Adjuvant prophylactic human papillomavirus vaccination for prevention of recurrent high-grade cervical intraepithelial neoplasia lesions in women undergoing lesion surgical treatment (VACCIN): a multicentre, phase 4 randomised placebo-controlled trial in the Netherlands

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Summary

Background The loop electrosurgical excision procedure (LEEP) is considered the gold standard for treating high-grade cervical intraepithelial neoplasia (CIN 2–3), but the risk of recurrence persists. Human papillomavirus (HPV) vaccines are highly effective for primary prevention of CIN in women who are HPV naive. Evidence exploring adjuvant prophylactic HPV vaccination after LEEP to reduce recurrent CIN lesions has not been conclusive, and, up to now, no placebo-controlled trials have been done. We aimed to investigate whether adjuvant HPV vaccination after LEEP can reduce the risk of recurrent CIN 2–3 lesions.

Methods In this multicentre, double-blind, placebo-controlled, randomised phase 4 trial at 16 secondary and tertiary hospitals in the Netherlands, we recruited women aged 18 years or older who had histological diagnoses of primary CIN 2–3 and who were treated with LEEP. Participants were randomly allocated (1:1) using a web-based system to three intramuscular injections of either nonavalent HPV vaccine or placebo, administered at enrolment, 2 months, and 6 months. The primary outcome was CIN 2–3 recurrence at 24 months of follow-up. Treatment effect was estimated as relative risk (RR) with 95% CIs in the intention-to-treat group, which comprised all participants who were randomly assigned and received at least one vaccination. Within 2 weeks after each vaccination a safety assessment was done by telephone. This trial is registered with the Dutch trial registry (NL-OMON22561) and the International Clinical Trial Registry Platform (EUCTR2018-002764-94-NL). Recruitment and data collection have been completed.

Findings Between Dec 19, 2019, and Jan 31, 2022, 840 participants were enrolled, of whom 29 withdrew their consent and two were excluded for not meeting inclusion criteria before LEEP and vaccination. Outcome data were obtained for 809 participants, of whom 402 (50%) were allocated HPV vaccination and 407 (50%) were allocated placebo vaccination, comprising the intention-to-treat group. Over the 24-month follow-up period, recurrences of CIN 2–3 were reported in 23 (6%) of 402 participants in the HPV-vaccinated group versus 35 (9%) of 407 participants in the placebo group (RR 0·67 [95% CI 0·40–1·11], p=0·11). No vaccine-related serious adverse events were reported.

Interpretation The results of this study did not show superiority of adjuvant HPV vaccination over placebo in women treated for CIN 2–3. Routine administration of adjuvant HPV vaccination did not contribute to the prevention of CIN recurrence in these women. Adjuvant HPV vaccination after treatment should be administered exclusively within trials to study subgroups that could potentially benefit.

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Introduction

Cervical dysplasia, a precursor to cervical cancer, is a serious health concern with an estimated prevalence of 4·3% worldwide,[1] and is caused by certain high-risk types of human papillomavirus (HPV).[2,3] Of all HPV types, approximately 15 are considered to be high risk, with HPV types 16 and 18 accounting for 70% of cervical cancers.[4] Although the primary treatment for high-grade cervical intraepithelial neoplasia (CIN 2–3, also known as high-grade squamous intraepithelial lesion) often involves surgery, such as the loop electrosurgical excision procedure (LEEP), a persistent risk of recurrence of up to 17% has been reported.[5] With recurrent or residual dysplasia, repeated treatment can be necessary, and LEEP (particularly multiple attempts) is associated with adverse obstetric events, such as premature birth.[6]

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See **Online** for appendix 1

Research in context

Evidence before this study

Before this trial, we searched the following databases from inception until Dec 1, 2017: PubMed, MEDLINE, and the Cochrane Library. The search strategies contained various combinations of keywords and MeSH headings: "papillomavirus vaccines", "HPV vaccine", "human papillomavirus vaccination", "papillomaviridae", "HPV", "LEEP", "conization", "CIN", "surgical therapy", and "biopsy". The search was restricted to English or Dutch language manuscripts. 27 potentially eligible articles were found, of which five were useful for our question and used in this review. A 2013 study suggested that adjuvant prophylactic human papillomavirus (HPV) vaccination could be considered in reducing recurrence after treatment of high-grade cervical intraepithelial neoplasia (CIN 2–3). Three studies published between 2012 and 2016 reported on vaccine efficacy, with varying outcomes; however, none of these trials was designed or intended to address the effect of vaccination after loop electrosurgical excision procedure. Furthermore, the vaccination was not administered during the loop electrosurgical excision procedure treatment period. No level 1 evidence was available on the efficacy of adjuvant HPV vaccination in women who are treated for cervical dysplasia.

Added value of this study

To our knowledge, this is the largest double-blinded, placebo-controlled trial on prophylactic HPV vaccination in women treated for CIN 2–3. Although previous studies, including meta-analyses and observational studies, have shown that adjuvant HPV vaccination reduces the recurrence of cervical dysplasia after surgical treatment, our trial suggests that adjuvant HPV vaccination is not effective in reducing the recurrence of CIN 2–3 lesions, contradicting the conclusions of previous works.

Implications of all the available evidence

Some clinical guidelines recommend additional prophylactic HPV vaccination after treating CIN lesions based on a lower level of evidence. Our study provides level 1 evidence that contradicts these current guideline recommendations. Although our study has limitations, guidelines should be more reticent in recommending additional prophylactic HPV vaccination after treating CIN lesions.

The safety of HPV vaccines is widely proven. HPV vaccination effectively reduces the risk of developing cervical dysplasia, and the most recent evidence also suggests a massive effect on decreasing cervical cancer prevalence by approximately 88%.[7–10] Although these studies show a major risk reduction, less is known about the effectiveness of HPV vaccination in women already infected with HPV or those who have already developed cervical dysplasia. Recent studies have explored the potential for adjuvant HPV vaccination as a supplementary measure to reduce the risk of recurrent CIN lesions, with results suggesting a promising effect in decreasing the likelihood of recurrent cervical dysplasia.[11–15] However, most of these studies were based on retrospective data[11,15] or were not designed for this research question.[13,14] Although multiple reviews have been conducted,[16–19] there is still insufficient evidence from randomised controlled trials to guide clinical practice.

There are multiple reasons to consider HPV vaccination after LEEP treatment. Vaccination could enhance the immune response against existing HPV infections, provide protection against new HPV infections, and possibly protect against viral reactivation of latent HPV infections, thereby reducing the overall risk of HPV-related diseases.[20] Although evidence is currently minimal, professional guidelines in some countries already recommend concurrent adjuvant HPV vaccination at surgical treatment for previously unvaccinated individuals undergoing treatment for cervical dysplasia.[21,22]

We aimed to evaluate the efficacy (superiority) of adjuvant nonavalent HPV vaccination compared with placebo on CIN 2–3 recurrence over 2 years of follow-up in women with a CIN 2–3 lesion who were planned for LEEP. We also aimed to assess the recurrence of any CIN, the effect on HPV presence and Pap smear results, the number of LEEPs, quality of life, and side-effects or adverse events associated with vaccination.

For the **study protocol** see https://zorgevaluatienederland-api-production.s3.eu-central-1.amazonaws.com/media/7927/C1.-Onderzoeksprotocol-versie-3.0-d.d.-13jan21_Geredigeerd.pdf

Methods

Study design

The Vaccination and Cervical Intraepithelial Neoplasia (VACCIN) study is a multicentre, double-blind, randomised, placebo-controlled phase 4 trial done in 16 secondary and tertiary hospitals in the Netherlands (appendix 2 p 4). Details of the study design have been published previously.[23] The study protocol is available online. Ethics committee approval was received from the Erasmus MC research ethics committee (MEC-2019-0067). This trial was registered with the Dutch trial registry (NL-OMON22561) and the International Clinical Trial Registry Platform (EUCTR2018-002764-94-NL). The Dutch patient foundation Olijf (Utrecht, Netherlands) was involved in the design of the trial. All participants gave written informed consent. The study was conducted in accordance with the Declaration of Helsinki.

Participants

Eligible participants were women aged 18 years and older with primary, histologically proven CIN 2–3 that was diagnosed in outpatient colposcopy clinics in one of the participating hospitals in the Netherlands, and who were planned for LEEP treatment or had a see-and-treat

procedure, with final pathology results showing CIN 2 or CIN 3. Exclusion criteria were previous HPV vaccination, invasive or microinvasive carcinoma, immunocompromised individuals (defined as status after organ transplantation, HIV, immune deficiencies, or indication for glucocorticoid stress schedule), pregnancy, previous treatment for CIN lesions, allergy to vaccine components, or insufficient understanding of the Dutch language. Participants were asked to use reliable contraceptives during the vaccination period (ie, for 6 months). In the case of pregnancy during the vaccination period, participants were discontinued from further study participation because HPV vaccination during pregnancy is not recommended. Nonetheless, data from these participants were still collected.

Randomisation and masking

Participants were randomly assigned (1:1) to either adjuvant HPV vaccination or placebo using a web-based randomisation module (Castor EDC, Ciwit BV, Amsterdam, Netherlands). An independent employee of the clinical trials office from the Dutch Society for Obstetrics and Gynaecology generated the randomisation sequence. Following this process, an automated email was sent to the local pharmacy indicating the assigned intervention group. The treating physician informed the pharmacy to associate the participant data with the corresponding study number. Following this process, the pharmacy was able to prepare the masked medication. Identical syringes, coated with tape to mask the suspension, were used in both groups to maintain masking. Randomisation was stratified by three different age groups: 18–29 years, 30–44 years, and 45 years and older. Health-care providers and participants were masked to the treatment allocation throughout the study process.

Procedures

Participants allocated to active vaccination Gardasil 9 (MSD, Rahway, NJ, USA) received three intramuscular doses containing HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58. Participants allocated to placebo received three intramuscular doses of sterile 0·9% NaCl saline solution. The vaccinations followed a regular three-dose schedule (enrolment, 2 months, and 6 months). The first vaccination was planned to be administered by a research nurse on the day of LEEP treatment, but could be accepted up to 4 weeks after LEEP when pathology was confirmed in a see-and-treat case (immediate treatment after visual inspection, without punch biopsy). 2 weeks after each vaccination, the participant had a telephone appointment with a research nurse or their treating physician to determine side-effects or adverse events. A standardised set of items was pre-assessed, each rated on a 0–10 scale to indicate severity. These items included pain, redness, headache, fatigue, and other symptoms (not specified). Additionally, participants were asked about self-reported adverse events. All reported items were recorded as if they were linked to the vaccination. A complete overview of timepoints for administering medication and collecting data is included in the study protocol.[23]

All participants received care according to Dutch guidelines for cervical dysplasia.[24] The pathology review was done by a local pathologist, who was masked to the random assignment. Pathology reviews were done at the discretion of the pathologists; no standard p16 staining was done. HPV testing was done 6 months and 24 months after treatment to check for high-risk HPV with an HPV DNA assay, without any restrictions on HPV assays. Cytology (Pap smear) was also done at 6 months and 24 months. Unscheduled visits occurred as needed. If HPV testing and cytology at 24 months were negative, the participant could return to regular screening. If one of the co-tests (at 6 months or 24 months) was abnormal, further investigation was required according to the guidelines; this could involve repeating the cytology test or performing a colposcopy, depending on the cytology result and HPV status. All participants with a cytology result of Pap 2 or higher (some abnormal cells; eg, atypical squamous cells of undetermined significance) or a positive HPV test at 24 months were to have colposcopy with biopsy for the histological endpoint. A normal cytology result (ie, Pap 1) in the absence of HPV was considered a proxy for no CIN (appendix 2 p 9). Participants had the choice to continue with masked vaccination if the final pathology review revealed an unexpected adenocarcinoma in situ (AIS) or carcinoma.

See Online for appendix 2

An independent data safety monitoring board monitored the safety of trial participants. Monitoring was done by means of safety reviews. There was no statistical interim analysis for efficacy.

Outcomes

The primary outcome was pathologically confirmed CIN 2–3 cumulative incidence over the 24-month follow-up period. Secondary outcomes were the recurrence of any CIN at 6 months and 24 months, HPV DNA presence (measured by testing at 6 months, 24 months, and unscheduled visits), Pap smear results (measured by testing at 6 months, 24 months, and unscheduled visits), number of LEEP reinterventions over the 24-month follow-up period, quality of life (measured using the EuroQol five-dimension, five-level questionnaire), and side-effects and adverse events (as described in Procedures). A cost-effectiveness analysis was also prespecified but will be reported separately.

Statistical analysis

To show a reduction of CIN 2–3 after LEEP from 8·0% in the placebo group to 3·0% in the HPV vaccine group using an α of 5% and target power of 80%, 646 participants had to be enrolled. The sample size was calculated based on a previous study by Kang and

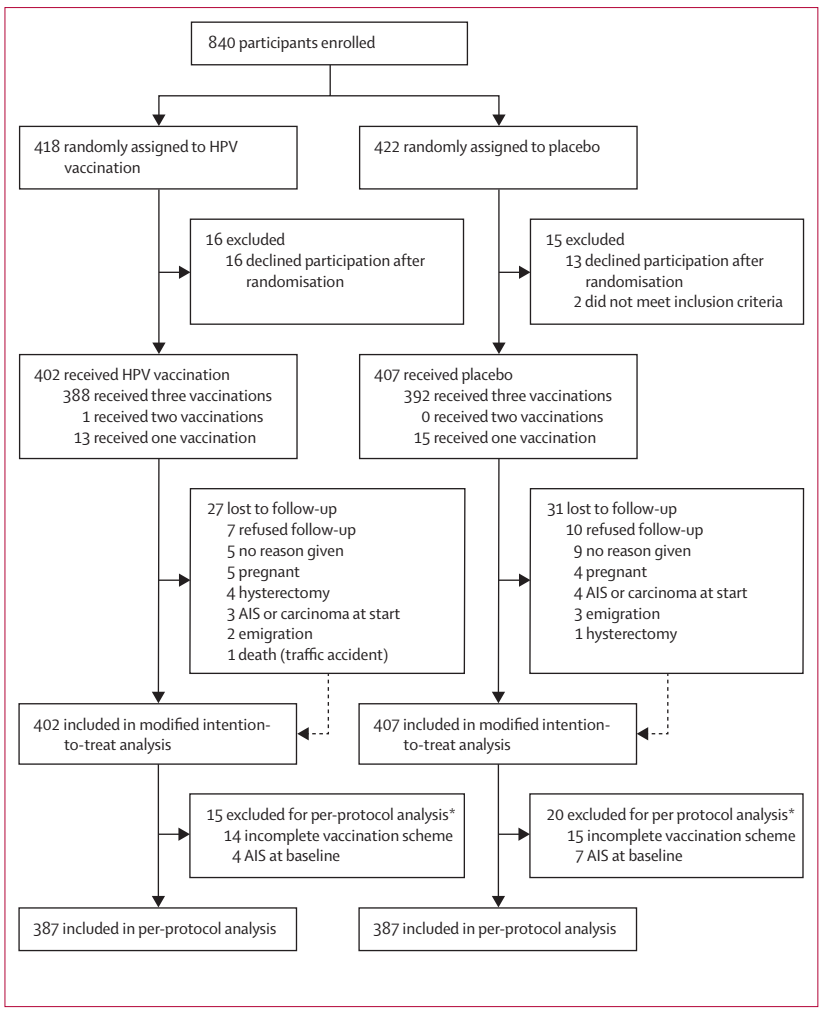

Figure 1: **Trial profile**
AIS=adenocarcinoma in situ. HPV=human papillomavirus. *AIS and incomplete vaccination can go hand in hand.

colleagues.[11] Clinical practice shows that the proportion of women who do not attend follow-up visits in this population is about 15%. Therefore, the target sample size was set at 750 participants.

For the primary outcome, a post-hoc modified intention-to-treat analysis was done to estimate the relative risk (RR) with 95% CIs for CIN 2–3 up to 24 months, using a generalised linear model with log-link and binomial distribution and accounting for the stratification by age group as a covariable. Inferential testing was done using a χ^2 test. A modified intention-to-treat analysis was done on all participants who were randomly allocated and received at least one vaccination or placebo dose. Vaccine effectiveness was defined as the absence of recurrent CIN 2–3 after vaccination and was estimated from the RR as 1 minus (disease incidence with the HPV vaccine divided by disease incidence with the placebo vaccine). The effect of missing primary outcome data was assessed using multiple imputation (appendix 2 p 7, appendix 3 pp 1–19). Data were imputed using fully conditional specification under a missing-at-random assumption 25 times, and results were weighted using Rubin's rules.

See Online for appendix 3

A preplanned per-protocol analysis of the primary outcome was also done. We defined participants for the per-protocol analysis as any participant who received all three doses of the study medication (either active or placebo) or those who received fewer doses but in whom CIN 2–3 recurred before the last doses would have been administered, and excluded participants with AIS or carcinoma at final pathology diagnosis at baseline.

Preplanned subgroup analyses for heterogeneity of treatment effects over age groups (18–29 years, 30–44 years, and ≥45 years) were conducted using the analysis model for the primary outcome with an interaction term for age groups added to the regression model. The p value for type III effects was used to assess interaction. A preplanned time-to-event analysis of the primary outcome was conducted using Kaplan–Meier curves.

RRs for the dichotomous secondary outcomes, including harms, were estimated using the same generalised linear models with binomial distribution and log-link as for the primary outcome. Where appropriate, Fisher's exact test was used, as indicated in the results. Continuous secondary data were described using means (SD), and mean differences (95% CI) were estimated using ANOVA. The time to reach HPV-negative status was studied using Kaplan–Meier curves. Quality of life at 24-month follow-up was analysed as mean scores with a difference in means with bootstrapped CIs (1000 samples).

A p value of less than 0·05 on a two-sided test was considered significant. All analyses were done using the software packages SPSS version 28.0 and SAS version 9.4.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between Dec 19, 2019, and Jan 31, 2022, 840 participants were enrolled, of whom 418 (50%) were allocated to HPV vaccination and 422 (50%) were allocated to placebo (figure 1). Before treatment and vaccination, 16 participants in the HPV vaccination group and 13 in the placebo group withdrew their consent, and two participants in the placebo group did not meet the inclusion criteria and were excluded. Of the 809 participants who had LEEP and had the study treatment (intention-to-treat group), 402 (50%) were allocated to the HPV vaccine and 407 (50%) were allocated to placebo. All participants had histologically proven CIN 2–3 at the time of vaccination. More participants

	Placebo (n=407)	HPV vaccine (n=402)
Age		
Mean, years	39·4 (8·6)	39·2 (8·7)
18–29 years	16 (4%)	20 (5%)
30–44 years	277 (68%)	267 (66%)
≥45 years	114 (28%)	115 (29%)
HPV history		
Vulvar intraepithelial neoplasia	3 (1%)	0
Vaginal intraepithelial neoplasia	1 (<1%)	2 (<1%)
Genital warts	8 (2%)	8 (2%)
Anus carcinoma	0	0
HPV-related oropharyngeal diseases	1 (<1%)	1 (<1%)
Smoker	99 (24%)	93 (23%)
1–10 cigarettes per day	46 (11%)	49 (12%)
11–20 cigarettes per day	32 (8%)	27 (7%)
>20 cigarettes per day	6 (1%)	3 (1%)
Use of contraceptives		
Condoms	35 (9%)	28 (7%)
Oral contraceptives	112 (28%)	132 (33%)
Levonorgestrel IUD	98 (24%)	82 (20%)
Copper IUD	12 (3%)	14 (3%)
Implant or injectable contraceptive	12 (3%)	11 (3%)
Other (not specified)	16 (4%)	17 (4%)
Condom complementary contraceptive	50 (12%)	33 (8%)
Pregnancy—nulliparous	138 (34%)	135 (34%)
Desire to have children in the future		
Yes	87 (21%)	99 (25%)
No	262 (64%)	251 (62%)
Do not know	57 (14%)	52 (13%)
Indication cytology testing (multiple answers possible)		
Population screening	258 (63%)	259 (64%)
Complaints	38 (9%)	34 (8%)
Follow-up	109 (27%)	108 (27%)
Other	4 (1%)	6 (1%)
(Table 1 continues in next column)		
(Continued from previous column)		
Cytology for colposcopy referral		
Pap 1	2 (<1%)	0
Pap 2	41 (10%)	43 (11%)
Pap 3a1	73 (18%)	72 (18%)
Pap 3a2	191 (47%)	170 (42%)
Pap 3b	93 (23%)	111 (28%)
Pap 4	6 (1%)	2 (<1%)
Pap 5	1 (<1%)	2 (<1%)
Unknown	0	2 (<1%)
HPV status at cytology		
Positive	390 (96%)	394 (98%)
Negative	3 (1%)	2 (<1%)
Unknown	14 (3%)	6 (1%)
Biopsy colposcopy result		
CIN 2	193 (47%)	183 (46%)
CIN 3	165 (41%)	163 (41%)
See-and-treat	49 (12%)	56 (14%)
LEEP result		
No CIN	6 (1%)	8 (2%)
CIN 1	27 (7%)	32 (8%)
CIN 2	180 (44%)	160 (40%)
CIN 3	187 (46%)	197 (49%)
Carcinoma or adenocarcinoma in situ	7 (2%)	4 (1%)
Not assessable	0	1 (<1%)
LEEP margins		
Positive	34 (8%)	43 (11%)
Negative	29 (7%)	34 (8%)
Not assessed	344 (85%)	324 (81%)

Data are mean (SD) or n (%). HPV=human papillomavirus. IUD=intra-uterine device. CIN=cervical intraepithelial neoplasia. LEEP=loop electrosurgical excision procedure.

Table 1: **Baseline characteristics**

were enrolled than initially planned due to direct withdrawal of consent or those with an AIS or carcinoma after randomisation. According to Good Clinical Practice guidelines, we could not withhold study participation from participants who had already consented before histology results were obtained.

Baseline characteristics of the 809 participants in the intention-to-treat group, including pathological details and results of primary intervention, showed even distribution between groups (table 1). All participants underwent LEEP as primary treatment. A biopsy was taken in 704 (87%) participants before LEEP, whereas the other 105 participants (13%) received so-called see-and-treat colposcopy. 11 (1%) participants were found to have unexpected AIS or carcinoma after randomisation. Seven (<1%) participants with AIS or cervical carcinoma discontinued the study after the first vaccination, and no follow-up data were available. According to the intention-to-treat principle, all participants were analysed according to their random assignment. The full vaccination schedule was completed by 781 (97%) participants, with no difference between treatment groups.

The median follow-up time was 749 days (IQR 711–781). After 24 months of follow-up, 23 (6%) of 402 participants in the vaccine group had a recurrence of CIN 2–3 versus 35 (9%) of 407 participants in the placebo group (RR 0·67 [95% CI 0·40–1·11], p=0·11; table 2). Strictly, the primary outcome was defined as cumulative recurrence of CIN grade 2–3. One participant was diagnosed with squamous cell carcinoma in the placebo group, and two participants in the HPV group were diagnosed with an AIS in the follow-up period. All three of these participants were excluded from the primary outcome. Including these cases in the primary

	Placebo (n=407)	HPV vaccine (n=402)	RR	p value
Primary outcome				
CIN grade 2 or 3	35 (9%)	23 (6%)	0·67 (0·40–1·11)	0·11
Intention-to-treat	35 (9%)	23 (6%)	0·67 (0·40–1·11)	0·11
Per-protocol (387 *vs* 387)	34 (8%)	22 (5%)	0·65 (0·17–1·09)	..
Pathology, highest grade diagnosed at 6-month follow-up				
No CIN	2 (<1%)	1 (<1%)	0·47 (0·04–5·10)	0·53
CIN 1	6 (1%)	4 (1%)	0·62 (0·18–2·18)	0·46
CIN 2	11 (3%)	4 (1%)	0·35 (0·11–1·07)	0·072
CIN 3	14 (3%)	10 (2%)	0·67 (0·30–1·49)	0·33
AIS or carcinoma	0	0	..	..
Pathology, highest grade diagnosed at 24-month follow-up				
No CIN	283 (70%)	282 (70%)	1 (ref)	..
CIN 1	15 (4%)	15 (4%)	1·00 (0·50–2·02)	0·99
CIN 2	16 (4%)	10 (2%)	0·64 (0·30–1·39)	0·25
CIN 3	19 (5%)	13 (3%)	0·70 (0·35–1·39)	0·31
AIS or carcinoma	1 (<1%)	2 (<1%)	2·00 (0·18–54·4)	1·00*
HPV status for 6-month follow-up				
Negative	275 (68%)	293 (73%)	1 (ref)	..
Positive	120 (29%)	100 (25%)	0·84 (0·67–1·05)	0·12
Unknown	12 (3%)	11 (3%)	0·87 (0·39–1·93)	0·72
HPV status for 24-month follow-up				
Negative	253 (62%)	269 (67%)	1 (ref)	..
Positive	148 (36%)	127 (32%)	0·87 (0·72–1·05)	0·15
Unknown	6 (1%)	6 (1%)	..	..
Cytology for 6-month follow-up				
Pap 1	325 (80%)	338 (84%)	1 (ref)	..
Pap 2	28 (7%)	27 (7%)	0·93 (0·56–1·55)	0·79
Pap 3a1	13 (3%)	8 (2%)	0·60 (0·25–1·43)	0·25
Pap 3a2	21 (5%)	14 (3%)	0·66 (0·34–1·27)	0·21
Pap 3b	12 (3%)	7 (2%)	0·57 (0·23–1·43)	0·23
≥Pap 4	0	0	..	..
Missing	8 (2%)	8 (2%)	0·96 (0·37–2·53)	0·94
(Continued from previous column)				
Highest cytology for 24-month follow-up				
Pap 1	298 (73%)	311 (77%)	1 (ref)	..
Pap 2	45 (11%)	37 (9%)	0·81 (0·54–1·22)	0·31
Pap 3a1	18 (4%)	17 (4%)	0·91 (0·48–1·73)	0·77
Pap 3a2	25 (6%)	22 (5%)	0·85 (0·49–1·48)	0·57
Pap 3b	15 (4%)	10 (2%)	0·65 (0·30–1·43)	0·28
≥Pap 4	0	0	..	..
Missing	6 (1%)	5 (1%)	..	..
LEEP at 6-month follow-up	22 (5%)	12 (3%)	0·54 (0·27–1·08)	0·085
Additional LEEP during 24-month follow-up†	35 (9%)	23 (6%)	0·87 (0·56–1·39)	0·57

Data are n (%), RR (95% CI), or p. HPV=human papillomavirus. RR=relative risk. CIN=cervical intraepithelial neoplasia. AIS=adenocarcinoma in situ. LEEP=loop electrosurgical excision procedure. *Fisher's exact test. †Seven participants had a third LEEP (four in the HPV vaccine group and three in the placebo group).

Table 2: **Primary and secondary outcomes**

outcome, the RR is estimated at 0·70 (0·43–1·15; p=0·16). The primary outcome for the per-protocol analysis is reported in table 2.

The time to CIN 2–3 or AIS is shown in figure 2A. The results of analysis for the primary outcome after multiple imputations are shown in appendix 2 (p 7). With respect to subgroup effects, no recurrences were observed in the youngest age stratum, and no heterogeneity of treatment effects was observed for ages 30–44 years compared with ages 45 years and older (appendix 2 p 8). There was no evidence of heterogeneity of treatment effects.

According to our study protocol, a biopsy should be performed irrespective of colposcopy findings when HPV is positive or cytology is abnormal at 24 months. Histology was missing for 95 (12%) of 809 participants due to the treating physician not performing biopsies according to the protocol. Of the 95 participants with histology missing, 75 (80%) had normal cytology on Pap smear, and 10 (11%) had a Pap 2 or higher with a positive HPV test (appendix 2 p 1). 58 (7%) of 809 participants did not complete the follow-up schedule as planned. Of these, 46 (79%) participants were lost to follow-up, and 12 (21%) did not receive colposcopy due to an AIS or carcinoma at the start of the study or had a hysterectomy for a reason other than CIN lesions. Of the 46 participants lost to follow-up, 40 (87%) had normal cytology (Pap 1) at the last hospital visit. Of these 40 participants, 33 (83%) were HPV negative (appendix 2 p 1).

Nine (1%) participants underwent surgery other than LEEP for cervical dysplasia (eight hysterectomies and one cervical amputation), and 65 additional LEEPs were performed, including seven participants who had a third LEEP (four in the HPV vaccine group and three in the placebo group; appendix 2 p 1).

During the 24-month follow-up period, any CIN (CIN 1–3) recurrence was reported in 38 (9%) participants in the HPV vaccine group versus 50 (12%) participants in the placebo group (RR 0·77 [95% CI 0·52–1·15], p=0·20; appendix 2 p 1). At 6 months, 18 (4%) participants in the HPV vaccine group had any CIN recurrence, versus 31 (8%) in the placebo group (RR 0·59 [0·33–1·03], p=0·055; appendix 2 p 2).

At 6-month follow-up, 100 (25%) participants were HPV positive in the HPV vaccine group versus 120 (29%) in the placebo group (RR 0·84 [95% CI 0·67–1·05], p=0·12). In the HPV vaccine group, 338 (84%) participants had normal cytology versus 325 (80%) in the placebo

group, and 33 (8%) participants had a formal indication for colposcopy (≥Pap 3a2) in the placebo group versus 21 (5%) in the HPV vaccination group (RR 0·63 [0·37–1·08], p=0·091; table 2).

34 additional LEEPs were performed at the 6-month follow-up, 12 (35%) in the HPV vaccine group versus 22 (65%) in the placebo group (RR 0·54 [95% CI 0·27–1·08], p=0·076). Three participants underwent a hysterectomy for dysplasia after colposcopy. In post-hoc analysis, the RR of a secondary treatment (LEEP or hysterectomy) at 6 months following abnormal cytology was 0·52 (95% CI 0·27–1·00; p=0·053). Three participants in the placebo group followed an expected management with a CIN 2 at 6 months without additional treatment during the study period. One of these participants had a normal cytology and a negative HPV status at 24-month follow-up. One participant had missing data, and one participant was HPV positive and had Pap 2 cytology at 24 months. 18 additional LEEPs were performed during the follow-up period after 6 months and before 24 months. Ten (56%) of these LEEPs were in the HPV vaccination group and eight (44%) were in the placebo group (appendix 2 pp 1–3).

During the 24-month follow-up period, HPV positivity was recorded in 127 (32%) participants in the HPV vaccination group versus 148 (36%) participants in the placebo group (RR 0·87 [95% CI 0·72–1·05], p=0·15; table 2). The time to HPV negativity is shown in figure 2B. The cumulative number of LEEPs at 24-month follow-up was 35 (9%) in the placebo group versus 23 (6%) in the HPV vaccine group (RR 0·87 [0·56–1·39], p=0·57; table 2).

The number of respondents to the EuroQol-5D-5L questionnaire was 749 (93%) of 809 at baseline, 714 (88%) at 2 months, 714 (88%) at 6 months, and 631 (78%) at 24 months. No significant difference was reported in average self-reported health status. 24 months after treatment, the average EuroQol score was 0·89 in the HPV vaccination group compared with 0·88 in the placebo group (mean difference 0·009 [95% CI –0·01 to 0·03], p=0·42).

Two participants discontinued vaccination due to presumed side-effects. One participant in the placebo group had a rash a couple of weeks after the second vaccination and withdrew themselves for vaccination. Another participant in the HPV vaccine group discontinued after local pain at the injection site. Redness and pain at the injection site were significantly more common in the HPV vaccination group (redness RR 29·44 [95% CI 4·03–215·05], p<0·0001; pain RR 7·84 [4·33–14·47], p<0·0001; table 3). One participant died due to a traffic accident a couple of weeks after the last dose. No other deaths occurred. Fever, headache, and tiredness were not significantly more common in the HPV vaccination group after the first vaccination (table 3). A list of adverse events reported by each participant is available in appendix 2 (pp 5–6).

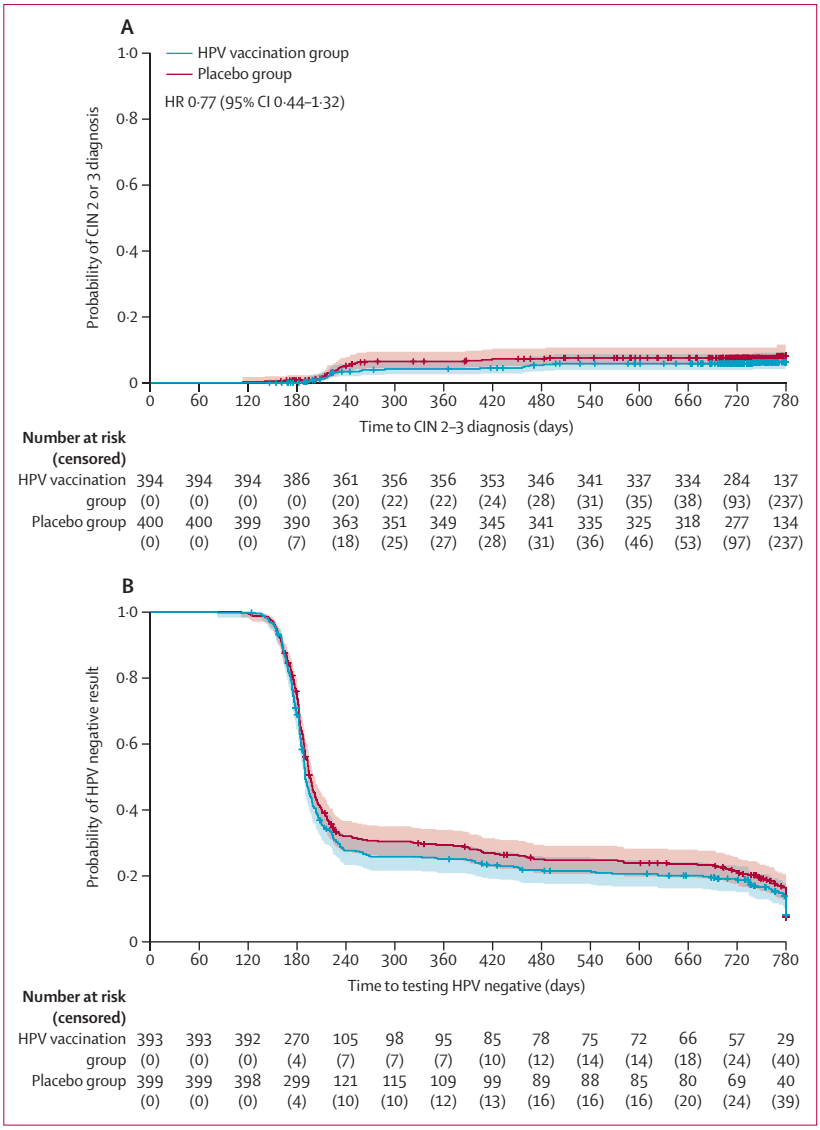

Figure 2: **Kaplan–Meier curves to CIN diagnosis (A) and HPV negative test (B) for all participants**
CIN diagnosis (A) and HPV negative test (B). CIN=cervical intraepithelial neoplasia. HPV=human papillomavirus.

Discussion

To our knowledge, this is the largest randomised, double-blinded, placebo-controlled trial investigating the effects of adjuvant HPV vaccination in women who have received LEEP. After 24 months, the study showed no benefit of HPV vaccination in reducing CIN 2–3 recurrence and no major differences in the need for additional treatments (LEEP or hysterectomies) or HPV positivity compared with placebo. At the 6-month follow-up, there was a small difference in the number of colposcopies and a slight difference in HPV negativity in favour of HPV vaccination without any histologic difference after biopsy or LEEP treatment. Redness and pain at the injection site were more common in the group that received HPV vaccination. Such reactions are

	Placebo (n=407)	HPV vaccine (n=402)	RR	p value
Discontinued for adverse effects	1 (<1%)	1 (<1%)	NA	..
Redness	1 (<1%)	29 (7%)	29·44 (4·03–215·05)	<0·0001
Pain	11 (3%)	85 (21·1)	7·84 (4·33–14·47)	<0·0001
Pain score	5 (2–6)	3 (2–4)	..	0·30*
Fever	10 (2%)	4 (1%)	0·74 (0·30–1·82)	0·51
Headache	28 (7%)	29 (7%)	1·05 (0·64–1·73)	0·84
Tiredness	24 (6%)	24 (6%)	1·02 (0·59–1·76)	0·96

Data are n (%), median (IQR), RR (95% CI), or p. HPV=human papillomavirus. RR=relative risk. NA=not applicable. *Mann–Whitney test.

Table 3: **Reported side-effects**

typical side-effects associated with vaccinations and, as such, are anticipated. One participant from each group discontinued vaccination due to these side-effects, indicating that they were generally well tolerated. Despite the increased reporting of side-effects, we observed no difference in quality of life.

There has only been one randomised, placebo-controlled trial conducted before that studied additional HPV vaccination on CIN 2–3 lesions in women living with HIV.[25] That study by Firnhaber and colleagues also showed no added benefit of adjuvant HPV vaccination. This result might be explained by the immunocompromised status of these women, which affects viral control and vaccine response.[26,27] Two other randomised controlled trials examined the efficacy of a prophylactic HPV vaccine in reducing disease recurrence. Karimi-Zarchi and colleagues focused on women with recurrent CIN,[13] whereas the randomised controlled trial by Pieralli and colleagues involved women who were HPV negative and were assessed 3 months after LEEP.[14] Both studies showed a positive outcome associated with HPV vaccination; however, LEEP was not done at the time of vaccination. Most non-randomised studies also pointed to a positive effect of additional HPV vaccination.[11,15,28,29] These studies are likely to have important biases: women had to pay for their vaccination, or vaccination was administered up to 1 year after surgical treatment. The most extensive study on HPV vaccination after treatment was performed by Sand and colleagues.[30] Using Danish nationwide registries of women who received HPV vaccination 3 months before to 1 year after surgical treatment, this study reported a non-significant decrease in grade 2 or worse CIN in women who were HPV vaccinated.

The strength of our study is the randomisation and double-blinded design. Both the participant and physician were unaware of the treatment allocation throughout the trial. Furthermore, this is a high-volume, nationwide trial that adequately represents the population diagnosed with CIN 2–3 in general. The results of recurrence rates after LEEP treatments of roughly 8% are consistent with the literature and show a good reflection of daily practice. The high number of participants who received all three vaccinations indicates high compliance.

The primary endpoint had to be obtained from definitive pathological results after colposcopy. Biopsies for histologic endpoint were used if testing was positive for high-risk types of HPV or the cytology result was Pap 2 or higher. In 95 (12%) participants, histology was not obtained on the basis of the treating physician's decision, but this should have been diagnosed pathologically. Only ten (1%) of these participants had a Pap 2 or higher with a positive HPV test. Therefore, the likelihood of missing high-grade lesions is minimal.

Limitations of the VACCIN study were that the HPV tests used were not standardised or protocolised, and HPV typing was not mandatory. Most hospitals used Aptima (Hologic, Marlborough, MA, USA) HPV assays, testing on 14 types of high-risk HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) or the cobas 4800 system (Roche, Basel, Switzerland). This test specifies only between 16, 18, or another high-risk type of HPV. As a result, there is a limitation to the specification of HPV types and the effect of vaccination on the presence of specific HPV types in relation to the strains in the vaccine. Unfortunately, the funding for HPV typing was insufficient. Separate funding has been requested to perform HPV typing post hoc for all samples and study these types. HPV typing was reported sporadically, but it was insufficiently representative for this paper.

HPV positivity is the most valuable predictor for recurrence, but the second-best predictor is the margins of the LEEP. Another limitation of our study is that the assessment of LEEP margins is not mandatory for pathology review in the Netherlands, as it has no clinical consequence according to Dutch guidelines. Additional resection is not routinely performed when margins are positive after LEEP. The number of missing reported LEEP margins was 83% in our study. No statistical analysis was performed with these numbers of non-reported data.

One of the reasons to prevent a secondary LEEP is the possibility of adverse obstetric outcomes, such as premature labour. Although 513 (63%) of the 809 participants in our study said that they did not want to conceive in the future, even a smaller effect could still be beneficial for the subgroup that plans to have children. In a future study, we will examine a potential impact on pregnancies.

The safety and side-effects of HPV vaccination have been broadly investigated in previous studies. HPV vaccination is safe and well tolerated.[31] Even though side-effects are primarily local reactions such as pain or swelling, they occurred significantly more frequently in the HPV vaccination group. Without a positive protective effect, the benefit–risk balance would be negative. Given

the low burden of adjuvant vaccination with LEEP and the high burden and cost associated with disease recurrence, a smaller risk reduction could be relevant.

The effect of vaccination was less than the 62·5% reduction we expected on the basis of our sample size calculation. This trial indicates that there might be a lower reduction of CIN 2–3 after treatment; however, this finding requires a confirmation trial with a revised power calculation. Based on the data from this study, a new trial with approximately 2850 women will be required. We have no information on whether women received HPV vaccinations outside the study. Whether the 33·7% reduction (from 8·6% to 5·7%) in recurrence is clinically relevant is up for debate. Cost-effectiveness will be the most important factor. Further research is needed to investigate smaller positive effects or to identify a group that could benefit from additional HPV vaccination.

In conclusion, the effect of additional HPV vaccination in women treated with LEEP for cervical dysplasia is smaller than anticipated and not significant after 2 years of follow-up. Superiority over placebo could not be shown. Therefore, we do not support routine administration of additional HPV vaccination in women treated for CIN 2–3. Further research is needed to investigate smaller positive effects or to identify a group that could benefit from additional HPV vaccination.

Contributors

RLOvdL, WH, RLMB, and HJvB conceptualised the study and acquired funding. RLOvdL, WH, HJvB, and RGD designed the study. RGD designed the methodology. RLOvdL and WH were responsible for project administration. RLOvdL, WH, RLMB, HPMS, GMN-dB, and the VACCIN study group performed data collection. RLOvdL and RGD directly accessed and verified the data. RLOvdL, RGD, and HJvB analysed the data and wrote the original draft. WH, RLMB, HPMS, and GMN-dB reviewed and edited the manuscript. RLOvdL had final responsibility for submitting the data for publication. All authors had full access to all the data in the study and were responsible for the decision to submit for publication.

Declaration of interests

RLMB reports a speaker fee from MSD. All other authors declare no competing interests.

Data sharing

De-identified individual participant data from this trial, including a data dictionary, that underlie the results reported in this article will be shared upon reasonable request to the corresponding author, beginning 1 year after publication of this paper.

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