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# Human Papillomavirus neutralizing and cross-reactive antibodies induced in HIV-positive subjects after vaccination with quadrivalent and bivalent HPV vaccines

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ABSTRACT

Ninety-one HIV-infected individuals (61 men and 30 women) were randomized to vaccination either with quadrivalent (Gardasil™) or bivalent (Cervarix™) HPV vaccine. Neutralizing and specific HPV-binding serum antibodies were measured at baseline and 12 months after the first vaccine dose. Presence of neutralizing and binding antibodies had good agreement (average Kappa for HPV types 6, 11, 16, 18, 31, 33 and 45 was 0.65). At baseline, 88% of subjects had antibodies against at least one genital HPV. Following vaccination with Cervarix™, all subjects became seropositive for HPV16 and 18. After Gardasil™ vaccination, 96% of subjects seroconverted for HPV16 and 73% for HPV18. Levels of HPV16-specific antibodies were <1 international unit (IU) in 87% of study subjects before vaccination but >10 IU in 85% of study subjects after vaccination. Antibodies against non-vaccine HPV types appeared after Gardasil™ vaccination for >50% of vaccinated females for HPV 31, 35 and 73 and for >50% of Cervarix™-vaccinated females for HPV 31, 33, 35, 45, 56 and 58. Cross-reactivity with non-genital HPV types was also detected. In conclusion, HIV-infected subjects responded to HPV vaccination with induction of neutralizing antibodies against both vaccine and non-vaccine types.



## 1. Introduction

Globally, about 10% of human cancers are caused by Human Papillomavirus (HPV) infection. The majority of HPV-related cancers occur in the anogenital tract and in the oropharynx. Both the bivalent Cervarix™ and the quadrivalent Gardasil™ vaccine have demonstrated efficacy up to 100% against persistent infections with HPV 16 and HPV 18 and safety [1]. These 2 viruses are responsible for causing about 70% of all HPV-related cancers. Besides these types, several other HPV types are also established as carcinogenic to human (HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59) with HPV 68 classified as a probable carcinogen [2]. The bivalent vaccine induces significant protection also against non-vaccine HPV types HPV 31, 33, 45, 51 and the quadrivalent vaccine against HPV 31, through induction of cross-protective antibody responses [3]. The Distribution of carcinogenic HPV types differs between regions of the world. For example in East Asia HPV 52 and 58 are prevalent and a 9-valent HPV vaccine including HPV types 6/11/16/18/31/33/45/52/58 has the potential to prevent up to 90% of cervical cancer cases, in these region as well [4].

Vaccine immunogenicity analyses are important for several reasons. They help to determine the range and duration of responses and can be used in bridging studies to extend vaccination recommendations to groups that are difficult to evaluate specifically in efficacy trials. For example, clinical outcomes for children cannot be measured in realistic time frames. Also, because of the large amount of data on efficacy of vaccines, it is no longer ethical to use placebo groups who do not receive vaccine and could acquire oncogenic HPV infection and develop HPV-associated diseases. Therefore, induction of HPV specific antibodies has emerged as a most important outcome measure for HPV vaccine research [5]. So far, all large scale HPV vaccine efficacy trials have used

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only in-house standards and methods to measure immunogenicity [6,7], such as Enzyme-linked Immunosorbent Assays (ELISAs) that employ virus-like particles as antigen, *in vitro* neutralization assays that measure the biologically relevant subset of capsid-binding antibodies that can prevent infection or competitive Luminex Immunoassays (cLia) that measure the subset of antibodies that compete with a type-specific neutralizing monoclonal antibody for binding to an epitope of the virus capsid. WHO has been standardizing HPV serology, for both ELISA and for the *in vitro* neutralization assay [8]. To evaluate efficacy of next generation vaccines, high-throughput methods with wide HPV type coverage are necessary [9]. The neutralization assay has been automated to a high-throughput scale to simultaneously measure neutralizing antibodies to HPV 16, 18, 31, 33 and 45 [10]. Merck has developed a cLia for monitoring antibodies against the 9 HPV types included in Gardasil-9 [11]. A multiplexed serology method based on mammalian cell-derived pseudovirions (Pseudovirion-Luminex) [12] can simultaneously measure antibodies to 21 different HPV types (15 of these are sexually-transmitted anogenital HPVs). The antibodies measured with this method correlate well with natural HPV infection [13]. We wished to employ this methodology in HPV vaccine research. To ensure reliable outcome measurements, we also measured neutralizing antibodies on the same samples [14,15]. We now report the results of an HPV vaccine trial investigating the effect of vaccination with either Cervarix™ or Gardasil™ in HIV-positive subjects, as evaluated using the HPV immunogenicity endpoint.

## 2. Methods

### 2.1. Study population

The study design is described in detail by Toft et al. [14]. Briefly, it was a double-blind clinical trial (NCT01386164) in which HIV-infected adults were randomized to vaccination with either Cervarix™ or Gardasil™. The study was conducted at the Department of Infectious Diseases, Aarhus University Hospital, Denmark. The adult volunteers received 3 doses of HPV vaccine at days 0, 45, and 180 and were followed up to months 7 and 12 post receipt of the first vaccine dose. In the present study, samples from the baseline (day 0) and the final visit (12 months after first vaccine dose) were analyzed. Altogether 91 individuals (61 men and 30 women, average age 46 years, 82% Caucasians) were vaccinated and tested for HPV DNA and HPV serum antibodies.

### 2.2. HPV DNA testing

The HPV DNA testing has been described by Bonde et al. [16] and Toft et al. [14]. Briefly, anal and cervical swabs were collected at baseline and at month 7. HPV DNA was purified using MagNA Pure Nucleic Acid Isolation Kit (Roche Diagnostics, Switzerland). HPV genotyping was done at Aarhus University, Denmark, using the Genomica CLART HPV2 Genotyping micro array (Genomica), which detects 35 different types of HPV.

### 2.3. Pseudovirion Luminex

Detection of HPV specific IgG serum antibodies was performed as described [13]. We produced pseudovirions for 17 HPV types belonging to species alpha (HPV 3, 6, 11, 16, 18, 31, 32, 33, 35, 39, 45, 52, 56, 58, 59, 68, 73) and for 4 HPV types belonging to species beta (HPV 5, 15, 38, 76) as well as pseudovirions for a control virus (Merkel cell polyomavirus (MCV)). Pseudovirions were generated by transfection of 293TT cells, as described [17]. VLPs from another control virus (JC polyomavirus (JCV)) were a kindly provided from Dr. K. Sasnauskas. Serum samples from baseline and final visit (12 months after the first vaccine dose) were analyzed in dilution 1:150. Cut-off values to define seropositivity were calculated independently for each HPV type by analysing the mean fluorescence intensity unit (MFI) values obtained from 106 children's sera (≤12 years old). The cut-off algorithm was as recommended by the global HPV LabNet (mean MFI value of a negative control serum panel plus 3 standard deviations) [18]. If this cut-off value was unreasonably low (less than 400 MFI) we used 400 MFI as cut-off to have sensitivity and specificity similar to classical ELISA [12]. HPV 16 specific antibody levels were calculated into international units (IU) using International Standard Serum for HPV 16 (10 IU) and wPLLmodel [19].

### 2.4. Neutralization assay for HPV 6 and 11

A conventional neutralization assay was performed using pseudovirions (PsV) carrying secreted alkaline phosphotase (SEAP) reporter gene, generated by transfection of 293TT cells as described [17]. For HPV 6 and 11, the neutralization assay protocol by Pastrana et al. [20] was followed using a serum dilution of 1:500. Chemoluminescence was read for 0.2 s per well using a Wallac Victor 1420 Multilabel counter. A serum was considered neutralizing, if the secreted alkaline phosphatase signal was reduced by more than 50%.

### 2.5. High-throughput pseudovirion-based neutralization assay (HT-PBNA) for HPV 16, 18, 31, 33, 45

An automated pseudovirion-based neutralization assay was performed as described [10,14,15]. Bovine Papillomavirus type1 (BPV 1) was used as a control. The mean $ED_{50}$ value plus 3 standard deviations of the serum samples reacting with the negative control (BPV 1) was used as a cut-off ($ED_{50}$ = 160).

### 2.6. Statistics

Agreement of categorical values between assays was quantified using Kappa values, calculated using GraphPad QuickCalcs online calculator. $R^2$ was calculated using Microsoft Excel to compare continuous results from neutralization and Pseudovirion-Luminex assays. Sensitivity and specificity of Pseudovirion-Luminex assays were calculated using the neutralization assay as the golden standard. GraphPad Prism software and a Fisher's exact test were applied to evaluate differences of seroconversion by vaccine groups and gender. Mann–Whitney and Kruskal–Wallis tests were applied to estimate HPV 16 antibody level differences between groups vaccinated with Cervarix™ or Gardasil™.

## 3. Results

### 3.1. Validation of immunogenicity measurements

Categorical data obtained with the neutralization assay was compared with data from the antibody binding (Pseudovirion-Luminex) assay (Table 1). The baseline ($N$ = 91) and 12 months follow up (final visit) serum samples ($N$ = 90) were all tested with Pseudovirion-Luminex and automated neutralization assays (HT-PBNA). As the non-automated neutralization assay is highly laborious, the HPV 6 and 11 neutralization assay was performed only for a subset of samples (35 Gardasil™ and 10 Cervarix™ recipients, all 10 were HPV 6 and 11 DNA negative at baseline). The cut-off levels for the different assays have been established in previous studies. Briefly, they were based on a negative serum panel (Pseudovirion-Luminex) [13], on a bovine papillomavirus type 1 control antigen (PBNA) [10] and on the secreted alkaline

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