPV 18	9.91%	2.14%	0.66%	0.11%
	HPV 16 or 18	27.62%	8.46%	3.16%	0.59%
Sample size (both arms), *N*	HPV 16	554	1740	4557	24 215
	HPV 18	1166	5790	19 006	93 342
	HPV 16 or 18	352	1386	3888	20 454
Reduction in sample size relative to CIN2+	HPV 16	97.7%	92.8%	81.2%	Ref.
	HPV 18	98.8%	93.8%	79.6%	Ref.
	HPV 16 or 18	98.3%	93.2%	81.0%	Ref.
Ratio of *N* to the reciprocal percentage of cases	HPV 16	107	119	123	126
	HPV 18	116	124	125	102
	HPV 16 or 18	97	117	123	120

CIN2+, cervical intraepithelial neoplasia of grade 2 or worse (CIN3 and cancer); HPV, human papillomavirus; Ref., referent group.

[a] 3736 women and 19 503 women-years of follow-up.

[b] Based on 90% power, 5% α error, and 50% effect size (vaccine efficacy) (using formulae in Blackwelder [7]).

[c] Counts and associated proportions reflect cumulative frequencies, i.e. each cell represents the sum of all end-points downstream from it (to the right of it in the table row) and conditioned on the HPV type shown.

Finally, the savings translate also in terms of duration. An RCT based on 1-year type-specific persistence would require at most 2 years of follow-up to accommodate the vaccination administration regimen and the follow-up phase after the last dose. In contrast, an RCT designed with CIN2/3 (a step with a long latency period) as end-point would require an extra 2–3 years to accrue a sufficient number of cases to be able to attain the required statistical power.

3.4 Benchmarking protection against HPV 16 and 18

Nearly three quarters of all cervical cancers are caused by HPV 16 or 18, the two types against which there are two clinically validated vaccines, Gardasil (from Merck) and Cervarix (from GlaxoSmithKline). The initial successes of trials with these vaccines have established benchmarks of performance in terms of efficacy and duration of protection against both virological and lesion end-points. Protection against these two HPV types and their associated lesions is very high at ≥ 95% (depending on the type of analysis and restrictions to the source cohorts in the RCTs). Future vaccines will have to be evaluated against these benchmarks. The second generation of vaccines may be based on VLPs containing L1 for multiple types (a nonavalent candidate has already completed clinical studies; see below), whereas third- and fourth-generation candidate vaccines may be based on L1 capsomers

and/or L2 capsid protein to enhance the range of types against which protection can be attained. The immunogenicity of these future vaccines may also be enhanced with new adjuvants being developed (see Chapter 2).

Obviously, the goal in developing new HPV vaccines is to extend the range of protection to cervical cancers other than those caused by HPV 16 and 18, a question that is best addressed by sample size projections based on a superiority trial framework. The implications of a superiority comparison were discussed above and shown in Table 3.1 to illustrate the impact of choosing a virological end-point on the costs and duration of a trial. However, in addition to the concerns about properly measuring the incremental gain in protection via a candidate vaccine with enhanced HPV immunogenic properties, one also has to maintain the original protection that has been demonstrated against HPV 16 and 18. The same is also true for HPV 6 and 11 for any new vaccine whose expanded range of protection includes these two types.

An RCT of a candidate nonavalent vaccine, whose results were recently announced [8], compares a new vaccine that contains nine types as individual VLP components against Gardasil, the control arm vaccine, which includes four types (HPV 6, 11, 16, and 18). A key question of clinical and public health relevance is: How much extra protection against cervical lesion outcomes will this nonavalent vaccine provide relative to what is expected with quadrivalent Gardasil alone? Will the addition of five high-risk types (HPV31, 33, 45, 52, and 58) as immunogens bring extra protection in preventing cervical lesions while the “core” protection against HPV 16/18 and HPV 6/11 (and their associated lesions) is maintained? Having recruited 14 215 women aged 16–26 years, Merck’s trial for its V503 (the provisional name) vaccine was designed to measure the extra protection with sufficient power for a superiority trial while permitting ample power to assess non-inferiority for the protection against HPV 16/18 and HPV 6/11.

The definition of statistical boundaries for equivalency of efficacy becomes critical for types in which an expectation exists as to how well a vaccine should perform. For the sake of argument, a proportional gain of an extra 20% protection in absolute terms relative to an original protection of 70% when only HPV 16/18 outcomes are prevented would raise the population-level protection to 90% of all cervical lesions. Hypothetically speaking, however, it is possible that this gain may be obscured by a small loss of protection against outcomes associated with HPV 16 and 18. For instance, the net 90% overall protection may be the result of a lower VE against HPV 16/18 outcomes for the new vaccine (relative to quadrivalent Gardasil) that ends up being compensated for by the expanded protection against lesions caused by the other five high-risk types. Stakeholders may have different opinions about whether this is an acceptable trade-off. There is sufficient evidence to indicate that lesions caused by HPV 16 and 18 are worse in prognosis and tend to occur earlier than those caused by other high-risk types. In terms of the potential for preventing cervical cancers, it could be argued that preventing an HPV 16 infection (high PPV and high PAF) is far more important than preventing an infection with, say, HPV 52 (low PPV and low PAF).

In addition to the above-mentioned concerns related to quantitative expectations about gains or equivalency in efficacy, one must also qualify the type of outcome. From a prevention standpoint, a new vaccine will ideally reduce the incidence of the pre-specified validated end-point, whether it is a defined measure of persistent HPV infection of a given type (as proposed in the above-mentioned arguments) or CIN2/3 and cancer (as broadly accepted by regulators). For non-inferiority of efficacy against HPV 16/18, the sample size requirements based on incidence rates are more onerous than those based on calculations of equivalency of immunogenicity, which can be defined as comparability of geometric mean serological titres by trial arm at the end of the vaccination regimen. Using immunogenicity instead of

preventive efficacy permits earlier assessment of non-inferiority for outcomes that have been accepted a priori as validated bridging end-points. Such a strategy was used to justify vaccinating girls aged 9–11 years and by Merck in its V503 trial of the nonavalent HPV vaccine. The non-inferiority of the V503 candidate (nonavalent) vaccine against the established Gardasil (quadrivalent) vaccine taken as control was decided on the basis of immunogenicity against the four HPV types that were common to both vaccines (HPV 6, 11, 16, and 18). Such a strategy permitted the company to satisfy regulatory agencies with a trial of the size of the pivotal efficacy study (N ~ 14 000). Were non-inferiority against the above-mentioned four types to be based on incidence of type-specific virological and lesion outcomes, the sample size requirements would have been much larger. At the end of the trial, there were only 3 cases of CIN among the per-protocol population (N ~ 11 500), 2 among controls, and 1 in the V503 arm [8]. It suffices to take a look at the estimates of trial size in Table 3.1 to see that even a study as large and costly as Merck's V503 trial would not have permitted adequate statistical resolution between the efficacy of the new, nonavalent vaccine and that of the established, quadrivalent one.

In summary, the implications suggested above regarding the influence of the frequency of the end-point on the size and duration of an RCT also apply to the equivalence or non-inferiority framework. The sample size requirements for both situations are similarly affected. In theory, the required sample size to detect equal VE is proportional to the expected number of events in both superiority and equivalence/non-inferiority trials.

3.5 Conclusions

From a regulator's perspective, conservatism tends to be favoured in deciding about acceptable end-points, to protect the public against medical products that do not exert a beneficial effect on clinically relevant outcomes. However, in the specific case of HPV 16 and 18 infection and cervical cancer, there is abundant and sufficient evidence that relying on a virological end-point such as persistent type-specific infection determined with validated and quality-assured assays will be technically acceptable and pragmatic. Use of immunogenicity outcomes provides a sound basis for assessing the equivalence of protection against types in new vaccine formulations that are common to those in standard-of-care vaccines already approved and taken as controls.

Keeping a dogmatic preference for a less common downstream end-point will result in overly stringent sample size and trial duration requirements, which will increase costs of clinical development of new HPV vaccines enormously as they reach ever-broader levels of protection against multiple Alphapapillomavirus types. This, in turn, will inhibit continual improvement of the technology because of the economic disincentive to the industry.

Acknowledgements

The authors are indebted to Brian Befano, Christine Fermo, and Fang Hui Zhao for assistance with data analysis and discussions, and to Suzanne Garland for editorial comments on an earlier draft of this paper.

References

1. Wacholder S (2013). Precursors in cancer epidemiology: aligning definition and function. *Cancer Epidemiol Biomarkers Prev*. 22(4):521–7. http://dx.doi.org/10.1158/1055-9965.EPI-13-0084 PMID:23549395
2. Prentice RL (1989). Surrogate endpoints in clinical trials: definition and operational criteria. *Stat Med*. 8(4):431–40. http://dx.doi.org/10.1002/sim.4780080407 PMID:2727467

3. Kjaer SK, Frederiksen K, Munk C, Iftner T (2010). Long-term absolute risk of cervical intraepithelial neoplasia grade 3 or worse following human papillomavirus infection: role of persistence. *J Natl Cancer Inst*. 102(19):1478–88. http://dx.doi.org/10.1093/jnci/djq356 PMID:20841605
4. Franco EL, Tota J (2010). Invited Commentary: Human papillomavirus infection and risk of cervical precancer – using the right methods to answer the right questions. *Am J Epidemiol*. 171(2):164–8. http://dx.doi.org/10.1093/aje/kwp392 PMID:20007675
5. Eklund C, Forslund O, Wallin KL, Zhou T, Dillner J; WHO Human Papillomavirus Laboratory Network (2012). The 2010 global proficiency study of human papillomavirus genotyping in vaccinology. *J Clin Microbiol*. 50(7):2289–98. http://dx.doi.org/10.1128/JCM.00840-12 PMID:22535980
6. Herrero R, Wacholder S, Rodríguez AC, Solomon D, González P, Kreimer AR *et al.*; Costa Rica Vaccine Trial Group (2011). Prevention of persistent human papillomavirus infection by an HPV16/18 vaccine: a community-based randomized clinical trial in Guanacaste, Costa Rica. *Cancer Discov*. 1(5):408–19. http://dx.doi.org/10.1158/2159-8290.CD-11-0131 PMID:22586631
7. Blackwelder WC (1993). Sample size and power for prospective analysis of relative risk. *Stat Med*. 12(7):691–8. http://dx.doi.org/10.1002/sim.4780120708 PMID:8511445
8. Joura E on behalf of the V503-001 study team (2013). Efficacy and immunogenicity of a novel 9-valent HPV L1 virus-like particle vaccine in 16- to 26-year-old women. EUROGIN Congress 2013, Florence, Italy. Abstract SS 8-4, p. 102.

Chapter 4. Trials of vaccine efficacy against cervical outcomes associated with HPV types other than HPV 16 and 18

Silvia Franceschi and Silvia de Sanjosé Llongueras

4.1 Introduction

The primary efficacy end-points endorsed by WHO and required by regulatory authorities for the first phase III trials of the licensed human papillomavirus (HPV) 16/18 vaccines were high-grade histologically confirmed lesions, i.e. cervical intraepithelial neoplasia of grade 2 or 3 (CIN2/3), adenocarcinoma in situ, or worse lesions (hereafter referred to, for brevity, as CIN2/3+) as best surrogates of invasive cervical cancer. Therefore, large and long-duration trials had to be carried out to reach sufficient statistical power to demonstrate vaccine efficacy (VE) against CIN2/3+ associated with the vaccine types HPV 16/18 or with any high-risk (HR) HPV types. Successful trials were facilitated by high VE and high incidence rates of HPV 16/18-associated CIN2/3+ in women in the age group 15–26 years. In fact, trials targeting older women showed significant VE against HR HPV persistent infection, and all CIN, but they did not reach statistically significant results against CIN2/3+ in women aged 24–45 years [1]. Vaccines that are currently in preparation or under clinical evaluation (e.g. based on virus-like particles [VLPs], capsomers, L2 vaccine) target HR HPV types other than HPV 16 and 18 (hereafter referred to as “other HR HPVs”) in addition to HPV 16/18. To facilitate the evaluation and accelerate the availability of broader-spectrum or cheaper vaccines, it is important to consider possible modifications in trial protocols, notably alternative end-points, in particular for VE against other HR HPVs.

Our present understanding of the natural history of other HR HPVs is more limited than our understanding of HPV 16 and, to some extent, HPV 18 on account of the relative rarity of other HR HPVs in CIN3 and invasive cervical cancer. Here, we will refer, wherever possible, to 13 other HR HPVs, i.e. those that were categorized as “carcinogenic” (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59) or “probably carcinogenic” (HPV 68) to humans [2]. Other types of doubtful carcinogenic potential (e.g. HPV 26, 53, 66, 67, 70, 73, and 82) [2] and HPV 6 and 11 will not be discussed here.

A comprehensive review of the knowledge about other HR HPVs is beyond our present aims, but we summarize key information from a selection of large studies. Data from meta-analyses of worldwide HR HPV prevalence [3–5], cohort studies [6–10], and screening studies [11, 12] provided information on the involvement of other HR HPVs in the development of CIN of all grades and invasive cervical cancer. Two meta-analyses of cohort studies [13, 14] are especially useful to evaluate the frequency and duration of persistent infection with other HR HPVs and compare them with those of HPV 16/18. In addition, HPV vaccine trials enable evaluation of the correlation between different VE end-points, notably for non-vaccine types that showed evidence of cross-protection, i.e. HPV 31, 33, and (bivalent vaccine only) HPV 45 (Allan Hildesheim, personal communication; for a review, see [15]).

The rarity of other HR HPVs in cancers of the anogenital tract (other than the cervix) and in the head and neck is also described [16–18]. Essential questions and answers in relation to different VE end-points for other HR HPVs are proposed (Table 4.1).

Table 4.1. Main questions and answers in relation to vaccine end-points associated with HPV types other than HPV 16 and 18

Question	Answer
Question 1. Are CIN2/3 end-points the best option for vaccine efficacy against HR HPV types other than HPV 16/18?	No
Question 2. Do we have enough data on the natural history of HR HPV types other than HPV 16/18 to consider alternative end-points?	Yes, at least for the cervix uteri
Question 3. Can persistent infection be used as a surrogate end-point to express vaccine efficacy against HR HPV types other than HPV 16/18?	Yes
Question 4. Can serology be used as a surrogate end-point to express vaccine efficacy against HR HPV types other than HPV 16/18?	Yes, but only for selected aims

CIN2/3, cervical intraepithelial neoplasia of grade 2 or 3; HPV, human papillomavirus; HR, high-risk.

4.2 Main open issues

4.2.1 CIN2/3 end-points for vaccine efficacy

A large meta-analysis including polymerase chain reaction (PCR)-based data published up to November 2011 from all world regions [4] can help to elucidate the implications of retaining CIN2/3+ end-points if VE against other HR HPVs is to be evaluated. Table 4.2 shows the prevalence of each of 13 HR HPV types among HPV-positive women by cervical disease grade. The last two columns in Table 4.2 show HPV 16/other HR HPV prevalence ratios separately in CIN2 and CIN3. Ratios give an approximate idea of the increase in a trial's women-years that would be required to achieve the same statistical power for individual other HR HPVs as for HPV 16. Ratios ≤ 5 were found for HPV 33, 58, 31, 52, and 51 when CIN2 was used as end-point, but only for HPV 31 when CIN3 was considered. In CIN3, many HPV 16/other HR HPV ratios were ≥ 10.

The number of CIN2/3 cases would obviously increase if VE were evaluated for several other HR HPVs all together. The sum of the prevalence of HPV 31, 33, 45, 52, and 58 in CIN3 (43.6%) is larger than the sum of the prevalence of HPV 16 and 18. This sum, however, is greatly inflated by double-counting of HR HPV types in multiple infections (see Answer 3 in Table 4.1). In the PATRICIA trial, one third of CIN3 among control women were related to HPV types other than HPV 16 and 18 [15].

The evaluation of VE against vulvovaginal [16, 17], anal [17], and glandular cervical pre-neoplastic lesions [4] would be even more difficult than for squamous cell lesions of the cervix on account of the larger predominance of HPV 16- and 18-associated disease than in CIN2/3+.

4.2.2 Natural history

Other HR HPVs are frequently acquired in sexually active women [8], can persist over time [13, 14], and are linked to the development of CIN2/3+ lesions [9], including invasive cervical cancer [19].

The cumulative incidence of infection with other HR HPVs in 3731 women aged 18–25 years in Guanacaste, Costa Rica, was 29.4%, 43.0%, and 51.3% at 12, 24, and 51 months, respectively [8]. It ranged between 1.7% for HPV 33 and 6.3% for HPV 52. Type-specific cumulative incidence of other HR HPVs corresponded closely with type-specific prevalence, whereas HPV 16 was more prevalent than predicted by incidence, confirming greater persistence. Vast data on type-specific prevalence showed, however, that the prevalence of

individual other HR HPVs varies substantially across world regions in women with normal cytology but that the variation decreases as the severity of cervical lesion increases [3, 7, 10].

Strong evidence that other HR HPVs can often persist is derived from two meta-analyses [13, 14] that included 41 and 86 cohort studies, respectively. The median duration of HR HPV infection was on average 9.8 months [14]. The longest durations were found for HPV 16, 31, 33, and 52 (≥ 11 months). Longer duration of infection, wider testing intervals, and concurrent presence of high-grade lesions were associated with larger relative risks of CIN2/3+ [13]. However, repeated detection of HR HPV was a robust predictor of CIN2/3+ despite different definitions of persistence, HPV detection method, interval, and so on [13].

The probability of progression of persistent infection of other HR HPVs to CIN2/3+ has been reported in many cohort studies. Kjaer *et al*. [9], for instance, followed up 8256 women aged 20–29 years for an average time of 13.4 years. Women with type-specific persistent infection with other HR HPVs had a substantial probability of developing CIN2+ (ranging from 4.3% for HPV 68 to 18.3% for HPV 33) and CIN3+ (ranging from 5% for HPV 45 to 32% for HPV 58). Findings from cohort studies also allowed a comparison between HPV 16 and/or 18 and other HR HPVs. In the study of Castle *et al*. [6], 3-year incidence rates of CIN2/3+ after persistent infection were 40.8%, 17.5%, and 10.0% for HPV 16, 18, and an average of other HR HPVs, respectively. Schiffman *et al*. [20] distinguished type-specific viral persistence from neoplastic progression to CIN3+ given persistence. Compared with low-risk types, other HR HPVs were not particularly persistent, but they could cause neoplastic progression, although at lower rates than HPV 16, if they persisted.

Several cervical screening trials showed the association between persistent infection and CIN2/3+, but few provided detailed information on other HR HPVs individually. The VUSA trial in the Netherlands [11] and the ATHENA study [12] reported cumulative CIN2/3+ risk over 3 years among other HR HPV-positive women of 12.7 and 4.6 per 100, respectively.

A caveat is required about the natural history of other HR HPVs outside the cervix. HPV 16 accounts for most of HPV-associated non-cervical high-grade intraepithelial lesions and cancer [16–18]. Persistent infection and neoplastic progression of other HR HPVs in the anus, penis, vulva, and vagina, and in the head and neck are little understood and difficult to evaluate.

4.2.3 Persistent infection as a surrogate end-point to express vaccine efficacy

Persistent infection with other HR HPVs was found to be strongly associated with CIN2/3+ in many prospective studies that assessed individual HPV types or groups of types defined by phylogenetic species (see Answer 2 in Table 4.1). Newly acquired infections were associated with lower risk of CIN2/3+ than were long-duration/prevalent infections, regardless of age [21]. Data on the correlation between persistent infection and CIN2/3+ from HPV vaccine trials are scantier for other HR HPVs than for HPV 16/18. However, the estimates of VE against other HR HPVs were similar using CIN2/3+ and 6-month persistent infection as end-points, in agreement with what was reported for HPV 16/18 [15]. Unpublished data from the Costa Rica Vaccine Trial of the bivalent vaccine show a good correlation between VE estimates based on CIN2/3+ and on 12- or 6-month persistence for the combination of HPV 31, 33, 45, i.e. the other HR HPVs that showed significant cross-protection (Table 4.3) (Allan Hildesheim, personal communication). However, the use of 6-month persistent HPV 31/33/45 infection allowed > 10-fold more events to be observed than the use of CIN2/3+ associated with the corresponding types. The estimates of VE against the combination of HPV 31/33/45 (64.1%; 95% confidence interval [CI], 49.7–74.8%) were similar to but slightly more conservative than VE estimates based on CIN2/3+ (77.3%; 95% CI, 4.8–96.7%). For the combination of HPV types 35, 39, 51, 52, 56, 58, 59, and 68/73, the use of CIN2/3+ as

end-point led to a much larger VE than the use of persistent infection, on account of the difficulty of adjudicating cervical lesions to individual HPV types. In fact, persistent infection is clearly superior to CIN2/3+ in the evaluation of multiple-type cervical infections (see Answer 1 in Table 4.1).

The choice and interpretation of persistent infection as an end-point for other HR HPVs are more complicated in (i) trials in women above a certain age, for example 35 years, because of the probable rarity of neoplastic progression of new cervical infections with other HR HPVs in older women [22] and (ii) trials in men and/or targeting cancer of the head and neck, because of the rarity of other HR HPVs in HPV-associated malignancies other than cervical cancer [16–18].

4.2.4 Role of serology

Prospective studies and HPV vaccine trials provided limited information on the correlation between seropositivity and CIN2/3+ or persistent infection for other HR HPVs [23–25]. In addition, quantitative serological methods for other HR HPVs (see, for instance, problems with HPV 52 [23, 24]) require additional cross-validation and standardization (see the work of WHO HPV LabNet [26]). New broader-spectrum vaccines may imply additional problems, for example between-type immune interference (see Chapter 2).

Nevertheless, the evaluation of high levels of antibodies against any HPV is more robust among vaccinated individuals than the evaluation of much lower levels arising from natural infection. Immunogenicity associated with persistent infection with vaccine HPV types at a given time, for example 2 or 3 years, can, therefore, help in establishing non-inferiority of new VLP vaccines versus first-generation VLP vaccines. Immunogenicity will also remain essential for bridging studies in adolescent girls and boys and in children, as soon as it will be possible to envisage HPV vaccination. It is, however, difficult at present to endorse serology as primary VE end-point, notably in the case of other HR HPVs.

4.3 Discussion

The use of persistent infection with other HR HPVs as a proxy of CIN2/3+ in HPV vaccine trials is supported by strong evidence that high-grade cervical lesions are preceded by persistent infection for all HR HPV types. The use of persistent infection will allow gains in terms of statistical power and accuracy in the assessment of VE in the presence of multiple-type infections. In fact, the use of persistent infection would make the study size necessary to evaluate VE against any HR HPV types (including HPV 18 but not HPV 16) much smaller than the use of CIN2/3. In addition, persistent infection seems to be, if anything, a more conservative VE end-point than CIN2/3+.

The majority of persistent infections with HR HPVs other than HPV 16 or 18 do not evolve into CIN2/3+. If VE of any new vaccine against persistent infection is low, it may be argued that those infections that are not prevented may be the ones that progress to CIN2/3+ (see Chapter 2). In addition, the probability of progression is associated with infection duration. Six-month persistent infections in women who are HPV-negative at baseline will be associated with a lower risk of progression than what has been reported in the literature, which mainly includes data on persistent infections of unknown duration.

HPV testing can be more objective and reproducible than cytology or histology, but the choice of persistent infection as vaccine trial end-point will oblige trials to put in place strict quality standards for testing methods (see the work of WHO HPV LabNet [27]). That especially applies to other HR HPVs, for which the accuracy of some available HPV testing methods can be lower than the accuracy for HPV 16/18, especially in the presence of multiple infections. For instance, the proficiency study by Eklund *et al*. [27] reported lower

sensitivity for HPV 52, 56, and 59 compared with HPV 16 and 18 and a certain frequency of false positives.

To improve the feasibility and efficiency of trials of new HPV vaccines, we may also wish to reconsider other aspects of trial protocols, notably the length of the interval between visits. The shorter the interval, the more accurate will be the estimates of persistence duration [14]. Very labour-intense follow-up may, however, threaten the feasibility of HPV vaccine trials. Eventually, the evaluation of very broad-spectrum vaccines may overcome the need for genotyping and allow us, therefore, to rely on simpler and more robust diagnostic kits, for example Hybrid Capture 2 or PCR with generic primers. If differential bias in the evaluation of the vaccine arm and control arm can be excluded, a trade-off between large trial size and the strictness of protocol requirements may be envisaged.

Finally, it is worth bearing in mind that, as a global indicator, the number of persistent infections with other HR HPVs avoided by any vaccine will exaggerate the number of CIN2/3+ avoided to a much greater extent for HPV 16/18 (Table 4.2). VE very close to 100% may, therefore, correspond to the prevention of relatively few severe lesions and much fewer cervical cancers than VE against HPV 16/18. Because of their substantial contribution to HPV infections and cytological/histological abnormalities, the prevention of other HR HPVs will be very useful and, possibly, cost-effective in settings in which cervical screening is in place, even if new multivalent vaccines will be more expensive. However, VE against HPV 16/18 infection will continue to be of paramount importance in the prevention of cervical cancer and, to an even greater extent, cancers in other sites.

Acknowledgements

The authors thank Dr Allan Hildesheim and Dr Aimee Kreimer for providing unpublished data.

References

1. Castellsagué X, Muñoz N, Pitisuttithum P, Ferris D, Monsonego J, Ault K *et al.* (2011). End-of-study safety, immunogenicity, and efficacy of quadrivalent HPV (types 6, 11, 16, 18) recombinant vaccine in adult women 24–45 years of age. *Br J Cancer*. 105(1):28–37. http://dx.doi.org/10.1038/bjc.2011.185 PMID:21629249
2. IARC (2012). Biological agents. *IARC Monogr Eval Carcinog Risks Hum*, 100B:1–441. PMID:23189750
3. Guan P, Howell-Jones R, Li N, Bruni L, de Sanjosé S, Franceschi S *et al.* (2012). Human papillomavirus types in 115,789 HPV-positive women: a meta-analysis from cervical infection to cancer. *Int J Cancer*. 131(10):2349–59. http://dx.doi.org/10.1002/ijc.27485 PMID:22323075
4. Guan P, Clifford GM, Franceschi S (2013). Human papillomavirus types in glandular lesions of the cervix: a meta-analysis of published studies. *Int J Cancer*. 132(1):248–50. http://dx.doi.org/10.1002/ijc.27663 PMID:22684765
5. Serrano B, Alemany L, Tous S, Bruni L, Clifford GM, Weiss T *et al.* (2012). Potential impact of a nine-valent vaccine in human papillomavirus related cervical disease. *Infect Agent Cancer*. 7(1):38. http://dx.doi.org/10.1186/1750-9378-7-38 PMID:23273245
6. Castle PE, Rodríguez AC, Burk RD, Herrero R, Wacholder S, Alfaro M *et al.*; Proyecto Epidemiológico Guanacaste (PEG) Group (2009). Short term persistence of human papillomavirus and risk of cervical precancer and cancer: population based cohort study. *BMJ*. 339:b2569. http://dx.doi.org/10.1136/bmj.b2569 PMID:19638649
7. Chen HC, Schiffman M, Lin CY, Pan MH, You SL, Chuang LC *et al.*; CBCSP-HPV Study Group (2011). Persistence of type-specific human papillomavirus infection and increased

long-term risk of cervical cancer. *J Natl Cancer Inst*. 103(18):1387–96. http://dx.doi.org/10.1093/jnci/djr283 PMID:21900119
8. Clarke M, Schiffman M, Wacholder S, Rodriguez AC, Hildesheim A, Quint W; Costa Rican Vaccine Trial Group (2013). A prospective study of absolute risk and determinants of human papillomavirus incidence among young women in Costa Rica. *BMC Infect Dis*. 13(1):308. http://dx.doi.org/10.1186/1471-2334-13-308 PMID:23834901
9. Kjaer SK, Frederiksen K, Munk C, Iftner T (2010). Long-term absolute risk of cervical intraepithelial neoplasia grade 3 or worse following human papillomavirus infection: role of persistence. *J Natl Cancer Inst*. 102(19):1478–88. http://dx.doi.org/10.1093/jnci/djq356 PMID:20841605
10. Muñoz N, Hernandez-Suarez G, Méndez F, Molano M, Posso H, Moreno V *et al.*; Instituto Nacional de Cancerología HPV Study Group (2009). Persistence of HPV infection and risk of high-grade cervical intraepithelial neoplasia in a cohort of Colombian women. *Br J Cancer*. 100(7):1184–90. http://dx.doi.org/10.1038/sj.bjc.6604972 PMID:19293802
11. Rijkaart DC, Berkhof J, van Kemenade FJ, Coupe VM, Rozendaal L, Heideman DA *et al.* (2012). HPV DNA testing in population-based cervical screening (VUSA-Screen study): results and implications. *Br J Cancer*. 106(5):975–81. http://dx.doi.org/10.1038/bjc.2011.581 PMID:22251922
12. Wright TC Jr, Stoler MH, Sharma A, Zhang G, Behrens C, Wright TL; ATHENA (Addressing THE Need for Advanced HPV Diagnostics) Study Group (2011). Evaluation of HPV-16 and HPV-18 genotyping for the triage of women with high-risk HPV+ cytology-negative results. *Am J Clin Pathol*. 136(4):578–86. http://dx.doi.org/10.1309/AJCPTUS5EXAS6DKZ PMID:21917680
13. Koshiol J, Lindsay L, Pimenta JM, Poole C, Jenkins D, Smith JS (2008). Persistent human papillomavirus infection and cervical neoplasia: a systematic review and meta-analysis. *Am J Epidemiol*. 168(2):123–37. http://dx.doi.org/10.1093/aje/kwn036 PMID:18483125
14. Rositch AF, Koshiol J, Hudgens MG, Razzaghi H, Backes DM, Pimenta JM *et al.* (2013). Patterns of persistent genital human papillomavirus infection among women worldwide: a literature review and meta-analysis. *Int J Cancer*. 133(6):1271–85. http://dx.doi.org/10.1002/ijc.27828 PMID:22961444
15. Lehtinen M, Dillner J (2013). Clinical trials of human papillomavirus vaccines and beyond. *Nat Rev Clin Oncol*. 10(7):400–10. http://dx.doi.org/10.1038/nrclinonc.2013.84 PMID:23736648
16. de Sanjosé S, Alemany L, Ordi J, Tous S, Alejo M, Bigby SM *et al.*; HPV VVAP study Group (2013). Worldwide human papillomavirus genotype attribution in over 2000 cases of intraepithelial and invasive lesions of the vulva. *Eur J Cancer*. 49(16):3450–61. http://dx.doi.org/10.1016/j.ejca.2013.06.033 PMID:23886586
17. De Vuyst H, Clifford GM, Nascimento MC, Madeleine MM, Franceschi S (2009). Prevalence and type distribution of human papillomavirus in carcinoma and intraepithelial neoplasia of the vulva, vagina and anus: a meta-analysis. *Int J Cancer*. 124(7):1626–36. http://dx.doi.org/10.1002/ijc.24116 PMID:19115209
18. Kreimer AR, Clifford GM, Boyle P, Franceschi S (2005). Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. *Cancer Epidemiol Biomarkers Prev*. 14(2):467–75. http://dx.doi.org/10.1158/1055-9965.EPI-04-0551 PMID:15734974
19. Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV *et al.*; International Agency for Research on Cancer Multicenter Cervical Cancer Study Group (2003). Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med*. 348(6):518–27. http://dx.doi.org/10.1056/NEJMoa021641 PMID:12571259
20. Schiffman M, Herrero R, Desalle R, Hildesheim A, Wacholder S, Rodriguez AC *et al.* (2005). The carcinogenicity of human papillomavirus types reflects viral evolution. *Virology*. 337(1):76–84. http://dx.doi.org/10.1016/j.virol.2005.04.002 PMID:15914222

21. Maucort-Boulch D, Plummer M, Castle PE, Demuth F, Safaeian M, Wheeler CM *et al.* (2010). Predictors of human papillomavirus persistence among women with equivocal or mildly abnormal cytology. *Int J Cancer*. 126(3):684–91. http://dx.doi.org/10.1002/ijc.24752 PMID:19609952
22. Plummer M, Peto J, Franceschi S; International Collaboration of Epidemiological Studies of Cervical Cancer (2012). Time since first sexual intercourse and the risk of cervical cancer. *Int J Cancer*. 130(11):2638–44. http://dx.doi.org/10.1002/ijc.26250 PMID:21702036
23. Draper E, Bissett SL, Howell-Jones R, Edwards D, Munslow G, Soldan K *et al.* (2011). Neutralization of non-vaccine human papillomavirus pseudoviruses from the A7 and A9 species groups by bivalent HPV vaccine sera. *Vaccine*. 29(47):8585–90. http://dx.doi.org/10.1016/j.vaccine.2011.09.021 PMID:21939712
24. Draper E, Bissett SL, Howell-Jones R, Waight P, Soldan K, Jit M *et al.* (2013). A randomized, observer-blinded immunogenicity trial of Cervarix(®) and Gardasil(®) human papillomavirus vaccines in 12–15 year old girls. *PLoS One*. 8(5):e61825. http://dx.doi.org/10.1371/journal.pone.0061825 PMID:23650505
25. Lang Kuhs KA, Porras C, Schiller J, Rodriguez AC, Schiffman M, Gonzalez P *et al*. (2014). The effect of differing serological assays and HPV DNA positivity criteria on HPV vaccine efficacy in HPV-naive subcohorts. *Am J Epidemiol* (submitted).
26. Ferguson M, Heath A, Johnes S, Pagliusi S, Dillner J; Collaborative Study Participants (2006). Results of the first WHO international collaborative study on the standardization of the detection of antibodies to human papillomaviruses. *Int J Cancer*. 118(6):1508–14. http://dx.doi.org/10.1002/ijc.21515 PMID:16184553
27. Eklund C, Zhou T, Dillner J; WHO Human Papillomavirus Laboratory Network (2010). Global proficiency study of human papillomavirus genotyping. *J Clin Microbiol*. 48(11):4147–55. http://dx.doi.org/10.1128/JCM.00918-10 PMID:20844222

Table 4.2. Prevalence rate[a] of HPV 16 and other high-risk HPV types (X) as a percentage of HPV-positive women, in CIN2 and CIN3[b]

HPV type	Normal		ASCUS		LSIL		HSIL		CIN1		CIN2		CIN3		ICC		CIN2	CIN3
	%	2 SD	%	2 SD	%	2 SD	%	2 SD	%	2 SD	%	2 SD	%	2 SD	%	2 SD	HPV 16/X[c]	HPV 16/X[c]
HPV 16	20.4	3.6	22.9	2.9	25.1	2.8	47.5	5.5	27.6	4.3	39.8	5.0	58.2	4.1	62.6	2.2	Ref.	Ref.
HPV 18	8.4	1.1	9.0	1.6	8.7	1.3	9.6	1.6	9.0	1.7	10	1.0	7.4	1.2	15.7	2.9	4.0	7.9
HPV 45[d]	4.8	0.9	5.8	1.3	4.3	0.9	4.5	1.3	4.2	1.3	5	1.7	3.6	1.0	5.3	0.7	8.0	16.2
HPV 33[d]	4.7	0.8	5.9	1.4	6.1	1.2	8.4	1.2	6.1	2.0	8.3	1.3	9.1	1.2	4.5	0.8	4.8	6.4
HPV 58[d]	6.3	1.0	7.8	1.9	7.1	1.1	7.9	1.7	9.6	2.5	12.1	4.3	9.0	2.7	4.4	2.2	3.3	6.5
HPV 31[d]	8	2.0	9.4	1.3	9.5	1.4	11	1.6	11.3	3.9	11.6	2.7	11.7	1.5	4.0	0.4	3.4	5.0
HPV 52[d]	8	1.9	10.1	2.2	8.1	2.0	9.6	2.0	13.8	3.6	16.4	5.2	10.2	3.0	3.5	0.9	2.4	5.7
HPV 35	3.4	0.7	5.6	1.4	4.7	1.2	5.6	1.6	4.1	1.4	4.9	2.1	3.6	1.1	1.7	0.3	8.1	16.2
HPV 39	5.2	1.1	7.7	1.4	6.8	1.8	4.2	1.1	6.8	2.2	5.4	1.6	3.3	1.4	1.4	0.3	7.4	17.6
HPV 59	3.2	0.7	5.8	1.5	5.3	1.9	2.9	1.3	5.1	1.8	4.7	1.6	2.3	1.0	1.3	0.3	8.5	25.3
HPV 51	7.1	1.7	8.8	1.8	11.8	2.2	6.7	1.6	10.8	2.5	9.7	2.0	5.5	1.6	1.1	0.3	4.1	10.6
HPV 56	5.2	1.7	6.4	1.3	8.6	2.1	3.4	0.9	7.7	1.5	4.3	1.0	2.5	0.9	0.9	0.2	9.3	23.3
HPV 68	3.1	1.4	3.4	0.9	2.7	1.0	2.0	0.8	3.3	1.6	2.9	1.0	2.0	0.8	0.6	0.1	13.7	29.1

ASCUS, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; ICC, invasive cervical cancer; LSIL, low-grade squamous intraepithelial lesion; Ref., referent group; SD, standard deviation.

[a] In HPV-positive women, type-specific prevalence includes that contributed by multiple infections.

[b] In order of decreasing prevalence in invasive cervical cancer (ICC).

[c] Ratio of prevalence of HPV 16 to prevalence of other high-risk HPV types (X).

[d] The sum of the high-risk HPV types included in the nonavalent vaccine (HPV 45, 33, 58, 31, 52) is 53.4% in CIN2 and 43.6% in CIN3, but this is a severe overestimate on account of a large number of multiple infections, including, in many instances, HPV 16/18.

Source: Modified from Guan *et al*. [4]. This material is adapted with permission of John Wiley & Sons, Inc.

Table 4.3. Effect of selection of end-point on vaccine efficacy against (i) HPV 31/33/45, (ii) other non-HPV 16/18 carcinogenic HPV types, and (iii) all carcinogenic HPV types, in an according-to-protocol (ATP) analytic cohort[a]

End-point	HPV 31/33/45			Other non-HPV 16/18 carcinogenic HPV types[b]			Any carcinogenic HPV types		
	No. of women, V:C	No. of events, V:C	VE (95% CI)	No. of women, V:C	No. of events, V:C	VE (95% CI)	No. of women, V:C	No. of events, V:C	VE (95% CI)
CIN2+	2642:2695	2:9	77.3% (4.8% to 96.7%)	2643:2697	9:25	63.3% (22.9% to 83.7%)	2643:2697	11:33	66.0% (34.0% to 83.5%)
12-month persistence	2642:2695	38:82	52.7% (30.9% to 68.1%)	2643:2697	230:209	–12.3% (–35.5% to 6.9%)	2643:2697	268:334	18.1% (3.9% to 30.3%)
6-month persistence	2642:2695	44:125	64.1% (49.7% to 74.8%)	2643:2697	353:345	–4.4% (–21.1% to 10.0%)	2643:2697	390:510	22.0% (11.0% to 31.6%)
One-time detection	2642:2695	140:385	62.9% (55.1% to 69.5%)	2643:2697	985:1019	1.4% (–7.7% to 9.6%)	2643:2697	1055:1238	13.0% (5.6% to 19.9%)

CI, confidence interval; CIN2+, cervical intraepithelial neoplasia of grade 2 or worse; HPV, human papillomavirus; V:C, vaccine:control; VE, vaccine efficacy.

[a] The according-to-protocol (ATP) cohort for efficacy analyses was defined as all subjects who received three doses within the protocol-defined windows, whose timing between doses was respected (21–90 days between doses 1 and 2; 90–210 days between doses 2 and 3), who were HPV DNA-negative (by PCR) at months 0 and 6 for the corresponding HPV type considered in the analysis, who did not have a biopsy or treatment (loop electrosurgical excisional procedure [LEEP]) during the vaccination phase, for whom there was no investigational new drug safety report during the vaccination period, and who otherwise complied with the protocol during the vaccination period.

[b] Includes HPV types 35, 39, 51, 52, 56, 58, 59, and 68/73.

Source: Allan Hildesheim, personal communication.

Chapter 5. Evaluation of durability of protection of HPV prophylactic vaccines

John Schiller and Joakim Dillner

5.1 Introduction

Durability of protection is a key question for evaluating the usefulness of a vaccine. This is certainly true for human papillomavirus (HPV) prophylactic vaccines that are recommended for administration to early adolescents, some time before the major force of HPV infections. However, duration of protection has generally not been a key issue for initial licensure of vaccines by regulatory agencies such as the United States Food and Drug Administration (FDA) and the European Medicines Agency (EMA) [1, 2]. Pivotal trials for licensure were designed to have the power to show efficacy, with reasonably narrow confidence intervals, over the entire course of the trial. They were not designed to have the power to compare efficacy over shorter intervals within the formal trial, or thereafter. With the possibility of concomitant administration of HPV vaccine and other licensed childhood vaccines [3] and the emergence of second-generation vaccines [4], the initial focus on evaluating efficacy and safety for a new vaccine is likely to be expanded to also consider issues of durability of protection when comparing vaccines.

5.2 Durability of protection

Trials designed to study protection over longer periods of time or to compare shorter intervals within an efficacy trial (e.g. comparing the first and last year of the trial) would obviously need to be more extensive than trials designed to demonstrate significant efficacy over the entire course of a trial of relatively short duration. The expense of such trials might be prohibitive, depending on the end-point that is chosen to evaluate efficacy. The net effect could be the abandonment of, or at least a delay in the introduction of, potentially more effective or less expensive vaccine candidates or vaccination protocols, to the detriment of public health [4]. Conversely, licensure of a vaccine that ultimately proves to have only a short duration of protection might lead to many vaccinees losing protective immunity over time and consequently requiring periodic booster doses or new vaccination with a vaccine that confers long-term protection. There is good evidence that the current virus-like particle (VLP) vaccines induce memory B cells that can generate potent anamnestic antibody responses to a booster dose [5, 6]. However, a requirement for booster doses after the initial immunization series to maintain protection would substantially increase the cost and complexity of a vaccination programme.

The phase III efficacy trials that led to licensure of Gardasil and Cervarix for prevention of cervical cancer and other genital neoplasia in young women were 4 years in duration [7]. However, both vaccines were licensed based on interim analyses of shorter duration, with the proviso that the trials would be completed. In up to 4 years of additional follow-up of the trial subjects, no increase in breakthrough infections [8, 9] or incident cervical precancers [10] by the vaccine-targeted types has been detected. Formal analyses to determine the degree of protection for specific time intervals during follow-up have in some instances been hampered by insufficient power and crossover vaccination of subjects who initially received placebo control. However, in the Nordic countries, population-based, concomitant enrolment of vaccinated (with HPV vaccine or control vaccine) and unvaccinated cohorts in 2002–2005 has resulted in passive cancer registry-based follow-up of the sizable phase III trial cohorts [10]. The pivotal

phase III trial in young men that led to FDA licensure of Gardasil for prevention of external genital lesions, anal intraepithelial neoplasia, and anal cancer was 3 years in duration [11, 12].

Both Gardasil and Cervarix have demonstrated significant cross-protection against HPV types not included in the vaccines. However, as the protections seen have only been partial and as the cross-neutralizing antibody levels induced have been much lower (typically more than an order of magnitude less than the antibody levels induced against the HPV vaccine types) [13], the issue of duration of protection is particularly relevant when comparing the cross-protective ability of different vaccines. The extent and duration of cross-protection does affect the cost-effectiveness estimations of programmes. Because of the lower antibody levels and incomplete degrees of protection seen in cross-protection analyses, cross-protection may be particularly interesting to analyse as an early surrogate end-point of duration of protection.

Although no immune surrogate marker of protection has been defined in clinical trials, the results of animal studies, particularly passive transfer of protection in VLP immune sera, make it very likely that protection after Gardasil or Cervarix vaccination is antibody-mediated [6]. A biphasic decrease in antibody titres is well documented for both vaccines, using several different in vitro assays, including those that assess VLP binding and pseudovirus neutralization. There is a relatively rapid, approximately 10-fold, decrease in antibody levels over the first 2 years, followed by a plateauing of the levels. Thereafter, the antibody levels have essentially remained unchanged, or decreased very slowly, in most vaccinees [14]. Constant antibody levels once a plateau phase is reached have been observed for almost 10 years since vaccination [9]. Antibody levels that plateau in response to other vaccines (mostly live attenuated viral) or natural virus infections are often maintained for decades, or even lifelong [15]. Based on these data and our current understanding of the biology of antibody-producing plasma cells, it is reasonable to anticipate that antibody levels after the primary vaccination series with Gardasil or Cervarix will continue to be durable. Thus, the immunogenicity analyses, coupled with the low number of breakthrough infections and disease in the trial subjects that continue to be followed, have led to an optimistic projection for the durability of protection by the current licensed vaccines. Linkages between cancer and maternity cohort registries may soon yielding confirmatory data from women vaccinated as HPV-naive adolescents [16].

It is unclear whether durable antibody responses and protection will be induced by other vaccine candidates, or with the current vaccines in other (especially immunosuppressed) populations or using alternative vaccination protocols. Stabilization of antibody levels is not routinely observed in immunocompetent humans after vaccination with simple subunit vaccines such as tetanus or diphtheria toxoids, and booster doses are frequently necessary [17]. The multivalent engagement of B-cell receptors by the repetitive epitopes on the VLPs of the current vaccine are thought to send especially strong signals to the interacting B cell that promotes the long-term survival of the resulting plasma cells [18]. It is possible that antibody levels will not stabilize after vaccination with HPV vaccine candidates that are not based on VLPs, such as L1 capsomeres or L2 fusion protein vaccines, which in turn might result in vaccines with shorter durations of efficacy.

5.3 Central questions

Given this background, we believe that the following are the central questions regarding licensure of future prophylactic HPV vaccine candidates from a perspective of durability of protection: (i) Is duration of protection a more critical issue for licensure of HPV vaccines than for other microbial vaccines where it hasn't been a primary consideration? (ii) Are post-licensure studies of long-term protection warranted to inform regulatory and/or public health decision-

makers? (iii) Is it feasible, or practical, to conduct trials that would reasonably assess duration of protection?

5.3.1 Is duration of protection a more critical issue for licensure of HPV vaccines than for other microbial vaccines where it hasn't been a primary consideration?

HPV prophylactic vaccines are not unique in terms of the importance of durability of protection. Other vaccine-preventable diseases, such as measles, mumps, polio, tetanus, and diphtheria, are concerns throughout life, and the vaccines to prevent them are routinely administered in childhood immunization programmes. Therefore, they also need to provide long-term protection, either from the initial immunization series or via booster doses. The duration of protection for these vaccines was determined long after they were licensed. Similarly, for more recently introduced vaccines (e.g. the meningococcal, rotavirus, and current HPV vaccines), the data that supported initial licensure did not include end-points on long-term protection; the duration of protection remains to be determined. Second-generation HPV vaccines should be held to similar regulatory standards.

Pivotal trials with virological or immunological primary end-points could potentially be of short duration, perhaps as short as 1–2 years. It should be noted that trials using placebo controls may no longer be considered ethical and so trials of second-generation vaccines will likely be using a trial design based on non-inferiority to an already licensed vaccine. It is reasonable to consider requiring that non-inferior protection and immunogenicity be demonstrated in a trial of at least a minimum duration, for example 4 years, to conform to the phase III trials of the licensed vaccines. However, such long trials might tax the resources of manufacturers, particularly those in developing countries, and could delay the implementation of potentially valuable vaccine programmes.

5.3.2 Are post-licensure studies of long-term protection warranted to inform regulatory and/or public health decision-makers?

From a public health perspective, it would be highly desirable to evaluate duration of protection of both novel and currently used vaccines or vaccination protocols for longer periods in post-licensure studies. Such studies would provide important information to public health decision-makers on programme cost-effectiveness and programme design, for example on whether to adopt new strategies or continue to use the current vaccines and protocols. However, it is important to consider that long-term protection from infection and disease will reflect both the individual's immune status and "herd immunity" in the population. Herd effects can substantially reduce risk of infection in individuals whose own immunity has waned below protective levels (or who remain unvaccinated). Thus, long-term effectiveness in a vaccination programme with high coverage rates might substantially exceed the duration of efficacy in a clinical trial. A vaccine that facilitates high coverage might, in some circumstances, be attractive even if it has a shorter, or uncertain, duration of protection.

Nevertheless, companies should be encouraged to develop plans for long-term follow-up in their phase III study designs. Ideally, this would be accomplished in collaboration with regulatory authorities to establish pre-specified end-points that would support updates to the product's label to describe the data. A particularly important consideration is the plan to follow subjects who participate in the initial pivotal studies. For example, being able to recruit adequate numbers of such subjects would facilitate the conduct of a follow-up study in which subjects are re-randomized to booster or control. In addition, independent phase IV studies of long-term effectiveness should be considered/encouraged. However, the interpretation of all such trials

might be complicated by herd immunity if there is substantial uptake of the current vaccines in the study population.

5.3.3 Is it feasible, or practical, to conduct trials that would reasonably assess duration of protection?

It might be feasible to assess durability of protection in a controlled clinical trial, at least over a limited number of years. However, the practicality of assessing this question will depend on many factors, particularly efficacy end-point and basic trial design. Assessing protection from incident cervical infection by the vaccine-targeted types in aggregate over the last year of a 4-year trial would be much more feasible than assessing protection against cervical intraepithelial neoplasia of grade 2 or higher (CIN2+) against individual types over this time period, because there would be many more events. When appropriate end-points are considered, the fact that demonstrating non-inferiority to a licensed vaccine in the later years of a trial would require more subjects than demonstrating significant efficacy in a placebo-controlled trial over the same time period should be taken into account.

5.4 Conclusions

Although the pivotal trials of both currently licensed vaccines have reported on both virological and immunogenicity end-points, the end-points used for licensure have so far been clinical end-points [7]. The trial databases clearly demonstrate that protection against HPV infection is strongly correlated with subsequent protection against disease caused by HPV. Use of infection as an end-point for trials has several advantages for assessing duration of vaccine efficacy, including a smaller number of subjects and no ambiguity when attributing a case of disease to a specific infection. However, a major obstacle for the use of both virological and immunogenicity end-points is the fact that international criteria for evaluating performance of virological and immunogenicity assays are not fully established. WHO initiated work towards establishing such international criteria in 2006. Currently, there is a WHO HPV laboratory manual that describes the required quality assurance. There also exist International Standards (IS) and International Units (IU) for HPV 16 and 18 DNA and for HPV 16 antibodies. Candidate standards for HPV 31, 33, 35, 52, 56, and 58 DNA and for HPV 18 antibodies, as well as regularly issued international proficiency panels for HPV DNA genotyping, are also available [19]. However, the work towards a set of internationally recognized standards that would allow the routine use of virological and immunogenicity end-points in the development of second-generation vaccines, as well as for evaluating new administration schemes and programme strategies, has unfortunately not been completed.

The analysis of antibody responses might be used to help predict the duration of efficacy of VLP-based vaccines. Since antibody levels stabilize in the 18–24-month range after VLP vaccination, it should be possible to determine whether alternative vaccine candidates induce similar responses in trials of 3–4 years' duration, provided that sera are sampled at sufficient frequency, for example every 6 months. If efficacy for a virological or disease end-point was demonstrated in years 3–4, and antibody titres had stabilized, then there would be a strong likelihood that protection would persist.

It would be difficult to predict the likelihood of long-term protection if antibody levels continued to decline over time, since the minimum level of serum antibodies needed for protection is unknown and, at least for L1 antibodies, the protective level may be less than can be reproducibly measured with current in vitro assays [20]. A complicating factor is that it is uncertain whether an anamnestic antibody response induced by exposure to the virus can

provide protection from clinical disease [14]. It therefore remains possible, although in our opinion unlikely, that the presence of vaccine-specific memory B cells will provide a better measure of long-term protection than circulating antibody levels. The quantitative relationship between L2 vaccine-induced antibodies and protection from clinical disease or infection is not understood and so, at present, L2 antibody levels cannot be used to predict duration of protection.

References

1. Baylor NW, Marshall VB (2013). Regulation and testing of vaccines. In: Plotkin SA, Orenstein WA, Offit PA, editors. *Vaccines*, 6th ed. Philadelphia: Saunders Elsevier, pp. 1427–46.
2. Ehmann F, Kurz X, Cavaleri M, Arlett PR (2013). Regulation of vaccines in Europe. In: Plotkin SA, Orenstein WA, Offit PA, editors. *Vaccines*, 6th ed. Philadelphia: Saunders Elsevier, pp. 1447–53.
3. Vesikari T, Van Damme P, Lindblad N, Pfletschinger U, Radley D, Ryan D *et al.* (2010). An open-label, randomized, multicenter study of the safety, tolerability, and immunogenicity of quadrivalent human papillomavirus (types 6/11/16/18) vaccine given concomitantly with diphtheria, tetanus, pertussis, and poliomyelitis vaccine in healthy adolescents 11 to 17 years of age. *Pediatr Infect Dis J*. 29(4):314–8. http://dx.doi.org/10.1097/INF.0b013e3181c177fb PMID:19952980
4. Gersch ED, Gissmann L, Garcea RL (2012). New approaches to prophylactic human papillomavirus vaccines for cervical cancer prevention. *Antivir Ther*. 17(3):425–34. http://dx.doi.org/10.3851/IMP1941 PMID:22293302
5. Olsson SE, Villa LL, Costa RLR, Petta CA, Andrade RP, Malm C *et al.* (2007). Induction of immune memory following administration of a prophylactic quadrivalent human papillomavirus (HPV) types 6/11/16/18 L1 virus-like particle (VLP) vaccine. *Vaccine*. 25(26):4931–9. http://dx.doi.org/10.1016/j.vaccine.2007.03.049 PMID:17499406
6. Stanley M, Pinto LA, Trimble C (2012). Human papillomavirus vaccines – immune responses. *Vaccine*. 30 Suppl 5:F83–7. http://dx.doi.org/10.1016/j.vaccine.2012.04.106 PMID:23199968
7. Schiller JT, Castellsagué X, Garland SM (2012). A review of clinical trials of human papillomavirus prophylactic vaccines. *Vaccine*. 30 Suppl 5:F123–38. http://dx.doi.org/10.1016/j.vaccine.2012.04.108 PMID:23199956
8. Romanowski B (2011). Long term protection against cervical infection with the human papillomavirus: review of currently available vaccines. *Hum Vaccin*. 7(2):161–9. http://dx.doi.org/10.4161/hv.7.2.13690 PMID:21307652
9. Roteli-Martins CM, Naud P, De Borba P, Teixeira JC, De Carvalho NS, Zahaf T *et al.* (2012). Sustained immunogenicity and efficacy of the HPV-16/18 AS04-adjuvanted vaccine: up to 8.4 years of follow-up. *Hum Vaccin Immunother*. 8(3):390–7. http://dx.doi.org/10.4161/hv.18865 PMID:22327492
10. Rana MM, Huhtala H, Apter D, Eriksson T, Luostarinen T, Natunen K *et al.* (2013). Understanding long-term protection of human papillomavirus vaccination against cervical carcinoma: cancer registry-based follow-up. *Int J Cancer*. 132(12):2833–8. http://dx.doi.org/10.1002/ijc.27971 PMID:23180157
11. Giuliano AR, Palefsky JM, Goldstone S, Moreira ED Jr, Penny ME, Aranda C *et al.* (2011). Efficacy of quadrivalent HPV vaccine against HPV infection and disease in males. *N Engl J Med*. 364(5):401–11. http://dx.doi.org/10.1056/NEJMoa0909537 PMID:21288094
12. Palefsky JM, Giuliano AR, Goldstone S, Moreira ED Jr, Aranda C, Jessen H *et al.* (2011). HPV vaccine against anal HPV infection and anal intraepithelial neoplasia. *N Engl J Med*. 365(17):1576–85. http://dx.doi.org/10.1056/NEJMoa1010971 PMID:22029979

13. Draper E, Bissett SL, Howell-Jones R, Edwards D, Munslow G, Soldan K *et al.* (2011). Neutralization of non-vaccine human papillomavirus pseudoviruses from the A7 and A9 species groups by bivalent HPV vaccine sera. *Vaccine*. 29(47):8585–90. http://dx.doi.org/10.1016/j.vaccine.2011.09.021 PMID:21939712
14. Stanley M (2010). Potential mechanisms for HPV vaccine-induced long-term protection. *Gynecol Oncol*. 118(1) Suppl:S2–7. http://dx.doi.org/10.1016/j.ygyno.2010.04.002 PMID:20494220
15. Amanna IJ, Slifka MK (2010). Mechanisms that determine plasma cell lifespan and the duration of humoral immunity. *Immunol Rev*. 236(1):125–38. http://dx.doi.org/10.1111/j.1600-065X.2010.00912.x PMID:20636813
16. Lehtinen M, Herrero R, Mayaud P, Barnabas R, Dillner J, Paavonen J *et al.* (2006). Chapter 28: Studies to assess the long-term efficacy and effectiveness of HPV vaccination in developed and developing countries. *Vaccine*. 24 Suppl 3:233–41. http://dx.doi.org/10.1016/j.vaccine.2006.05.109 PMID:16950012
17. Amanna IJ, Carlson NE, Slifka MK (2007). Duration of humoral immunity to common viral and vaccine antigens. *N Engl J Med*. 357(19):1903–15. http://dx.doi.org/10.1056/NEJMoa066092 PMID:17989383
18. Bachmann MF, Jennings GT (2010). Vaccine delivery: a matter of size, geometry, kinetics and molecular patterns. *Nat Rev Immunol*. 10(11):787–96. http://dx.doi.org/10.1038/nri2868 PMID:20948547
19. Eklund C, Forslund O, Wallin K-L, Zhou T, Dillner J; WHO Human Papillomavirus Laboratory Network (2012). The 2010 global proficiency study of human papillomavirus genotyping in vaccinology. *J Clin Microbiol*. 50(7):2289–98. http://dx.doi.org/10.1128/JCM.00840-12 PMID:22535980
20. Longet S, Schiller JT, Bobst M, Jichlinski P, Nardelli-Haefliger D (2011). A murine genital-challenge model is a sensitive measure of protective antibodies against human papillomavirus infection. *J Virol*. 85(24):13253–9. http://dx.doi.org/10.1128/JVI.06093-11 PMID:21976653

Chapter 6. HPV VLP vaccines – alternative dosage schedules and immunization in immunosuppressed subjects

Margaret Stanley and Cosette Wheeler

6.1 HPV vaccines

Human papillomavirus (HPV) vaccines are subunit vaccines consisting of virus-like particles (VLPs) made of only one protein – the major HPV coat or capsid protein L1. HPV VLPs are made using sophisticated recombinant technology in which the L1 gene is expressed in recombinant yeast or baculovirus vectors. The chemistry of the expressed protein is such that it spontaneously assembles into VLPs that are morphologically and, more importantly, immunologically similar to the native virus but lack DNA and are therefore non-infectious.

Two prophylactic HPV VLP vaccines have been developed: Cervarix, a bivalent HPV 16/18 (bHPV) product from GlaxoSmithKline, and Gardasil (also known as Silgard), a quadrivalent HPV 6/11/16/18 (qHPV) product from Merck. Both vaccines have undergone large phase III double-blind placebo-controlled randomized trials that have demonstrated remarkable efficacy in individuals naive for the HPV types in the relevant vaccines at trial entry and after completion of the three-dose immunization regimen [1–5]. Follow-up studies of qHPV-vaccinated cohorts in the Nordic countries are still in progress and have extended so far for up to 7 years with no breakthrough cases of cervical intraepithelial neoplasia of grade 2 or 3 (CIN2/3) caused by vaccine HPV types [6].

6.2 HPV vaccination programmes

HPV vaccination programmes have now been introduced in many countries. These programmes target adolescent girls aged 9–15 years, with or without catch-up for older adolescents and young women. Genital HPV is a sexually transmitted infection with peak incidence occurring just after the onset of sexual activity [7]. To achieve optimal vaccine effectiveness, immunization ideally should be completed before the start of sexual activity, and therefore the target group for immunization is perimenarchal girls. In view of the overwhelming data on efficacy from the randomized controlled trials and the emerging data on population effectiveness, the debates about the current vaccines are no longer about efficacy but rather about implementation, access, and affordability.

6.3 Alternative dosing schedules: modified three-dose schedules

Flexibility in the dosing schedules could be important for national vaccination policies. The current schedules of 0, 1 or 2, and 6 months are, in immunological terms, prime-prime-boost schedules. Such schedules in which the immune system is challenged with optimal antigen dose at two time intervals close together – priming – and then again with a third dose after an extended time interval – boosting – are designed to generate optimal immune responses in terms of both antibody concentration and long-term immune memory. Rigid adherence to these schedules may be impractical in certain locations, and modifications of the schedules have been assessed for immunogenicity and reactogenicity in several studies. An open-label cluster randomized non-inferiority study conducted between October 2007 and January 2010 assessed four schedules of the qHPV vaccine Gardasil in girls aged 11–13 years in Viet Nam [8, 9]. Vaccines were delivered to 903 girls via schools using either the standard schedule of 0, 2, and 6 months or an alternative schedule of 0, 3, and 9 months, 0, 6, and 12 months, or 0, 12, and

24 months. Serum neutralizing type-specific HPV antibody titres were measured with the competitive Luminex immunoassay (cLIA) at 1 month and 2 years after the third and final vaccine dose. The outcome measure was non-inferiority of the alternative regimens against the standard schedule if the lower bound of the multiplicity-adjusted confidence interval of the type-specific geometric mean titre (GMT) ratio for HPV 16 and 18 was > 0.5. At 1 month after dose 3, the non-inferiority criterion was met for the schedules of 0, 3, and 9 months and 0, 6, and 12 months but not for the schedule of 0, 12, and 24 months. However, when GMTs were measured at 2 years after dose 3, all three alternative schedules met the non-inferiority criterion. Interestingly, the data gathered indicated that two doses of HPV vaccine delivered at 0 and 12 months might afford similar protection to three doses delivered at 0, 6, and 12 months.

Non-inferiority of the immune response to the qHPV vaccine administered as a modified regimen of 0, 2, and 12 months was assessed in 200 college women in the USA aged 18–23 years [10]. Type-specific neutralizing antibody was measured using the cLIA at 2–6 weeks after dose 3. GMTs achieved in the modified schedule were non-inferior to those in the standard schedule of 0, 2, and 6 months. In another study, 200 female sex workers in Peru were randomized to receive the qHPV vaccine in the standard regimen of 0, 2, and 6 months and a modified regimen of 0, 3, and 6 months [11]. GMTs measured by cLIA 1 month after dose 3 were non-inferior in the alternative schedule. As might be expected in this cohort, baseline seropositive rates were high and GMTs after vaccination for both schedules were higher in the baseline seropositive subjects than in the seronegative subjects.

Immunogenicity and safety of the bHPV vaccine Cervarix when administered at 0, 1, and 12 months compared with 0, 1, and 6 months in women aged 15–25 years were evaluated in a multicentre randomized study in Europe [12]. GMTs measured by enzyme-linked immunosorbent assay (ELISA) 1 month after dose 3 were non-inferior in the modified schedule compared with the standard schedule. Data from all reported studies show that the immunization schedule for these vaccines is flexible and that extending the interval between the second and third doses does not adversely affect the antibody response in women.

6.4 Alternative dosing schedules: reduction from three to two doses

In the context of access and affordability, discussion of alternative dosing schedules in the young adolescent cohort, from the licensed prime-prime-boost schedules of three doses over 6 months to a prime-boost schedule of two doses over 6 months, is now a key issue. It is argued that there is a compelling public health case for considering alternatives to the three-dose regimen. The vast burden of HPV-associated malignant and indeed benign disease is in developing countries without effective screening programmes and with poor access to medical services [13]. There is the view that there are significant logistical challenges in administering three doses over 6 months to young adolescents and young women since in most countries there is no infrastructure for vaccination of adolescents. Even in developed countries, with notable exceptions where school programmes have been implemented [14], immunization programmes do not successfully deliver the full three-dose regimen, and vaccination coverage varies hugely [15].

VLP vaccines, even at discounted cost, are beyond the health budget for a large number of developing countries that are not eligible for support from the GAVI Alliance. A case could be made that with a two-dose regimen, implementation of HPV vaccination in such countries would be programmatically easier and faster, would achieve higher coverage, and would be a more effective use of the available resources. The reduction in costs achieved by implementing a two-dose schedule in terms of administration and vaccine price make this a hugely attractive option

to public health authorities, but the key question concerns the robustness of the evidence base for a change from the licensed schedule.

6.5 Vaccine immunogenicity: licensed three-dose regimens

At present, the assumption is that the major basis for the protection afforded by the VLP vaccines is neutralizing antibody, although other mechanisms cannot be ruled out [16]. This assumption is supported by animal models that demonstrate protection against viral challenge in animals immunized by passive transfer of antibody from VLP-immunized individuals [17–19]. The licensed administration schedules for the two vaccines include three doses delivered by intramuscular injection at 0, 2, and 6 months for the qHPV vaccine and at 0, 1, and 6 months for the bHPV vaccine. In the pivotal randomized controlled trials FUTURE I and II for the qHPV vaccine [20] and PATRICIA for the bHPV vaccine [21], virtually all subjects (women aged 15–26 years) seroconverted. This is in contrast to natural infection, in which seroconversion can be shown in only 50–70% of women with an incident HPV infection [22]. GMTs of HPV type-specific antibody at 1 month after the third vaccine dose (month 7) were 2–4 logs greater than those measured in natural infection. Furthermore, after 18 months GMTs remain 10-fold higher than those recorded with natural infection, and these levels appear to be preserved over time [23, 24].

Non-inferiority immunogenicity bridging studies have been conducted for both vaccines. For the qHPV vaccine, these studies were conducted in boys and girls aged 9–15 years with the objective of bridging the efficacy findings in young women to pre-adolescents and adolescents. GMTs of HPV-specific antibody were non-inferior in adolescents and 1.7–2.7-fold higher than in the efficacy group of women aged 16–23 years [25]. Preliminary data from the adolescent extension study of these cohorts (P018) at 6 years shows no breakthrough cases of infection or disease related to vaccine HPV types 6/11/16/18 in the per-protocol population of girls (Alfred Saah, personal communication). There were no breakthrough cases of infection related to HPV types 6/11/16/18 reported in boys. Disease end-points are not yet available in boys at this interim analysis. In an immunobridging study, the bHPV vaccine induced GMTs in girls aged 10–14 years that were 2.1–2.5-fold higher than those induced in women aged 15–25 years [26].

The robust antibody response after HPV VLP immunization is attributed to the route of immunization. Natural HPV infections are exclusively intraepithelial. There is no viraemia as far as is known. Virus is shed sporadically from mucosal surfaces. In consequence, virus particles and capsid protein have limited access to lymphoid organs (draining lymph nodes and spleen) and systemic antibody and cellular immune responses are weak. In contrast, VLPs are delivered intramuscularly, allowing rapid access of antigen to vascular channels and lymphatics and thus to lymph nodes and spleen. To date, there is no immune correlate for vaccine-induced protection against infection or disease [15]. The minimal level of antibody needed for such protection, the long-term durability of neutralizing antibody, and the role of B-cell memory if antibody wanes have yet to be established in vaccinated subjects.

6.6 Randomized controlled trials of alternative dosage schedules

The robust immunogenicity of VLP vaccines and the higher responses in young adolescents, together with potential cost savings, have evoked intense interest in testing the notion that alternative dosing regimens could be efficacious in young adolescents.

6.6.1 Bivalent vaccine

Immunogenicity and safety of the bHPV vaccine administered as a two-dose schedule compared with the licensed three-dose schedule has been assessed in a partially blind controlled randomized trial (NCT00541970) sponsored by the manufacturer. In this study, in addition to the licensed formulation of 20 μg of each antigen (20/20F), an alternative formulation of 40 μg of each antigen (40/40F) was delivered. The subjects were 961 healthy young women stratified by age (9–14, 15–19, or 20–25 years) and randomized to receive two doses of the licensed vaccine 20/20F at 0 and 6 months, two doses of the alternative formulation 40/40F at 0 and 6 months, two doses of the alternative formulation 40/40F at 0 and 2 months, or three doses of the licensed vaccine 20/20F at 0, 1, and 6 months.

The study objectives were to evaluate immunogenicity and reactogenicity of each two-dose schedule compared with the three-dose regimen. The three-dose regimen would be considered to be superior to a two-dose schedule if the lower bound of the 95% confidence interval around the GMT ratio was < 0.5. For both HPV 16 and 18, at months 7, 24, and 48 the two-dose schedule in girls aged 9–14 years was non-inferior to the three-dose schedule in women aged 15–25 years in whom efficacy had been demonstrated [27].

Immunogenicity and safety of the bHPV vaccine when administered according to alternative two-dose schedules (0 and 6 months and 0 and 12 months) in healthy girls aged 9–14 years compared with the standard three-dose schedule (0, 1, and 6 months) in women aged 15–25 years is being assessed in a current trial (NCT01381575).

6.6.2 Quadrivalent vaccine

At present, there are two randomized controlled trials assessing immunogenicity or immunogenicity and effectiveness of two doses compared with three doses of the qHPV vaccine in adolescent cohorts. The manufacturer has donated vaccine to and/or performs antibody testing for these trials but is not a sponsor.

6.6.2.1 British Columbia GOV01 trial

This two-dose versus three-dose vaccine study was a phase III post-licensure randomized controlled multicentre study (NCT00501137) in three Canadian provinces – British Columbia, Quebec, and Nova Scotia – with three parallel groups in two age strata receiving open-label qHPV vaccine: girls aged 9–13 years receiving two doses of vaccine, at 0 and 6 months; girls aged 9–13 years receiving three doses of vaccine, at 0, 2, and 6 months; and women aged 16–26 years receiving three doses of vaccine, at 0, 2, and 6 months.

The study objectives were to determine whether the antibody responses to HPV 16 and 18 were non-inferior at month 7 after a two-dose regimen compared with a three-dose regimen. Primary end-points of the study were differences in GMT of antibodies to HPV 16 and 18 at month 7 between the three arms. Non-inferiority was declared if the lower bounds of the adjusted 95% confidence interval of GMT ratios for HPV 16 and 18 were > 0.5. Secondary outcomes were GMTs for HPV 6 and 11 at 7 months, seroconversion rates, and specific B-cell and T-cell memory responses at 0 and 7 months. Two sero-assays were used to measure neutralizing antibody concentration. These were a pseudovirion1 neutralizing assay that measures all neutralizing antibodies for each vaccine HPV VLP [28] and a proprietary cLIA (Merck cLIA) that measures a single type-specific neutralizing epitope for each of the vaccine HPV types [29].

Non-inferiority as assessed only at month 7 may not be informative when long-term duration of protection is the crucial issue, and this study was extended. At 7, 18, and 24 months after a two-dose regimen in girls aged 9–13 years, HPV 16, 18, 6, and 11 antibody responses using Merck cLIA were non-inferior compared with the three-dose regimens in young adult women and girls aged 9–13 years. There was 100% seroconversion in all groups at month 7. At 36 months, HPV 16, 18, 6, and 11 antibody responses using Merck cLIA in both two-dose and three-dose regimens in girls aged 9–13 years remained non-inferior to the three-dose regimen in young adult women. In girls aged 9–13 years, the two-dose regimen compared with the three-dose regimen was non-inferior for HPV 16 and 11, but for HPV 6 or 18 the lower bounds of the 95% confidence interval were < 0.5 [30].

6.6.2.2 WHO/IARC multicentre randomized trial of two versus three doses of the quadrivalent vaccine in India

A multicentre cluster-randomized trial to evaluate comparative efficacy of two versus three doses of HPV vaccine in preventing persistent HPV infection in cervical neoplasia was initiated in India in September 2009 (principal investigator, Rengaswamy Sankaranarayanan; http://screening.iarc.fr/hpvvaccine.php), supported by WHO/IARC and the Bill & Melinda Gates Foundation. This trial aimed to recruit 20 000 unmarried girls aged 10–18 years from different regions in India, randomized to groups of 10 000 girls each to receive either two doses of qHPV vaccine, at 0 and 6 months, or three doses, at 0, 2, and 6 months. All girls were to be followed up during the 5 years of the project to document outcomes with follow-up visits at months 12, 24, 36, and 48. Blood was to be collected from all girls at month 7 and from a cohort of 15% of girls at months 12, 24, 36, and 48 to evaluate seroconversion and HPV 16 and 18 antibody titre. Cervical cells were to be collected for HPV testing and typing from the participants 6 months after delivery of the first child or after 18 months of marriage and once annually thereafter.

Regrettably, progress in this trial has been impeded after vaccination in all clinical trials of HPV vaccination in India was suspended on 8 April 2010 by the Director-General of the Indian Council of Medical Research. The trials were suspended while the Indian authorities investigated reports of deaths of girls participating in a separate trial sponsored by the nongovernmental organization PATH. After consideration, these deaths were determined to be coincidental to, and not caused by, vaccination. Despite no causal link being identified, the authorities remained cautious. A large proportion of the subjects in the WHO/IARC trial had already been enrolled and vaccinated with at least one dose when the trial was halted. Vaccination courses in many subjects could not be completed, and vaccination schedules were disrupted, with second or third doses given outside the 2-month or 6-month window in the different schedules.

However, vaccinated subjects are being followed up and serological data from this trial have been presented at medical meetings (Rengaswamy Sankaranarayanan, personal communication). In those subjects who completed vaccination per protocol, immunogenicity of two doses was non-inferior to the three-dose schedule at month 7 and month 18 after the first dose. The immunogenicity of the three-dose (days 1, 60, and 180–800), two-dose (days 1 and 180), and two-dose (days 1 and 180–800) schedules were non-inferior to the per-protocol three-dose schedule (days 1, 60, and 180). Immunogenicity of a single dose (girls who missed further doses) was inferior to that of two doses at days 1 and 60 (girls who missed the third dose at day 180) at 12 months and 18 months after the first dose. In the per-protocol groups (two-dose and three-dose), immunogenicity of girls aged 10–14 years was non-inferior to that of those aged 15–18 years.

6.7 Efficacy of two-dose versus three-dose schedules

At present, there are no data on vaccine effectiveness either for infection or disease in the adolescent two-dose and three-dose cohorts in any of the trials for either the qHPV or bHPV vaccine. These cohorts are being prospectively followed. The only evaluation of efficacy for fewer than three doses has been reported in adult women for the Costa Rica Vaccine Trial using the bHPV vaccine [31]. In this trial, women (aged 18–25 years) were randomly assigned to receive three doses of vaccine at 0, 1, and 6 months, but 20% received fewer than three doses. Analysis of the clinical efficacy data at 48 months provides suggestive evidence that two doses or even one dose is efficacious in the prevention of incident HPV 16 and 18 infections that persist for 12 months or more. Unexpectedly, protection against infection was 100% with one dose (n = 196 HPV vaccine vs n = 188 control vaccine), 84% with two doses (n = 422 HPV vs n = 380 control), and 81% with three doses (n = 2957 HPV vs n = 3010 control). These data are intriguing, but the study was not designed to analyse clinical efficacy of schedules of fewer than three doses, and those receiving fewer than three doses did so overwhelmingly because of pregnancy and/or referral to colposcopy and thus represent a selected population. Interestingly, in this study although cross-protection against HPV 31, 33, and 45 incident infection over 1 year could be shown in the three-dose group, this was not observed in the two-dose group, suggesting differences in the antibody species generated after two compared with three doses. However, the numbers are small and the results of this study should not be overinterpreted.

6.8 Global experience: use of off-licence dosing schedules

An alternative, off-licence dosing regimen for HPV vaccines is now being implemented in several countries for young adolescent age groups. Some examples are given here.

In Mexico, for girls aged 9 years, an extended three-dose schedule, at 0, 6, and 60 months, is being used [15]. In Canada, the province of Quebec has an amended three-dose schedule for schoolgirls: in grade 4, at age 9 years (doses 1 and 2), and in the third year of secondary school, at age 14 years (dose 3) [32], and the province of British Columbia commenced with a schedule of 0, 6, and 60 months for schoolgirls but recently switched to a schedule of 0 and 6 months.

In Switzerland, the Swiss Federal Vaccination Commission and the Swiss Federal Office of Public Health recommend two doses at an interval of 4–6 months for girls aged 11–14 years, where the first dose is administered before the 15th birthday. A third dose could be used for a subsequent booster if this should prove necessary. This decision was based on the assumptions (i) that age, and not number of doses, is the main driver of immunoglobulin G responses and plasma cell and memory B cell formation, and (ii) that two doses at an interval of 6 months would therefore provide similar protection to the licensed three-dose schedule [33]. However, no surveillance system has been implemented to date to identify recipients of two doses or three doses, in case of breakthrough disease; there could be a perception of vaccine failure, if the vaccine was not administered in accordance with the label. The vaccination schedule remains unchanged (three doses) for young girls and women from age 15 years.

6.9 Unresolved issues

An important question is whether the duration of protection provided by two doses of vaccine is equivalent to that provided by three doses in the adolescent cohorts. In adolescents who are immunized at age 12 or 13 years, protection will have to be maintained for the following 2–4 decades. Duration of protection is a key issue for public health bodies since the cost-effectiveness analyses [34] on which policy decisions have been based have used estimates of

the duration of protection based on the randomized controlled trials and the long-term follow-up studies currently in progress. At present, the duration of protection against disease (CIN2/3) provided by the three-dose schedule of the bHPV vaccine is up to 8.4 years [35], by the qHPV vaccine in women aged 16–23 years is up to 7 years [6], and by the HPV 16 L1 VLP of the qHPV vaccine is up to 9.5 years [36]. However, there is no immune correlate – no antibody concentration or any other measurable immune marker that correlates with protection against clinical disease.

The current evidence is that very low serum antibody concentrations are protective [19, 37], but the importance of antibody affinity and avidity in relation to this is not known. The classical prime-prime-boost schedule with the extended interval of several months between prime and boost is designed to generate high-affinity, high-avidity antibodies and a large memory B-cell pool [38]. The importance of avidity to antibody-mediated protection to viruses has been demonstrated in experimental systems [39], but there are limited data on avidity either in natural HPV infections [40, 41] or after vaccination [41–43]. The available serological assays provide only a partial characterization of the immune status in vaccinated individuals, and current understanding of this response is superficial. The kinetics of the response [44] and specific details – avidity, affinity, and epitope specificity, all of which are central to memory [45] – are either poorly known or not known [16].

6.10 Memory B cells

Since immune memory is central to the long-term protection afforded by vaccines, it is feasible that data on the circulating HPV-specific memory B cell (MBC) population after differing dosage schedules could be informative. In an early study using the bHPV vaccine, Giannini and colleagues [46] showed a significantly increased HPV 16 MBC population at month 7 after the third dose of vaccine compared with that after the second dose. HPV 18-specific MBCs were increased after the third dose, but this was not significant ($P > 0.05$). MBCs at month 7 and month 24 have been measured in the comparative immunogenicity study of Einstein and colleagues [47] and show comparable responses for HPV 16-specific MBCs for both vaccines but higher levels of HPV 18-specific MBCs generated by the bHPV vaccine. In a study using an unadjuvanted HPV 16 vaccine, antigen-specific MBCs measured at 7 months correlated with antibody concentration, but avidity was unrelated to either [44].

B-cell and T-cell memory responses measured by modified enzyme-linked immunosorbent spot (ELISPOT) assays at 0 and 7 months have been reported from the British Columbia GOV01 trial [48]. These preliminary data show that a higher percentage of HPV-specific MBCs at month 7 compared with month 0 was observed for each HPV subtype in all vaccine groups, but this was not statistically significant for HPV 18 in the group of girls aged 9–13 years receiving two doses. Significantly higher B-cell memory responses were observed in girls aged 9–13 years receiving three doses compared with the three-dose adult group. T-cell memory responses were dose-related. Both adult and paediatric three-dose groups had similar T-cell memory responses to each HPV type, but the group of girls aged 9–13 years receiving two doses had significantly lower responses to HPV 6, 16, and 18.

The B-cell ELISPOT methodology used in these studies is the same (with minor variations) and measures circulating B cells that have been activated and respond rapidly to the recall antigen [44]. In mice and humans there are several subsets of these cells, defined by surface markers, and their contribution to long-term memory remains to be defined [49]. Furthermore, there is considerable heterogeneity in the lifespan of circulating MBCs, with only a subpopulation destined to be long-lived [50]. The studies that have quantified HPV-specific MBCs after VLP

vaccination do not give any information on the longevity of these cells, the key determinant for long-term protection.

However, antibody “memory” is bipartite [51] and has two forms of MBCs circulating in the blood (discussed above) and serological memory – antibodies circulating in the blood derived from long-lived plasma cells residing in special niches in bone marrow and spleen [52]. Long-lived plasma cells are selected for the ability to secrete high-affinity antibody, a process that takes place in the germinal centre of lymph nodes as a result of somatic hypermutation and selection. This affinity maturation extends over several months after priming, and avidity maturation (the sum of the affinities of the vaccine-induced antibodies) is a measure of this process. There is only rudimentary information about avidity maturation with any dosage schedule for HPV VLP vaccine, with only the study of Dauner and colleagues [44] showing avidity maturation over time, indicating selection for high-affinity clones.

The current understanding of the immune response engendered after HPV VLP immunization is rudimentary. Antibody concentration is the only consistently measured immune parameter; the serological assays differ between trials, they are relatively insensitive, and there is no immune correlate of protection. The clinical development programme of the licensed HPV vaccines was based on clinical efficacy data for a three-dose prime-prime-boost regimen. This has led to the current indications for these vaccines approved by regulatory authorities around the world. The evidence from the clinical trials for two-dose prime-boost schedules in adolescents aged 9–13/14 years is restricted to immunogenicity but is consistent. In all studies, reported antibody concentrations after two doses (0 and 6 months) in adolescents aged 9–14 years were non-inferior to three doses (0, 1, and 6 months) in women aged 16–26 years, the group in which efficacy has been demonstrated. At present, there is no evidence from the trials in adolescents demonstrating clinical efficacy or duration of protection. There is no published evidence from any randomized controlled trial in women aged 15–26 years that shows clinical efficacy against disease for a two-dose schedule.

The adoption of an alternative to the licensed three-dose regimen in the absence of robust data on efficacy and duration of protection is a risk, and the question then arises: How much and how significant is the risk? If public health and regulatory authorities wish to adopt or recommend alternatives to the licensed three-dose regimens, they will need to address this question, make risk assessments based on the evidence, and devise risk management strategies for worst-case scenarios to minimize any impact on cancer prevention strategies and other immunization programmes.

6.11 Immunization schedules in immunosuppressed subjects

Small studies have been conducted in HIV-infected subjects to determine immunogenicity and safety of the licensed HPV vaccines. HIV-infected children aged 7–12 years with CD4% ≥ 15 and on stable antiretroviral therapy (ART) if the CD4% was ≤ 25 were randomized to receive placebo or the qHPV vaccine at 0, 2, and 6 months [53]. Antibodies were assayed by cLIA, and seroconversion to all four antigens occurred in > 96% of recipients with GMTs 27–262 times the cut-off levels. After a further 72 weeks, a subset of children were given a fourth dose of vaccine [54]. At the time of the fourth dose, type-specific antibodies to HPV 6, 11, and 16 were detected in > 94% and to HPV 18 in 76% of children. The fourth dose of vaccine induced an anamnestic response, with > 96% of recipients becoming seropositive for all vaccine HPV types. Kahn and colleagues [55] enrolled 99 HIV-infected women aged 16–23 years in a phase 2 open-label multicentre trial. Subjects were immunized with the qHPV vaccine at 0, 2, and 6 months. Seroconversion rates for all four vaccine antigens of 100% were shown in subjects on ART and

> 92% in non-ART subjects. GMTs for HPV 16 were lower in non-ART subjects. The qHPV vaccine has been evaluated in HIV-infected men and shown to be immunogenic and safe [56].

The safety and immunogenicity of the bHPV vaccine has been evaluated in 120 asymptomatic HIV-infected women aged 18–25 years in South Africa and a non-HIV-infected control group [57]. All subjects irrespective of HIV status seroconverted and remained seropositive at month 12 after dose 1. Overall, these studies show that HPV VLP vaccines are immunogenic and safe in HIV-infected subjects, but the duration of protection requires mature studies for evaluation.

Immunogenicity of the qHPV vaccine was evaluated in a cohort of young adult transplant recipients [58]. Seroconversion rates at 1 month after dose 3 for HPV 6, 11, 16, and 18 were 63%, 68%, 63%, and 52%, respectively. Reduced immunogenicity in this cohort correlated with short interval since transplant, lung transplantation, and tacrolimus levels, suggesting that the degree of immunosuppression is the important factor. Further studies are necessary to determine optimal immunization schedules for this patient group. Immunogenicity and safety of the bHPV vaccine has been evaluated in patients with juvenile systemic lupus erythematosus [59] and juvenile idiopathic arthritis [60]. In the group with juvenile idiopathic arthritis, all subjects seroconverted, but antibody responses were lower in patients than in controls. The kinetics of HPV 16/18 MBC responses were comparable between patients and controls, but the magnitude of B-cell responses at 7 and 12 months appeared lower in patients. No relevant differences in adverse events were found. HPV vaccination did not aggravate disease.

References

1. Lehtinen M, Paavonen J, Wheeler CM, Jaisamrarn U, Garland SM, Castellsagué X *et al.*; HPV PATRICIA Study Group (2012). Overall efficacy of HPV-16/18 AS04-adjuvanted vaccine against grade 3 or greater cervical intraepithelial neoplasia: 4-year end-of-study analysis of the randomised, double-blind PATRICIA trial. *Lancet Oncol*. 13(1):89–99. http://dx.doi.org/10.1016/S1470-2045(11)70286-8 PMID:22075171
2. Kjaer SK, Sigurdsson K, Iversen OE, Hernandez-Avila M, Wheeler CM, Perez G *et al.* (2009). A pooled analysis of continued prophylactic efficacy of quadrivalent human papillomavirus (types 6/11/16/18) vaccine against high-grade cervical and external genital lesions. *Cancer Prev Res (Phila)*. 2(10):868–78. http://dx.doi.org/10.1158/1940-6207.CAPR-09-0031 PMID:19789295
3. Dillner J, Kjaer SK, Wheeler CM, Sigurdsson K, Iversen OE, Hernandez-Avila M *et al.*; FUTURE I/II Study Group (2010). Four year efficacy of prophylactic human papillomavirus quadrivalent vaccine against low grade cervical, vulvar, and vaginal intraepithelial neoplasia and anogenital warts: randomised controlled trial. *BMJ*. 341:c3493. http://dx.doi.org/10.1136/bmj.c3493 PMID:20647284
4. Muñoz N, Manalastas R Jr, Pitisuttithum P, Tresukosol D, Monsonego J, Ault K *et al.* (2009). Safety, immunogenicity, and efficacy of quadrivalent human papillomavirus (types 6, 11, 16, 18) recombinant vaccine in women aged 24–45 years: a randomised, double-blind trial. *Lancet*. 373(9679):1949–57. http://dx.doi.org/10.1016/S0140-6736(09)60691-7 PMID:19493565
5. Palefsky JM, Giuliano AR, Goldstone S, Moreira ED Jr, Aranda C, Jessen H *et al.* (2011). HPV vaccine against anal HPV infection and anal intraepithelial neoplasia. *N Engl J Med*. 365(17):1576–85. http://dx.doi.org/10.1056/NEJMoa1010971 PMID:22029979
6. Krüger-Kjaer S, Nygård M, Dillner J, Marshall B, Hansen BT, Sigurdardottir LG *et al*. (2012). Long-term effectiveness of Gardasil™ in the Nordic countries. Abstract, 28th International Papillomavirus Conference, San Juan, Puerto Rico, 30 November–6 December 2012.

Available at hpv2012pr.org/HPV2012_PUERTO_RICO_Abstracts_Epidemiology_Public_Health.pdf.
7. Moscicki AB, Schiffman M, Kjaer S, Villa LL (2006). Chapter 5: Updating the natural history of HPV and anogenital cancer. *Vaccine*. 24 Suppl 3:42–51. http://dx.doi.org/10.1016/j.vaccine.2006.06.018 PMID:16950017
8. Neuzil KM, Canh G, Thiem VD, Janmohamed A, Huong VM, Tang Y *et al.* (2011). Immunogenicity and reactogenicity of alternative schedules of HPV vaccine in Vietnam: a cluster randomized noninferiority trial. *JAMA*. 305(14):1424–31. http://dx.doi.org/10.1001/jama.2011.407 PMID:21486975
9. Lamontagne DS, Thiem VD, Huong VM, Tang Y, Neuzil KM (2013). Immunogenicity of quadrivalent HPV vaccine among girls 11 to 13 years of age vaccinated using alternative dosing schedules: results 29 to 32 months after third dose. *J Infect Dis*. 208(8):1325–34. http://dx.doi.org/10.1093/infdis/jit363 PMID:23901077
10. Zimmerman RK, Nowalk MP, Lin CJ, Fox DE, Ko FS, Wettick E *et al.* (2010). Randomized trial of an alternate human papillomavirus vaccine administration schedule in college-aged women. *J Womens Health (Larchmt)*. 19(8):1441–7. http://dx.doi.org/10.1089/jwh.2009.1753 PMID:20629576
11. Brown B, Blas MM, Cabral A, Byraiah G, Guerra-Giraldez C, Sarabia-Vega V *et al.* (2012). Human papillomavirus prevalence, cervical abnormalities and risk factors among female sex workers in Lima, Peru. *Int J STD AIDS*. 23(4):242–7. http://dx.doi.org/10.1258/ijsa.2011.011193 PMID:22581946
12. Esposito S, Birlutiu V, Jarcuska P, Perino A, Man SC, Vladareanu R *et al.* (2011). Immunogenicity and safety of human papillomavirus-16/18 AS04-adjuvanted vaccine administered according to an alternative dosing schedule compared with the standard dosing schedule in healthy women aged 15 to 25 years: results from a randomized study. *Pediatr Infect Dis J*. 30(3):e49–55. http://dx.doi.org/10.1097/INF.0b013e318206c26e PMID:21273939
13. Arbyn M, Castellsagué X, de Sanjosé S, Bruni L, Saraiya M, Bray F *et al.* (2011). Worldwide burden of cervical cancer in 2008. *Ann Oncol*. 22(12):2675–86. http://dx.doi.org/10.1093/annonc/mdr015 PMID:21471563
14. Garland SM, Skinner SR, Brotherton JM (2011). Adolescent and young adult HPV vaccination in Australia: achievements and challenges. *Prev Med*. 53 Suppl 1:S29–35. http://dx.doi.org/10.1016/j.ypmed.2011.08.015 PMID:21962468
15. Hernandez-Avila M, Lazcano-Ponce E, Martinez-Montanez O (2011). Public policies for HPV screening and vaccination in Mexico. Abstract 0-04.04, 27th International Papillomavirus Conference and Clinical Workshop, Berlin, 17–22 September 2011.
16. Stanley M, Pinto LA, Trimble C (2012). Human papillomavirus vaccines – immune responses. *Vaccine*. 30 Suppl 5:F83–7. http://dx.doi.org/10.1016/j.vaccine.2012.04.106 PMID:23199968
17. Breitburd F, Kirnbauer R, Hubbert NL, Nonnenmacher B, Trin-Dinh-Desmarquet C, Orth G *et al.* (1995). Immunization with viruslike particles from cottontail rabbit papillomavirus (CRPV) can protect against experimental CRPV infection. *J Virol*. 69(6):3959–63. PMID:7745754
18. Suzich JA, Ghim SJ, Palmer-Hill FJ, White WI, Tamura JK, Bell JA *et al.* (1995). Systemic immunization with papillomavirus L1 protein completely prevents the development of viral mucosal papillomas. *Proc Natl Acad Sci U S A*. 92(25):11553–7. http://dx.doi.org/10.1073/pnas.92.25.11553 PMID:8524802
19. Day PM, Kines RC, Thompson CD, Jagu S, Roden RB, Lowy DR *et al.* (2010). In vivo mechanisms of vaccine-induced protection against HPV infection. *Cell Host Microbe*. 8(3):260–70. http://dx.doi.org/10.1016/j.chom.2010.08.003 PMID:20833377

20. Joura EA, Kjaer SK, Wheeler CM, Sigurdsson K, Iversen OE, Hernandez-Avila M *et al.* (2008). HPV antibody levels and clinical efficacy following administration of a prophylactic quadrivalent HPV vaccine. *Vaccine*. 26(52):6844–51. http://dx.doi.org/10.1016/j.vaccine.2008.09.073 PMID:18930097
21. Harper DM, Franco EL, Wheeler CM, Moscicki AB, Romanowski B, Roteli-Martins CM *et al.*; HPV Vaccine Study Group (2006). Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomised control trial. *Lancet*. 367(9518):1247–55. http://dx.doi.org/10.1016/S0140-6736(06)68439-0 PMID:16631880
22. Carter JJ, Madeleine MM, Shera K, Schwartz SM, Cushing-Haugen KL, Wipf GC *et al.* (2001). Human papillomavirus 16 and 18 L1 serology compared across anogenital cancer sites. *Cancer Res*. 61(5):1934–40. PMID:11280749
23. Villa LL, Ault KA, Giuliano AR, Costa RL, Petta CA, Andrade RP *et al.* (2006). Immunologic responses following administration of a vaccine targeting human papillomavirus types 6, 11, 16, and 18. *Vaccine*. 24(27–28):5571–83. http://dx.doi.org/10.1016/j.vaccine.2006.04.068 PMID:16753240
24. Schwarz TF, Spaczynski M, Schneider A, Wysocki J, Galaj A, Schulze K *et al.* (2011). Persistence of immune response to HPV-16/18 AS04-adjuvanted cervical cancer vaccine in women aged 15–55 years. *Hum Vaccin*. 7(9):958–65. http://dx.doi.org/10.4161/hv.7.9.15999 PMID:21892005
25. Block SL, Nolan T, Sattler C, Barr E, Giacoletti KE, Marchant CD *et al.*; Protocol 016 Study Group (2006). Comparison of the immunogenicity and reactogenicity of a prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in male and female adolescents and young adult women. *Pediatrics*. 118(5):2135–45. http://dx.doi.org/10.1542/peds.2006-0461 PMID:17079588
26. Pedersen C, Petaja T, Strauss G, Rumke HC, Poder A, Richardus JH *et al.*; HPV Vaccine Adolescent Study Investigators Network (2007). Immunization of early adolescent females with human papillomavirus type 16 and 18 L1 virus-like particle vaccine containing AS04 adjuvant. *J Adolesc Health*. 40(6):564–71. http://dx.doi.org/10.1016/j.jadohealth.2007.02.015 PMID:17531764
27. Romanowski B, Schwarz TF, Ferguson LM, Peters K, Dionne M, Schulze K *et al.* (2011). Immunogenicity and safety of the HPV-16/18 AS04-adjuvanted vaccine administered as a 2-dose schedule compared with the licensed 3-dose schedule: results from a randomized study. *Hum Vaccin*. 7(12):1374–86. http://dx.doi.org/10.4161/hv.7.12.18322 PMID:22048171
28. Krajden M, Cook D, Yu A, Chow R, Mei W, McNeil S *et al.* (2011). Human papillomavirus 16 (HPV 16) and HPV 18 antibody responses measured by pseudovirus neutralization and competitive Luminex assays in a two- versus three-dose HPV vaccine trial. *Clin Vaccine Immunol*. 18(3):418–23. http://dx.doi.org/10.1128/CVI.00489-10 PMID:21248158
29. Opalka D, Lachman CE, MacMullen SA, Jansen KU, Smith JF, Chirmule N *et al.* (2003). Simultaneous quantitation of antibodies to neutralizing epitopes on virus-like particles for human papillomavirus types 6, 11, 16, and 18 by a multiplexed Luminex assay. *Clin Diagn Lab Immunol*. 10(1):108–15. http://dx.doi.org/10.1128/CDLI.10.1.108-115.2003 PMID:12522048
30. Dobson SR, McNeil S, Dionne M, Dawar M, Ogilvie G, Krajden M *et al.* (2013). Immunogenicity of 2 doses of HPV vaccine in younger adolescents vs 3 doses in young women: a randomized clinical trial. *JAMA*. 309(17):1793–802. http://dx.doi.org/10.1001/jama.2013.1625 PMID:23632723
31. Kreimer AR, Rodriguez AC, Hildesheim A, Herrero R, Porras C, Schiffman M *et al.*; CVT Vaccine Group (2011). Proof-of-principle evaluation of the efficacy of fewer than three doses of a bivalent HPV16/18 vaccine. *J Natl Cancer Inst*. 103(19):1444–51. http://dx.doi.org/10.1093/jnci/djr319 PMID:21908768

32. National Advisory Committee on Immunization (2012). Update on human papillomavirus (HPV) vaccines. *Can Commun Dis Rep*. 38:ACS-1. Available at http://www.phac-aspc.gc.ca/publicat/ccdr-rmtc/12vol38/acs-dcc-1/index-eng.php.
33. INFOVAC (2012). Vaccination against HPV: change from a three-dose schedule to a two-dose schedule for adolescents under 15 years of age. *Bulletin*. Feb. Available at http://www.infovac.ch.
34. Jit M, Demarteau N, Elbasha E, Ginsberg G, Kim J, Praditsitthikorn N *et al.* (2011). Human papillomavirus vaccine introduction in low-income and middle-income countries: guidance on the use of cost-effectiveness models. *BMC Med*. 9(1):54. http://dx.doi.org/10.1186/1741-7015-9-54 PMID:21569406
35. Roteli-Martins CM, Naud P, De Borba P, Teixeira JC, De Carvalho NS, Zahaf T *et al.* (2012). Sustained immunogenicity and efficacy of the HPV-16/18 AS04-adjuvanted vaccine: up to 8.4 years of follow-up. *Hum Vaccin Immunother*. 8(3):390–7. http://dx.doi.org/10.4161/hv.18865 PMID:22327492
36. Rowhani-Rahbar A, Mao C, Hughes JP, Alvarez FB, Bryan JT, Hawes SE *et al.* (2009). Longer term efficacy of a prophylactic monovalent human papillomavirus type 16 vaccine. *Vaccine*. 27(41):5612–9. http://dx.doi.org/10.1016/j.vaccine.2009.07.027 PMID:19647066
37. Longet S, Schiller JT, Bobst M, Jichlinski P, Nardelli-Haefliger D (2011). A murine genital-challenge model is a sensitive measure of protective antibodies against human papillomavirus infection. *J Virol*. 85(24):13253–9. http://dx.doi.org/10.1128/JVI.06093-11 PMID:21976653
38. McHeyzer-Williams M, Okitsu S, Wang N, McHeyzer-Williams L (2012). Molecular programming of B cell memory. *Nat Rev Immunol*. 12(1):24–34. http://dx.doi.org/10.1038/nri3128 PMID:22158414
39. Bachmann MF, Kalinke U, Althage A, Freer G, Burkhart C, Roost H *et al.* (1997). The role of antibody concentration and avidity in antiviral protection. *Science*. 276(5321):2024–7. http://dx.doi.org/10.1126/science.276.5321.2024 PMID:9197261
40. Namujju PB, Hedman L, Hedman K, Banura C, Mbidde EK, Kizito D *et al.* (2011). Low avidity of human papillomavirus (HPV) type 16 antibodies is associated with increased risk of low-risk but not high-risk HPV type prevalence. *BMC Res Notes*. 4(1):170. http://dx.doi.org/10.1186/1756-0500-4-170 PMID:21645376
41. Scherpenisse M, Schepp RM, Mollers M, Meijer CJ, Berbers GA, van der Klis FR (2013). Characteristics of HPV-specific antibody responses induced by infection and vaccination: cross-reactivity, neutralizing activity, avidity and IgG subclasses. *PLoS One*. 8(9):e74797. http://dx.doi.org/10.1371/journal.pone.0074797 PMID:24058629
42. Kemp TJ, Safaeian M, Hildesheim A, Pan Y, Penrose KJ, Porras C *et al.* (2012). Kinetic and HPV infection effects on cross-type neutralizing antibody and avidity responses induced by Cervarix(®). *Vaccine*. 31(1):165–70. http://dx.doi.org/10.1016/j.vaccine.2012.10.067 PMID:23123024
43. Petráš M, Sýkora T, Andrýs C, Drahošová M (2012). Post-vaccination anti-human papillomavirus antibody seroprevalence among Czech teenaged girls and women. *Int J Gynaecol Obstet*. 119(2):178–81. http://dx.doi.org/10.1016/j.ijgo.2012.06.007 PMID:22906959
44. Dauner JG, Pan Y, Hildesheim A, Harro C, Pinto LA (2010). Characterization of the HPV-specific memory B cell and systemic antibody responses in women receiving an unadjuvanted HPV16 L1 VLP vaccine. *Vaccine*. 28(33):5407–13. http://dx.doi.org/10.1016/j.vaccine.2010.06.018 PMID:20591543
45. Tarlinton D, Good-Jacobson K (2013). Diversity among memory B cells: origin, consequences, and utility. *Science*. 341(6151):1205–11. http://dx.doi.org/10.1126/science.1241146 PMID:24031013

46. Giannini SL, Hanon E, Moris P, Van Mechelen M, Morel S, Dessy F *et al.* (2006). Enhanced humoral and memory B cellular immunity using HPV16/18 L1 VLP vaccine formulated with the MPL/aluminium salt combination (AS04) compared to aluminium salt only. *Vaccine*. 24(33-34):5937–49. http://dx.doi.org/10.1016/j.vaccine.2006.06.005 PMID:16828940
47. Einstein MH, Baron M, Levin MJ, Chatterjee A, Fox B, Scholar S *et al.*; HPV-010 Study Group (2011). Comparative immunogenicity and safety of human papillomavirus (HPV)-16/18 vaccine and HPV-6/11/16/18 vaccine: follow-up from months 12–24 in a Phase III randomized study of healthy women aged 18–45 years. *Hum Vaccin*. 7(12):1343–58. http://dx.doi.org/10.4161/hv.7.12.18281 PMID:22048173
48. Smolen KK, Gelinas L, Franzen L, Dobson S, Dawar M, Ogilvie G *et al.* (2012). Age of recipient and number of doses differentially impact human B and T cell immune memory responses to HPV vaccination. *Vaccine*. 30(24):3572–9. http://dx.doi.org/10.1016/j.vaccine.2012.03.051 PMID:22469863
49. Shlomchik MJ, Weisel F (2012). Germinal center selection and the development of memory B and plasma cells. *Immunol Rev*. 247(1):52–63. http://dx.doi.org/10.1111/j.1600-065X.2012.01124.x PMID:22500831
50. Rosado MM, Scarsella M, Pandolfi E, Cascioli S, Giorda E, Chionne P *et al.* (2011). Switched memory B cells maintain specific memory independently of serum antibodies: the hepatitis B example. *Eur J Immunol*. 41(6):1800–8. http://dx.doi.org/10.1002/eji.201041187 PMID:21469123
51. Wrammert J, Ahmed R (2008). Maintenance of serological memory. *Biol Chem*. 389(5):537–9. http://dx.doi.org/10.1515/BC.2008.066 PMID:18953719
52. Crotty S, Felgner P, Davies H, Glidewell J, Villarreal L, Ahmed R (2003). Cutting edge: long-term B cell memory in humans after smallpox vaccination. *J Immunol*. 171(10):4969–73. PMID:14607890
53. Levin MJ, Moscicki AB, Song LY, Fenton T, Meyer WA 3rd, Read JS *et al.*; IMPAACT P1047 Protocol Team (2010). Safety and immunogenicity of a quadrivalent human papillomavirus (types 6, 11, 16, and 18) vaccine in HIV-infected children 7 to 12 years old. *J Acquir Immune Defic Syndr*. 55(2):197–204. http://dx.doi.org/10.1097/QAI.0b013e3181de8d26 PMID:20574412
54. Weinberg A, Song LY, Saah A, Brown M, Moscicki AB, Meyer WA 3rd *et al.*; IMPAACT/PACTG P1047 Team (2012). Humoral, mucosal, and cell-mediated immunity against vaccine and nonvaccine genotypes after administration of quadrivalent human papillomavirus vaccine to HIV-infected children. *J Infect Dis*. 206(8):1309–18. http://dx.doi.org/10.1093/infdis/jis489 PMID:22859825
55. Kahn JA, Xu J, Kapogiannis BG, Rudy B, Gonin R, Liu N *et al.* (2013). Immunogenicity and safety of the human papillomavirus 6, 11, 16, 18 vaccine in HIV-infected young women. *Clin Infect Dis*. 57(5):735–44. http://dx.doi.org/10.1093/cid/cit319 PMID:23667266
56. Wilkin T, Lee JY, Lensing SY, Stier EA, Goldstone SE, Berry JM *et al.* (2010). Safety and immunogenicity of the quadrivalent human papillomavirus vaccine in HIV-1-infected men. *J Infect Dis*. 202(8):1246–53. http://dx.doi.org/10.1086/656320 PMID:20812850
57. Denny L, Hendricks B, Gordon C, Thomas F, Hezareh M, Dobbelaere K *et al.* (2013). Safety and immunogenicity of the HPV-16/18 AS04-adjuvanted vaccine in HIV-positive women in South Africa: a partially-blind randomised placebo-controlled study. *Vaccine*. 31(48):5745–53. http://dx.doi.org/10.1016/j.vaccine.2013.09.032 PMID:24091311
58. Kumar D, Unger ER, Panicker G, Medvedev P, Wilson L, Humar A (2013). Immunogenicity of quadrivalent human papillomavirus vaccine in organ transplant recipients. *Am J Transplant*. 13(9):2411–7. http://dx.doi.org/10.1111/ajt.12329 PMID:23837399
59. Heijstek MW, Scherpenisse M, Groot N, Wulffraat NM, Van Der Klis FRM (2013). Immunogenicity of the bivalent human papillomavirus vaccine in adolescents with juvenile

systemic lupus erythematosus or juvenile dermatomyositis. *J Rheumatol*. 40(9):1626–7. http://dx.doi.org/10.3899/jrheum.130246 PMID:23997002
60. Heijstek MW, Scherpenisse M, Groot N, Tacke C, Schepp RM, Buisman AM *et al.* (2013). Immunogenicity and safety of the bivalent HPV vaccine in female patients with juvenile idiopathic arthritis: a prospective controlled observational cohort study. *Ann Rheum Dis*. [Epub ahead of print]. http://dx.doi.org/10.1136/annrheumdis-2013-203429 PMID:23723319

Chapter 7. Trials of vaccine efficacy against non-cervical outcomes – prevention of anal HPV infection and anal cancer

Joel Palefsky

7.1 Introduction

In a placebo-controlled randomized clinical trial of HIV-negative heterosexual men and men who have sex with men (MSM) aged 16–26 years, it was shown that the quadrivalent human papillomavirus (HPV) vaccine prevented 86% of persistent infections with HPV 6, 11, 16, and 18 and 90% of external genital lesions related to these types in the per-protocol population [1]. Among the per-protocol population of MSM in this trial, it was shown in a separate substudy that the vaccine prevented 95% of anal HPV 6, 11, 16, or 18 infection, 77% of anal squamous intraepithelial lesions (ASILs), and 75% of anal high-grade squamous intraepithelial lesions (HSILs) [2]. HPV vaccination to prevent ASILs has not been studied in women, but in a post hoc analysis of women participating in the Costa Rica Vaccine Trial, using the bivalent vaccine, there was an 84% reduction in anal HPV 16 or 18 infection in the restricted cohort, similar to the magnitude of the reduction in cervical HPV 16 or 18 infection [3].

Based on these and other data, HPV vaccination is approved in some countries for prevention of anal cancer in both women and men. Although there is a growing body of information on the natural history of anal HPV infection and progression from anal HPV infection to anal cancer and its precursor, anal HSIL, much more is known about the natural history of cervical HPV infection than about anal HPV infection. To the degree that the biology of anal cancer and cervical cancer and the role of HPV in their pathogenesis are very similar, lessons learned from cervical HPV natural history and vaccine trials may guide vaccine trials for prevention of anal HSIL/cancer. At the same time, however, when attempting to extrapolate experience with cervical HSIL/cancer prevention trials to anal HSIL/cancer prevention trials, additional variables must be considered. These include potential differences in the way cervical and anal HPV infections are acquired, differences between the natural history of anal and cervical HSIL once HPV infection at these sites has occurred, and sex-based differences in the natural history of anal HPV infection. HPV vaccination is also under consideration for prevention of anal HSIL/cancer in populations other than those for whom it is currently approved. These include MSM and HIV-infected individuals older than 26 years, and those being vaccinated after ablation of anal HSIL to reduce the risk of recurrent or incident HSIL. This chapter summarizes the known information in these areas in an effort to determine whether prevention of anal HPV infection may serve as an acceptable biomarker for protection against anal HSIL/cancer in vaccine trials.

7.2 Anal cancer and cervical cancer share a similar association with HPV

The relationship between HPV and anal cancer is similar to that for cervical cancer but with a few differences. Unlike cervical cancer, the proportion of anal cancers associated with HPV approaches, but does not reach, 100%. In a meta-analysis, HPV DNA was found in 71% of anal cancers [4]. As was the case with cervical cancer, earlier studies generally showed lower proportions of HPV positivity, likely due to use of older HPV DNA detection technologies [5]. More recent studies show that 90% or more of anal cancers contain HPV. In a recent French study of 366 anal cancers, HPV was found in 97% of cases, with 72% associated with a single HPV type [6]. In another study, from the USA, analysis of 146 anal cancers from the USA Surveillance, Epidemiology, and End Results (SEER) registry showed that HPV was detected in

133 cases (91%); 129 (88%) contained at least one high-risk (HR) type, most (80%) as a single genotype [7]. Similarly, in a recent analysis of 43 cases of anal cancer from eastern Europe, the overall prevalence of HPV DNA was 91% [8].

Of the cancers that are positive for HPV, a higher proportion of anal cancers than cervical cancers are found to harbour HPV 16 or 18, particularly HPV 16 [4]. In the French study, HPV 16 was the most common HPV type (75%) [6]. In the study of anal cancer cases from the USA SEER registry, HPV 16 had the highest prevalence (77%) [7], and in the eastern European study, HPV 16 was found in 86% of tumours [8].

Despite the predominance of HPV 16 and 18 in anal cancers, it is clear that other HPV types, including some low-risk (LR) types, are also found by themselves or with other HPV types. Among the HR HPV types other than HPV 16 or 18, HPV 31 and 33 have been described in anal cancers [4, 6]. In one study from Germany, all 5 anal canal cancers contained HPV 16, compared with only 1 of 4 anal margin cancers [9]. HPV 31, 33, and 68 were found in the other 3 anal margin cancers. HPV 6 has been described in 5.1% of anal cancers, and HPV 11 has been described in 1.0% of anal cancers [4]. In the USA SEER anal cancer analysis, one or more LR types were found by themselves in 2.7% of the cancers (2 cases of HPV 6 alone, and 2 cases of HPV 26 alone) [7]. HPV 6 was found alone or in combination with another HPV type in 4.1% of cases, and HPV 11 in 3.4%. The finding of HPV 6 alone or HPV 11 alone has been confirmed in a small number of anal cancer cases using laser capture microscopy [10]. HPV 6 and 11 are also associated with a rare but distinct tumour known as Buschke–Löwenstein tumour [11]. These giant condyloma may progress to verrucoid carcinomas, although squamous cell cancers have also been described. Although these lesions rarely, if ever, metastasize, they may be locally aggressive. The exact role of HPV 6 and 11 in the pathogenesis of the cancers remains unclear.

7.3 The association between HPV infection and anal cancer is similar in men and women

An earlier study, from 1997, showed that 93% of cases from women contained HPV compared with 69% from men, a statistically significant difference, but that included both cancers and HSILs [5]. In two more recent studies, the differences between men and women were not statistically significant [6, 7]. In a meta-analysis, HPV prevalence in anal cancer was higher among women (91%) than among men (75%), but sex-based differences were not found in tumours from North America [12]. Overall, in more recent studies a high proportion of anal cancer is positive for at least one HPV type among both men and women, and in some but not all studies, that proportion is modestly higher in women than in men.

Taken together, the relationship between HPV and anal cancer is nearly as strong as that for cervical cancer. As in the cervix, over time the proportion of cancers found in association with HPV has increased, likely due to improvement in HPV DNA technology. An even higher proportion of HPV-positive tumours in the anus may contain HPV 16 than in the cervix. Both the quadrivalent and bivalent vaccines are designed to prevent initial infection with HPV 16, and high efficacy against HPV 16 infection might lead to a substantial reduction in anal cancers in the vaccinated population [2, 3]. Further, although LR types such as HPV 6 or 11 have been found in association with a small number of anal cancers, infection with these types may also be prevented through vaccination with the quadrivalent vaccine [1, 2]. Prevention of persistent HPV 6, 11, 16 or 18 infection, in which persistent infection is used as the biomarker of true infection versus carriage, is likely to reflect a true reduction in risk of incident anal cancer in both men and women. The same is likely to be true for other HPV types that are found occasionally in

anal cancer and that are present in the nonavalent vaccine, such as HPV 31 and 33, but this has not yet been shown in clinical trials.

7.4 Anal HSIL is the precursor to anal cancer

Substantial evidence points to anal HSIL as the precursor lesion to anal cancer, similar to the relationship between cervical HSIL and cervical cancer. Several lines of evidence point to a role for anal HSIL as the cancer precursor. The earliest assumptions that anal HSIL is an anal cancer precursor were based on our knowledge of cervical carcinogenesis and identifying anal HSILs adjacent to invasive anal cancers. More direct evidence came from descriptions of progression to anal cancer among patients with anal HSIL. In a report of 6 patients with untreated perianal HSIL who were immunocompromised due to autoimmune diseases or renal transplantation, 3 (50%) progressed to anal cancer at a median of 5 years after diagnosis of HSIL [13]. Another group reported progression in 8 (15%) of 55 patients with HSIL at a median time of 42 months and no progression among 17 patients with low-grade squamous intraepithelial lesions (LSILs) [14]. Neither of these reports documented that the cancer arose directly from the area of HSIL, but a recent report described 27 men in whom anal cancer developed at the same location as a previously biopsied site of HSIL [15]. Prevention of anal HSIL has been accepted as a biomarker of likely future reduction in the incidence of anal cancer [16].

7.5 High-risk anal HPV infection is associated with HSIL, and the association is similar in men and women

If prevention of anal HSIL is an acceptable biomarker for future efficacy in reducing the incidence of anal cancer, consideration of using prevention of persistent HPV infection as a biomarker requires an understanding of the relationship between anal HPV infection and anal HSIL. Similarities between HPV infection and HSIL in the anus and the cervix suggest that biomarkers of vaccine efficacy in the cervix will also apply to the anus.

Anal HPV infection is as commonly associated with ASILs as cervical HPV infection is with cervical squamous intraepithelial lesions (CSILs). In a meta-analysis of CSILs, 80% of cervical LSILs in North America and 70% in Asia were positive for HPV [17]. HPV 6 or 11 was found in 12% of HPV-positive LSILs. HPV 16 was the most common HPV type, found in 26% of cases. In another meta-analysis, 92% of cervical HSILs were positive overall for HPV [18]. HPV 16 was the most common HPV type in these lesions, found in 54% of lesions, followed by HPV 18, found in 5%. Likewise, a high proportion of anal LSILs and HSILs are positive for HPV DNA. Approximately 88–92% of anal LSILs and 91–94% of anal HSILs are positive for HPV [4, 12, 19]. Most studies that have investigated HPV types in ASILs are largely based on individuals at high risk for ASIL development, particularly immunosuppressed patients and MSM [20].

As in the cervix, LR HPV types are predominantly associated with anal LSIL. In one meta-analysis, HPV 6 and 11 were identified in anal swabs from 36.2% and 18.1% of anal LSILs, respectively [12]. HPV 6 was present in 10.0% of HSILs among HIV-positive patients, compared with 6.8% among those who were HIV-negative; HPV 11 infection was present in 13.6% of HSILs among HIV-positive patients, compared with 3.4% among those who were HIV-negative. There are few data on the distribution of HPV types in anal HSIL biopsies in men versus women. In one meta-analysis, almost all HSIL biopsies were positive for HPV in both men (91%) and women (93%), and HPV 16 was the most common type in both [4]. The proportion containing either HPV 16 or 18 was 67% in men and 75% in women. Similarly, in an Australian study, HSIL was more strongly associated with HR HPV types than LR HPV types [21]. Overall,

the relationship between cervical HPV infection and CSIL is similar to that seen between anal HPV infection and ASIL.

HPV testing was performed in samples from 27 patients with HSIL that progressed to anal cancer [15]. Nine matched pairs for which DNA from both HSIL and cancer were available were analysed. All were HPV-positive. Eight pairs (89%) contained HPV 16 DNA. One pair (11%) contained both HPV 18 and 51 in both samples. Of the eight HPV 16-positive pairs, all contained the same HPV 16 strain variant. Concordance of both the HPV type and strain variant in anal cancers and the HSIL from which they progressed further supports the role of anal HSIL as an anal cancer precursor as well as the importance of HPV in the pathogenesis of both HSIL and cancer.

Given the strong relationship between HR anal HPV infection, particularly HPV 16, and both anal HSIL and anal cancers, it is likely that prevention of persistent HPV infection will be a valid biomarker of reduced risk of incident HSIL and anal cancer. Similarities in the HPV type distribution in anal HSIL between men and women further suggest that this will be the case in both sexes. Similarities between HPV infection, HSIL, and cancer in the anus and cervix further indicate that biomarkers useful to predict vaccine efficacy in the cervix will also be useful in the anus.

7.6 Routes of anal HPV infection may differ between men and women but do not have an impact on consideration of biomarkers of vaccine efficacy

Although anal HSIL and anal cancer are clearly associated with anal HPV infection in both men and women, there are differences in how commonly HPV is found in these groups and, in some circumstances, how frequently it is associated with ASIL. In descending order of prevalence, anal HPV infection is found in HIV-positive MSM, HIV-positive women, HIV-negative MSM, HIV-negative women, and men who have sex with women (MSW) [22–28]. The differences in prevalence of anal HPV infection likely reflect a combination of immune response and sexual behaviours, in which MSM have the highest likelihood of exposure due to their number of sexual partners and number of episodes of receptive anal intercourse relative to the other groups. Studies of women and MSW also clearly show that anal HPV infection can be detected in the absence of a history of receptive anal intercourse [24–28]. Sources of infection in these groups may be external anal contact with oral or genital HPV-infected epithelium of sexual partners, insertion into the anus of inert objects that may carry infectious HPV for at least a short period of time, or spread from an individual's own HPV-infected genital epithelium. MSM may acquire anal HPV infection through all of these mechanisms but also through receptive anal intercourse. Women may acquire anal HPV infection through anal intercourse or through spread from the cervix and vulvovaginal area. Despite the wide variety of ways in which HPV infection may be introduced, at present there is no reason to believe that the biology of HPV entry, replication, and progression through the life-cycle and different stages of disease differs according to these varying modes of acquisition. Hence, the efficacy of vaccination to prevent anal HPV infection is likely to be similar among these groups.

7.7 Natural history of anal HPV infection may differ between men and women but does not have an impact on consideration of biomarkers of vaccine efficacy

Once anal HPV infection has been acquired, the natural history of progression from HPV infection to ASIL and cancer may vary between men and women, but this may reflect sexual behaviours more than true biological differences. The incidence of anal cancer is similar, although slightly higher among women than among men (predominantly MSW) in the general

population [29, 30]. Among women, it is possible that the anus is less transformable on a per-HPV infection basis than the cervix, given that HR anal HPV infection is at least as common as cervical HPV infection in women [22, 25, 26], yet the incidence of anal cancer in women is lower than that of cervical cancer even in settings in which there is screening for cervical cancer [29, 30].

The incidence of anal cancer is not always proportional to the prevalence of anal HPV infection in some groups. HIV-negative MSM have more anal HPV infection than HIV-negative MSW but may have a disproportionately higher incidence of anal cancer than the MSW [31]. The incidence of anal cancer is higher among HIV-positive MSM than among HIV-negative MSM, but again the difference in anal cancer incidence [32, 33] is disproportionately greater than the differences in anal HPV prevalence between these two groups. HIV-positive MSM and HIV-positive women both have very high rates of anal HPV infection [22–25], whereas the incidence of anal cancer among HIV-positive MSM is substantially higher [33].

Apart from potential differences in the biology of the anal canal between men and women, for which there is currently little evidence, it is possible that the difference in rates of cancer in these groups may reflect sexual behaviour. In general, HIV-positive MSM have more anal receptive partners and receptive anal intercourse episodes, and more anal sexually transmitted infections than do HIV-positive women. Risk factors such as chronic inflammation and other sexually transmitted infections may potentiate the risk of anal cancer [29] in conjunction with anal HPV infection. Lower CD4 level has been associated with increased incidence of anal HSIL [34, 35] and cancer [36], but the contribution of duration of HIV infection and CD4 nadir to the risk of anal cancer remains uncertain. One recent study showed that better control of HIV viral load was associated with lower risk of anal cancer [37].

Taken together, the data would suggest that the natural history of anal HPV infection is similar among women and MSW in the general population. However, it is possible that the natural history may be different in MSM and HIV-positive individuals, particularly HIV-positive MSM, who have the highest incidence of anal cancer of any group. Risk factors beyond HPV infection may play a role in risk of developing disease in MSM, including chronic inflammation, co-infection with other sexually transmitted agents, and other unmeasured risk factors. Among those with HIV infection, additional risk factors may include chronic immune suppression and dysregulation.

Regardless of the differences in how anal HPV infection is acquired between groups at risk for anal cancer, differences in the natural history of anal HPV infection once HPV is acquired, or differences in the natural history of anal HPV infection compared with cervical HPV infection, there is no evidence to suggest that anal HSIL or anal cancer will develop in the absence of persistent anal HPV infection in any of these groups. Therefore, prevention of persistent HPV infection should be an adequate biomarker of vaccine efficacy for prevention of anal HSIL or cancer across all these groups. To the degree that HPV 16 and 18 comprise a higher proportion of HPV-positive anal cancers than cervical cancers, demonstration of prevention of persistent infection with these two types is particularly important. It will also be important to consider prevention of other HPV types, including HPV 6 and 11, which have been shown to be present in the absence of co-infection with HR HPV types in a small percentage of anal cancers. Therefore, guidelines for measuring HPV types as biomarkers of vaccine study outcomes for cervical cancer prevention studies may be similarly applied for studies of prevention of anal disease, but should also include HPV 6 and 11.

7.8 HPV infection as a biomarker of the efficacy of HPV vaccination to prevent incident anal HSIL among individuals older than 26 years

Individuals older than 26 years are not approved for routine vaccination or catch-up vaccination, largely based on studies of women that show that there is sufficient prior exposure to vaccine types in this group that vaccination is unlikely to be cost-effective on a population basis. Vaccine studies of mid-adult women (older than 26 years) show that the cost-effectiveness of routine vaccination is not high enough in this group to warrant routine vaccination [38]. However, it is possible that men older than 26 years may benefit more from vaccination than similarly aged women. The prevalence of seropositivity to HPV 16 is lower among men than among women in the USA [39], and if men older than 26 years have more new sexual exposures than do women, they may remain at higher risk of new HPV infection than similarly aged women.

HPV vaccines are as likely to prevent anal HPV infection among naive individuals older than 26 years as they are for younger individuals. However, it is not yet clear that doing so is cost-effective in terms of prevention of HSIL and cancer. As in studies of vaccine efficacy for cervical disease in mid-adult women, vaccine studies for ASIL in those older than 26 years should include both HPV and disease markers. This applies as well to a substantial proportion of HIV-negative and HIV-positive MSM older than 26 years. Although many of these men are highly sexually experienced, a high proportion have been shown to be seronegative and HPV DNA-negative, and hence theoretically “naive” to those HPV types [40, 41]. These individuals may benefit from vaccination even if they are older than 26 years, since they continue to have a high incidence of anal HSIL, and despite the likelihood that a substantial number of these “naive” men were exposed to vaccine HPV types in the past and had seroreverted. The efficacy of HPV vaccination to prevent persistent incident infection in this population is not yet known, nor is the efficacy to prevent incident anal HSIL. Although it seems likely that prevention of incident persistent HPV infection would be accompanied by reduced risk of anal HSIL and cancer, there is sufficient lack of knowledge of the natural history of HPV infection and ASIL in this clinical setting. Consequently, vaccine studies of putatively naive men and women should include both HPV and HSIL. Studies of HSIL would also be important in these populations to examine the cost-effectiveness of routine vaccination.

7.9 HPV as a biomarker of the efficacy of HPV vaccination as a tool to prevent recurrent disease after treatment of anal HSIL

In addition to preventing initial anal HPV infection, there are some very preliminary data to suggest that among HIV-negative MSM with HR anal HPV infection, vaccination with the quadrivalent vaccine may reduce the risk of recurrent anal HSIL after ablative treatment for prevalent HSIL. In one retrospective chart review, the risk of recurrent HSIL after ablation among HIV-uninfected men with oncogenic HPV infection was approximately one half that among unvaccinated men at 2 years after study entry [42]. These results were consistent with studies in women after treatment of cervical lesions [43]. However, measurements of HPV were not done post-vaccination among the MSM, and there are no data indicating that clearance of HPV correlated with reduced risk of recurrent HSIL. Studies examining the effect of HPV vaccination as a post-treatment “adjuvant” to reduce the risk of recurrent HSIL, as well as reducing the risk of disease as a result of preventing new HPV infection, must examine both HPV and HSIL as markers of vaccine efficacy in this setting.

7.10 Conclusions

The role of HPV infection in the pathogenesis of anal HSIL and cancer is similar to that in cervical HSIL and cancer, and also in the pathogenesis of anal HSIL and cancer, between men

and women. Among men and women younger than 26 years, regardless of differences in the natural history of anal HPV infection once HPV has been acquired, prevention of incident persistent anal HPV infection with vaccine HPV types is likely to be associated with prevention of disease, and hence is a reasonable end-point for vaccine studies of anal cancer prevention in both men and women. Results from vaccine studies to prevent anal HPV infection in men should be applicable in women, and vice versa. These studies should include markers of HPV infection with all of the types in the vaccine, including HPV 6 and 11 if they are included in the vaccine mix. Studies of HIV-positive and HIV-negative individuals older than 26 years, including those who are seronegative and DNA-negative to vaccine HPV types, should include markers of prevention of both persistent anal HPV infection and disease biomarkers such as anal HSIL. Similarly, studies of the role of vaccine in reducing disease recurrence after treatment of HSIL should include both HPV and disease end-points.

References

1. Giuliano AR, Palefsky JM, Goldstone S, Moreira ED Jr, Penny ME, Aranda C *et al.* (2011). Efficacy of quadrivalent HPV vaccine against HPV Infection and disease in males. *N Engl J Med*. 364(5):401–11. http://dx.doi.org/10.1056/NEJMoa0909537 PMID:21288094
2. Palefsky JM, Giuliano AR, Goldstone S, Moreira ED Jr, Aranda C, Jessen H *et al.* (2011). HPV vaccine against anal HPV infection and anal intraepithelial neoplasia. *N Engl J Med*. 365(17):1576–85. http://dx.doi.org/10.1056/NEJMoa1010971 PMID:22029979
3. Kreimer AR, González P, Katki HA, Porras C, Schiffman M, Rodriguez AC *et al.*; CVT Vaccine Group (2011). Efficacy of a bivalent HPV 16/18 vaccine against anal HPV 16/18 infection among young women: a nested analysis within the Costa Rica Vaccine Trial. *Lancet Oncol*. 12(9):862–70. http://dx.doi.org/10.1016/S1470-2045(11)70213-3 PMID:21865087
4. Hoots BE, Palefsky JM, Pimenta JM, Smith JS (2009). Human papillomavirus type distribution in anal cancer and anal intraepithelial lesions. *Int J Cancer*. 124(10):2375–83. http://dx.doi.org/10.1002/ijc.24215 PMID:19189402
5. Frisch M, Glimelius B, van den Brule AJC, Wohlfahrt J, Meijer CJLM, Walboomers JMM *et al.* (1997). Sexually transmitted infection as a cause of anal cancer. *N Engl J Med*. 337(19):1350–8. http://dx.doi.org/10.1056/NEJM199711063371904 PMID:9358129
6. Abramowitz L, Jacquard AC, Jaroud F, Haesebaert J, Siproudhis L, Pradat P *et al.* (2011). Human papillomavirus genotype distribution in anal cancer in France: the EDiTH V study. *Int J Cancer*. 129(2):433–9. http://dx.doi.org/10.1002/ijc.25671 PMID:20839262
7. Steinau M, Unger ER, Hernandez BY, Goodman MT, Copeland G, Hopenhayn C *et al.* (2013). Human papillomavirus prevalence in invasive anal cancers in the United States before vaccine introduction. *J Low Genit Tract Dis*. 17(4):397–403. http://dx.doi.org/10.1097/LGT.0b013e31827ed372 PMID:23609590
8. Škamperle M, Kocjan BJ, Maver PJ, Seme K, Poljak M (2013). Human papillomavirus (HPV) prevalence and HPV type distribution in cervical, vulvar, and anal cancers in central and eastern Europe. *Acta Dermatovenerol Alp Panonica Adriat*. 22(1):1–5. PMID:23674179
9. Kreuter A, Potthoff A, Brockmeyer NH, Gambichler T, Swoboda J, Stücker M *et al.*; German Competence Network HIV/AIDS (2010). Anal carcinoma in human immunodeficiency virus-positive men: results of a prospective study from Germany. *Br J Dermatol*. 162(6):1269–77. http://dx.doi.org/10.1111/j.1365-2133.2010.09712.x PMID:20184584
10. Cornall AM, Roberts JM, Garland SM, Hillman RJ, Grulich AE, Tabrizi SN (2013). Anal and perianal squamous carcinomas and high-grade intraepithelial lesions exclusively associated with “low-risk” HPV genotypes 6 and 11. *Int J Cancer*. 133(9):2253–8. http://dx.doi.org/10.1002/ijc.28228 PMID:23616200

11. Trombetta LJ, Place RJ (2001). Giant condyloma acuminatum of the anorectum: trends in epidemiology and management: report of a case and review of the literature. *Dis Colon Rectum*. 44(12):1878–86. http://dx.doi.org/10.1007/BF02234473 PMID:11742180
12. Machalek DA, Poynten M, Jin F, Fairley CK, Farnsworth A, Garland SM *et al.* (2012). Anal human papillomavirus infection and associated neoplastic lesions in men who have sex with men: a systematic review and meta-analysis. *Lancet Oncol*. 13(5):487–500. http://dx.doi.org/10.1016/S1470-2045(12)70080-3 PMID:22445259
13. Scholefield JH, Castle MT, Watson NF (2005). Malignant transformation of high-grade anal intraepithelial neoplasia. *Br J Surg*. 92(9):1133–6. http://dx.doi.org/10.1002/bjs.4994 PMID:16044425
14. Watson AJ, Smith BB, Whitehead MR, Sykes PH, Frizelle FA (2006). Malignant progression of anal intra-epithelial neoplasia. *ANZ J Surg*. 76(8):715–7. http://dx.doi.org/10.1111/j.1445-2197.2006.03837.x PMID:16916390
15. Berry JM, Jay N, Cranston RD, Darragh TM, Holly EA, Welton ML *et al.* (2014). Progression of anal high-grade squamous intraepithelial lesions to invasive anal cancer among HIV-infected men who have sex with men. *Int J Cancer*. 134(5):1147–55. http://dx.doi.org/10.1002/ijc.28431 PMID:23934991
16. Centers for Disease Control and Prevention (2011). Recommendations on the use of quadrivalent human papillomavirus vaccine in males – Advisory Committee on Immunization Practices (ACIP), 2011. *MMWR Morb Mortal Wkly Rep*. 60(50):1705–8. PMID:22189893
17. Clifford GM, Rana RK, Franceschi S, Smith JS, Gough G, Pimenta JM (2005). Human papillomavirus genotype distribution in low-grade cervical lesions: comparison by geographic region and with cervical cancer. *Cancer Epidemiol Biomarkers Prev*. 14(5):1157–64. http://dx.doi.org/10.1158/1055-9965.EPI-04-0812 PMID:15894666
18. Insinga RP, Liaw KL, Johnson LG, Madeleine MM (2008). A systematic review of the prevalence and attribution of human papillomavirus types among cervical, vaginal, and vulvar precancers and cancers in the United States. *Cancer Epidemiol Biomarkers Prev*. 17(7):1611–22. http://dx.doi.org/10.1158/1055-9965.EPI-07-2922 PMID:18628412
19. De Vuyst H, Clifford GM, Nascimento MC, Madeleine MM, Franceschi S (2009). Prevalence and type distribution of human papillomavirus in carcinoma and intraepithelial neoplasia of the vulva, vagina and anus: a meta-analysis. *Int J Cancer*. 124(7):1626–36. http://dx.doi.org/10.1002/ijc.24116 PMID:19115209
20. Palefsky JM (2010). Human papillomavirus-related disease in men: not just a women's issue. *J Adolesc Health*. 46(4) Suppl:S12–9. http://dx.doi.org/10.1016/j.jadohealth.2010.01.010 PMID:20307839
21. Hillman RJ, van Leeuwen MT, Vajdic CM, McHugh L, Prestage GP, Botes LP *et al.* (2012). Prevalence and predictors of high-grade anal intraepithelial neoplasia in a community-based sample of homosexual men. *Sex Health*. 9(6):574–9. http://dx.doi.org/10.1071/SH11139 PMID:22951248
22. Palefsky JM, Holly EA, Ralston ML, Jay N (1998). Prevalence and risk factors for human papillomavirus infection of the anal canal in HIV-positive and HIV-negative homosexual men. *J Infect Dis*. 177:361–7. http://dx.doi.org/10.1086/514194 PMID:9466522
23. Chin-Hong PV, Vittinghoff E, Cranston RD, Buchbinder S, Cohen D, Colfax G *et al.* (2004). Age-specific prevalence of anal human papillomavirus infection in HIV-negative sexually active men who have sex with men: the EXPLORE study. *J Infect Dis*. 190(12):2070–6. http://dx.doi.org/10.1086/425906 PMID:15551204
24. Palefsky JM, Holly EA, Ralston ML, Da Costa M, Greenblatt RM (2001). Prevalence and risk factors for anal HPV infection in HIV-positive and high-risk HIV-negative women. *J Infect Dis*. 183:383–91. http://dx.doi.org/10.1086/318071 PMID:11133369
25. Kojic EM, Cu-Uvin S, Conley L, Bush T, Onyekwuluje J, Swan DC *et al.* (2011). Human papillomavirus infection and cytologic abnormalities of the anus and cervix among HIV-

infected women in the study to understand the natural history of HIV/AIDS in the era of effective therapy (the SUN study). *Sex Transm Dis*. 38(4):253–9. http://dx.doi.org/10.1097/OLQ.0b013e3181f70253 PMID:20966828
26. Goodman MT, Shvetsov YB, McDuffie K, Wilkens LR, Zhu X, Ning L *et al.* (2008). Acquisition of anal human papillomavirus (HPV) infection in women: the Hawaii HPV Cohort study. *J Infect Dis*. 197(7):957–66. http://dx.doi.org/10.1086/529207 PMID:18429348
27. Piketty C, Darragh TM, Da Costa M, Bruneval P, Heard I, Kazatchkine MD *et al.* (2003). High prevalence of anal human papillomavirus infection and anal cancer precursors among HIV-infected persons in the absence of anal intercourse. *Ann Intern Med*. 138(6):453–9. http://dx.doi.org/10.7326/0003-4819-138-6-200303180-00008 PMID:12639077
28. Nyitray AG, Carvalho da Silva RJ, Baggio ML, Lu B, Smith D, Abrahamsen M *et al.* (2011). Age-specific prevalence of and risk factors for anal human papillomavirus (HPV) among men who have sex with women and men who have sex with men: the HPV in men (HIM) study. *J Infect Dis*. 203(1):49–57. http://dx.doi.org/10.1093/infdis/jiq021 PMID:21148496
29. Nelson RA, Levine AM, Bernstein L, Smith DD, Lai LL (2013). Changing patterns of anal canal carcinoma in the United States. *J Clin Oncol*. 31(12):1569–75. http://dx.doi.org/10.1200/JCO.2012.45.2524 PMID:23509304
30. Joseph DA, Miller JW, Wu X, Chen VW, Morris CR, Goodman MT *et al.* (2008). Understanding the burden of human papillomavirus-associated anal cancers in the US. *Cancer*. 113(10) Suppl:2892–900. http://dx.doi.org/10.1002/cncr.23744 PMID:18980293
31. Daling JR, Weiss NS, Hislop TG, Maden C, Coates RJ, Sherman KJ *et al.* (1987). Sexual practices, sexually transmitted diseases, and the incidence of anal cancer. *N Engl J Med*. 317(16):973–7. http://dx.doi.org/10.1056/NEJM198710153171601 PMID:2821396
32. Shiels MS, Pfeiffer RM, Chaturvedi AK, Kreimer AR, Engels EA (2012). Impact of the HIV epidemic on the incidence rates of anal cancer in the United States. *J Natl Cancer Inst*. 104(20):1591–8. http://dx.doi.org/10.1093/jnci/djs371 PMID:23042932
33. Silverberg MJ, Lau B, Justice AC, Engels E, Gill MJ, Goedert JJ *et al.*; North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD) of IeDEA (2012). Risk of anal cancer in HIV-infected and HIV-uninfected individuals in North America. *Clin Infect Dis*. 54(7):1026–34. http://dx.doi.org/10.1093/cid/cir1012 PMID:22291097
34. Critchlow CW, Surawicz CM, Holmes KK, Kuypers J, Daling JR, Hawes SE *et al.* (1995). Prospective study of high grade anal squamous intraepithelial neoplasia in a cohort of homosexual men: influence of HIV infection, immunosuppression and human papillomavirus infection. *AIDS*. 9(11):1255–62. http://dx.doi.org/10.1097/00002030-199511000-00007 PMID:8561979
35. Palefsky JM, Holly EA, Ralston ML, Jay N, Berry JM, Darragh TM (1998). High incidence of anal high-grade squamous intra-epithelial lesions among HIV-positive and HIV-negative homosexual and bisexual men. *AIDS*. 12(5):495–503. http://dx.doi.org/10.1097/00002030-199805000-00011 PMID:9543448
36. Reekie J, Kosa C, Engsig F, Monforte Ad, Wiercinska-Drapalo A, Domingo P *et al.*; EuroSIDA Study Group (2010). Relationship between current level of immunodeficiency and non-acquired immunodeficiency syndrome-defining malignancies. *Cancer*. 116(22):5306–15. http://dx.doi.org/10.1002/cncr.25311 PMID:20661911
37. Chiao EY, Hartman CM, El-Serag HB, Giordano TP (2013). The impact of HIV viral control on the incidence of HIV-associated anal cancer. *J Acquir Immune Defic Syndr*. 63(5):631–8. http://dx.doi.org/10.1097/QAI.0b013e3182968fa7 PMID:23614995
38. Grant LA, Dunne EF, Chesson H, Markowitz LE (2011). Considerations for human papillomavirus (HPV) vaccination of mid-adult women in the United States. *Vaccine*. 29(13):2365–70. http://dx.doi.org/10.1016/j.vaccine.2011.01.032 PMID:21277406

39. Stone KM, Karem KL, Sternberg MR, McQuillan GM, Poon AD, Unger ER *et al.* (2002). Seroprevalence of human papillomavirus type 16 infection in the United States. *J Infect Dis*. 186(10):1396–402. http://dx.doi.org/10.1086/344354 PMID:12404154
40. Sharma R, Efird JT, Chein A, Holly EA, Krajden M, Berry JM *et al.* (2013). Prevalence and risk factors for neutralizing antibodies to human papillomavirus types 16 and 18 in HIV-positive men who have sex with men. *J Acquir Immune Defic Syndr*. 64(5):479–87. http://dx.doi.org/10.1097/QAI.0b013e3182a7acd2 PMID:24231786
41. Wilkin T, Lee JY, Lensing SY, Stier EA, Goldstone SE, Berry JM *et al.* (2010). Safety and immunogenicity of the quadrivalent human papillomavirus vaccine in HIV-1-infected men. *J Infect Dis*. 202(8):1246–53. http://dx.doi.org/10.1086/656320 PMID:20812850
42. Swedish KA, Factor SH, Goldstone SE (2012). Prevention of recurrent high-grade anal neoplasia with quadrivalent human papillomavirus vaccination of men who have sex with men: a nonconcurrent cohort study. *Clin Infect Dis*. 54(7):891–8. http://dx.doi.org/10.1093/cid/cir1036 PMID:22291111
43. Joura EA, Garland SM, Paavonen J, Ferris DG, Perez G, Ault KA *et al.*; FUTURE I and II Study Group (2012). Effect of the human papillomavirus (HPV) quadrivalent vaccine in a subgroup of women with cervical and vulvar disease: retrospective pooled analysis of trial data. *BMJ*. 344:e1401. http://dx.doi.org/10.1136/bmj.e1401 PMID:22454089

Chapter 8. HPV vaccines and potential prevention of HPV-positive head and neck cancer

Maura Gillison

8.1 Introduction

Human papillomavirus (HPV) is newly appreciated as the principal cause of a distinct subset of oropharyngeal squamous cell carcinomas that is rising in incidence in numerous countries around the world, predominantly among men [1, 2]. In the case of cervical cancer, prospective clinical trials have demonstrated that cervical premalignant lesions can be prevented by HPV vaccination and detected by screening for HPV infection. By contrast, no analogous clinical trials support the use of HPV vaccination or HPV detection for the primary or secondary prevention of oropharyngeal cancer. To some extent, this lack of data can be attributed to the fact that the elucidation of the HPV etiology of cervical cancer predates that for oropharyngeal cancer by approximately 25 years. Thus, natural history studies of oral HPV infection comparable to those performed for cervical HPV infection in the 1990s are currently in progress. Nevertheless, despite considerable homology between HPV-caused cervical and oropharyngeal cancers, some distinctions between the two explain current barriers to the use of HPV vaccines for the primary prevention of oropharyngeal cancer.

8.2 HPV etiology of cervical versus oropharyngeal cancers

Head and neck cancers are now known to be etiologically heterogeneous, with a predominant subset attributable to tobacco and alcohol use, and a distinct subset attributable to HPV infection [3]. In 2007, IARC stated that there is sufficient evidence to conclude that HPV 16 is a cause of oropharyngeal cancers [4].

The comparative epidemiology of HPV infection and cancers of the head and neck and cervix has recently been reviewed [5]. Epidemiological associations are quite similar for cervical and oropharyngeal cancers. Case–control studies have observed strong and consistent associations between sexual behaviour, oral HPV infection, serological measures of HPV exposure, and oropharyngeal cancer [6]. A clear difference between cervical and oropharyngeal cancers is that only a subset of oropharyngeal cancers are attributable to HPV infection. Although only HPV 16 currently satisfies the epidemiological criteria necessary to be classified as high-risk in the oral cavity, other high-risk HPV types (e.g. HPV 18, 31, 33, 35, and 45) have been detected in 5–10% of HPV-positive oropharyngeal cancers. Thus, a higher proportion of HPV-positive oropharyngeal cancers than cervical cancers are attributable to HPV 16 infection [7]. Molecular analysis of oropharyngeal cancers demonstrates similarities to cervical cancer with regard to chromosomal aberrations, gene expression, methylation, and microRNA profiles, indicating that these cancers share common carcinogenic pathways [6]. Importantly, expression of HPV oncogenes has been shown to be necessary for the malignant phenotype of HPV-positive cervical and oropharyngeal cancers [8]. Thus, HPV is unlikely to be a pathophysiologically insignificant passenger infection. Prevention of HPV infection of the oral cavity should prevent development of cancer.

Therefore, epidemiological and molecular associations between HPV and cervical and oropharyngeal cancers are quite similar. Important differences include the etiological heterogeneity of oropharyngeal cancers and the higher attributable fraction for HPV 16.

8.3 Sex-based differences in incidence of HPV-positive oropharyngeal cancers

In virtually all regions of the world, incidence rates of oropharyngeal cancer are significantly higher among men than among women [1]. In a recent analysis of the *Cancer Incidence in Five Continents* database, incidence rates of oropharyngeal cancer among women rose predominantly in countries where rates for oral cavity and lung squamous cell carcinomas were also increasing, consistent with a role for increased exposure to tobacco among women, rather than oral HPV infection [1]. In contrast, rates for oropharyngeal cancer were rising among men in regions where rates for oral cavity and lung squamous cell carcinomas were declining, consistent with an association with HPV. Analyses on archived tumour specimens in the USA [2], Sweden [9], and Australia [10] support the conclusion that this increase in incidence of oropharyngeal cancer among men is attributable to HPV infection. In the USA, a recent analysis of National Cancer Institute (NCI) Surveillance, Epidemiology, and End Results (SEER) cancer registry tissues revealed that the incidence rates of HPV-positive oropharyngeal cancer were significantly higher among men than among women, and rates were projected to continue to increase until 2030 among men but to remain stable among women [2].

Cross-sectional studies of oropharyngeal cancer have also consistently observed male sex to be associated with HPV-positive oropharyngeal cancer [11]. An analysis of oropharyngeal cancer specimens collected from NCI SEER registries from 1984 to 2004 showed that the prevalence of HPV in oropharyngeal cancers was significantly higher among men than among women [2]. Moreover, the prevalence of HPV in oropharyngeal cancers in the USA significantly increased for men, but not for women, from 1984 to 2004. Therefore, evidence to date suggests that incidence rates of oropharyngeal cancers attributable to HPV are rising in several regions around the world, predominantly among men.

At this time, there are limited data regarding the risk of head and neck cancer given HPV exposure among men and women. A nested case–control study in Nordic countries observed the risk of head and neck cancer associated with HPV 16 L1 seropositivity to be similar among men (odds ratio [OR], 2.3; 95% confidence interval [CI], 1.3–4.0) and women (OR, 3.5; 95% CI, 1.5–7.7) [12]. However, in a nested case–control study conducted in Europe, the 10-year cumulative risk of oropharyngeal cancer associated with E6 seropositivity appeared to be dramatically higher among men than among women [13], albeit non-significantly.

Tumour HPV status is the single greatest predictor of the clinical behaviour of head and neck cancer. HPV-positive tumours are associated with a dramatically better prognosis than HPV-negative cancers. There is no evidence that the biological behaviour of HPV-positive cancer with regard to better prognosis is different for men and women [14].

Therefore, incidence rates of HPV-positive oropharyngeal cancer are higher among men than among women. There are insufficient data to evaluate whether the risk of HPV-positive oropharyngeal cancer given HPV infection is different for men and women.

8.4 Sex-based differences in natural history of oral HPV infection

There is a growing body of evidence that the natural history of oral HPV infection is significantly different among men and women. Cross-sectional studies have consistently reported the prevalence of oral HPV infection to be higher in men than in women. In a recent population-based survey in the USA, men had an approximate 3-fold higher prevalence of oral HPV infection than women [15]. Prevalence of oral HPV 16 infection was > 5-fold higher in men. Oral HPV infection had a bimodal age distribution among men but not among women. In addition to

interactions with age, sex significantly affected associations with marital status and smoking. Intensity of current smoking significantly increased the odds of oral HPV infection among women but not among men, and married women, but not married men, had significantly lower odds of infection.

At this time, the possible contribution of a difference by sex in risk of acquisition or persistence of an oral HPV infection to the differences in prevalence by sex is unknown. However, natural history studies in progress are expected to elucidate sex differences in risk and persistence of infection. The overwhelming majority of cross-sectional surveys have observed associations between sexual behaviour and prevalent oral HPV infection among men and women [15]. However, cross-sectional studies have also observed associations between kissing [16], prevalent paronychial infection [17], and genital infections [18], possibly consistent with oral-to-oral transmission and auto-inoculation from genital infection. Recent studies in which a small number of incident oral HPV infections were detected have inconsistently observed associations between sexual behaviour and incident infection [19, 20]. At this time, there is insufficient evidence to conclude that the risk factors for acquiring an oral HPV infection are different by sex. Nevertheless, both the prevalence of infection and the incidence of disease are higher among men than among women, consistent with natural history differences yet to be elucidated, and therefore HPV vaccine efficacy for oropharyngeal cancer prevention will be particularly important for men.

8.5 Histopathological progression of cervical cancer versus oropharyngeal cancer

The histopathological progression of oral cavity squamous cell carcinomas is quite analogous to that for cervical cancer: an accumulation of genetic alterations is associated with histopathological progression from mild and moderate dysplasia to severe dysplasia/carcinoma in situ [21]. However, the majority of HPV-positive oropharyngeal cancers arise from deep within the tonsillar crypt epithelium, and thus are neither accessible to visual inspection nor detectable by superficial brush biopsy. A single study has shown that HPV 16 detection is strongly associated with squamous cell carcinoma when oropharyngeal lesions are visible and accessible to brush biopsy [22]. In contrast, HPV 16 infection is not associated with abnormal cytology in superficial exfoliated tonsillar cytology specimens from individuals without detectable lesions [22]. Therefore, the histopathological progression of HPV-positive oropharyngeal cancer in particular remains poorly defined.

In contrast, the cervical epithelium is *relatively* readily accessible to brush biopsy, and therefore the biological and histological continuum of cervical HPV infection through carcinoma in situ has been prospectively observed in clinical trials. We note that HPV-positive high-grade dysplasias have been detected in surgical resection specimens from patients with head and neck cancers [23]. Thus, an invasive procedure such as bilateral tonsillectomy and laser excision of the base of tongue could, in theory, detect HPV-positive oropharyngeal precancers in healthy subjects. This is neither clinically feasible nor ethical, which remains a substantial barrier to both natural history studies and vaccine prevention trials. Regulatory agencies have historically required a histopathological disease end-point in clinical trials designed to evaluate the efficacy of HPV vaccines.

8.6 Prevention of oral HPV infection and associated cancers

There is no reason to believe that the molecular mechanism underlying vaccine efficacy in the head and neck would be different from that in the anogenital tract. The animal models that provided proof-of-concept studies that virus-like particle (VLP) vaccines could protect against

the development of papillomavirus-associated malignancies were oral cancer models. Models of canine oral papillomavirus (COPV)-associated oral cancer demonstrated COPV L1 vaccination to protect against development of oral cancer and that passive transfer of serum immunoglobulin from vaccinated dogs is protective [24].

Salivary antibodies are largely serum-derived, and antibodies to HPV 16 L1 have been detected among women who have received the HPV vaccine [25]. Compelling preliminary evidence of the potential utility of HPV vaccination for prevention of oral HPV infection was recently published [26]. In a cross-sectional study nested within a randomized controlled trial of bivalent vaccine for prevention of cervical dysplasia, oral HPV 16/18 infections were less common 4 years after HPV vaccination in women in the vaccine arm versus the placebo arm. There are no analogous data in men. Thus, data support a hypothesis that VLP vaccination would generate serum immunoglobulin-derived salivary immunoglobulin with potential to prevent incident oral HPV infection.

In all prospective randomized controlled clinical trials of HPV VLP vaccines completed to date, prevention of incident and persistent HPV 16/18 infection has been shown to be a conservative estimate of efficacy against HPV 16/18-positive moderate to severe dysplasias. Therefore, this outcome should be acceptable in future trials of VLP vaccines. In the absence of such precedent, such a surrogate end-point would not be acceptable for vaccine technologies that are substantially different from VLP vaccines (e.g. not thought to generate neutralizing antibodies). In that instance, proof-of-principle studies with disease end-points may be reasonably required from regulatory agencies.

Although serum-based studies have demonstrated HPV exposure to be associated with risk of oropharyngeal cancer [12, 13], nested, case–control studies are in progress to determine whether oral HPV infection is associated with risk.

Although natural history studies of oral HPV infection are currently under way, these studies will never be sufficiently large as to demonstrate an association between oral HPV infection persistence and incident oropharyngeal cancer. Because it is not currently clinically or ethically feasible to detect subclinical HPV-positive oropharyngeal dysplasias in healthy subjects, there is unlikely to be evidence, in the near or distant future, that persistent oral HPV infection is an acceptable surrogate for risk of progression to an oropharyngeal dysplasia or oropharyngeal cancer.

In summary, it is not possible to determine from empirical observational data whether oral HPV infection persistence can be used as an acceptable surrogate for risk of HPV-positive oropharyngeal cancer.

Nevertheless, it is universally accepted that it is not possible for an individual to develop HPV-positive oropharyngeal cancer in the absence of a preceding oral HPV 16 infection. It is also highly implausible that oral HPV 16 infections that are undetectable are associated with significant risk, given the strong associations between oral HPV 16 infection and oropharyngeal cancer in case–control studies.

In conclusion, oropharyngeal cancers are etiologically heterogeneous. Although HPV infection is not necessary for the development of oropharyngeal cancer, HPV infection is now accepted as necessary for the development of the HPV-positive subset. Oropharyngeal cancer incidence rates are increasing in numerous geographical regions around the world, and HPV infection has

been implicated as the underlying cause, predominantly among men. The risk of HPV-positive oropharyngeal cancer is higher in men than in women in all geographical regions investigated to date. The prevalence of oral HPV infection is significantly higher in men than in women.

There is increasing evidence that sex strongly influences the natural history of oral HPV infection. Whether the risk of oropharyngeal cancer given oral HPV 16 infection is different in men than in women is unknown. Current-generation HPV VLP vaccines (HPV 16/18) have the potential to prevent > 90% of HPV-positive oropharyngeal cancers. Clinical trials designed to evaluate the efficacy of HPV VLP vaccines for prevention of incident and persistent oral HPV 16 infection should include men and perhaps women. At this time, the best (and only feasible) surrogate end-point for risk of HPV-positive oropharyngeal cancer in a clinical trial would be prevention of incident and persistent oral HPV 16 infection.

References

1. Chaturvedi AK, Anderson WF, Lortet-Tieulent J, Curado MP, Ferlay J, Franceschi S *et al.* (2013). Worldwide trends in incidence rates for oral cavity and oropharyngeal cancers. *J Clin Oncol*. 31(36):4550–9. http://dx.doi.org/10.1200/JCO.2013.50.3870 PMID:24248688
2. Chaturvedi AK, Engels EA, Pfeiffer RM, Hernandez BY, Xiao W, Kim E *et al.* (2011). Human papillomavirus and rising oropharyngeal cancer incidence in the United States. *J Clin Oncol*. 29(32):4294–301. http://dx.doi.org/10.1200/JCO.2011.36.4596 PMID:21969503
3. Gillison ML, D'Souza G, Westra W, Sugar E, Xiao W, Begum S *et al.* (2008). Distinct risk factor profiles for human papillomavirus type 16-positive and human papillomavirus type 16-negative head and neck cancers. *J Natl Cancer Inst*. 100(6):407–20. http://dx.doi.org/10.1093/jnci/djn025 PMID:18334711
4. IARC (2007). Human papillomaviruses. *IARC Monogr Eval Carcinog Risks Hum*. 90:1–636. PMID:18354839
5. Gillison ML, Castellsagué X, Chaturvedi A, Goodman MT, Snijders P, Tommasino M *et al.* (2014). EUROGIN roadmap: comparative epidemiology of HPV infection and associated cancers of the head and neck and cervix. *Int J Cancer*. 134(3):497–507. http://dx.doi.org/10.1002/ijc.28201 PMID:23568556
6. Gillison ML, Alemany L, Snijders PJ, Chaturvedi A, Steinberg BM, Schwartz S *et al.* (2012). Human papillomavirus and diseases of the upper airway: head and neck cancer and respiratory papillomatosis. *Vaccine*. 30 Suppl 5:F34–54. http://dx.doi.org/10.1016/j.vaccine.2012.05.070 PMID:23199965
7. Kreimer AR, Clifford GM, Boyle P, Franceschi S (2005). Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. *Cancer Epidemiol Biomarkers Prev*. 14(2):467–75. http://dx.doi.org/10.1158/1055-9965.EPI-04-0551 PMID:15734974
8. Rampias T, Sasaki C, Weinberger P, Psyrri A (2009). *E6* and *E7* gene silencing and transformed phenotype of human papillomavirus 16-positive oropharyngeal cancer cells. *J Natl Cancer Inst*. 101(6):412–23. http://dx.doi.org/10.1093/jnci/djp017 PMID:19276448
9. Hammarstedt L, Lindquist D, Dahlstrand H, Romanitan M, Dahlgren LO, Joneberg J *et al.* (2006). Human papillomavirus as a risk factor for the increase in incidence of tonsillar cancer. *Int J Cancer*. 119(11):2620–3. http://dx.doi.org/10.1002/ijc.22177 PMID:16991119
10. Hong AM, Grulich AE, Jones D, Lee CS, Garland SM, Dobbins TA *et al.* (2010). Squamous cell carcinoma of the oropharynx in Australian males induced by human papillomavirus vaccine targets. *Vaccine*. 28(19):3269–72. http://dx.doi.org/10.1016/j.vaccine.2010.02.098 PMID:20226244

11. Gillison ML, Koch WM, Capone RB, Spafford M, Westra WH, Wu L *et al.* (2000). Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. *J Natl Cancer Inst*. 92(9):709–20. http://dx.doi.org/10.1093/jnci/92.9.709 PMID:10793107
12. Mork J, Lie AK, Glattre E, Hallmans G, Jellum E, Koskela P *et al.* (2001). Human papillomavirus infection as a risk factor for squamous-cell carcinoma of the head and neck. *N Engl J Med*. 344(15):1125–31. http://dx.doi.org/10.1056/NEJM200104123441503 PMID:11297703
13. Kreimer AR, Johansson M, Waterboer T, Kaaks R, Chang-Claude J, Drogen D *et al.* (2013). Evaluation of human papillomavirus antibodies and risk of subsequent head and neck cancer. *J Clin Oncol*. 31(21):2708–15. http://dx.doi.org/10.1200/JCO.2012.47.2738 PMID:23775966
14. Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tân PF *et al.* (2010). Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med*. 363(1):24–35. http://dx.doi.org/10.1056/NEJMoa0912217 PMID:20530316
15. Gillison ML, Broutian T, Pickard RK, Tong ZY, Xiao W, Kahle L *et al.* (2012). Prevalence of oral HPV infection in the United States, 2009–2010. *JAMA*. 307(7):693–703. http://dx.doi.org/10.1001/jama.2012.101 PMID:22282321
16. D'Souza G, Agrawal Y, Halpern J, Bodison S, Gillison ML (2009). Oral sexual behaviors associated with prevalent oral human papillomavirus infection. *J Infect Dis*. 199(9):1263–9. http://dx.doi.org/10.1086/597755 PMID:19320589
17. Edelstein ZR, Schwartz SM, Hawes S, Hughes JP, Feng Q, Stern ME *et al.* (2012). Rates and determinants of oral human papillomavirus infection in young men. *Sex Transm Dis*. 39(11):860–7. http://dx.doi.org/10.1097/OLQ.0b013e318269d098 PMID:23064535
18. Steinau M, Hariri S, Gillison ML, Broutian TR, Dunne EF, Tong ZY *et al.* (2014). Prevalence of cervical and oral human papillomavirus infections among US women. *J Infect Dis*. [Epub ahead of print]. PMID:24319284
19. Kreimer AR, Pierce Campbell CM, Lin HY, Fulp W, Papenfuss MR, Abrahamsen M *et al.* (2013). Incidence and clearance of oral human papillomavirus infection in men: the HIM cohort study. *Lancet*. 382(9895):877–87. http://dx.doi.org/10.1016/S0140-6736(13)60809-0 PMID:23827089
20. Pickard RK, Xiao W, Broutian TR, He X, Gillison ML (2012). The prevalence and incidence of oral human papillomavirus infection among young men and women, aged 18–30 years. *Sex Transm Dis*. 39(7):559–66. http://dx.doi.org/10.1097/OLQ.0b013e31824f1c65 PMID:22706220
21. Forastiere A, Koch W, Trotti A, Sidransky D (2001). Head and neck cancer. *N Engl J Med*. 345(26):1890–900. http://dx.doi.org/10.1056/NEJMra001375 PMID:11756581
22. Fakhry C, Rosenthal BT, Clark DP, Gillison ML (2011). Associations between oral HPV16 infection and cytopathology: evaluation of an oropharyngeal "Pap-test equivalent" in high-risk populations. *Cancer Prev Res (Phila)*. 4(9):1378–84. http://dx.doi.org/10.1158/1940-6207.CAPR-11-0284 PMID:21836021
23. Begum S, Cao D, Gillison M, Zahurak M, Westra WH (2005). Tissue distribution of human papillomavirus 16 DNA integration in patients with tonsillar carcinoma. *Clin Cancer Res*. 11(16):5694–9. http://dx.doi.org/10.1158/1078-0432.CCR-05-0587 PMID:16115905
24. Suzich JA, Ghim SJ, Palmer-Hill FJ, White WI, Tamura JK, Bell JA *et al.* (1995). Systemic immunization with papillomavirus L1 protein completely prevents the development of viral mucosal papillomas. *Proc Natl Acad Sci U S A*. 92(25):11553–7. http://dx.doi.org/10.1073/pnas.92.25.11553 PMID:8524802
25. Rowhani-Rahbar A, Carter JJ, Hawes SE, Hughes JP, Weiss NS, Galloway DA *et al.* (2009). Antibody responses in oral fluid after administration of prophylactic human

papillomavirus vaccines. *J Infect Dis*. 200(9):1452–5. http://dx.doi.org/10.1086/606026 PMID:19698077

26. Herrero R, Quint W, Hildesheim A, Gonzalez P, Struijk L, Katki HA *et al.*; CVT Vaccine Group (2013). Reduced prevalence of oral human papillomavirus (HPV) 4 years after bivalent HPV vaccination in a randomized clinical trial in Costa Rica. *PLoS One*. 8(7):e68329. http://dx.doi.org/10.1371/journal.pone.0068329 PMID:23873171

Disclosures of interests

Dr Jack Cuzick reports receiving personal consultancy fees from Merck, Abbott, Cepheid, and Becton, Dickinson and Company; Dr Cuzick reports that his unit at the Wolfson Institute of Preventive Medicine, Queen Mary, University of London benefits from research funding from Qiagen, Abbott, Hologic, Roche, Cepheid, Genera, OncoHealth, and Trovagene.

Dr Silvia de Sanjosé Llongueras reports that her research group at the Catalan Institute of Oncology benefits from research funding from GlaxoSmithKline, Merck, and Qiagen; Dr de Sanjosé Llongueras reports non-financial support (personal) from Sanofi, Qiagen, and Merck.

Dr Joakim Dillner reports that his unit at the Karolinska Institute receives research grants from Merck and Sanofi Pasteur MSD.

Dr Eduardo Franco reports that his unit at McGill University receives research support from Merck.

Dr Suzanne Garland reports that her unit at the Royal Women's Hospital benefited from research funding from CSL, Merck, and GlaxoSmithKline; Dr Garland reports benefiting from personal speaker's fees and support for travel from CSL, Merck, Sanofi Pasteur, and GlaxoSmithKline.

Dr Maura Gillison reports she received personal consultancy fees from Merck and GlaxoSmithKline; Dr Gillison reports that her unit at The Ohio State University received research funding from Merck.

Dr Anna Giuliano reports that her unit at the H. Lee Moffitt Cancer Center and Research Institute benefited from research funding from GlaxoSmithKline; Dr Giuliano reports that her institution currently benefits from research funding from Merck; Dr Giuliano's institution receives consultancy fees from Merck, through a three-way agreement.

Dr Allan Hildesheim reports that his research group at the United States National Cancer Institute (NCI) received vaccine and limited support related to regulatory submission needs for the NCI-sponsored Costa Rica HPV 16/18 Vaccine Trial.

Dr Susanne Krüger-Kjaer reports that her unit at the Danish Cancer Society benefited from unrestricted research funding from Sanofi Pasteur MSD and is currently benefiting from unrestricted research funding from Merck; Dr Krüger-Kjaer reports that she is receiving personal consultancy fees and personal speaker's fees from Merck and Sanofi Pasteur MSD.

Dr Matti Lehtinen has obtained grants for his HPV vaccination studies through his employer, the University of Tampere, from Merck and GSK.

Dr Douglas Lowy reports that as part of his United States government-supported research at the National Cancer Institute (NCI)/National Institutes of Health (NIH), he is an inventor of technology that underlies the L1-based prophylactic virus-like particle (VLP) HPV vaccine and technology that underlies an L2-based candidate prophylactic HPV vaccine. The NIH has licensed the technology for the L1 VLP vaccine to Merck, the manufacturer of Gardasil, to

GlaxoSmithKline, the manufacturer of Cervarix, and to Indian Immunologicals Ltd. The L2-based vaccine technology is the subject of a cooperative research and development agreement between the NCI, Johns Hopkins University, and Shantha Biotech, and has been licensed to Shantha, PanVax, Acambis Inc., and GlaxoSmithKline. United States Federal law entitles Dr Lowy to a limited share of royalties the NIH receives for these technologies.

Dr Jorma Paavonen reports that he has received research grants from Merck and GlaxoSmithKline through the Helsinki University Hospital Research Institute, to conduct clinical trials on HPV vaccination.

Dr Joel Palefsky reports that his research unit at the University of California, San Francisco benefited from research funding from Merck and Hologic; Dr Palefsky reports that he benefited from support for travel from Merck; Dr Palefsky reports that he has received honoraria for a research presentation at Qiagen; Dr Palefsky reports that he testified on behalf of Merck on the vaccine efficacy in males.

Dr Mark Schiffman reports that his research group at the United States National Cancer Institute (NCI) received vaccine and limited support related to regulatory submission needs for the NCI-sponsored Costa Rica HPV 16/18 Vaccine Trial.

Dr John Schiller reports that as part of his United States government-supported research at the National Cancer Institute (NCI)/National Institutes of Health (NIH), he is an inventor of technology that underlies the L1-based prophylactic virus-like particle (VLP) HPV vaccine and technology that underlies an L2-based candidate prophylactic HPV vaccine. The NIH has licensed the technology for the L1 VLP vaccine to Merck, the manufacturer of Gardasil, to GlaxoSmithKline, the manufacturer of Cervarix, and to Indian Immunologicals Ltd. The L2-based vaccine technology is the subject of a cooperative research and development agreement between the NCI, Johns Hopkins University, and Shantha Biotech, and has been licensed to Shantha, PanVax, Acambis Inc., and GlaxoSmithKline. United States Federal law entitles Dr Schiller to a limited share of royalties the NIH receives for these technologies.

Dr Margaret Stanley reports that she received personal consultancy fees from Sanofi Pasteur MSD, GlaxoSmithKline, and MSD; Dr Stanley reports that she received personal speaker's fees from MSD.

Dr Cosette Wheeler reports that her research unit and institution benefit from grants, contracts, and research agreements awarded to the University of New Mexico by the United States National Institutes of Health, GlaxoSmithKline, and Roche Molecular Systems, respectively.