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Immunogenicity and safety of a mixed vaccination schedule with one dose of nonavalent and one dose of bivalent HPV vaccine versus two doses of nonavalent vaccine – a randomized clinical trial

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Abstract

Background—Limited data is available on the use of different HPV vaccines in the same subjects. We evaluated the immunogenicity and safety of a mixed vaccination schedule with one dose of nonavalent (9vHPV) and one dose of bivalent vaccine (2vHPV) administered in different order versus two doses of 9vHPV vaccine.

Methods—371 girls and boys aged 9–10 years were randomized (1:1) to receive (I) two doses of 9vHPV or (II) a mixed schedule of 2vHPV+9vHPV or 9vHPV+2vHPV with a 6 month interval. Antibodies to HPV were tested by ELISA in blood samples collected one or six months post-first dose and one month post-second dose.

Results—Post-first dose of 9vHPV 99.4–100% of subjects were seropositive to 9 HPV types included in the vaccine. GMTs varied from 5.0 to 73.6 IU(AU)/ml depending on HPV type. Post-first dose of 2vHPV all subjects were seropositive to HPV16 and 18 (GMTs 16.7 and 11.7 IU/ml, respectively) and 50.0–76.7% were seropositive to 7 types not included in 2vHPV (GMTs varied from 0.3 to 17.5 AU/ml depending on type). Post-second dose all subjects, regardless of the study group, were seropositive to 9 HPV types included in 9vHPV. Anti-HPV16 and 18 GMTs were higher in subjects with the mixed schedule and for the other 7 HPV types higher in subjects who received two doses of 9vHPV vaccine. A higher proportion of subjects who received 2vHPV reported local or systemic adverse events than those who received 9vHPV as the first dose. Post-second dose there were no differences in reported adverse events between the two vaccines.

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Conclusions—The results show the mixed HPV vaccination schedules used in this study are immunogenic and have an acceptable safety profile. Although the seroprotective threshold of antibodies remains unknown the 100% seropositivity to all 9 HPV types included in 9vHPV and the increase of GMTs observed in all study groups post-second dose administration are reassuring and suggest protection might be achieved regardless of the schedule used.

Keywords

Human papillomavirus (HPV); bivalent vaccine; nonavalent vaccine; mixed schedule

Introduction

Human papillomaviruses (HPV) are responsible for the great majority of anogenital and oropharyngeal cancers as well as anogenital warts(1). Safe and highly protective HPV vaccines have been available for more than a decade(2). The high immunogenicity and efficacy of the HPV vaccines has led to the reduction of the initial three-dose to a two-dose vaccination schedule. By 2017, more than half of the countries which implemented HPV vaccination programs adopted a two-dose schedule and several other countries and jurisdictions are planning to switch to this reduced schedule in 2018(3,4).

Presently, HPV vaccines are produced by two different manufacturers. Although all available HPV vaccines are recombinant and non-infectious, their manufacturing process and content are different. The bivalent vaccine (Cervarix®, 2vHPV) contains recombinant L1 proteins from HPV types 16 and 18 assembled as virus-like particles (VLPs). These antigens are prepared by recombinant DNA technology using a Baculovirus expression system in *Trichoplusia ni* cells. The antigens are adjuvanted with AS04. AS04, is composed of 3-*0*-desacyl-4'-monophosphoryl lipid A (MPL) adsorbed onto aluminum (as hydroxide salt)(5). The nonavalent vaccine (Gardasil®9, 9vHPV) which is replacing the quadrivalent vaccine (Gardasil®, 4vHPV), is prepared from virus-like particles (VLPs) of the L1 protein of HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58. These L1 proteins are produced by recombinant technology using *Saccharomyces cerevisiae*. The purified VLPs are adsorbed on aluminum-containing adjuvant (Amorphous Aluminum Hydroxyphosphate Sulfate)(6).

Despite the long term worldwide use of these HPV vaccines only limited data is available on the use of different vaccines in the same individuals. The existing data is limited to the use of 4vHPV for primary vaccination and 2vHPV or 9vHPV as a booster dose(s) given a few years later(7–9), and to our knowledge no data exist regarding the immune response when administering two different HPV vaccines in different order. Availability and price of each of the HPV vaccine formulations varies by country and region. Data from this study could provide guidance on flexible vaccination schedules in case of vaccine shortages and may offer an alternative to the current paradigm of using the same product throughout multi-dose schedules that offers optimal protection at a better cost. While current guidance indicates the series may be completed with any approved HPV vaccine formulation, the study would be the first to provide data addressing this question.

The main objective of this clinical trial was to evaluate the immunogenicity and safety of two doses of 9vHPV vaccine versus one dose of 9vHPV and one dose of 2vHPV vaccine.

The secondary objectives were (I) to assess the seropositivity rates and anti-HPV geometrical mean titers (GMTs) one and six month post-first dose of 9vHPV vaccine, (II) to assess the immunogenicity of 9vHPV and 2vHPV vaccines when administered in different order, and (III) to evaluate comparative safety/reactogenicity profile of 9vHPV and 2vHPV vaccines. Our main, *a priory* hypothesis, was that after the second dose of vaccine more than 98% of subjects in each study group would have detectable antibodies to 9 HPV types included in 9vHPV vaccine.

Methods

Study ethics and registration statement

The study protocol, the informed consent and the assent for minor subjects were approved by the Research Ethics Board of CHU de Québec – Université Laval. The study is registered with ClinicalTrials.gov: NCT02567955.

Study area and design

This prospective randomized (1:1) clinical trial was conducted in the Quebec City area, Canada. The randomization lists were generated by using a standard Statistical Analysis System (SAS) program. Half of the subjects were allocated to receive two doses of 9vHPV and half to receive a mixed schedule of one dose of 9vHPV and one dose of 2vHPV vaccine. Both study groups were randomized a second time (1:1): the standard two dose 9vHPV group to have the blood sample collected 1 or 6 month post-first dose; and the mixed schedule group to receive the 9vHPV and the 2vHPV vaccines in different order (9vHPV +2vHPV or 2vHPV+9vHPV). Per protocol interval between vaccine doses was 6 months.

Subjects and study procedures

Healthy girls and boys aged 9–10 years living in the Quebec City area were eligible to participate. Potential subjects were recruited by using invitation letters addressed to their parents. The eligibility criteria for potential subjects were 1) no immunosuppression; 2) no coagulation problems; 3) no previous HPV vaccination; 4) no allergy to any vaccine component, and 5) not planning to move away from the Quebec City area during the next three years. Parents or legal representatives of the subjects had signed the informed consent and each subject had signed an assent prior to any study intervention. The vaccines were administered by an accredited nurse according to manufacturers' recommendations: 0.5 ml of vaccine in the deltoid muscle. Blood samples (5.0 ml) were collected 1 or 6 months post-first dose of 9vHPV, 6 months (but not 1 month) post-first dose of 2vHPV and 1 month post-second dose of either vaccine.

Immunogenicity assessment

Laboratory assays were performed at the Centers for Disease Control and Prevention (CDC, Atlanta, USA) using multiplex direct IgG ELISA to HPV L1+L2 virus-like particles (VLPs) on Meso Scale Discovery platform as previously described with minor modification(10).

The M9ELISA used VLPs for HPV6, 11, 16, 18, 31, 33, 45, 52 and 58 pre-coated on 10-spot standard plates (Meso Scale Discovery, MSD, Gaithesburg, MD). Test sera were 3.16

fold serially-diluted for at least 3 dilutions starting at 1:100 or higher. Dilutions of reference sera were used in each plate to allow titer determination using the parallel line method (PLL). PLL analysis was performed as described in the WHO HPV Labnet Manual, using raw signal for each HPV type. Cut-off values (COV) were determined using serum samples from children (n=50, Gift from Dr. J Dillner, Lund University).

Antibody titers were measured in International Units (IU/ml) for HPV16 and 18. In the absence of international standards for the other 7 HPV types, Arbitrary Units (AU/ml) were used. Test samples were considered positive if they passed PLL conditions as well as were above Median+2 Standard Deviations of the PLL/titer generated from the children sera. Cut off value for HPV6, 11, 16, 18, 31, 33, 45, 52 and 58 were 0.1 AU/ml, 0.1 AU/ml, 0.5 IU/ml, 0.4 IU/ml, 0.5 AU/ml, 1.3 AU/ml, 2.5 AU/ml, 0.7 AU/ml and 1.2 AU/ml, respectively.

Safety assessment

Solicited local and general adverse events observed during the first 4 days post-each vaccine dose administration were collected on standardized diaries. Subjects and their parents were blinded to which group they were allocated until one month post-second dose (period during which the safety profile was assessed). Parents were asked to report any event which demanded a medical intervention during the entire study period.

Statistical methods

We measured and compared the proportion of anti-HPV seropositive subjects, titers distribution and GMTs among study groups post-first and post-second dose of vaccine. Fisher's exact test was used for the comparison of proportions, Wilcoxon test for continuous variables and Kolmogorov-Smirnov test for comparison of titers distribution. All statistical tests were 2-tailed. P values of 0.05 or less were considered significant. SAS Institute software version 9.2 (Cary, NC, USA) was used for statistical analysis. An intention-to-treat (ITT) and an according-to-protocol analysis (ATP) were conducted.

The sample size was calculated based on previous results showing ≈98% seropositivity rates after two doses of HPV vaccines (7, 8, 12). Assuming a power of at least 80% and a level of significance of 5% (α=5%) with a maximal difference between groups of 4% (Δ=4%) a sample size of 145 subjects per group was needed. As the study includes a follow-up for three years, with an expected 5%−7% annual loss of subjects, enrollment of 185 subjects in each study group was needed in order to have 145 evaluable subjects in the final analysis.

Results

Enrollment and demographics

Invitation letters were sent to about 2000 parents. The recruitment was stopped when 371 subjects had been enrolled and randomized to receive two doses of 9vHPV or one dose of 9Vhpv and one dose of 2vHPV vaccine (Figure 1). Subjects' socio-demographic characteristics by randomization group are presented in Table 1. The subjects were 9 or 10 year old (mean 9.6 years) at the time of the first vaccine dose administration and 50% were girls. The interval between two vaccine doses varied from 173 to 203 days (mean 183 days;

median 182 days). The first blood sample was collected 28–38 days (mean&median 31 days) post-first dose of 9vHPV vaccine in 91 subjects and 173–202 days (mean&median 183 days) post-first dose of 9vHPV or 2vHPV vaccine in 267 subjects. The second blood sample was collected 11–45 days post-second dose of vaccine (mean 30 days; median 29 days). Inadvertently, 6 subjects had their second blood sample collected between 11 and 19 days post-second dose and were excluded from the according-to-protocol (ATP) immunogenicity analysis. The ATP analysis excluded another 16 subjects who refused one of the two per protocol blood samples. No statistically significant difference was observed between the ITT (N=367) and ATP (N=345) analysis results. Here we present the ATP analysis results for immunogenicity and ITT analysis for safety/reactogenicity (all reported adverse events included).

Immune response to the first dose of 9vHPV vaccine

Post-first dose of 9vHPV vaccine administration one subject was negative (0.6%) to HPV45, otherwise all subjects were seropositive to all 9 HPV types included in the vaccine. No difference in seropositivity or antibody GMTs was observed between subjects tested 1 or 6 month post-first dose administration ($p>0.05$). Depending on HPV type the antibody GMTs varied from 4.6 to 75.1 IU(AU)/ml (Table 2).

Immune response to the first dose of 2vHPV vaccine

Six months post-first dose of 2vHPV, all subjects were seropositive to vaccine types HPV16 and 18 and between 50% and 76.7% were also seropositive to the seven other HPV types only included in the 9vHPV vaccine. Antibody GMTs were 16.7 and 11.7 IU/ml for HPV16 and 18, respectively. For the other seven HPV types the antibody GMTs varied from 0.3 to 7.9 AU/ml (Table 2)

Immune response to the second dose

Irrespective of the vaccination schedule, one month post-second dose all subjects were seropositive to the 9 HPV types included in the 9vHPV vaccine (same results in ITT analysis – not presented). Compared to the post-first dose GMTs, there was a statistically significant 1.3 to 143-fold increase in antibody GMTs after the second dose in all study groups for all 9 HPV types, except for HPV11 in the 9vHPV+2vHPV group (1.2-fold GMT increase; $p=0.08$). The GMTs to HPV16 were significantly higher in subjects who received the 2vHPV vaccine as the first dose and to HPV18 in both study sub-groups that received two different vaccines when compared to those who received two doses of 9vHPV vaccine. Among those who received two different vaccines, GMTs to HPV16 were higher when 2vHPV was the first dose. The GMTs to HPV6, 11, 31, 33, 45, 52 and 58 were higher in subjects who received two doses of 9vHPV (Table 3). In the group which received a dose of 9vHPV followed by a dose of 2vHPV the GMTs to HPV 16, 18, 31, 33, 45, 52 and 58 were higher in girls (81.9–1820.7 IU(AU)/ml depending on HPV type) when compared to boys (41.2–1001.3 IU(AU)/ml) ($p<0.05$ for each specific HPV type). The immunogenicity results were similar in boys and girls when 2vHPV was followed by a dose of 9vHPV and in those who received two doses of 9vHPV.

Safety and reactogenicity

Post-first dose, adverse events were more frequent with 2vHPV compared with 9vHPV both for at least one local (87.1% vs. 67.4%; P<0.001) and systemic reaction (66.7% vs. 49.8%; P=0.006). Post-second dose there was no statistically significant differences between the two vaccines. Grade 3 systemic reactions post-first and post-second dose were reported by 4.3 vs.4.0% (p=1.00) and 3.3 vs. 1.8% (p=0.42), and local reactions by 8.6% vs 1.8% (p=0.005) and 6.7 vs. 3.3% (p=0.22) post-2vHPV and post-9vHPV, respectively (Table 4). No statistically significant difference was observed in the safety profile of 9vHPV when given in standard (9vHPV+9vHPV) or mixed schedule (9vHPV+2vHPV or 2vHPV+9vHPV). The safety profile was comparable (all p>0.05) when 2vHPV was given as first or second dose in the mixed schedule (Table 4). Post-first dose of 2vHPV administration a higher proportion of girls reported at least one systemic adverse event when compared to boys (78.3% vs. 55.3%; p=0.03). No subject withdrew from the study due to adverse events.

Discussion

To our knowledge, this is the first clinical trial to compare the immunogenicity and safety of two doses of 9vHPV vaccine versus mixed schedules of 2vHPV and 9vHPV vaccines. The virtually 100% seropositivity post-first dose of 9vHPV is in line with previously reported data after a single dose of 2vHPV(11) and 4vHPV vaccines (8,12) which show similar seropositivity rates to HPV types included in the respective vaccines. The seropositivity rates to HPV 31, 33, 45, 52 and 58 after a dose of 9vHPV in our study are slightly higher when compared to those reported after a single dose of 9vHPV vaccine given to 12–26 year-old subjects previously vaccinated with three doses of 4vHPVvaccine (9). The slightly higher seropositivity rates (1.4–5.7% higher) to HPV31, 33, 52 and 58 types and 33% higher to HPV45 in our study when compared to the above mentioned study with 9vHPV vaccine might be due to different serological assay used (ELISA versus cLIA) and/or to the difference in subjects' age.

The 100% seropositivity to HPV16 and 18 after a single dose of 2vHPV vaccine in this study is consistent with rates reported in previous studies(11–13). Interestingly, an important proportion (50 to 77%) of subjects vaccinated with a single dose of 2vHPV were seropositive to 7 HPV types not included in the vaccine. Similar observations were reported in some previous studies(14–18). In a study by Faust et al.(14), cross-reactive neutralizing antibodies against non-vaccine types HPV31, 35 and 73 were present in >50% of subjects vaccinated with 4vHPV and to HPV31, 33, 35, 45, 56 and 58 in >50% of subjects vaccinated with 2vHPV. In a study by Toft et al.(15), both 4vHPV and 2vHPV vaccine induced anti-HPV31, 33, 45 neutralizing antibodies in subjects who were seronegative and HPV-DNA negative for those types at study entry. In a study by Einstein(16), the immune response induced by 4vHPV and 2vHPV vaccine against HPV31 and 45 was compared up to month 18 post-vaccination. GMTs measured by pseudovirion-based neutralization assay and enzyme-linked immunosorbent assay were similar between vaccines. However, the circulating antigen-specific CD4+ T-cell frequencies were higher in 2vHPV group for HPV31 and 45: ratio 2.0 and 2.6, respectively(16).

In our study, GMTs after a single dose of either vaccine were relatively low when compared to those reported after 2 or 3 doses of the respective vaccine. This is consistent with previous reports(8,9,13). Post-second dose GMTs were generally higher to HPV16 and 18 in the group which received two different vaccines and higher for other 7HPV types in the group which received two doses of 9vHPV. The GMTs increased considerably (14–148-fold) after the administration of the second dose of 9vHPV vaccine following either a dose of 9vHPV or 2vHPV. The highest GMTs increase was observed to HPV16 and 18 after the administration of 9vHPV to subjects who received the 2vHPV as the first dose (148- and 112-fold, respectively). The amplitude of GMTs increase to HPV6, 11, 31, 33, 45, 52 and 58 was lower after a dose of 2vHPV given to subjects primed with 9vHPV (1.2–8.6-fold), however, these increases were statistically significant ($p<0.05$) for all HPV types except HPV11 ($p=0.08$). Despite the differences in post/pre-second dose ratios GMTs increase when using two vaccines in different order the final GMTs were comparable except for anti-HPV16 which was higher in the group primed with 2vHPV vaccine compared to 9vHPV (2476 vs. 1541 IU/ml; $p<0.001$). The clinical importance of higher antibody titers is not well understood and existing data suggest that very low post-vaccination antibody titers protect against disease. Moreover, the absence of detectable antibodies or their loss with time after vaccination is not a reliable indicator of loss of protection(19–21). In fact, several studies, mainly in non-compliant subjects, have shown that despite lower antibody titers after one compared to two or three doses there were no or small differences in vaccine efficacy(12,22–24). Ecological registry-based studies which evaluated the impact of different vaccination schedules (1, 2 or 3 doses) on HPV prevalence(25–27), anogenital warts(28,29) and cervical lesions prevalence(30–34) had more divergent results, with several of them showing higher efficacy of three-dose schedule. However, these studies are subject to potential biases, mainly due to differences in vaccinees' characteristics and approaches used to calculation of time at risk. Namely, (I) subjects who received fewer than 3 doses were older and began or may have begun sexual activities earlier in life which put them at higher risk of infection before vaccination and (II) the probability of clearing prevalent pre-vaccination infections is higher when assessment begins 1 month post-third or post-second dose (given at 6 months interval) when compared to one month post-first dose. Without a similar interval of 7 months for natural clearing of prevalent infection, one dose can erroneously appear less effective than schedules using an interval of 6 months between the first and the last dose of vaccine. The above is at least partially confirmed by the important reduction in HPV prevalence and HPV related diseases independently of the number of doses when vaccinating young girls who were at a low risk of expose to the virus before vaccination(27) and by the high efficacy of one, two and three doses of HPV vaccine against high grade cervical abnormalities(35). The later observation is in line with the commonly recognized fact that an important proportion of HPV infections, including prevalent ones pre-vaccination, are eventually cleared without leading to medical complications.

The results of the above mentioned studies, the observed high rates of seropositivity to 9 HPV types after the administration of a single dose of 2vHPV, as well as the observed increase in antibody titers to HPV types included in the 9vHPV vaccine after giving the 2vHPV to subjects primed with 9vHPV vaccine lead us to believe that vaccination with a dose of 9vHPV and a dose of 2vHPV should ensure protection against related diseases.

HPV16 and 18 are the most important causes of cervical cancers (70–75% of cases globally) (2,36) and are responsible for virtually all HPV related cancers in men. Accepting that the bivalent vaccine alone has substantial cross-protection against types 31, 33, 45, which are associated with 13% of cervical cancer cases, then potentially, the incremental benefit in cancer protection with 9vHPV compared to 2vHPV may relate only to the 5% of disease associated globally with types 52 and 58(3,36). Given that in our study all subjects were seropositive to HPV52 and 58 and that GMTs in subjects who received two different vaccines were relatively high (59–106 AU/ml) the probability of breakthrough diseases with these two HPV types should be minimal if any.

Noteworthy, a recent Cochrane Systematic review mentions that the cumulative incidence of HPV16 and 18 infections among women who received one dose or two doses were similarly low compared to those who received the three doses(35).

If a mixed dose schedule is introduced, the lower antibody titers to HPV6 and 11 in the groups that received two different vaccines may warrant attention through active type-specific and genital wart surveillance. However, the strong and highly significant reduction of the occurrence of genital warts in those vaccinated with a single dose of 4vHPV at a younger age(27) and the observed increase in antibody titers when administering 2vHPV to vaccinees primed with 4vHPV(7) or 9vHPV in the present study are reassuring and make unlikely the risk of breakthrough infections with HPV6 and 11.

The results of vaccines' safety assessment are also congruent with previously reported data showing that all three vaccines presently available have an acceptable safety profile(2,34,37,38). In our study, the 2vHPV vaccine induced a higher proportion of adverse events when compared to 4vHPV or 9vHPV vaccines but these reactions were transient and the great majority of them did not require any medical intervention(2). This is similar to findings of other studies using 2vHPV alone (5). Interestingly, in our study we observed a higher proportion of local and systemic adverse events when 2vHPV vaccine was administered as the first but not the second dose. This might have some biological explanation related to the first or repetitive presentation of antigens to the immune system or might indicate that both children and their parents paid more attention to adverse events post-first dose and were more reassured about vaccine safety after the administration of the second dose of vaccine.

Our study has some limitations. First, 92% of our subjects were Caucasians which is representative of the population of the study area. While this theoretically limits the capacity to extrapolate results to other ethnicities, previous studies have consistently shown that virtually 100% of vaccinees develop antibodies regardless of ethnicity. Second, 18.6% of children whose parents were sent an invitation letter participated in the study. This participation rate is unlikely to have biased results as recruitment was stopped as soon as the *a priory* calculated sample size was reached. Third, we measured antibody titers 1 and 6 month post-first dose of 9vHPV but only 6 months post-first dose of 2vHPV. Several previous studies reported 100% seropositivity 1 and 6 months post a single dose of 2vHPV but no such public data were available after a single dose of 9vHPV given to naïve (previously unvaccinated) preadolescents. The additional randomisation in two sub-groups

bled at 1 or 6 months post-first dose should have no impact on overall study results, as the results were similar in each sub-goup. Finally, this phase of the study assessed only short-term antibody response. Persistence of antibodies will be further assessed after three more years.

In summary, the results of this study suggest that a mixed schedule with 9vHPV and 2vHPV vaccines induces an immune response to all 9 HPV types included in the 9vHPV vaccine. This schedule induces higher antibody titers to HPV16 and 18 and lower antibody titers to the other 7 HPV types when compared to two doses of 9vHPV vaccines. As one dose of vaccine seems to induce protection against HPV types included in the vaccines the administration of the second dose of either vaccine which increases the amplitude of the immune response might be seen as a safety insurance of obtaining the desired protection.

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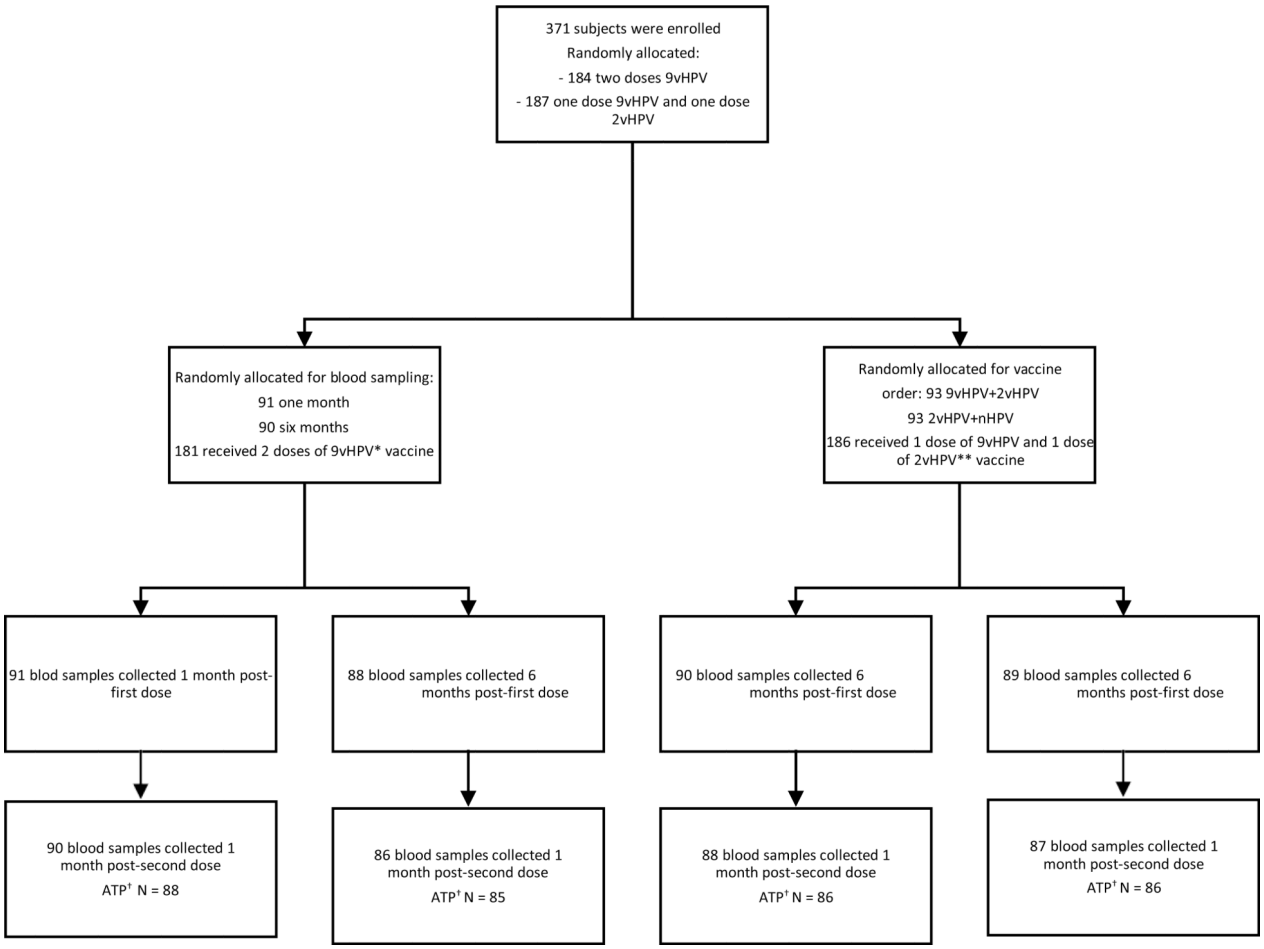

Fig 1.

Study design and participation

*9vHPV – nonavalent HPV vaccine; **2vHPV – bivalent HPV vaccine; ATP† – according to protocol analysis

Table 1.

Subjects' socio-demographic characteristics by randomisation group

Characteristics	Two doses of 9vHPV (N=184)		9vHPV (1st dose) and 2vHPV (2nd dose) (N=94)		2vHPV (1st dose) and 9vHPV (2nd dose) (N=93)		Total (N=371)	
	n	%	n	%	n	%	n	%
Girls	92	50.0	47	50.0	46	49.5	185	49.9
Boys	92	50.0	47	50.0	47	50.5	186	50.1
Mean age at the 1st visit ± SD	9.6 ± 0.3		9.6 ± 0.4		9.6 ± 0.3		9.6 ± 0.3	
Median age at the 1st visit (min-max)	9.6 (9.0–10.7)		9.6 (9.0–10.9)		9.6 (9.0–10.9)		9.6 (9.0–10.9)	
Caucasian	168	91.3	83	88.3	91	97.8	342	92.2
Other ethinicity	16	8.7	11	11.7	2	2.2	29	7.8

Table 2.

Anti-HPV seropositivity and geometrical mean titers post-first dose of 9vHPV and post-first dose of 2vHPV vaccine

HPV type	1 month post-first dose of 9vHPV* (n=88)		6 months post-first dose of 9vHPV (n=171)		6 months post-first dose of 2vHPV** (n=86)	
	% seropositive (95% CI)	GMT (95% CI)	% seropositive (95% CI)	GMT (95%CI)	% seropositive (95% CI)	GMT (95% CI)
HPV6	100.0 (95.9–100.0)	4.6 (3.7–5.6)	100.0 (97.9–100.0)	6.4 (5.6–7.3)	76.7 (66.4–85.2)	0.3 (0.2–0.4)
HPV11	100.0 (95.9–100.0)	5.1 (4.3–6.0)	100.0 (97.9–100.0)	6.9 (6.0–7.9)	67.4 (56.5–77.2)	0.3 (0.2–0.4)
HPV16	100.0 (95.9–100.0)	31.4 (25.8–38.1)	100.0 (97.9–100.0)	30.3 (27.1–33.8)	100.0 (95.8–100.0)	16.7 (13.3–21.0)
HPV18	100.0 (95.9–100.0)	8.8 (7.1–10.9)	100.0 (97.9–100.0)	13.7 (12.2–15.3)	100.0 (95.8–100.0)	11.7 (9.4–14.7)
HPV31	100.0 (95.9–100.0)	23.9 (19.8–29.0)	100.0 (97.9–100.0)	22.6 (19.9–25.7)	70.9 (60.1–80.2)	1.6 (1.2–2.1)
HPV33	100.0 (95.9–100.0)	33.3 (28.0–39.6)	100.0 (97.9–100.0)	36.8 (32.9–41.2)	50.0 (39.0–61.0)	4.0 (3.0–5.3)
HPV45	100.0 (95.9–100.0)	31.9 (25.9–39.3)	99.6 (96.8–99.99)	26.0 (23.0–29.5)	50.0 (39.0–61.0)	7.9 (6.0–10.4)
HPV52	100.0 (95.9–100.0)	21.7 (18.1–26.0)	100.0 (97.9–100.0)	39.1 (33.8–45.1)	54.7 (43.6–65.4)	3.6 (2.5–5.1)
HPV58	100.0 (95.9–100.0)	75.1 (60.8–92.7)	100.0 (97.9–100.0)	70.3 (62.9–78.5)	52.3 (41.3–63.2)	4.1 (3.1–5.5)

*9vHPV – nonavalent HPV vaccine

**2vHPV – bivalent HPV vaccine

Table 3.

Anti-HPV seropositivity and geometrical mean titers one month post-second dose of 9vHPV or 2vHPV vaccine

HPV type	Two doses of 9vHPV* (n=173)			9vHPV (1st dose) and 2vHPV (2nd dose)** (n=86)			2vHPV (1st dose) and 9vHPV (2nd dose) (n=86)		
	seropositive (95% CI)	GMT (95% CI)	Post/pre second dose GMT ratio (95% CI)	% seropositive (95% CI)	GMT (95%CI)	Post/pre second dose GMT ratio (95% CI)	% seropositive (95% CI)	GMT (95% CI)	Post/pre second dose GMT ratio (95% CI)
HPV6	100.0 (97.9–100.0)	375.9 (334.6–422.2)	71.0 (59.0–85.4)	100.0 (95.8–100.0)	8.9 (7.4–10.6)	1.3 (1.03–1.7)	100.0 (95.8–100.0)	8.3 (6.8–10.1)	30.5 (22.1–42.2)
HPV11	100.0 (97.9–100.0)	525.2 (470.1–586.8)	90.9 (76.6–107)	100.0 (95.8–100.0)	9.0 (7.47–10.8)	1.2 (0.97–1.60)	100.0 (95.8–100.0)	9.2 (7.54–11.2)	32.0 (22.7–45.1)
HPV16	100.0 (97.9–100.0)	1174.5 (1049–1315)	39.5 (33.3–46.8)	100.0 (95.8–100.0)	1541 (1289–1841)	47.2 (37.4–59.5)	100.0 (95.8–100.0)	2476 (2130–2878)	148 (112–194)
HPV18	100.0 (97.9–100.0)	593.9 (527.7–668.3)	53.8 (44.7–64.8)	100.0 (95.8–100.0)	969 (793–1183)	72.2 (56.7–92.1)	100.0 (95.8–100.0)	1321 (1130–1545)	112 (85.9–147.0)
HPV31	100.0 (97.9–100.0)	1163.0 (1033–1309)	50.4 (42.2–60.3)	100.0 (95.8–100.0)	104 (89.1–121)	4.50 (3.57–5.7)	100.0 (95.8–100.0)	123 (101–148)	77.3 (56.8–105.0)
HPV33	100.0 (97.9–100.0)	1970.6 (1746–2224)	57.7 (48.6–68.6)	100.0 (95.8–100.0)	79.4 (68.0–92.6)	2.1 (1.7–2.5)	100.0 (95.8–100.0)	58.4 (47.9–71.2)	14.5 (10.3–20.3)
HPV45	100.0 (97.9–100.0)	1230 (1085–1395)	42.0 (34.7–50.7)	100.0 (95.8–100.0)	216 (174–268)	8.6 (6.59–11.1)	100.0 (95.8–100.0)	167 (137–203)	21.1 (15.1–29.5)
HPV52	100.0 (97.9–100.0)	1095 (981–1222)	39.1 (32.7–46.8)	100.0 (95.8–100.0)	63.2 (54.2–73.7)	1.5 (1.17–1.94)	100.0 (95.8–100.0)	59.0 (42.7–60.7)	14.1 (10.0–20.0)
HPV58	100.0 (97.9–100.0)	1859 (1673–2065)	25.8 (21.7–30.6)	100.0 (95.8–100.0)	106.0 (90.7–123.0)	1.5 (1.20–1.82)	100.0 (95.8–100.0)	77.8 (65.5–92.3)	18.8 (13.8–25.6)

*9vHPV – nonavalent HPV vaccine

**2vHPV – bivalent HPV vaccine

Table 4.

Vaccine safety profile (Intention to Treat Analysis)

Adverse Reaction (AR)†	Dose 1		Dose 2	
	2vHPV** vaccine	9vHPV* vaccine	2vHPV vaccine	9vHPV vaccine
	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)
	n=93	n=274	N= 90¥	N=271¥
Local AR				
Pain	83.9 (74.8–90.6)	65.2 (59.2–70.8)	81.1 (71.4–88.5)	67.5 (61.6–73.0)
Redness	34.4 (24.8–44.9)	13.6 (9.73–18.1)	38.9 (28.7–49.7)	27.7 (22.4–33.4)
Swelling	17.2 (10.1–26.4)	7.7 (4.8–11.5)	16.7 (9.64–26)	17.3 (13.0–22.3)
At least one local AR	87.1 (78.5–93.1)	67.4 (61.4–72.9)	82.2 (72.7–89.4)	73.8 (68.1–78.9)
Local Grade 3 AR				
Pain grade 3	7.5 (3.1–14.9)	1.8 (0.6–4.2)	5.6 (1.83–12.4)	2.2 (0.82–4.8)
Redness &/or Swelling >50 mm	1.1 (0.03–5.9)	0.0	1.1 (0.03–6.0)	1.1 (0.01–2.6)
At least one local grade 3 AR	8.6 (3.8–16.2)	1.8 (0.6–4.2)	6.7 (2.49–13.9)	3.3 (1.5–6.2)
Systemic AR				
Fever (≥37.5°C)	6.5 (2.4–13.5)	7.0 (4.24–10.6)	4.4 (1.2–10.9)	3.7 (1.8–6.7)
Fever (≥38°C)	2.2 (0.3–7.6)	2.2 (0.81–4.7)	0.0	0.7 (0.1–2.6)
Fatigue	37.6 (27.7–8.2)	26.4 (21.2–32.0)	27.8 (18.8–38.2)	28.4 (23.1–34.1)
Headache	22.6 (14.5–2.4)	20.9 (16.2–26.1)	15.6 (8.77–24.7)	20.7 (16–25.9)
Gastro-Intestinal	9.7 (4.52–17.5)	10.3 (6.92–14.4)	6.7 (2.49–13.9)	11.1 (7.6–15.4)
Arthralgia	7.5 (3.08–14.9)	7.0 (4.24–10.6)	10.0 (4.68–18.1)	8.1 (5.16–12.0)
Myalgia	43.0 (32.7–53.6)	23.8 (18.8–29.3)	26.7 (17.8–37.0)	22.9 (18.0–28.3)
Rash	6.5 (2.4–13.5)	1.8 (0.6–4.22)	5.6 (1.83–12.4)	4.4 (2.3–7.6)
Urticaria	0.0	1.8 (0.6–4.2)	2.2 (0.27–7.8)	2.2 (0.8–4.8)
At least one systemic AR	66.7 (56.1–76.1)	49.8 (43.7–55.9)	45.6 (35.0–56.4)	52.0 (45.9–58.1)
Systemic Grade3 AR				
Fatigue	2.2 (0.3–7.6)	2.2 (0.8–4.7)	0.0	1.1 (0.2–3.2
Headache	0.0	1.8 (0.6–4.2)	3.3 (0.7–9.4)	1.1 (0.2–3.2)

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Adverse Reaction (AR)†	Dose 1		Dose 2		
	2vHPV** vaccine	9vHPV* vaccine	2vHPV vaccine		9vHPV vaccine
	% (95% CI)	% (95% CI)	% (95% CI)		% (95% CI)
	n=93	n=274	N= 90¥		N=271¥
Gastro-intestinal	0.0	0.4 (0.01–2.0)	0.0		1.5 (0.4–3.7)
Arthralgia	0.0	0.0	0.0		0.0
Myalgia	3.2 (0.7–9.1)	0.7 (0.1–2.6)	1.1 (0.03–6.0)		0.4 (0.01–2.0)
Rash&/or Urticaria	0.0	0.8 (0.01–2.0)	0.0		0.0
At least one systemic grade 3 AR	4.3 (1.2–10.6)	4.0 (2.0–7.1)	3.3 (0.7–9.4)		1.8 (0.6–4.3)

† AR – adverse reaction

* 9vHPV – nonavalent HPV vaccine

** 2vHPV – bivalent HPV vaccine

¥ the difference in number of subjects is due to non- return of 6 AR standardized diaries by parents.