30 May 2024
EMA/275870/2024
Human Medicines Division

Assessment report for paediatric studies submitted according to Article 46 of the Regulation (EC) No 1901/2006

Cervarix

human papillomavirus vaccine [types 16, 18] (recombinant, adjuvanted, adsorbed)

Procedure no: EMEA/H/C/000721/P46/100

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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Status of this report and steps taken for the assessment				
Current step	**Description**	**Planned date**	**Actual Date**	**Need for discussion**
☐	Start of procedure	01/04/2024	14/03/204	☐
☐	CHMP Rapporteur Assessment Report	06/05/2024	06/05/2024	☐
☐	CHMP members comments	21/05/2024	21/05/2024	☐
☐	Updated CHMP Rapporteur Assessment Report	23/05/2024	23/05/2024	☐
☒	CHMP adoption of conclusions:	30/05/2024	30/05/2024	☐

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1. Introduction

On 14 March 2024 the MAH submitted the final study report for study EPI-HPV-101-VE DB (221785) for Cervarix, in accordance with Article 46 of Regulation (EC) No1901/2006, as amended.

The MAH conducted a systematic literature review and meta-regression analysis in order to assess efficacy/effectiveness of Cervarix against advanced cervical intraepithelial neoplasia or worse (CIN3, CIN3+) and cervical cancer.

Long-term efficacy and immunogenicity information is already part of Cervarix´s label. However, as national immunization programs with universal Cervarix vaccination are being rolled out, and observational studies are being developed, real-world and long-term follow-up of clinical trials data on the long-term effects of Cervarix are accruing and becoming available. In addition, analysis may provide estimates of the effect size while adjusting for important covariates such as age at first vaccination, follow-up time to vaccination, study design, and other relevant variables. The EPI-HPV-101-VE DB study (study 221785) was thus conducted with the aim of compiling all published evidence, given that new available data had not been generated by GSK. Study 221785 is not part of any Paediatric Investigation Plan.

The MAH hereby submits the final study report for study EPI-HPV-101-VE DB (221785), entitled: "Efficacy/effectiveness of Cervarix against grade 3 cervical intraepithelial neoplasia or worse (CIN3, CIN3+) and cervical cancer. A systematic review and meta-regression analysis."

The MAH concluded that results are in line with the approved product information for Cervarix in the EU. Therefore, no additional changes to the Summary of Product Characteristics (SmPC) for Cervarix are considered necessary. This is agreed.

Among studies used in the systematic literature review, some were sponsored by GSK (the MAH), and some were sponsored by other sponsors. Among the studies sponsored by GSK, two studies included a paediatric population. These two GSK studies were submitted to EMA, not under the scope of article 46 but under the below scopes:

- Study 580299/008 or HPV-008 (NCT00122681) was submitted as follow up measure (EMEA/H/C/000721/FUM014, EMEA/H/C/000721/FUM 14.1, EMEA/H/C/000721/FUM028.2) and as a variation to update the SmPC and PL (EMEA/H/C/000721/II/011 and EMEA/H/C/000721/II/0020)
- Study 580299/012 or HPV-012 (NCT00169494) was submitted as follow up measure (EMEA/H/C/000721/FUM/018/019/020, EMEA/H/C/000721/FU2 18.1, EMEA/H/C/000721/FU2 18.2)

Some of the data have been already submitted to EMA in previous procedures. The current data of this systematic review and meta-regression analysis could bring different conclusions/outcome.

2. Scientific discussion

2.1. Information on the development program

The MAH stated that study *Efficacy/effectiveness of CERVARIX against grade 3 cervical intraepithelial neoplasia or worse (CIN3, CIN3+) and cervical cancer. A systematic review and meta-regression analysis.* 221785 (EPI-HPV-101 VE DB) is a standalone study.

Study 221785 is not part of a Paediatric Investigation Plan. To comply with the requirements of the Article 46 of the Regulation (EC) No 1901/2006, the MAH is submitting the final Clinical Study Report as agreed with EMA.

2.2. Information on the pharmaceutical formulation used in the studies

CERVARIX is composed of recombinant C-terminally truncated HPV 16 L1 and HPV 18 L1 proteins, assembled into VLPs adjuvanted with AS04. The HPV 16 L1 VLP and HPV 18 L1 VLP proteins constitute the active ingredient of the vaccine and are produced with a recombinant Baculovirus expression system. The AS04 adjuvant is composed of an aluminum salt, Al(OH)3 and MPL. The MPL immunostimulant is a detoxified derivative of the lipopolysaccharide of the gram-negative bacterium Salmonella Minnesota R595 strain.

The authorized indication is for use from the age of 9 years for the prevention of premalignant ano-genital lesions (cervical, vulvar, vaginal and anal) and cervical and anal cancers causally related to certain oncogenic Human Papillomavirus (HPV) types.

A two dose (0.5 ml each) immunization schedule is recommended in children 9 to and including 14 years of age (the second dose is given between 5 and 13 months after the first dose) while a three dose (0.5 ml each) regimen is recommended in case of individuals ≥15 years of age given at 0, 1, and 6 months.

2.3. Clinical aspects

2.3.1. Clinical study 221785 (EPI-HPV-101 VE DB)

Efficacy/effectiveness of CERVARIX against grade 3 cervical intraepithelial neoplasia or worse (CIN3, CIN3+) and cervical cancer. A systematic review and meta-regression analysis.

Description

Research question

What is the efficacy/effectiveness of the human papillomavirus vaccination with CERVARIX in girls and women against human papillomavirus on cervical cancer and grade 3 CIN or worse?

Objectives

To conduct a meta-analysis and meta-regression analyses on the efficacy/effectiveness of CERVARIX on cervical cancer and CIN3 or worse (CIN3+) to provide estimates of the effect size adjusting by covariates such as age at vaccination, time since vaccination, study design, or analytical cohort (HPV baseline status of participants).

The analysis will be designed to respond to the following 6 questions:

1. What is the combined efficacy/effectiveness of CERVARIX on CIN3+ caused by vaccine HPV types?
2. What is the combined efficacy/effectiveness of CERVARIX on CIN3+ caused by any HPV type?
3. **What is the efficacy of CERVARIX on CIN3+ caused by vaccine HPV types?**

4. **What is the effectiveness of CERVARIX on CIN3+ caused by vaccine HPV types? (Observational studies only)**
5. What is the efficacy of CERVARIX on CIN3+ caused by any HPV type?
6. What is the effectiveness of CERVARIX on CIN3+ caused by any HPV type? (Observational studies only)

Methods

Study Design

This study has been conceived as a systematic review to collect non-GSK data stemming mainly from long-term follow-up studies of RCTs, long-term observational studies and data from national surveillance from countries that implemented CERVARIX in their NIPs and that have been accruing over time. The aim was to analyze these data in a systematic and synthetic manner.

The objective of the present study is to determine effectiveness of CERVARIX (and not comparative effectiveness vs. any other HPV vaccine).

Study Population and Setting

Eligibility criteria

Studies were eligible if they compared the protection conferred by CERVARIX to prevent cervical cancer and CIN3 or worse (CIN3+) between CERVARIX vaccinated and non-vaccinated participants, be it a comparator arm in the case of RCTs (efficacy), or unvaccinated participants in case of observational/population-based surveillance/longitudinal studies (effectiveness). Vaccination has been considered if participants received at least one dose of the vaccine. No geographical limits, or race restrictions applied to the selection of articles.

Inclusion criteria

All studies that meet the following criteria were included:

• Studies that report CERVARIX efficacy (randomized controlled trials, RCTs) or effectiveness (observational studies) against cervical cancer and/or CIN3 or worse (CIN3+).

• Studies that have a comparator group receiving either placebo or another vaccine, or a control group of unvaccinated participants.

• The intervention group was considered as vaccinated if participants received at least one dose of the vaccine.

• Studies published in journal articles between 1 January 2000 to 21 June 2022. The following databases were screened: PubMed, EMBASE, Scopus, and Cochrane CENTRAL.

• Studies with the following design could be included: randomized controlled trials and observational studies (cohort, cross-sectional, case-control, longitudinal, population-based surveillance)

Exclusion criteria

Systematic reviews, reviews, modelling, economic studies (including cost-effectiveness and comparative effectiveness), letters to the editor, case reports, and case series were excluded. Conference abstracts and proceedings were excluded. Studies that have unreliable data for the extraction were excluded. Grey literature was not included.

Variables

Outcome definition

The outcome for this study is the vaccine efficacy/effectiveness of CERVARIX to prevent cervical cancer and CIN3 or worse (CIN3+) as provided by the retrieved publications.

However, when vaccine efficacy or effectiveness results were not available in the selected papers but a measure of effect was provided instead, vaccine efficacy/effectiveness was estimated (including 95% confidence interval) from the relevant measure of effect: i.e., OR, IRR, as cervical cancer below 25 years of age is rare [Teixeira, 2021] and therefore, these measures of effect offer a reasonable approximation of the RR [Viera, 2008]. In those cases, vaccine efficacy/effectiveness was calculated as VE=(1-OR)*100, or VE=(1-IRR)*100.

In those studies where HPV type was determined, relevant and specific DNA sequencing and bioinformatic techniques were used. Cytology and histopathology for CIN cases were mainly reviewed by an independent pathology committee, usually masked to the vaccine allocation.

Endpoints

CIN3, CIN3+, AIS, invasive cervical cancer (ICC)

Confounders and effect modifiers

Post hoc studies of clinical trials and observational and longitudinal studies stemming from surveillance of NIPs were likely subject to the following confounders/effect modifiers :

- Age at first vaccination (HPV acquisition, prevalent infection, or baseline HPV status).
- Sexual behavior (HPV acquisition, prevalent infection, or baseline HPV status).
- Time since vaccination or time of follow-up (immunogenicity, duration of protection).
- Age at first cervical screening.
- Healthcare seeking behavior.
- Socioeconomic factors.

Residual confounding cannot be completely ruled out.

Please also refer to quality assessment subheading.

The following variables were designed to be included in the meta-regression analyses with the aim to allow for certain known confounders/effect modifiers:

• TVC/TVC naïve: This is a binary variable and reflects whether the analytical cohort was the total vaccinated cohort (irrespective of the baseline HPV status) or the total vaccinated cohort naïve (HPV-negative at baseline).

• Age at first vaccination (known confounders/effect modifiers) : This variable represents the age at which the participant received the first vaccine dose. Age will be modelled as a continuous variable. Nonlinearity will be checked.

• Time since vaccination (time of follow-up) (known confounders/effect modifiers): This variable is the time that passed between when the participant received the first dose of the vaccine and the conduct of the study.

• HPV type: vaccine efficacy/effectiveness is expressed against different HPV types. Including those that are vaccine-specific (i.e., HPV 16/18) or non-vaccine types, or even composite indexes (i.e., 12

high-risk HPV types). For the purpose of this study, the meta-analysis and meta-regression will be planned to answer research questions that entail two scenarios concerning HPV type: "HPV 16/18" or "Irrespective of HPV type"

• Study design (known confounders/effect modifiers): This variable will have two values: RCT and observational that includes observational studies such as cohort studies and longitudinal population-based surveillance studies.

• Study correlation: This is a dummy variable created to adjust for potential correlation in studies. For instance, some study may contribute data from participants vaccinated at different age groups and two different analysis approaches (i.e., TVC naïve and TVC); in other instances, different studies may provide data from the same population but with different analytical approach (TVC naïve and TVC, respectively) and different components (RCT and observational study for vaccine efficacy and vaccine effectiveness, respectively) or combinations of both.

Exposure definitions

In this study, the exposure is vaccination with CERVARIX. For this study, a participant is considered as vaccinated if received at least one dose of the vaccine.

Selected RCTs in this systematic literature review had an intervention arm giving CERVARIX and an active comparator arm. Since the hepatitis A vaccine is not supposed to have any effect on CIN3+, subjects receiving this vaccine will be considered as non-exposed.

In the case of observational studies, the comparator arm used to determine vaccine effectiveness was a control group of unvaccinated participants, who will also be considered as non-exposed.

In the RCTs in this systematic review, CERVARIX vaccination was the intervention of the trial. Therefore, vaccination was registered within the trial. In observational studies that were post-hoc studies of clinical trials, the same procedure was followed. For longitudinal studies corresponding to surveillance of national immunization programs, individual vaccination status was retrieved from national registers and in some instances, when individual vaccination status was not available, researchers modelled the specific probability that a woman was vaccinated from the official national statistics for vaccination with three doses in the general population (i.e., [Rebolj, 2022]).

Data sources

The literature search is shown in the following table.

Table 1. Search terms for the different databases

Keyword	MeSH (PubMed)	Emtree (EMBASE)	Scopus
papillomavirus vaccine	"Papillomavirus Vaccines"[Mesh]	Human papilloma virus vaccine'/exp	papillomavirus vaccine
papillomavirus vaccination	No MeSH term	No Emtree term	papillomavirus vaccination
HPV vaccine	"Papillomavirus Vaccines"[Mesh]	No Emtree term	HPV vaccine
HPV vaccination	"Papillomavirus Vaccines"[Mesh]	'hpv vaccination'/exp	HPV vaccination
CERVARIX	No MeSH term	No Emtree term	CERVARIX
bivalent human papillomavirus vaccine	No MeSH term	'bivalent human papillomavirus vaccine'	"bivalent human papillomavirus vaccine"
program* evaluation	"Program Evaluation"[Mesh]	'program evaluation'/exp	"program* evaluation"
population surveillance	"Population Surveillance"[Mesh]	'population surveillance'/exp	"population surveillance"
sentinel surveillance	"Sentinel Surveillance"[Mesh]	'sentinel surveillance'/exp	"sentinel surveillance"
vaccine efficacy	"Vaccine Efficacy"[Mesh]	'vaccine efficacy'	"vaccine efficacy"
vaccine effectiveness	"Vaccine Efficacy"[Mesh]	'vaccine effectiveness'/exp	"vaccine effectiveness"
cervical intraepithelial neoplasia grade 3	"Cervical Intraepithelial Neoplasia"[Mesh] "Uterine Cervical Neoplasms"[Mesh]	'cervical intraepitelial neoplasia 3'/exp	cervical intraepithelial neoplasia
cervical severe dysplasia	"Cervical Intraepithelial Neoplasia"[Mesh] "Uterine Cervical Dysplasia"[Mesh]	'uterine cervix dysplasia'/exp	cervical dysplasia
cervical severe dyskariosis	"Cervical Intraepithelial Neoplasia"[Mesh]	No Emtree term	cervical severe dyskariosis
uterine cervical neoplasm	"Uterine Cervical Neoplasms"[Mesh]	'uterine cervix cancer'/exp	uterine cervical neoplasm
uterine cervical carcinoma	No MeSH term	uterine cervical carcinoma	uterine cervix carcinoma
high-grade CIN	No MeSH term	No Emtree term	high-grade CIN
high-grade squamous intraepithelial lesion	"Squamous Intraepithelial Lesions"[Mesh]	'high grade squamous intraepithelial lesion of the cervix'/exp	high-grade squamous intraepithelial lesion
high-grade cervical intraepithelial neoplasia	No MeSH term	'uterine cervix carcinoma in situ'/exp	high-grade cervical intraepithelial neoplasia
cervical carcinoma in-situ	"Cervical Intraepithelial Neoplasia"[Mesh]	'uterine cervix carcinoma in situ'/exp	cervical carcinoma in-situ
CIN 3	"Cervical Intraepithelial Neoplasia"[Mesh]	No Emtree term	CIN 3
cervical invasive carcinoma/cancer	No MeSH term	'uterine cervix cancer'/exp	cervical invasive carcinoma/cancer
HSIL	"Cervical Intraepithelial Neoplasia"[Mesh]	No Emtree term	HSIL

Abbreviations: CIN 3= Cervical intraepithelial neoplasia grade 3, EMBASE= Excerpta medica database, HSIL= High-grade squamous intraepithelial lesion, HPV= Human papillomavirus.

Search strategy and search strings were reviewed by the Medical Librarian at GSK. A reviewer searched in the databases. Two independent reviewers extracted the data (DN, MM), and the extracted data were cross-checked and confirmed. Discrepancies were resolved by consensus discussion and there were no final disagreements.

Study size

Not Applicable. This meta-regression analysis was not conceived as a confirmatory study. There is not a prior hypothesis to test and therefore it is not necessary to establish a sample size that has sufficient power to reject the null hypothesis. However, since two of the observational studies are nationwide surveillance studies (including several birth cohorts) and the other observational and follow-up of RCTs studies included high number of participants that allowed statistically significant vaccine efficacy/effectiveness estimates, precision of the estimates produced by the meta-regression results is expected to be sufficient. The cohort sizes for the different studies included in the meta-regression and the correspondent vaccine effect estimates and precision intervals are presented in Table 5.

Data analysis

Meta-regression is a generalization of the meta-analysis that allows assessing the relationship between specific study-level covariates, such as age or time since vaccination, and the effect size. In particular, it may take into account the heterogeneity of the results that may come from different levels of covariates of the different studies.

Heterogeneity among selected studies is expected to be large, given the differences in settings (e.g., time at first vaccination, time of follow-up, study design, etc.) that are known to influence vaccine efficacy/effectiveness but a decision was made to pursue a quantitative synthesis exercise. To consider these factors in the calculation of global estimates, meta-regression models will be fitted. They will provide summary point estimates for vaccine efficacy/effectiveness for every scenario while adjusting for relevant covariates (i.e., correcting for study differences due to different levels of covariates). Residual heterogeneity not explained by the multiparametric model will be shown in the statistical outputs. If this heterogeneity is still large, it will be discussed and acknowledged among the limitations of the study.

Meta-regression allows the effects of multiple factors to be investigated simultaneously. It examines if characteristics of studies are associated with the magnitude and direction of the effect in the selected studies. The outcome variable will be the effect estimate. The explanatory variables are characteristics of studies that might influence the size of the effect. These are often called "potential effect modifiers" or covariates. For this analysis, the outcome variable will be the effect estimate (CERVARIX efficacy/effectiveness). The explanatory variables will be study design (RCT/observational), age at first vaccination, the type of analytical cohort, and time since vaccination. Note that to increase the precision of the estimates, when possible, the MAH will split studies in different sub-studies given differences in terms of covariates. The correlations between the different sub-studies of a study will be taken into account in all subsequent analyses.

Meta-regression models will be fitted using a frequentist approach. For each question considered, the following strategy will be used:

• First a meta-analysis will be fitted (using the rma.mv function from R) using a REML estimation procedure allowing for Random Effect).

• Univariate meta-regressions (with Random Effect and REML) will be fitted to assess the impact of each covariate independently.

• A multivariate meta-regression (with Random Effect and REML) will then be considered.

<u>Regression</u>

Regression is a statistical method that assesses the relationship between covariates and the dependent variable in a particular study**.** In this study, meta-regression will always have random effects and vaccine efficacy/effectiveness will be modelled as the log of the relative risk [log(1-VE)] as normally distributed. In this whole analysis, the statistical significance will be at p=0.05. However, this study is not considered as confirmatory and no prior hypothesis has been formulated. There is no intention to adjust for multiplicity. Confidence intervals will be two-sided and will be at a 95% level.

Primary analysis

Main Analytical approach

The following scheme will be followed to answer the research questions and scenarios.

Multiparametric meta-regressions adjusting for the following covariates: age at first vaccination, study design (RCT vs observational), analytical cohort (TVC vs TVC naïve), and time since vaccination (time of follow-up). An AIC (estimator of prediction error) approach will be used to assess the quality of the models for every given dataset allowing a data-driven selection of the best model.

One model will be selected for each of the 6 questions assessed:

1. What is the combined efficacy/effectiveness of CERVARIX on CIN3+ caused by vaccine HPV types?
2. What is the combined overall efficacy/effectiveness of CERVARIX on CIN3+ caused by any HPV type?
3. What is the efficacy of CERVARIX on CIN3+ caused by vaccine HPV types?
4. What is the effectiveness of CERVARIX on CIN3+ caused by vaccine HPV types? (Observational studies only)
5. What is the efficacy of CERVARIX on CIN3+ caused by any HPV type?
6. What is the effectiveness of CERVARIX on CIN3+ caused by any HPV type? (Observational studies only)

However, prior to this, the following preliminary analyses will be considered:

1. Classical random effect meta-analysis without adjusting for covariates.

2. Univariate meta-regression with random effect of each potential covariate, e.g., age at first vaccination, study design (RCT vs observational), analytical cohort (TVC vs TVC naïve), and time since vaccination (time of follow-up)

Sensitivity analyses

The analysis will be conducted following different scenarios (i.e., analyzing RCTs or observational studies independently, and pooling together data corresponding to both study designs) to assess how different values of the independent variables affect the outcome variable. In addition, uni-, and multivariate models will be considered.

Quality control and Quality Assurance

The systematic review was conducted in accordance with the PRISMA checklist /and in compliance to the Cochrane Handbook of Systematic Review of Interventions [Higgins, 2023; PRISMA, 2023] and the Joanna Briggs Institute Manual for Evidence Synthesis [Jordan, 2019].

Expected sources of bias for observational studies are

• Selection bias: selection of participants could be influenced by participant´s characteristic or outcome.
• Information bias: bias related to measurements in the intervention and of the outcome (methods for the identification of the outcome, time between vaccination and outcome and baseline status to rule out outcomes due to pre-existing infection at a given dose)
• confounding: assessing the probability of differences between the two study groups.

The risk of bias was assessed by two different tools:

• Cochrane risk of bias for randomized controlled trails (RoB2) [The Cochrane Collaboration, 2022a]
• Cochrane ROBINS-I tool for observational epidemiological studies specifically designed for use in systematic reviews [The Cochrane Collaboration, 2022b].

Limitations of the research methods

Related to Data

- **Methodology of Systematic literature review (SLR)**

A SLR suffers from intrinsic limitations. It can only review what is found, and an element of publication bias is always present, which will reflect in the meta-analysis.

- **Data availability**

Absence of data about important covariates (needed for the meta-regression) can be a major limitation in the assessment of heterogeneity in meta-regressions.

The data included in the analysis is based on a systematic literature review. As such the analysis is limited by the detail and granularity of the data provided in published manuscripts.

- **Number of studies and power of analysis**

In a meta-regression framework, the unit of analysis is the study, so the regression performance is determined by the number of studies in the meta-analysis, which is sometimes relatively low. Consequently, one should not expect much statistical power from the meta-regression, depending on the number of covariates included in the model [Bartolucci, 1994]. The power of a statistical analysis is limited, i.e., based on the available data. Consequently, if a covariate is not found to be significant, we cannot conclude that there is no effect of that covariate. i.e., there may be a true effect but there may be insufficient evidence to demonstrate the effect with the available data.

- **Assessment of publication bias**

Publication bias occurs when published studies differ systematically from all conducted studies in relation with a topic. Publication bias arises when papers with statistically significant or positive results in a certain direction are more likely to be published than papers with non-statistically significant or negative results [Jordan, 2019], translating into a threat to the validity of the systematic review.

The minimal number of studies recommended for assessment of publication bias with existing tools (i.e., funnel plot, statistical test for funnel plot asymmetry, etc.) should be at least ten to ensure sound statistical power [Higgins, 2023; Jordan, 2019].

Related to Methodology

- **Interpretation of associations and confounding variables**

The associations derived from meta-regression are observational and have a less rigorous interpretation than the associations obtained within a single study, particularly when averages of patients' characteristics are used as covariates in the regression.

• Aggregation bias occurs when the relationship with patient averages across trials may not be the same as the relationship with patients within trial.

• Bias by confounding (association with one of the study characteristics that reflects a true association with another known or unknown correlated characteristic) is a particular problem in meta-regression.

- **Assumptions of linearity and normality**

In the majority of meta-regressions, there is no attempt to verify the underlying assumptions of normality of the residuals, or the linearity of covariates.

- **Assumptions on creation of age groups**

The data included in the analysis is based on a systematic literature review. As such the analysis is limited by the detail and granularity of the data provided in published manuscripts.

- **Potential post-hoc data dredging**

The principal pitfall in meta-regression is data-dredging.

• There are only a few studies included, and many characteristics that can explain heterogeneity. Each of these characteristics could potentially be analyzed, until associations are found. Such multiple or post hoc analyses lead to a high chance of false positive conclusions.

• Post hoc conclusions should be regarded as hypothesis generating, to be investigated in other data sets. However, in meta-analysis, the totality of evidence has been accumulated and there is no such external validation.

• Pre-specification of the covariates (prior to the literature search) to be investigated helps protecting against false positive conclusions. However, in order to be truly prespecified, a protocol should be drawn up without knowledge of any of the relevant literature, which is not really achievable in practice since experts have already strong scientific rationales.

• The number of covariates should be limited, to limit the false positive conclusions. Also a possibility is Bonferroni adjustment to the significance level for each covariate inclusion [Wasserstein, 2016].

• Unfortunately, in practice, after pre-specifying covariates, researchers often discover that for the originally chosen covariates, the information is not available, or that other new important covariates that have not been pre-specified should be included in the analysis.

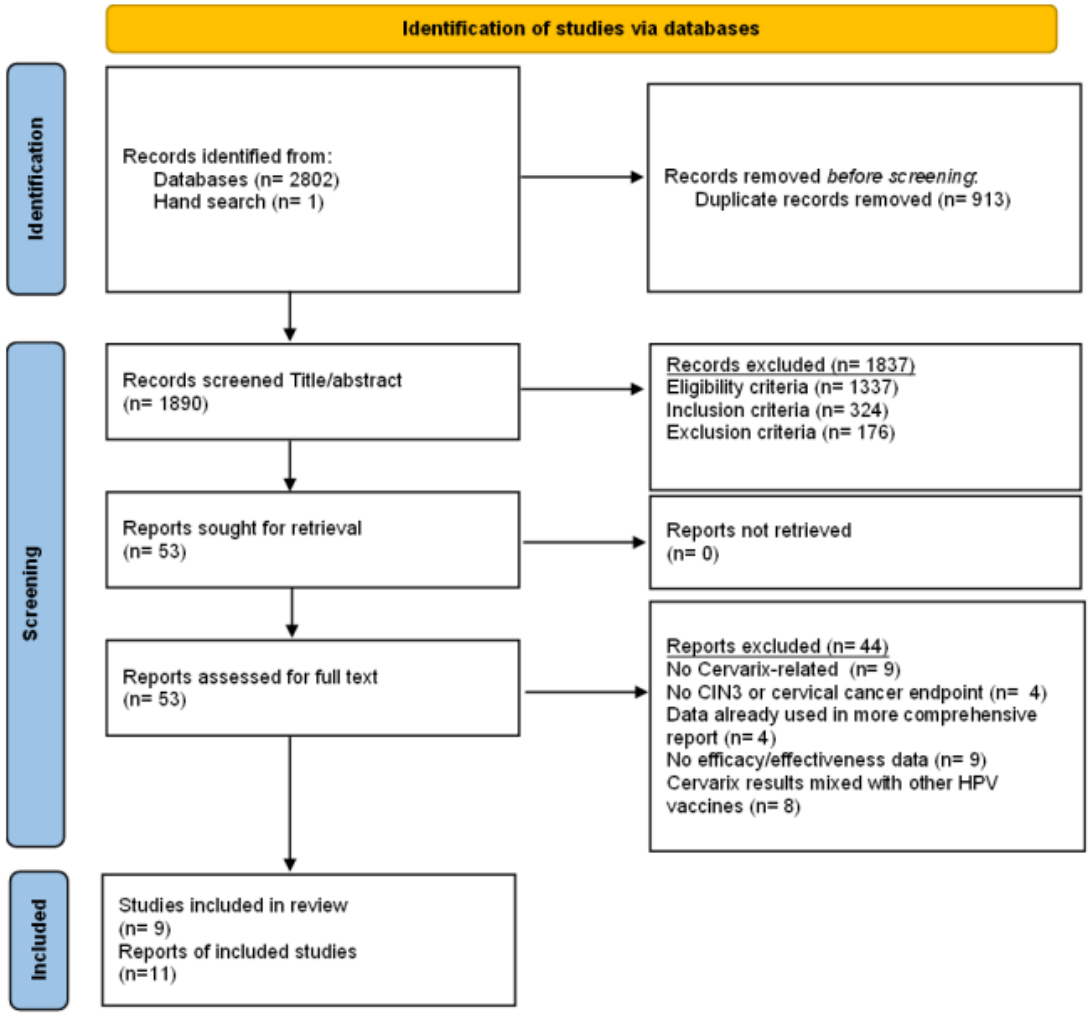
This study is exploratory and should not be regarded as more than hypothesis generating.

Results

Search results and characteristics of selected studies

Across the searches through the databases, 2803 potentially eligible articles were identified (including one article retrieved by hand search), Figure 2. Of them, 913 duplicates were removed. Records were screened (n=1890) and n=1837 were excluded based on the eligibility, inclusion, or exclusion criteria. After title and abstract screening, n=53 papers were included for full-text review. Of these, 9 met the inclusion criteria. Of them, 5 studies were follow-up of RCTs [Wheeler, 2012; Lehtinen, 2012; Konno, 2014; Porras, 2020; Shing, 2022], 3 studies were retrospective population-based registry linked studies [Palmer, 2019; Falcaro, 2021; Rebolj, 2022] and 1 study was an observational post-hoc long-term follow-up of an RCT [Lehtinen, 2017] Table 6 and Table 7. Two of the RCTs had also an observational component [Porras, 2020; Shing, 2022]. Studies were conducted in Japan [Konno, 2014], Costa Rica [Porras, 2020; Shing, 2022], Finland [Lehtinen, 2017], Scotland [Palmer, 2019], England [Falcaro, 2021; Rebolj, 2022], and in multicountry sites [Lehtinen, 2012; Wheeler, 2012).

Figure 1. PRISMA flow diagram



Abbreviations: CIN3= Cervical intraepithelial neoplasia grade 3, n=number of reports, HPV= Human Papillomavirus, PRISMA= Preferred Reporting Items for Systematic Reviews and Meta-Analyses.
Note: Two papers (i.e., Porras, Shing) included reports both on vaccine efficacy and vaccine effectiveness (observational component) [Porras, 2020; Shing, 2022].

The following parameters were considered for the inclusion of the studies in the meta-analysis/ meta-regression analyses:

1. Endpoint: CIN3+. Results on other endpoints [i.e., CIN3, AIS, or cervical cancer were reported by very few papers (one, one, and two papers respectively)]. Therefore CIN3+ was selected as endpoint for the meta-regression [Lehtinen, 2012; Konno, 2014; Lehtinen, 2017; Palmer, 2019; Porras, 2020; Shing, 2022; Rebolj, 2022].

2. Outcome: Vaccine efficacy/Vaccine effectiveness as reported by the different studies. In study by Palmer et al., vaccine effectiveness is calculated for as (1- OR)*100 (the measure of effect provided by the paper is OR) [Palmer, 2019]. In a study by Falcaro et al., vaccine effectiveness is calculated as (1-IRR)*100 (the measure of effect provided by the paper is IRR) [Falcaro, 2021].

3. Number of doses. The information for the number of doses injected needed to be reported in the paper. For the analysis, the groups vaccinated with "3 doses" and "At least 1 dose" from different studies will be pooled together if at least 75% of the participants of the "At least 1 dose" group received 3 doses of the vaccine.

4. Age at first vaccination. The decision was to stratify by age in those studies with this data available to increase the number of observations allowing a more robust model. This is to use the most granular results at the level of the studies in terms of age groups. For example, if the VE was reported for 3 age categories in a study, the three VE results will be used in the meta-regression.

5. Time since vaccination (time from the analysis to vaccination or time of follow-up).

6. CIN3+, HPV31/33/35/39/45/51/52/56/58/59/66/68 (common non-vaccine types endpoint), Vaccine effectiveness/vaccine efficacy. There are very few papers reporting on non-vaccine types, one is an RCT and the other is an observational study. A decision was made not to pursue meta-regression for this endpoint of non-vaccine types. Description of the findings will be presented in the narrative review.

7. Cervical cancer, Histological diagnosis (no HPV testing results), Vaccine effectiveness. There are only two papers [Falcaro, 2021; Rebolj, 2022] referring to the same population and there is certain possibility of overlapping in the birth cohorts of interest. In addition, vaccine effectiveness against cervical cancer in a study [Rebolj, 2022] did not reach statistical significance due to the small number of cases. Therefore, results from these papers referring to the outcome cervical cancer will not be included in the meta-regression and will be included in the narrative alone.

8. For the CIN3+ vaccine effectiveness meta-regression focusing on the endpoint "Irrespective of the HPV type" the decision is to also include a study as the vaccine effectiveness is calculated as overall since the endpoints are histology-based (no direct HPV testing of the samples) [Palmer, 2019]. Another study will also be included since vaccine effectiveness refers to 14 high-risk HPV types, which are considered the most relevant oncogenic types and responsible for cervical cancer (up to 99% of cervical cancer is caused by the high-risk HPV types) [Rebolj, 2022]. Therefore, this paper will be considered that reports the outcome "irrespective of the HPV type) [Dunne, 2007].

9. Choice of the analysis group: TVC cohort and TVC-naïve cohort. The TVC cohort is the cohort closest to the real world (regardless of their HPV baseline status) and more relevant from the public health perspective. However, differences for vaccine efficacy/effectiveness between both cohorts are significant. Therefore, a decision was made to conduct meta-regression having each of them independently (binary covariate) to highlight how important it is for increased protection to vaccinate girls and teenagers before sexual debut (the natural path of acquiring an HPV infection). A decision was made to determine summary point estimates for RCTs and observational studies alone, and also the combined effects of RCTs and observational data pooled together. This approach allows a sensitivity analysis considering the different scenarios: different study design and different vaccine outcomes (vaccine efficacy/effectiveness against vaccine types HPV16/18 or irrespective of HPV type).

A total of seven studies were selected for the quantitative synthesis [Lehtinen, 2012; Konno, 2014; Lehtinen, 2017; Palmer, 2019; Porras, 2020; Shing, 2022; Rebolj, 2022] whereas two studies remained for the narrative review alone since there were not enough individual records to conduct a quantitative synthesis [Wheeler, 2012; Falcaro, 2021] (Table 4). Refer to Table 6 showing the final outcomes and endpoints for the meta-regression analysis.

The majority of studies stratified by age at first vaccination, although age groups varied considerably.

Table 2. Summary of characteristics of selected studies

Author, Year	Country	Study period	Study design	Study population	Age at first vaccination	Vaccine coverage	Number of doses	Type of outcomes	Case-counting start
Wheeler, 2012	Multi-country (US, Australia, Belgium, Brazil, Canada, Finland, Germany, Italy, Philippines, Spain, Taiwan, Thailand, UK)	June 2004-June 2008	RCT (4-year follow-up)	Females with no more than 6 lifetime sexual partners (not applied in Finland), regardless of their baseline HPV DNA status, HPV-16 or HPV-18 serostatus, or cytology. N=16 114, 11 644, and 18 644 women were included in the ATP-E (vaccine n=8067, control n=8047), TVC-naive (Vaccine n=5824, control n=5820), and TVC cohorts (Vaccine n=9319, control n=9325), respectively. 16% of participants (3034 of 18 644) were lost to follow-up by the end of the study	15-25 y	NA	Participants considered for the analysis, 3 doses-ATP-E cohort at least 1 dose: TVC-naïve and TVC	Vaccine efficacy	Day after 1st vaccination for TVC-naïve and TVC, and the day after 3rd vaccination for ATP-E cohort
Lehtinen, 2012	Multi-country (US, Australia, Belgium, Brazil, Canada, Finland, Germany, Italy, Philippines, Spain, Taiwan, Thailand, UK)	June 2004-June 2008	RCT (4-year follow-up)	Females with no more than 6 lifetime sexual partners (not applied in Finland), regardless of their baseline HPV DNA status, HPV-16 or HPV-18 serostatus, or cytology. Completed study: TVC, n= 7798 HPV arm, n=7811 control arm TVC-naïve, n= 1879 HPV arm, n= 2315 control arm ATP-E, n= 6815 HPV arm, n=6769 control arm	15-25 y	NA	Participants considered for the analysis, •3 doses-ATP-E cohort •at least 1 dose: TVC-naïve and TVC	Vaccine efficacy	Day after 1st vaccination for TVC-naïve and TVC, and the day after 3rd vaccination for ATP-E cohort
Konno, 2014	Japan	October 2009- April 2013	RCT (4-year follow-up)	Healthy females not screened before enrollment with respect to baseline serological, cytological, or HPV DNA status. TVC-combined, n=519 HPV arm, n=521 control arm ATP cohort for efficacy-combined, n=499 HPV arm, n=498 control arm TVC naïve-combined, n=281 HPV arm, n=284 control arm	20-25 y	NA	Participants considered for the analysis if at least 1 dose: TVC-naïve and TVC	Vaccine efficacy	Day after receipt of the first vaccine dose for the TVC-naïve and TVC (up to 4 y follow-up) and the day after 3rd vaccination for ATP-E cohort
Lehtinen, 2017	Finland	Enrolment: June 2003/2005 and May 2004 to April 2005. Follow-up: 2009 to 2015	Cohort study	18-19 y unvaccinated women n=15627 16-17 y vaccinated women n=2401 PATRICIA trial 16-17 y vaccinated women N= 64 HPV-012 trial	15-25 y PATRICIA trial 10-25 y HPV-012 trial	NA	Participants considered for the analysis if at least 1 dose (TVC)	Vaccine effectiveness	Day after first vaccination (up to 10 years post vaccination follow-up)
Porras, 2020	Costa Rica	June 2004-December 2005 (RCT); Follow-up March 2009-July 2012 (Total 11 years)	RCT (up to year 4) and Cohort study (no randomization) (up to year 11)	Healthy women (HPV 16/18 DNA-negative at months 0 and 6, who did not have biopsy or LEEP during the vaccination phase) n= 2635 in HPV vaccine group n=2677 in control group (0-4 y RCT) n=2073 HPV vaccine group and n=2530 unvaccinated group in cohort analysis (7-11 y)	18-25 y	NA	3 doses	Vaccine efficacy Vaccine effectiveness	Day after first vaccination (up to year 11 of follow-up)

Author, Year	Country	Study period	Study design	Study population	Age at first vaccination	Vaccine coverage	Number of doses	Type of outcomes	Case-counting start
Shing, 2022	Costa Rica	June 2004-December 2005 (RCT); Follow-up March 2009-July 2012 (Total 11 years)	RCT (up to year 4) and Cohort study (no randomization) (up to year 11)	n= 3491 in HPV vaccine group and n=3512 in control arm (CIN3+ endpoint, years 1-4 follow-up) n= 2826 in HPV vaccine group and n=2592 unvaccinated control arm (CIN3+ endpoint, years 7-11 follow-up) Note: Analyses included all participants with at least one follow-up visit in the respective period and excluded participants with a previous endpoint (CIN2+, CIN3+) (i.e., modified intention-to-treat cohort).	18-25 y	NA	At least 1 dose (mITT)	Vaccine efficacy Vaccine effectiveness	Day after first vaccination (up to year 11 of follow-up)
Palmer, 2019	Scotland (UK)	Between 1 January 1988 and 5 June 1996 for screening. Extraction date August 2017	Retrospective population-based study	Routine vaccinated girls 12-13 y (born between 1 January 1988 and 5 June 1996); catch-up campaign vaccinated women (born 1991-94, age 14-17 at vaccination); unvaccinated women (born 1988-90, age 18-20 in 2008) screened at age 20. N= 138692 screened women at age 20	12-13 y 14 y 15 y 16 y 17 y ≥ 18 y	90% at age 13 (1995 birth cohort)	3, 2, or 1 dose	OR	NA

Author, Year	Country	Study period	Study design	Study population	Age at first vaccination	Vaccine coverage	Number of doses	Type of outcomes	Case-counting start
Falcaro, 2021	England (UK)	January 2006-June 2019, data extraction on 26 January 2021	Retrospective population-based database study	Vaccine eligible women (7 birth cohorts), Unvaccinated cohort (born between 1 May 1989 and 31 August 1990) 13·7 million-years of follow-up of women aged 20 years to younger than 30 years in the three vaccinated cohorts.	12-13 y 14-16 y 16-18 y	Routine cohort: 85.9%- 90.6% for 2008-09 and 2011-12 Catch-up cohort: 55.6% to 81.9% 1 dose: 60.5% to 88.7% 3 doses: 44.8% to 84.9%	At least 1 dose, 3 doses	Adjusted IRR	NA
Rebolj, 2022	England (UK)	2013-2018	Retrospective population-based database study	Women eligible for catch-up vaccination (14-17 y) and received HR-HPV test at 25 y N=64274 overall results of women tested; N=42384 genotyped results	Vaccinated cohort 24-25 years; Unvaccinated cohort 26-29 y	40%-75% depending on the birth cohort	Data on individual vaccination status unavailable	Vaccine effectiveness	NA

Abbreviations: ATP-E= According-to-protocol for efficacy cohort, DNA= Deoxyribonucleic acid, HPV= Human papillomavirus, HR HPV= High-risk human papillomavirus, IRR= Incident relative risk (or Risk Ratio), mITT= Modified intention to treat, N= Total (Overall), n= number of participants in each arm, NA= Not applicable, UK=United Kingdom, US=United States, RCT= Randomized controlled trial, y=years.

Table 3. Final outcomes and endpoints for the meta-regression analyses

Author, Year	Endpoint	HPV type	N of doses	Age at first vaccination	Time since vaccination (y)
Analysis 1_CIN3+, HPV16/18 RCT/Observational combined					
RCT, Vaccine efficacy					
Lehtinen, 2012	CIN3+	HPV 16/18	At least 1 dose (TVC and TVC naïve)	15-17 y 18-20 y 21-25 y	0-4
Porras, 2020	CIN3+	HPV 16/18	At least 1 dose (TVC naïve)	18-25 y	0-4
Observational; population-based surveillance, Vaccine effectiveness					
Shing, 2022	CIN3+	HPV 16/18	At least 1 dose (TVC)	18-25 y	7-11
Rebolj, 2022	CIN3+	HPV 16/18	3 doses	14-17 y	7-11
Analysis 2_CIN3+, Irrespective of HPV type RCT/Observational combined					
RCT, Vaccine efficacy					
Konno, 2014	CIN3+	Irrespective of HPV type	At least 1 dose (TVC and TVC naïve)	20-25 y	0-4
Lehtinen, 2012	CIN3+	Irrespective of HPV type	At least 1 dose (TVC and TVC naïve)	15-17 y 18-20 y 21-25 y	0-4
Observational; population-based surveillance, Vaccine effectiveness					
Palmer, 2019	CIN3+	Histological diagnosis (no HPV testing results). Considered as "irrespective of HPV type"	3 doses	12-13 y 14 y 15 y 16 y 17 y ≥18 y	0-8 0-6 0-5 0-4 0-3 0-2
Shing, 2022	CIN3+	Irrespective of HPV type	At least 1 dose	18-25 y	7-11
Rebolj, 2022	CIN3+	HR-HPV (16,18 ,31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68). Considered as "irrespective of HPV type".	3 doses	14-17 y	7-11
Analysis 3_CIN3+, HPV16/18, RCT					
RCT, Vaccine efficacy					
Lehtinen, 2012	CIN3+	HPV 16/18	At least 1 dose (TVC and TVC naïve)	15-17 y 18-20 y 21-25 y	0-4
Porras, 2020	CIN3+	HPV 16/18	At least 1 dose (TVC naïve)	18-25 y	0-4
Shing, 2022	CIN3+	HPV 16/18	At least 1 dose (TVC)	18-25 y	0-4
Analysis 4_CIN3+, HPV16/18, Observational, Vaccine effectiveness					
Lehtinen, 2017	CIN3+	HPV16/18	At least 1 dose	16-17 y	0-10
Shing, 2022	CIN3+	HPV16/18	At least 1 dose	18-25 y	7-11
Rebolj, 2022	CIN3+	HPV16/18	3 doses	14-17 y	7-11
Analysis 5_CIN3+, Irrespective of HPV type, RCT, Vaccine efficacy					
Konno, 2014	CIN3+	Irrespective of HPV type	At least 1 dose (TVC and TVC naïve)	20-25 y	0-4
Lehtinen, 2012	CIN3+	Irrespective of HPV type	At least 1 dose (TVC and TVC naïve)	15-17 y 18-20 y 21-25 y	0-4
Shing, 2022	CIN3+	Irrespective of HPV type	At least 1 dose (TVC)	18-25 y	1-4
Analysis 6_CIN3+, Irrespective of HPV type, Observational, Vaccine effectiveness					
Lehtinen, 2017	CIN3+	Irrespective of HPV type	At least 1 dose	16-17 y	0-10
Palmer, 2019	CIN3+	Histological diagnosis (no HPV testing results). Considered as "irrespective of HPV type"	3 doses	12-13 y 14 y 15 y 16 y 17 y ≥18 y	0-8 0-6 0-5 0-4 0-3 0-2
Shing, 2022	CIN3+	Irrespective of HPV type	At least 1 dose	18-25 y	7-11
Rebolj, 2022	CIN3+	HR-HPV (16,18 ,31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68). Considered as "irrespective of HPV type"	3 doses	14-17 y	7-11

Abbreviations: HPV= Human papillomavirus, HR HPV= High-risk Human papillomavirus, TVC= Total vaccinated cohort, RCT= Randomized controlled trial, y=years.

None of the 9 studies mentioned above were excluded based on quality.

Quality assessment of randomized controlled trials

The Cochrane RoB2 tool was applied to the selected RCTs and three studies showed low risk bias whereas the study by Konno et al., presented some concerns in the randomization and deviations from intended intervention domain because a) this study is a post hoc follow-up of an RCT and the follow-up was not blinded. However, laboratory staff that assessed the outcome was blinded to the vaccination

status. Therefore, a great impact on the efficacy was not expected; b) the study was not powered to evaluate vaccine efficacy against CIN3+, the reason why this result showed wide confidence intervals. The latter will be addressed when conducting the adjusting in the meta-regression analysis [Konno, 2014; The Cochrane Collaboration, 2022a].

Overall, completeness of all follow-up studies was quite high, and losses were not selective, leaving both arms balanced at completion of the study.

Quality assessment of observational studies

The Cochrane ROBINS I tool for non-interventional studies was used to assess the risk of bias of observational studies and surveillance of national immunization programs studies [The Cochrane Collaboration, 2022b]. All the included studies were considered to have at least moderate risk of bias, and two of the five studies included were at high (serious) risk of bias. These two studies that were at serious risk of bias had one or two domains at high risk (mainly confounding and information of outcome) [Palmer, 2019; Rebolj, 2022]. Uptake of screening in fully vaccinated women aged 20 or 21 years was 51%, and only 23% in unvaccinated women and this may have overestimated vaccine effectiveness [Palmer, 2019]. On the other hand, authors adjusted by immunization status and age at which the first dose was administered, and by year of birth in unvaccinated women, respectively. The analysis also adjusted for socioeconomic status (deprivation and rurality score) [Palmer, 2019]. In the study by Rebolj et al., individual vaccination status was unknown. The age and calendar year specific probability that a woman was vaccinated was estimated from the official national statistics for vaccination with three doses in the general population, available by school cohort. However, these two studies were population-based retrospective cohort studies limiting the risk of selection bias. The overall judgement was that both studies addressed bias and confounding in an appropriate manner in the analytical phase considering the limitations of the retrospective population-based registry linked study design [Palmer, 2019; Rebolj, 2022].

An important source of confounding of observational studies is related to HPV acquisition. The population-based studies did not determine HPV-baseline status to assess for prevalent infection at the time of vaccination as pre-vaccination cervical screening is not standard of care. To address this, studies allowed for buffer time between the vaccination and outcome assessment (cervical screening). Other important source of confounding in observational studies determining HPV vaccine effectiveness is differences in risk of HPV acquisition between vaccinated and unvaccinated participants. In those observational studies other than stemming from national surveillance, baseline characteristics of the participants were assessed, most importantly in relation to sexual behavior and activity and adjusted for [Porras, 2020; Shing, 2022] and in other instances, sexual debut age was very similar between the vaccinated and unvaccinated arms [Lehtinen, 2017].

In any case, a decision was made not to discard any observational study, to adjust for covariates instead, and to acknowledge the limitations of the studies.

The covariates considered in meta-regression analysis were age at vaccination, time since vaccination, study design, or analytical cohort (Total Vaccinated Cohort (TVC, irrespective of HPV baseline status) and Total Vaccinated Cohort naïve (TVC-naïve, HPV-naïve at baseline)).

Individual study results

This systematic review and meta-analysis/meta-regression analysis included data on CERVARIX effects on CIN3+ from roughly 290 000 participants aged 12 to 25 years at vaccination, and up to 11 years of follow-up. Population-based HPV surveillance data from England added 13.7 million-years of follow-up in relation to VE against CIN3, and cervical cancer.

In the 4-year follow-up of the PATRICIA trial [Lehtinen, 2012], the overall vaccine efficacy (VE) against CIN3+ caused by HPV 16/18 reached its highest in the TVC-naïve [VE= 100% (95% CI, 85.5-100)], and ATP-E cohort [VE= 91.7% (95% CI, 66.6-99.1)] whereas, vaccine efficacy was lower [VE= 45.7% (95%CI, 22.9-62.2) in the TVC whose participants received at least one dose of CERVARIX and were sexually active (Table 5). When stratified by age, usually vaccine efficacy decreased as vaccination age of participants increased (Table 5).

Konno et al, determined vaccine efficacy against CIN3+ caused by any HPV type at 100% (95% CI, -417.0-100) in the TVC naïve cohort, and 36.4% (95% CI,- 57.8-75.7) in the TVC cohort [Konno, 2014] (Table 5).

The 4-year post-vaccination analysis of the Costa Rica Vaccine Trial that Porras et al. conducted, established vaccine efficacy against CIN3+ caused by HPV 16/18 at 66.4% (95% CI, -175-97.3), and VE in a post-hoc observational study up to 11 years of follow-up at 100% (95% CI, 78.8-100). The analytical cohort for the 4-year follow-up was composed of women that were HPV 16/18 naïve and did not have CIN2+ or any LEEP treatment at enrollment (Table 5).

With a different analytical approach of the Costa Rica Vaccine Trial, Shing and colleagues established vaccine efficacy against incident CIN3+ caused by HPV 16/18 at 52.9% (95% CI, 22.4-72.1) in the 4-year follow-up of the trial in the TVC. Vaccine efficacy was 25.2% (95% CI, -5.0-46.9), irrespective of the HPV type. The observational post-hoc 7-11 years post-vaccination follow-up found VE of 86.9% (95% CI, 65.3-91.1) against incident CIN3+ caused by HPV 16/18, whereas VE declined to 14.4% (95% CI, - 23.4-40.7) when it was caused by any HPV type (Table 5).

In the 10-year follow-up observational study of the Finnish component of the PATRICIA and HPV-012 trials, Lehtinen and colleagues [Lehtinen, 2017] determined VE against CIN3+ irrespective of HPV type at 66% (95%CI, 8.4-88) (Table 5). The VE against CIN3+ of three doses of CERVARIX in the population-based study carried out by Palmer and colleagues in Scotland [Palmer, 2019] was estimated at 86% (95%CI, 75-92) in the 12-13 years at vaccination age-group, decreasing in older vaccinated birth cohorts.

Table 4. Vaccine effects reported on different endpoints

Author, Year	N (overall)	Age at first vaccination (y)	N (age group)	Endpoints	Vaccine effects % (95%CI)
Wheeler, 2012	ATP-E[a], N=16114 vaccine arm, n=8067 control arm, n=8047 TVC[b], N=18644 vaccine arm, n=9319 control arm, n=9325 TVC-naïve[c], N=11644 vaccine arm, n=5824 control arm, n=5820	15-25	NA	Vaccine efficacy against CIN3+ associated with a composite of 12 non-vaccine HPV types, with or without HPV-16/18 co-infection, in the ATP-E cohort. Vaccine efficacy against CIN3+ associated with a composite of 12 non-vaccine HPV types excluding HPV-16/18 co-infection, in the ATP-E cohort. Vaccine efficacy against CIN3+ associated with a composite of 12 non-vaccine HPV types, with or without HPV-16/18 co-infection, in the TVC-naïve. Vaccine efficacy against CIN3+ associated with a composite of 12 non-vaccine HPV types, excluding HPV-16/18 co-infection, in the TVC-naive. Vaccine efficacy against CIN3+ associated with a composite of 12 non-vaccine HPV types, with or without HPV-16/18 co-infection, in the TVC. Vaccine efficacy against CIN3+ associated with a composite of 12 non-vaccine HPV type, excluding HPV-16/18 co-infection, in the TVC	73.8 (48.3, 87.9) 62.1 (21.8, 82.9) 91.4 (65.0, 99.0) 81.9 (17.1, 98.1) 47.5 (22.8, 64.8) 40.0 (1.1, 64.2)
Lehtinen, 2012	ATP-E, N=16114 vaccine arm, n=8067 control arm, n=8047 TVC, N=18644 vaccine arm, n=9319 control arm, n=9325 TVC-naïve, N=11644 vaccine arm, n=5824 control arm, n=5820	15-25 15-25 15-25 15-25 15-25 15-25 15-25 15-25 15-25 15-25 15-25 15-25 15-25 15-17 18-25 18-20 21-25 15-17 18-25	NA	Vaccine efficacy against CIN3+ associated with HPV-16/18 in TVC-naïve. Vaccine efficacy against CIN3+ associated with HPV-16/18 in TVC. Vaccine efficacy against CIN3+ associated with HPV-16/18 in ATP-E cohort. Vaccine efficacy against CIN3+ associated with HPV-16 in ATP-E cohort. Vaccine efficacy against CIN3+ associated with HPV-18 in ATP-E cohort. Vaccine efficacy against all CIN3+ (irrespective of HPV type in the lesion and including lesions with no HPV DNA detected) in the TVC-naïve. Vaccine efficacy against all CIN3+ (irrespective of HPV type in the lesion) in the TVC. Vaccine efficacy against all AIS HPV 16/18-related in the TVC-naïve. Vaccine efficacy against all AIS HPV 16/18-related in the TVC. Vaccine efficacy against all AIS irrespective of HPV DNA in the lesion in the TVC-naïve. Vaccine efficacy against all AIS irrespective of HPV DNA in the lesion in the TVC. Vaccine efficacy against AIS associated with HPV-16/18 in ATP-E cohort. Vaccine efficacy against AIS associated with HPV-16 in ATP-E cohort. Vaccine efficacy against AIS associated with HPV-18 in ATP-E cohort. Vaccine efficacy against CIN3+ associated with HPV-16/18 in TVC-naïve. Vaccine efficacy against CIN3+ associated with HPV-16/18 in TVC-naïve.	100 (85.5, 100) 45.7 (22.9, 62.2) 91.7 (66.6, 99.1) 90.2 (59.7, 98.9) 100 (-8.2, 100) 93.2 (78.9, 98.7) 45.6 (28.8, 58.7) 100 (15.5, 100) 70.0 (-16.6, 94.7) 100 (31.0, 100) 76.9 (16.0, 95.8) 100 (-8.6, 100) 100 (48.4, 100) 100 (-3768.9, 100) 100 (69.4, 100) 100 (67.8, 100)
		18-20 21-25 15-17 18-25 18-20 21-25 15-17 18-25 18-20 21-25		Vaccine efficacy against CIN3+ associated with HPV-16/18 in TVC-naïve. Vaccine efficacy against CIN3+ associated with HPV-16/18 in TVC-naïve. Vaccine efficacy against CIN3+ associated with HPV-16/18 in TVC. Vaccine efficacy against CIN3+ associated with HPV-16/18 in TVC. Vaccine efficacy against CIN3+ associated with HPV-16/18 in TVC. Vaccine efficacy against CIN3+ associated with HPV-16/18 in TVC. Vaccine efficacy against all CIN3+ (irrespective of HPV type in the lesion and including lesions with no HPV DNA detected) in the TVC-naïve. Vaccine efficacy against all CIN3+ (irrespective of HPV type in the lesion and including lesions with no HPV DNA detected) in the TVC-naïve. Vaccine efficacy against all CIN3+ (irrespective of HPV type in the lesion and including lesions with no HPV DNA detected) in the TVC-naïve. Vaccine efficacy against all CIN3+ (irrespective of HPV type in the lesion and including lesions with no HPV DNA detected) in the TVC-naïve. Vaccine efficacy against all CIN3+ (irrespective of HPV type in the lesion) in the TVC. Vaccine efficacy against all CIN3+ (irrespective of HPV type in the lesion) in the TVC. Vaccine efficacy against all CIN3+ (irrespective of HPV type in the lesion) in the TVC. Vaccine efficacy against all CIN3+ (irrespective of HPV type in the lesion) in the TVC.	100 (39.5, 100) 100 (-4.6, 100) 80.5 (55.6, 92.7) 24.2 (-14.1, 50.0) 56.3 (13.6, 79.1) -10.1 (-90.5, 36.1) 91.5 (65.9, 99.0) 95.1 (69.3, 99.9) 90.6 (35.5, 99.8) 100 (51.4, 100) 65.5 (42.5, 80.0) 33.1 (7.5, 51.9) 49.5 (13.9, 71.2) 19.5 (-22.7, 47.4)
Konno, 2014	TVC combined, N=1040 vaccine arm, n=519 control arm, n=521 TVC-naïve combined, N=565 vaccine arm, n=281 control arm, n=284	20-25	NA	Vaccine efficacy against CIN3+ irrespective of the HPV type in the TVC-naive (over the combined 4-y study period of initial and follow-up studies). Vaccine efficacy against CIN3+ irrespective of the HPV type in the TVC (over the combined 4-y study period of initial and follow-up studies).	100 (-417.0, 100) 36.4 (-57.8, 75.7)

Lehtinen, 2017	N=18092 vaccinated arm, n=2465 unvaccinated arm, n=15627	16-17	NA	Vaccine effectiveness against CIN3+ caused by HPV16. Vaccine effectiveness against CIN3+ caused by HPV18. Vaccine effectiveness against CIN3+ caused by HPV16/18. Vaccine effectiveness against CIN3+ caused by HPV16/31/33/35/52/58. Vaccine effectiveness against CIN3+ caused by HPV/31/33/35/52/58 (excluding co-infections with HPV16). Vaccine effectiveness against CIN3+ caused by A9=HPV31/33/35/52/58 and A7=HPV39/45/59/68, (excluding co-infections with 16/18). Vaccine effectiveness against CIN3+ caused by HPV31/33/45. Vaccine effectiveness against CIN3+ caused by HPV6/11/16/18/31/33/45/51/74 (all protected types). Vaccine effectiveness against CIN3+ caused by HPV6/11/31/33/45/51/74 (all protected types excluding co-infections with 16/18). Vaccine effectiveness against CIN3+ caused by HPV34/35/39/40/42/43/44/52/53/54/56/58/59/66/68/70/73 (all non-protected types excluding co-infections with 16/18), Vaccine effectiveness against CIN3+ caused by all detected HPV types. Vaccine effectiveness against CIN3+ caused by all detected HPV types (HPV positive and HPV negative baseline, excluding co-infections with 16/18). Vaccine effectiveness against CIN3+ caused by Total (original FCR registered CIN3+ diagnoses). Vaccine effectiveness against CIN3+ caused by Total All, irrespective of HPV type, this includes the re-review of histopathological block retrieval and re-analysis.	22 (-160, 73) 100 (-1500, 100) 27 (-140, 74) 53 (-48, 83) 100 (-65, 100) 100 (-55, 100) 100 (-120,100) 50 (-60, 82) 100 (-120, 100) 100 (-480, 100) 56 (-38, 84) 100 (-55, 100) 59 (-26, 85) 66 (8.4, 88)
Porras, 2020	Analytical cohort (0-4 y), N=5312 vaccine arm, n=2635 control arm, n=2677 Analytical cohort (7-11 y), N=4603 vaccinated arm, n=2073 unvaccinated arm, n=2530	18-25	NA	Vaccine efficacy against CIN3+ caused by HPV 16/18 at year 4 post-vaccination (analytical cohort with original control group) Vaccine effectiveness against CIN3+ caused by HPV 16/18 at year 7 post-vaccination (analytical cohort with unvaccinated new control group). Vaccine effectiveness against CIN3+ caused by HPV 16/18 at year 9 post-vaccination (analytical cohort with unvaccinated new control group). Vaccine effectiveness against CIN3+ caused by HPV 16/18 at year 11 post-vaccination (analytical cohort with unvaccinated new control group).	66.4 (-175, 97.3) 100 (-40.1, 100) 100 (44.0, 100) 100 (78.8, 100)
Shing, 2022	Analytical cohort (1-4 y), N=7003 vaccine arm, n=3491 control arm, n=3512 Analytical cohort (7-11 y), N=5418 vaccinated arm, n=2826 unvaccinated arm, n=2592	18-25	NA	Vaccine efficacy against incident CIN3+ irrespective of HPV type (combined 4-year period). Vaccine efficacy against incident CIN3+ caused by HPV16 or HPV18 (combined 4-year period). Vaccine efficacy against incident CIN3+ caused by HPV 31,33, or 45 (excluding HPV 16 or 18 coinfection) (combined 4-year period). Vaccine efficacy against incident CIN3+ caused by HPV types other than HPV 16, 18, 31, 33, or 45 (combined 4-year period). Vaccine effectiveness against incident CIN3+ irrespective of HPV type (combined years 7-11 period). Vaccine effectiveness against incident CIN3+ caused by HPV16 or HPV18 (combined years 7-11 period). Vaccine effectiveness against incident CIN3+ caused by HPV 31,33, or 45 (excluding HPV 16 or 18 coinfection) (combined years 7-11 period). Vaccine effectiveness against incident CIN3+ caused by HPV types other than HPV 16, 18, 31, 33, or 45 (combined years 7-11 period). Vaccine effectiveness against incident CIN3+ irrespective of HPV type (combined 11-year period). Vaccine effectiveness against incident CIN3+ caused by HPV16 or HPV18 (combined 11-year period). Vaccine effectiveness against incident CIN3+ caused by HPV 31,33, or 45 (excluding HPV 16 or 18 coinfection) (combined 11-year period). Vaccine effectiveness against incident CIN3+ caused by HPV types other than HPV 16, 18, 31, 33, or 45 (combined 11-year period).	25.2 (-5.0, 46.9) 52.9 (22.4, 72.1) -16.1 (-149.0, 45.3) -17.4 (-123.2, 37.8) 14.4 (-23.4, 40.7) 86.9 (65.3, 96.1) 36.9 (-36.2, 71.6) -135.0 (-329.8,-33.5) 19.5 (-3.3, 37.5) 67.9 (51.1, 80.4) 16.6 (-40.6, 52.4) -81.7 (-190.6, -19.9)
Palmer, 2019	N=138692 0 doses (unvaccinated) n=64026 1 dose, n=2051 2 doses, n=4135 3 doses, n=68480	12-13 14 15 16 17 ≥18 ≤17 12-13	N=16200 N=5409 N=16532 N=17511 N=8711 N=4117 N=15678 N=48348	Vaccine effectiveness against CIN3+[d, e] Vaccine effectiveness against CIN3+ Vaccine effectiveness against CIN3+ Vaccine effectiveness against CIN3+ Vaccine effectiveness against CIN3+ Vaccine effectiveness against CIN3+ Vaccine effectiveness against CIN3+, born ≥ 1991 (unvaccinated). Vaccine effectiveness against CIN3, born 1995-1996 (unvaccinated).	86 (75, 92) 82 (57, 93) 71 (56, 81) 73 (59, 82) 45 (17, 64) 15 (-37, 48) 18 (-7, 37) 100 (69, 100)

Falcaro, 2021	13.7 million-years of follow-up	12-13 14-16 16-18 12-13 14-16 16-18	NA	Vaccine effectiveness against CIN3[f] Vaccine effectiveness against CIN3 Vaccine effectiveness against CIN3 Vaccine effectiveness against cervical cancer Vaccine effectiveness against cervical cancer. Vaccine effectiveness against cervical cancer.	97 (96, 98) 75 (72, 77) 39 (36, 41) 87 (72, 94) 62 (52, 71) 34 (25, 41)
Rebolj, 2022	N=108138 vaccinated, n=64274 unvaccinated, n=43863	14-17	NA	Vaccine effectiveness against HR-HPV positive CIN3+ (HR-HPV+/cytology+ primary screening test)[g]. Vaccine effectiveness against HPV 16/18-related CIN3+. Vaccine effectiveness against CIN3+ by "Other" HPV-related (excludes co-infections with HPV 16/18)[h]. Vaccine effectiveness against cervical cancer.	79 (73, 83) 87 (80, 91) 57 (25, 75) 64 (-91, 93)

a Participants received 3 doses of vaccine and were HPV DNA negative at baseline.
b Participants received at least 1 dose of vaccine, irrespective of baseline HPV DNA status.
c Participants received at least 1 dose of vaccine and were HPV DNA negative at baseline.
d Vaccine effectiveness calculated as VE=(1-odds ratio)*100.
e Results for 3 doses of vaccine.
f Vaccine effectiveness calculated as VE=(1-IRR)*100. (Adjusted IRR model 3).
g 14 HR-HPV types: 16,18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68.
h "Other" 12 HR-HPV types: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68.
Abbreviations: AIS= Adenocarcinoma in situ, ATP-E= According-to-protocol for efficacy cohort, DNA= Deoxyribonucleic Acid, FCR= Finnish cancer registry, HPV= Human papillomavirus, HR HPV= High-risk human papillomavirus, IRR= Incident relative risk (or risk ratio), N= Total (overall), n= Number of participants in each arm, NA= Not applicable, TVC= Total vaccinated cohort UK=United Kingdom, US=United States, RCT= Randomized controlled trial, y=years.

Overall results of primary analyses

Using meta-regression of individual published point estimates for vaccine efficacy/effectiveness, the questions in the following table were addressed:

Table 5. Questions addressed using meta-regression analysis of selected studies

Questions	RCT studies		Observational studies	
	Study (author/year)	Analytic cohort	Study (author/year)	Analytic cohort
Question 1: What is the combined efficacy/effectiveness of *Cervarix* on CIN3+ caused by vaccine HPV types? (Combined RCT and Observational studies)	Lehtinen, 2012	TVC naïve and TVC	Shing, 2022	TVC
	Porras, 2020	TVC naïve	Rebolj, 2022	TVC
Question 2: What is the combined overall efficacy/effectiveness of *Cervarix* on CIN3+ caused by any HPV type? (Combined RCT and Observational studies).	Lehtinen, 2012	TVC naïve and TVC	Shing, 2022	TVC
	Konno, 2014	TVC and TVC naïve	Palmer, 2019	TVC
			Rebolj, 2022	TVC
Question 3: What is the efficacy of *Cervarix* on CIN3+ caused by vaccine HPV types? (RCTs only).	Lehtinen, 2012	TVC naïve and TVC	NA	NA
	Porras, 2020	TVC naïve		
	Shing, 2022	TVC		
Question 4: What is the effectiveness of *Cervarix* on CIN3+ caused by vaccine HPV types? (Observational studies only).	NA	NA	Shing, 2022	TVC
	NA	NA	Lehtinen, 2017	TVC
	NA	NA	Rebolj, 2022	TVC
Question 5: What is the efficacy of *Cervarix* on CIN3+ caused by any HPV type? (RCTs only).	Lehtinen, 2012	TVC naïve and TVC	NA	NA
	Konno, 2014	TVC and TVC-naïve	NA	NA
	Shing, 2022	TVC		
Question 6: What is the effectiveness of *Cervarix* on CIN3+ caused by any HPV type? (Observational studies only).	NA	NA	Shing, 2022	TVC
	NA	NA	Lehtinen, 2017	TVC
	NA	NA	Palmer, 2019	TVC
	NA	NA	Rebolj, 2022	TVC

Abbreviations: CIN3+=Cervical intraepithelial neoplasia grade 3 or worse, HPV=Human papillomavirus, NA=Not applicable, RCT=Randomized controlled trial, TVC=Total Vaccinated Cohort.

Table 6. Summary of analysis

Study	Study design	Analytical cohort		Age at first vaccination (y)	HPV type		Time since vaccination (y)	Analysis 1	Analysis 2	Analysis 3	Analysis 4	Analysis 5	Analysis 6
		TVC	TVC naïve		HPV 16/18	Irrespective HPV type							
[Lehtinen, 2012]	RCT	X		15-17	X	X	4	X	X	X		X	
[Lehtinen, 2012]	RCT	X		18-20	X	X	4	X	X	X		X	
[Lehtinen, 2012]	RCT	X		21-25	X	X	4	X	X	X		X	
[Lehtinen, 2012]	RCT		X	15-17	X	X	4	X	X	X		X	
[Lehtinen, 2012]	RCT		X	18-20	X	X	4	X	X	X		X	
[Lehtinen, 2012]	RCT		X	21-25	X	X	4	X	X	X		X	
[Konno, 2014]	RCT	X		20-25		X	4		X	X		X	
[Konno, 2014]	RCT		X	20-25		X	4		X			X	
[Porras, 2020]	RCT		X	18-25	X		4	X		X			
[Shing, 2022]	RCT	X		18-25	X	X	4			X		X	
[Lehtinen, 2017]	Observational	X		16-17	X	X	10				X		X
[Palmer, 2019]	Observational	X		12-13		X	7-8		X				X
[Palmer, 2019]	Observational	X		14		X	6		X				X
[Palmer, 2019]	Observational	X		15		X	5		X				X
[Palmer, 2019]	Observational	X		16		X	4		X				X
[Palmer, 2019]	Observational	X		17		X	3		X				X
[Palmer, 2019]	Observational	X		≥18		X	2		X				X
[Rebolj, 2022]	Observational	X		14-17	X	X	7-11	X	X		X		X
[Shing, 2022]	Observational	X		18-25	X	X	7-11	X	X		X		X

HPV: Human Papillomavirus, RCT: randomized control trial, TVC: Total Vaccinated Cohort.

The following graph represents the different pooled vaccine effect estimates (including 95% CI) obtained by simple meta-analysis and thus, without adjusting for covariates.

Results for all analyses are described in section 9.2 of the CSR. Statistical outputs for all analyses are included in Annex 2.

Irrespectively of the question, the unadjusted vaccine effect was large in every scenario (within a range of vaccine effect from 48% to 78%).

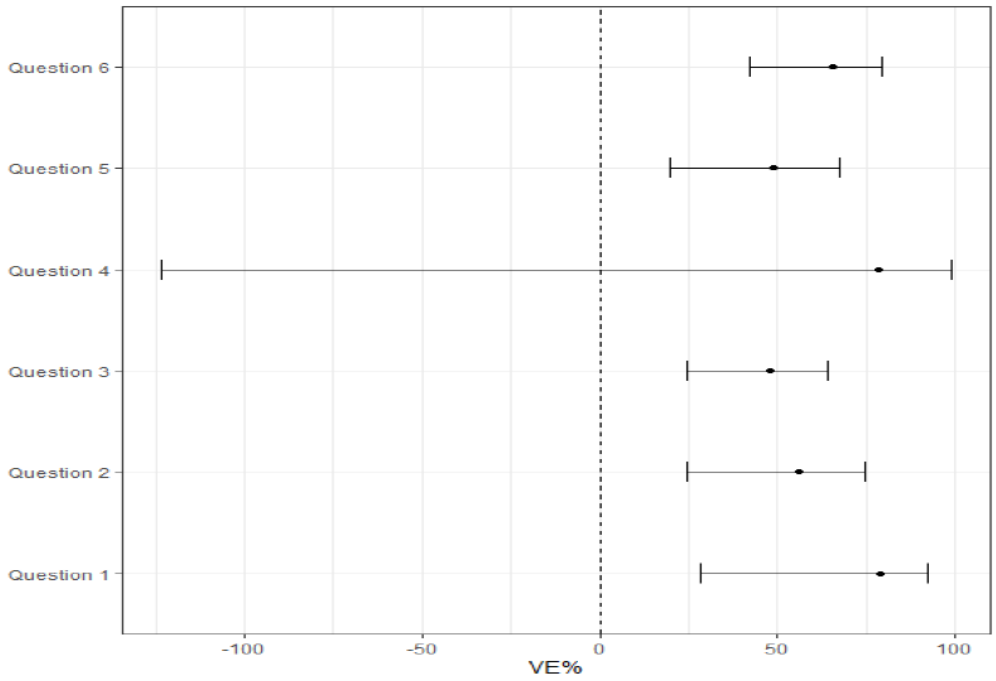
When adjusting for covariates:

1. Results are consistent across all the analyses, CERVARIX´s effects (either from RCTs, or observational studies, or both designs combined) against CIN3+ caused by HPV 16/18 types or by any HPV type. The CERVARIX´s effects were dependent of 3 covariates and were

- higher the younger the age at the first vaccination of the participants,
- higher in the TVC naïve population (HPV negative at baseline) compared to the TVC (irrespective of the HPV baseline status),
- shorter the follow-up (shorter time since vaccination).

2. These identified covariates explained most part of the heterogeneity leading to good predictions relevant for decision-making.

Figure 1. Pooled vaccine effects from unadjusted meta-analysis



Abbreviation: VE=Vaccine effect.

Table 7. Pooled long-term vaccine effects and impactful covariates

Analysis	Outcome	Meta-analysis Vaccine effect (95%CI)	Meta-regression Impactful covariates
HPV 16/18			
Analysis 1 (RCTs, Observational studies)	Combined VEs	76.78% (28.15-92.49)	Age at first vaccination. Analytic cohort[1]
Analysis 3 (RCTs)	Vaccine efficacy	47.84% (24.51-63.96)	Age at first vaccination. Analytic cohort[1]
Analysis 4 (Observational study)	Vaccine effectiveness	78.35% (-123.19-97.90)	NA[2]
Irrespective of HPV type			
Analysis 2 (RCTs, Observational study)	Combined VEs	56.19% (24.76-74.49)	Age at first vaccination. Analytic cohort[1]
Analysis 5 (RCTs)	Vaccine efficacy	48.89% (19.84-67.41)	Age at first vaccination. Analytic cohort[1]
Analysis 6 (Observational study)	Vaccine effectiveness	65.45% (42.02-79.41)	Age at first vaccination. Time since vaccination

Abbreviations: CI=Confidence interval, HPV=Human papillomavirus, NA=Not applicable, RCT=Randomized controlled trial.

1.Analytic cohorts included were Total Vaccinated Cohort (TVC, irrespective of HPV baseline status) and Total Vaccinated Cohort naïve (TVC-naïve, HPV-naïve at baseline)

2. The data-driven multiparametric meta-regression selection did not identify any stable model.

Secondary outcomes

The systematic literature review unveiled effects of CERVARIX on other endpoints (i.e., CIN3, AIS, cervical cancer), herd effects, and cross-protection. Since there were not enough individual records as to conduct a quantitative synthesis, a brief description of the findings is included.

Vaccine effects of CERVARIX on CIN3 and cervical cancer

Falcaro and colleagues [Falcaro, 2021] determined the VE of CERVARIX after its implementation as part of the NIP in England from 2008 to 2012. The immunization was deployed as a school-based routine vaccination program directed towards girls 12-13 years old and there were also catch-up campaigns for older adolescents (14-18 years). Results from this nationwide population-based study revealed a VE on CIN3 ranging from 97% (95% CI, 96%-98%) among the 12-13 years old vaccinated cohort, through 75% (95% CI, 72%-77%) in the 14-16 years old group, to 39% (95% CI, 36%-41%) in the 16-18 years old vaccinated cohort (Table 5).

Furthermore, the researchers estimated the VE of the program on cervical cancer at 87% (95% CI, 72%-94%) among students vaccinated at 12-13 years, through 62% (95% CI, 52%-71%) in the cohort vaccinated at 14-16 years, to 34% (95% CI, 25%-41%) in the 16- 18 years old vaccinated group [Falcaro, 2021].

The authors concluded that they observed a substantial reduction in the incidence of cervical cancer and CIN3 after the introduction of the universal vaccination program with CERVARIX in England, especially among women offered the vaccine at 12-13 years. They affirmed that the vaccine almost eliminated cervical cancer in women born since 01 September 1995. Part of this success was likely due to the high annual vaccine coverage in England that for 2008–09 and 2011–12 ranged between 85.9% and 90.6% in the routine cohorts [Falcaro, 2021]. These results are very important because this was the first time ever that real-world HPV VE on cervical cancer was reported for CERVARIX.

Rebolj et al, reported VE results on cervical cancer corresponding to 14-17 years old adolescents vaccinated in England through the catch-up campaign. Overall VE against cervical cancer among this population group was established at 64% (95% CI, -91%- 93%). However, results were not statistically significant (p=0.14) as number of cases was small (n=32). Vaccine coverage in the catch-up cohort ranged from 40% to 75%, depending on the birth cohort [Rebolj, 2022].

Vaccine effects of CERVARIX on CIN3+ caused by non-vaccine types.

Wheeler et al. investigated the vaccine efficacy on CIN3+ caused by non-vaccine types (a composite index of 12 HR HPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) as part of the PATRICIA trial (Table 5). The vaccine efficacy in the TVC-naïve (participants who were HPV negative at baseline and received at least one dose of the vaccine) was 81.9% (95% CI, 17.1-98.1) and 40.0% (95% CI, 1.1-64.2) in the TVC (participants who received at least one dose of the vaccine, irrespective of their HPV baseline status). VE against CIN3+ caused by non-vaccine types reached 62.1% (95% CI, 21.8-82.9) in the ATP-E cohort (participants who received three doses of the vaccine and were HPV negative at baseline). In all the above cases, the analysis excluded HPV 16/18 co-infection [Wheeler, 2012]. These results are relevant because underpin cross-protection and effectiveness of CERVARIX on advanced lesions and cervical cancer (CIN3+) caused by non-vaccine HPV types [Wheeler, 2012].

Vaccine effects of CERVARIX on AIS.

Lehtinen and colleagues reported vaccine efficacy against AIS HPV 16/18-related of 100% (95% CI, 15.5-100) in the TVC naïve whereas it was 70% (95% CI, -16.6-94.7) in the TVC. Vaccine efficacy against AIS irrespective of HPV DNA in the lesion in the TVC naïve and TVC, was 100% (95% CI, 31.0-100) and 76.9% (95% CI, 16.0-95.8), respectively [Lehtinen, 2012].

Other vaccine effects.

The systematic review also identified herd effects of CERVARIX on CIN3 as reported by Palmer et al. (Table 5). These authors investigated the impact of CERVARIX introduction in the NIP in Scotland among unvaccinated cohorts born in 1995 and 1996 (the same age than vaccine-eligible cohorts, 12-13 years old), and found a VE against CIN3 estimated at 100% (95% CI, 69-100) compared with unvaccinated women born in 1988-1990. Most likely these effects relate to high vaccine coverage as the vaccine uptake among the 1995 birth cohort (13 years at vaccination) was 90% [Palmer, 2019].

2.3.2. Discussion on clinical aspects

Cervical cancer cases are caused by persistent genital high-risk human papillomavirus (HPV) infection. Most HPV infections clear spontaneously but persistent infection with the oncogenic or high-risk types may cause cancer. Multiple modifiable risk-factors may contribute to the risk of acquiring HPV, including the age at sexual debut and the number of lifetime sexual partners. There is increased biological vulnerability in younger females associated with the cervical transformation zone that is undergoing active metaplastic changes. This, coupled with HPV-naïve immunogenicity and frequent sexual partner changes that are common among 15–24 year olds, makes this age group particularly vulnerable and correlates with observed HPV prevalence. The prevention of cervical neoplasms associated with HPV (especially related to HPV-16 and HPV-18) occurs through prophylactic vaccines, which, although safe and effective, cannot eliminate already established High-Grade Cervical Intraepithelial Neoplasia (CIN 2/3) lesions and have no effect on already established lesions caused by HPV[1]. Prophylactic HPV vaccines do not clear persistent high-risk HPV infection and/or cause regression of pre-cancerous lesions.

Recent systematic review and meta-analysis study results were published with important findings[2]. These demonstrated that the HPV vaccine is more effective when the vaccine series is initiated at younger ages in high-income settings. These data are consistent with data of a recently published meta-analysis[3]**.** In this meta-analysis 21 observational studies were identified that evaluated HPV vaccine effectiveness against different HPV-related disease outcomes by age at which the vaccine series was either initiated or completed. Seventeen of the 21 studies found the greatest vaccine effectiveness in the youngest age group evaluated with many of those studies also finding decreased vaccine effectiveness by later age at vaccine series initiation. Greater effectiveness of HPV vaccines at younger ages is likely due to administration of these prophylactic vaccines prior to natural exposure to HPV from sexual activity rather than a biologic mechanism independent of natural exposure. Though younger adolescents do produce higher levels of antibodies after vaccination, older adolescents and

[1] Deligeoroglou et al. HPV infection: Immunological aspects and their utility in future therapy. Infect. Dis. Obstet. Gynecol. 2013;2013:540850.
Bruni et al. Global estimates of human papillomavirus vaccination coverage by region and income level: A pooled analysis. Lancet Glob. Health. 2016;4:e453–e463.
Zhou, et al. Papillomavirus Immune Evasion Strategies Target the Infected Cell and the Local Immune System. *Front. ncol.* 2019, *9*, 682.
[2] Bartels, H.C.; Postle, J.; Rogers, A.C.; Brennan, D. Prophylactic human papillomavirus vaccination to prevent recurrence of cervical intraepithelial neoplasia: A meta-analysis. Int. J. Gynecol. Cancer 2020, 30, 777–782.
Brogden, D.R.L.; Walsh, U.; Pellino, G.; Kontovounisios, C.; Tekkis, P.; Mills, S.C. Evaluating the efficacy of treatment options for anal intraepithelial neoplasia: A systematic review. Int. J. Color. Dis. 2020, 36, 213–226.
Dion, G.R.; Teng, S.; Boyd, L.R.; Northam, A.; Mason-Apps, C.; Vieira, D.; Amin, M.R.; Branski, R.C. Adjuvant human papillomavirus vaccination for secondary prevention: A systematic review. JAMA Otolaryngol. Head Neck Surg. 2017, 143, 614–622.
Perkins RB, Humiston S, Oliver K. Evidence supporting the initiation of HPV vaccination starting at age 9: Collection overview. Hum Vaccin Immunother. 2023 Dec 15;19(3):2269026. doi: 10.1080/21645515.2023.2269026. Epub 2023 Oct 12. PMID: 37824444; PMCID: PMC10572037.
Kjaer SK, Dehlendorff C, Belmonte F, Baandrup L. Real-world effectiveness of human papillomavirus vaccination against cervical cancer. *J Natl Cancer Inst*. 2021;113(10):1329–35. doi: 10.1093/jnci/djab080.
Lei J, Ploner A, Elfström KM, Wang J, Roth A, Fang F, Sundström K, Dillner J, Sparén P. HPV vaccination and the risk of invasive cervical cancer. *N Engl J Med*. 2020;383(14):1340–8. doi: 10.1056/NEJMoa1917338.
[3] Mallory K. Ellingson, Hassan Sheikha, Kate Nyhan, Carlos R. Oliveira & Linda M. Niccolai (2023) Human papillomavirus vaccine effectiveness by age at vaccination: A systematic review, Human Vaccines & Immunotherapeutics, 19:2, 2239085

adults also have a robust immune response that produces antibody levels much higher than natural infection that likely confers substantial protection.

The HPV vaccine is recommended between ages 9 and 14 years for girls by the World Health Organization. However, many individuals do not initiate the recommended vaccine series in this window, starting vaccination later in adolescence or in young adulthood. Many studies used late adolescence (18–20 years of age) as a cutoff point between different age groups, likely reflecting the average age of sexual debut. While some studies did find that the vaccine was still effective when administered after the age of 18, in general, the vaccine was substantially more effective in those who received the vaccine prior to the age of 18 against all outcomes, reflecting findings from clinical trials that have demonstrated higher efficacy when the vaccine is administered prior to exposure to HPV.

Context of the study

Long-term efficacy and immunogenicity information is already a part of Cervarix´s label. However, the national immunization programs (NIPs) with universal Cervarix vaccination are being rolled out, observational studies are being conducted and real-world and long-term follow-up of clinical trials data on the long-term effects of Cervarix are accruing and becoming available.

The MAH conducted a systematic literature review and meta-analysis/meta-regression analysis (critical appraisal of the data to assess its quality, and robustness) with the aim of compiling all published data (including data not generated by the MAH).

Methods

The objectives of the study was to first conduct a systematic literature review of the long-term efficacy/effectiveness of Cervarix on cervical cancer and CIN3 or worse (CIN3+) and second, to perform a meta-analysis/meta-regression analysis to provide estimates of the effect size of Cervarix on cervical cancer and CIN3 or worse (CIN3+) while adjusting for covariates such as age at vaccination, time since vaccination (time of follow-up), or type of analytical cohort (HPV baseline status), and study design.

The overall research question was

What is the efficacy/effectiveness of the (HPV) vaccination with Cervarix in girls and women against HPV on (CIN3) or worse (CIN3+)?

Six specific research questions were investigated which considered the efficacy, the effectiveness and the combined efficacy/effectiveness of Cervarix on CIN3+ caused by vaccine HPV types and irrespective of HPV types.

To answer the research questions and scenarios, a main analytical step-wise approach considered as first step a classical random effect meta-analysis without adjusting for covariates. The second step was to conduct univariate meta-regression with random effect of each potential covariate and third, followed by multiparametric meta-regressions adjusting for the covariates. Finally, an estimator of prediction error approach was used to assess the quality of the models for every given dataset allowing a data-driven selection of the best model. One model was selected for each of the 6 questions assessed.

Meta-regression analysis allows assessing the relationship between specific study-level covariates, such as age at vaccination or time since vaccination, and the effect size. In particular, it takes into account the heterogeneity of the results that may come from different levels of covariates of the different studies. This meta-regression analysis was not conceived as a confirmatory study. There was not a prior hypothesis to test and therefore it was not necessary to establish a sample size that has sufficient power to reject the null hypothesis.

Results

Systematic review results

Nine publications met the inclusion criteria. Seven studies were selected for the quantitative synthesis. Of these, 4 studies were follow-up of RCTs [Lehtinen, 2012;Konno, 2014; Porras, 2020; Shing, 2022] and 3 observational studies of which 2 studies were retrospective population-based registry linked studies [Palmer, 2019; Rebolj, 2022] and one study was an observational post-hoc long-term follow-up of an RCT [Lehtinen, 2017]. Two of the RCTs had also an observational component [Porras, 2020; Shing, 2022]. The studies included in this systematic review were conducted in Japan, Costa Rica, Finland, Scotland, England, and multi-country sites. Around 290 000 participants aged 12 to 25 years at vaccination, and up to 11 years of follow-up were included for the meta-regression analysis.

The risk of bias of the systematic literature review was assessed by Cochrane risk of bias for randomized controlled trails (RoB2) tool and risk of bias in non-randomized studies of interventions (ROBINS-I) tool. None of the 9 studies mentioned above were excluded based on quality. Based on these results, the MAH decided to conduct meta-regression analyses adjusting for covariates and discussing and acknowledging the limitations of the studies. The covariates considered in meta-regression analysis were age at vaccination, time since vaccination, study design, or analytical cohort (Total Vaccinated Cohort (TVC, irrespective of HPV baseline status) and Total Vaccinated Cohort naïve (TVC-naïve, HPV-naïve at baseline)).

This systematic literature review suffers from intrinsic limitations mainly concerning the ability to retrieve all available actual data as it was done for publications published up to June 2022. All the observational studies included in this systematic review were considered to have some degree of risk of bias but this is a well-known limitation.

Meta-analysis results without adjusting for covariates

This meta-analysis/meta-regression analysis included data on Cervarix long-term effects (4 years for RCTs, and 10-11 years for observational studies) against CIN3+ from roughly 290 000 participants aged 12 to 25 years at vaccination. The different pooled vaccine effects estimates (including 95% CI) were obtained by simple meta-analysis. Irrespective of the question, the pooled vaccine effect was large in every scenario (within a range of vaccine effect from 48% [95% CI: 24.5, 64.0] to 78% [95% CI: -123.2, 97.9] - regardless of HPV DNA types.

Meta-regression analysis with adjusting for covariates

Overall, the meta-regression analysis of each question showed that the vaccine effects decreased with increase in age at first vaccination and was lower in the TVC population (irrespective of HPV baseline status of participants) compared to the TVC naïve (HPV negative at baseline). Vaccine effects were higher in younger age at first vaccination of the participants, in the TVC naïve population (HPV negative at baseline) compared to the TVC (irrespective of the HPV baseline status), and the shorter the time since vaccination (time of follow-up). Results of cervarix´s long-term effects (either from RCTs, or observational studies, or both designs combined) against CIN3+ caused by HPV 16/18 types or by any HPV type were consistent across all the analyses.

Results are consistent with findings from pivotal clinical trials that demonstrated higher efficacy when the vaccine was administered before exposure to HPV. The meta-regression analysis unveiled that age at vaccination was impactful on VE when pooling data from observational studies, irrespective of the HPV type. This was also evident in the population-based studies (Palmer, 2019) where participants vaccinated at 17 years were more than three times as likely to be diagnosed with CIN3+ than those vaccinated at 12-13 years (Odds ratio= 0.55 [95%CI, 0.36-0.83] vs. Odds ratio= 0.14 [95%CI, 0.08-0.25], respectively).

Further, results from the univariate models for combined effects (RCTs and observational studies) irrespective of HPV type (Analysis 2) showed higher vaccine effects for long-term follow-up observational studies compared to RCTs as most likely these results were driven by the large nationwide population-based studies in the dataset (Palmer, 2019; Rebolj, 2022).

In relation to the limitations of the statistical analysis, the regression performance was determined by the number of studies in the meta-analysis, which in this study was relatively low (9 studies). The power of the statistical analyses was limited mainly due to the small number of studies and their heterogeneity. Consequently, if a covariate is not found to be significant, it cannot be concluded that there is no true effect of that covariate as available data is plausibly insufficient. Hence, the precision of the estimates produced by the meta-regression results is insufficient to conclude on covariates effects. Furthermore, non-linearity for the covariate "age at first vaccination" was not checked given the small number of observations.

3. CONCLUSIONS

This meta-analysis/meta-regression analysis included data on *Cervarix* effects on CIN3+ from roughly 290 000 participants aged 12 to 25 years at vaccination, followed-up for at least 4 years up to 11 years. Overall, results were consistent across all the analyses including *Cervarix´s* long-term effects (either from RCTs, or observational studies, or both designs combined) against CIN3+ caused by HPV 16/18 types or by any HPV type. Moreover, the vaccine effects (vaccine efficacy and/or effectiveness) were higher, the younger the age at the first vaccination of the participants, in the TVC-naïve population (HPV negative at baseline) compared to the TVC (irrespective of the HPV baseline status), and the shorter the follow-up (shorter time since vaccination). The evidence generated supports higher efficacy when vaccination is administered at a younger age, and long-term protection against CIN3+ conferred by *Cervarix*.

The additional data generated with this study via modelling may suffer from several limitations that may impact the robustness of the results. Indeed, the overall number of studies on which the analyses are based is low while the studies are widely different, inducing a strong heterogeneity that may not be entirely controlled by the models. The analysis is also descriptive in nature.

Nevertheless, findings from this systematic review and meta-regression analysis are consistent with clinical trials results which are already captured in the PI, including vaccine efficacy against advanced cervical lesions (i.e., CIN3+). The data generated supports high efficacy when vaccination is administered at a younger age, and long-term protection conferred by Cervarix. These findings do not justify a PI update as they are confirming previous findings which were already mentioned with observed data. SmPC includes wordings that reflect the limitations of vaccine efficacy in older population in 4.4 with cross-references to data description in section 5.1. No changes to the PI for *Cervarix* are considered necessary.

4. Rapporteur’s overall conclusion and recommendation

☒ Fulfilled:

No regulatory action required.

ANNEX 2

9.2.1. Analysis 1: What is the combined efficacy/effectiveness of CERVARIX on CIN3+ caused by vaccine HPV types? (RCTs and Observational studies combined)

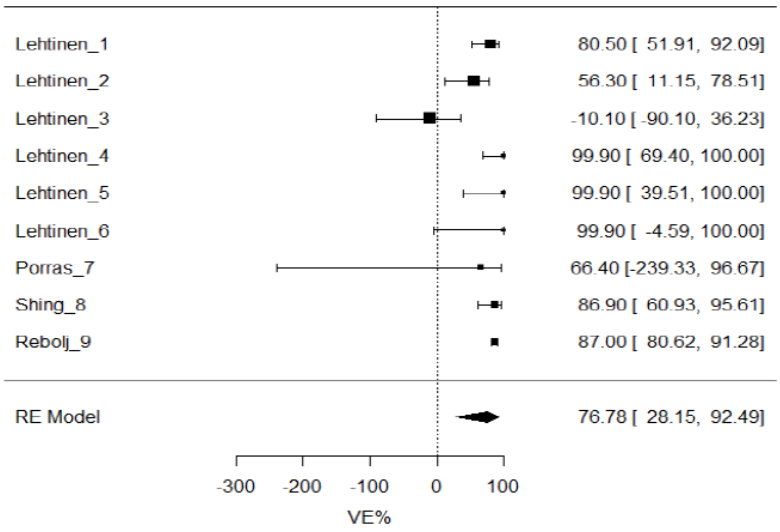
This analysis studied the combined effects of follow-up studies of RCT ([Lehtinen, 2012], including the TVC naïve and TVC; [Porras, 2020], TVC naïve) and observational studies ([Shing, 2022], TVC; [Rebolj, 2022], TVC) of CERVARIX on CIN3+ caused by HPV 16/18 types. The rationale behind the selection of studies for this dataset was to include RCTs and observational studies with outcome results on HPV 16/18 types. We excluded Lehtinen, 2017 from this analysis because participants partially overlapped with Lehtinen, 2012 [Lehtinen, 2012; Lehtinen, 2017]. The observational component of Shing, 2022 was included to consider the long-term follow-up of the CVT, although participants partially overlap with those of Porras, 2020, but with a different approach to the analytical cohort. The "study correlation" variable was used to account for the partial overlapping [Porras, 2020; Shing, 2022].

1. Meta-analysis*.

Pooled vaccine effects were determined at vaccine effect= 76.78 (95%CI, 28.15-92.49) (Figure 2).

*Meta-analysis was done on the log relative risk scale assuming normality. Then results were back transformed to the vaccine effect scale. Therefore, some differences may be found in 95%CI between the pooled vaccine effects provided in the datasets and those estimated in the meta-analysis.

Figure 2 Pooled estimated vaccine effects of CERVARIX on CIN3+ caused by HPV 16/18 types (Analysis 1)



Note for interpretation of graphs:
Lehtinen 1= Lehtinen 2012, age at first vaccination 15-17 years, TVC, time since vaccination 0-4 years.
Lehtinen 2= Lehtinen 2012, age at first vaccination 18-20 years, TVC, time since vaccination 0-4 years.
Lehtinen 3= Lehtinen 2012, age at first vaccination 21-25 years, TVC, time since vaccination 0-4 years.
Lehtinen 4= Lehtinen 2012, age at first vaccination 15-17 years, TVC naïve, time since vaccination 0-4 years.
Lehtinen 5= Lehtinen 2012, age at first vaccination 18-20 years, TVC naïve, time since vaccination 0-4 years.
Lehtinen 6= Lehtinen 2012, age at first vaccination 21-25 years, TVC naïve, time since vaccination 0-4 years.
Porras 7= Porras 2020, age at first vaccination 18-25 years, TVC naïve, time since vaccination 0-4 years.
Shing 8= Shing 2022, age at first vaccination 18-25 years, TVC, time since vaccination 7-11 years.
Rebolj 9= Rebolj 2022, age at first vaccination 14-17 years, TVC, time since vaccination 7-11 years.
Abbreviations: VE=Vaccine effects, RE=random effects.
Reference: [Lehtinen, 2012; Porras, 2020; Shing, 2022; Rebolj, 2022].

The most remarkable aspect of this meta-analysis was that the lower limit of the 95%CI for some individual vaccine effect estimates are negative. This was the case of the Porras, 2020 study that showed a very wide 95%CI. In case of the Lehtinen, 2012 study, the point estimate for vaccine effect corresponding to the age group 21-25 years at first vaccination (Lehtinen 3), was negative whereas vaccine effect=100% for all age groups in the TVC naïve cohort for this particular study, indicated within-trial variability[Lehtinen, 2012]. Negative lower limits occured only for wide confidence intervals and were not given much weight in the model given their uncertainty and thus, it does not largely contribute to the pooled effect. Therefore, the pooled estimate for vaccine effect of all RCTs and observational studies against CIN3+ caused by HPV 16/18 types was vaccine effect=76.78 (95%CI, 28.15-92.49).

2. Univariate meta-regression analysis.

Results from this analysis showed that the variables "age at first vaccination" (p=0.0086),

"study design" (RCT follow-up vs. observational study, p=0.0011) and "time since vaccination" (0-4 years vs. 7-11 years, p=0.0011) presented strong association with the outcome vaccine effect (i.e., small p values in the univariate meta-regression analysis model). All estimates suggests that the vaccine effect decreases with age at first vaccination, it is lower in randomized trials compared with observational studies, and it is larger when time since vaccination is "0-4" years (RCTs) compared to "7-11" years (observational studies). vaccine effect is also larger when the analytical cohort is the TVC naïve (participants HPV negative at baseline). Figures below show the observed and the predicted vaccine effect (black line with 95%CI) as a function of the univariate covariates. Size of the bubbles is proportional to the inverse of the variance (corresponds to the weight in classical meta-analysis).

Figure 3 Univariate effect of analytical cohort on vaccine efficacy/effectiveness (Analysis 1)

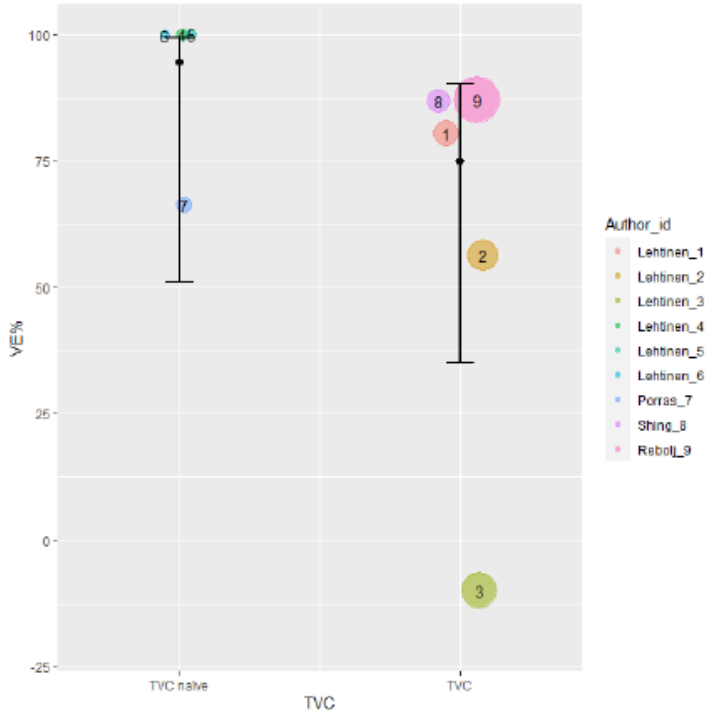


Figure 4 Univariate effect of study design on vaccine efficacy/effectiveness (Analysis 1)

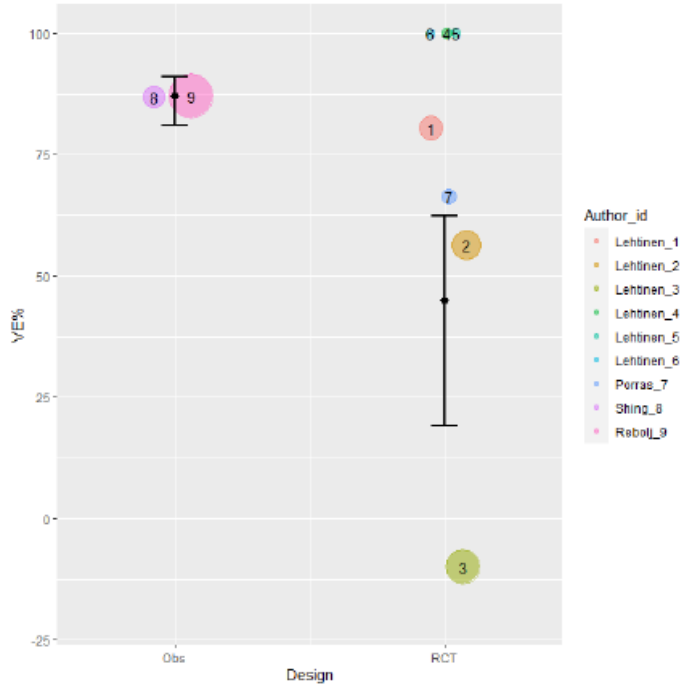


Figure 5 Univariate effect of age at first vaccination on vaccine efficacy/effectiveness (Analysis 1)

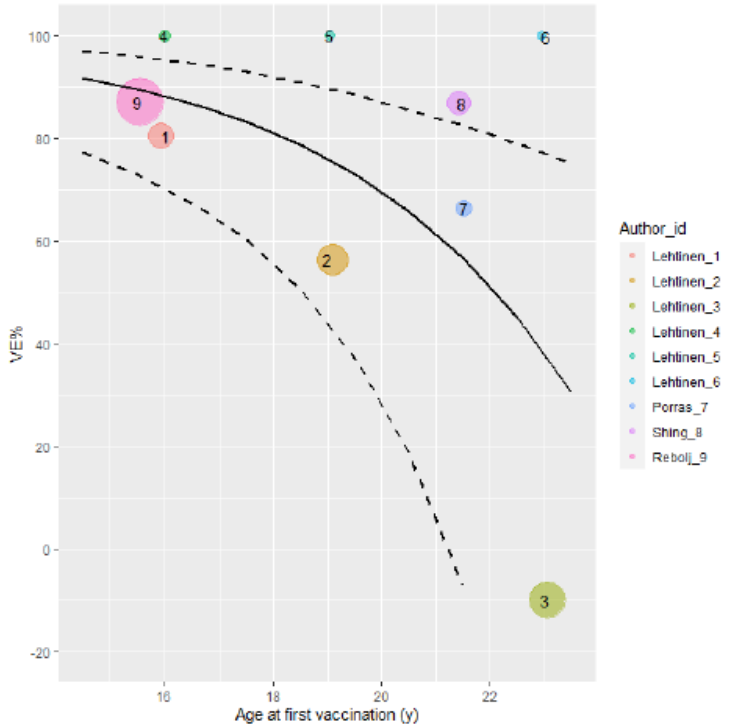
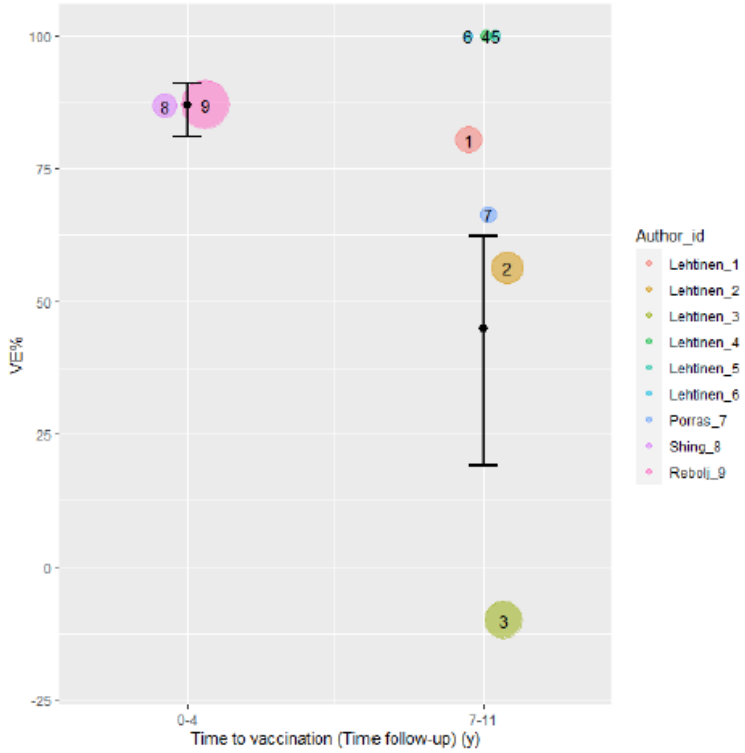


Figure 6 Univariate effect of time since vaccination (time of follow-up) on vaccine fficacy/effectiveness (Analysis 1)

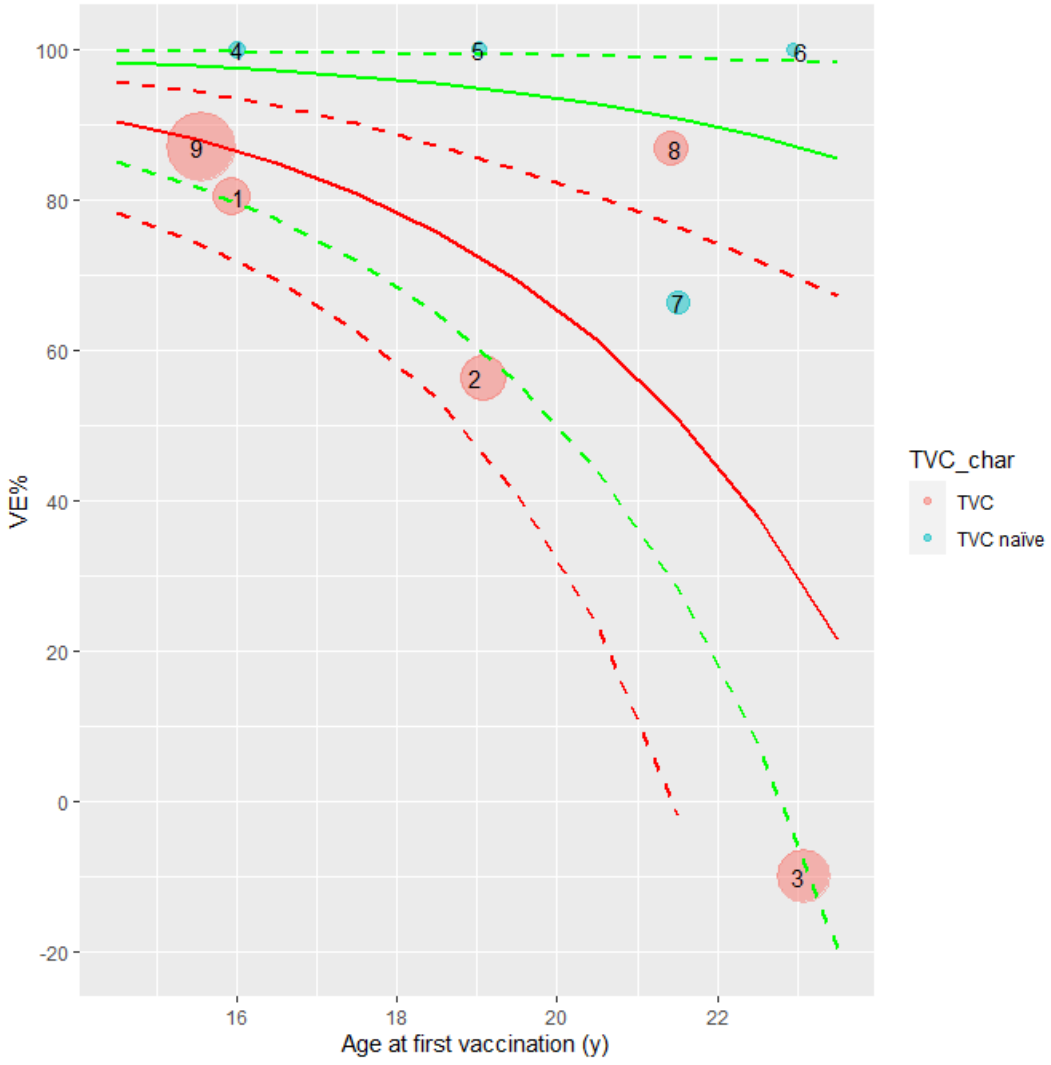


7. Multiparametric meta-regression analysis

All possible combinations of predictors were evaluated and compared using AIC (datadriven approach) to find the best model, and which predictors were the most important ones. This data-driven exploration conducted to a final model that includes the "age at first vaccination" and "analytical cohort" variables. After adjusting for the analytical cohort (TVC vs. TVC naïve), "age at first vaccination" resulted as the most impactful variable on the outcome (p=0.0092). It is also interesting to mention that time since vaccination was not selected as one of the two main explanatory factors, which may indicate persistence of the effect of the vaccine over time. The heterogeneity explained by the selected model was R^2*= 62.18%.

The following figure shows the predictions (with 95% Confidence intervals, dotted line) from this data-driven selected model adjusting for "age at first vaccination" and "analytical cohort". Red and green curves represent the predicted vaccine effect as a function of age for TVC and TVC naïve populations. Red and blue bubbles represent the observed Ves of the studies with TVC and TVC naïve population, respectively. Size of the bubbles is proportional to the inverse of the variance (it corresponds to the weight in a classical meta-analysis). Observed values seem to be relatively well approximated by the multiparametric model.

Figure 7 Results of data-driven multiparametric meta-regression analysis model (Analysis 1)



Note for interpretation of graphs:
Lehtinen 1= Lehtinen 2012, age at first vaccination 15-17 years, TVC, time since vaccination 0-4 years.
Lehtinen 2= Lehtinen 2012, age at first vaccination 18-20 years, TVC, time since vaccination 0-4 years.
Lehtinen 3= Lehtinen 2012, age at first vaccination 21-25 years, TVC, time since vaccination 0-4 years.
Lehtinen 4= Lehtinen 2012, age at first vaccination 15-17 years, TVC naïve, time since vaccination 0-4 years.
Lehtinen 5= Lehtinen 2012, age at first vaccination 18-20 years, TVC naïve, time since vaccination 0-4 years.
Lehtinen 6= Lehtinen 2012, age at first vaccination 21-25 years, TVC naïve, time since vaccination 0-4 years.
Porras 7= Porras 2020, age at first vaccination 18-25 years, TVC naïve, time since vaccination 0-4 years.
Shing 8= Shing 2022, age at first vaccination 18-25 years, TVC, time since vaccination 7-11 years.
Rebolj 9= Rebolj 2022, age at first vaccination 14-17 years, TVC, time since vaccination 7-11 years.
Abbreviations: VE=Vaccine effect, TVC=total vaccinated cohort, y=years.
Reference: [Lehtinen, 2012; Porras, 2020; Shing, 2022; Rebolj, 2022].

9.2.2. Analysis 2: What is the combined overall efficacy/effectiveness of CERVARIX on CIN3+ caused by any HPV type? (RCTs and Observational studies combined).

This analysis studied the combined effects of follow-up studies of RCT ([Lehtinen, 2012], including the TVC naïve and TVC; [Konno, 2014], TVC and TVC naïve) and observational studies ([Shing, 2022], TVC; [Palmer, 2019], TVC; [Rebolj 2022], TVC) of CERVARIX on CIN3+ caused by any HPV type. The rationale behind the selection of studies for this dataset was to include RCTs and observational studies with outcome results irrespectively of the causing HPV type. We excluded Lehtinen, 2017 of this analysis because participants partially overlapped with Lehtinen, 2012 [Lehtinen, 2012; Lehtinen, 2017]. The observational component of Shing, 2022 was included to consider the long-term follow-up of the CVT, as Porras, 2020 only reports on HPV 16/18 types [Porras, 2020; Shing, 2022].

1. Meta-analysis*.
Pooled vaccine effects were determined at vaccine effect= 56.19 (95%CI, 24.76-74.49) (Figure 8).

*Meta-analysis was done on the log relative risk scale assuming normality. Then results were back transformed to the vaccine effect scale. Therefore, some differences may be found in 95%CI between the pooled vaccine effects provided in the datasets and those estimated in the meta-analysis.

Figure 8 Pooled estimated vaccine effects of CERVARIX on CIN3+ caused by any HPV type (Analysis 2)

The most remarkable aspect of this meta-analysis is that the lower limit of the 95%CI for some individual vaccine effect estimates is negative. This is especially relevant for Konno, 2014 (TVC naïve cohort) (Konno 8), as already described in Section 7.8.1 [Konno, 2014]. However, the combined pooled estimate reached statistical significance and the lower limit is above "0" [vaccine effect= 56.19 (95%CI, 24.76-74.49].

2. Univariate meta-regression analysis

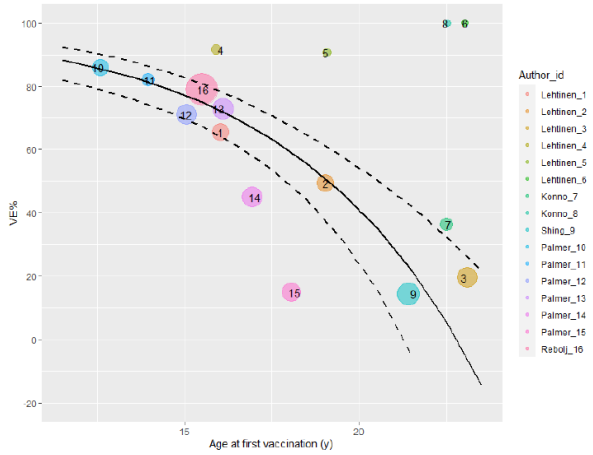
Results from this analysis showed that the variables "analytical cohort" (TVC vs. TVC naïve, p=0.0104), "age at first vaccination" (p<0.001), and "time since vaccination" (0-4 years vs. 7-11 years, p<0.001), presented strong association with the outcome vaccine effect (small p values in the univariate meta-regression analysis model). All estimates (with exception of "time since vaccination") suggests that the vaccine effect decreases with age at first vaccination, it is lower in randomized trials compared with observational studies, and it is higher as time since vaccination increases. vaccine effect is also clearly larger when the analytical cohort is the TVC naïve (participants HPV negative at baseline). Figures below show the observed and the predicted vaccine effect (black line with 95%CI) as a function of the univariate covariates. Size of the bubbles is proportional to the inverse of the variance (it corresponds to the weight in a classical meta-analysis).

Figure 9 Univariate effect of analytical cohort on vaccine efficacy/effectiveness (Analysis 2)

Figure 10 Univariate effect of study design on vaccine efficacy/effectiveness (Analysis 2)

Figure 11 Univariate effect of age at first vaccination on vaccine efficacy/effectiveness (Analysis 2)

Figure 11 **Univariate effect of age at first vaccination on vaccine efficacy/effectiveness (Analysis 2)**



Note for interpretation of graphs:
Lehtinen 1= Lehtinen 2012, age at first vaccination 15-17 years, TVC, time since vaccination 0-4 years.
Lehtinen 2= Lehtinen 2012, age at first vaccination 18-20 years, TVC, time since vaccination 0-4 years.
Lehtinen 3= Lehtinen 2012, age at first vaccination 21-25 years, TVC, time since vaccination 0-4 years.
Lehtinen 4= Lehtinen 2012, age at first vaccination 15-17 years, TVC naïve, time since vaccination 0-4 years.
Lehtinen 5= Lehtinen 2012, age at first vaccination 18-20 years, TVC naïve, time since vaccination 0-4 years.
Lehtinen 6= Lehtinen 2012, age at first vaccination 21-25 years, TVC naïve, time since vaccination 0-4 years.
Konno 7= Konno 2014, age at first vaccination 20-25 years, TVC, time since vaccination 0-4 years.
Konno 8= Konno 2014, age at first vaccination 20-25 years, TVC naïve, time since vaccination 0-4 years.
Shing 9= Shing 2022, age at first vaccination 18-25 years, TVC, time since vaccination 7-11 years.
Palmer 10= Palmer 2019, age at first vaccination 12-13 years, TVC, time since vaccination 0-8 years.
Palmer 11= Palmer 2019, age at first vaccination 14 years, TVC, time since vaccination 0-6 years.

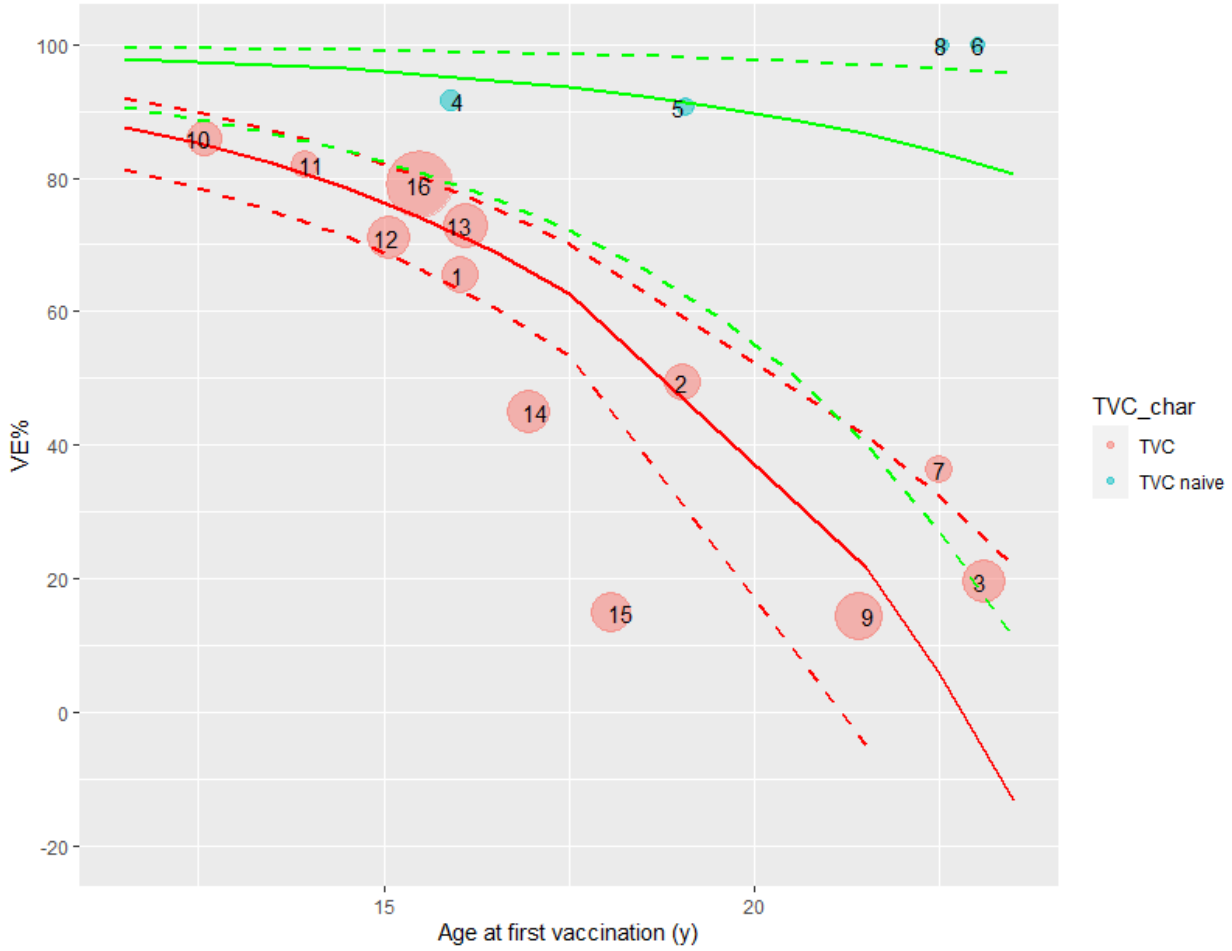
Figure 12 Univariate effect of time since vaccination (time of follow-up) on vaccine efficacy/effectiveness (Analysis 2)

3. Multiparametric meta-regression analysis

All possible combinations of predictors were evaluated and compared using AIC (datadriven approach) to find the best model, and which predictors were the most important ones. This data-driven exploration conducted to a final model that includes the "age at first vaccination" and "analytical cohort" variables. After adjusting for the analytical cohort (TVC vs. TVC naïve), "age at first vaccination" resulted as the most impactful variable on the outcome ($p<0.001$). The heterogeneity explained by the selected model was R^2*= 87.47%.

The following figure shows the predictions (with 95% Confidence intervals, dotted line) from this data-driven selected model adjusting for "age at first vaccination" and "analytical cohort". Red and green curves represent the predicted vaccine effect as a function of age for TVC and TVC naïve populations. Red and blue bubbles represent the observed vaccine effects of the studies with TVC and TVC naïve population, respectively. Size of the bubbles is proportional to the inverse of the variance (it corresponds to the weight in a classical meta-analysis).

Figure 13 Results of the data-driven multiparametric meta-regression analysis model (Analysis 2)



9.2.3. Analysis 3: What is the efficacy of CERVARIX on CIN3+ caused by vaccine HPV types? (RCTs only)

This analysis studied the combined effects of follow-up studies of RCT ([Lehtinen, 2012], including the TVC naïve and TVC; [Porras, 2020], TVC naïve; [Shing, 2022], TVC) of CERVARIX on CIN3+ caused by HPV 16/18 types. The rationale behind the selection of studies for this dataset was to include RCTs with outcome results on HPV 16/18 types. The RCT follow-up component of Shing, 2022 was included to consider the long-term follow-up of the CVT, although participants partially overlap with those of Porras, 2020, but with a different approach to the analytical cohort [Porras, 2020; Shing, 2022] . The "study correlation" variable was used to account for the partial overlapping.

1. Meta-analysis*.

Pooled vaccine efficacy was determined at vaccine efficacy= 47.84% (95%CI, 24.51-63.96) (Figure 14).

*Meta-analysis is done on the log relative risk scale assuming normality. Then results are back transformed to the vaccine effects scale. Therefore, some differences may be found in 95%CI between the pooled vaccine efficacy provided in the datasets and those estimated in the meta-analysis.

Figure 14 Pooled estimated Vaccine efficacy of CERVARIX on CIN3+ caused by HPV 16/18 types (Analysis 3)

The most remarkable aspect of this meta-analysis is that the lower limit of the 95%CI for some individual vaccine efficacy estimates is negative. This is the case of the Porras 2020 study that shows a very wide 95% CI. In case of the Lehtinen, 2012 study, the point estimate for vaccine efficacy corresponding to the age group 21-25 years at first vaccination (Lehtinen 3), is negative whereas vaccine efficacy=100% for all age groups in the TVC naïve cohort, indicating within-trial variability [Lehtinen, 2012]. Negative lower limits occur only for wide confidence intervals. As wide negative confidence intervals are not given much weight in the model, the corresponding studies do not largely contribute to the pooled effect. Therefore, the pooled estimate for vaccine efficacy of all RCTs against CIN3+ caused by HPV 16/18 types was vaccine efficacy= 47.84 (95%CI, 24.51-63.96).

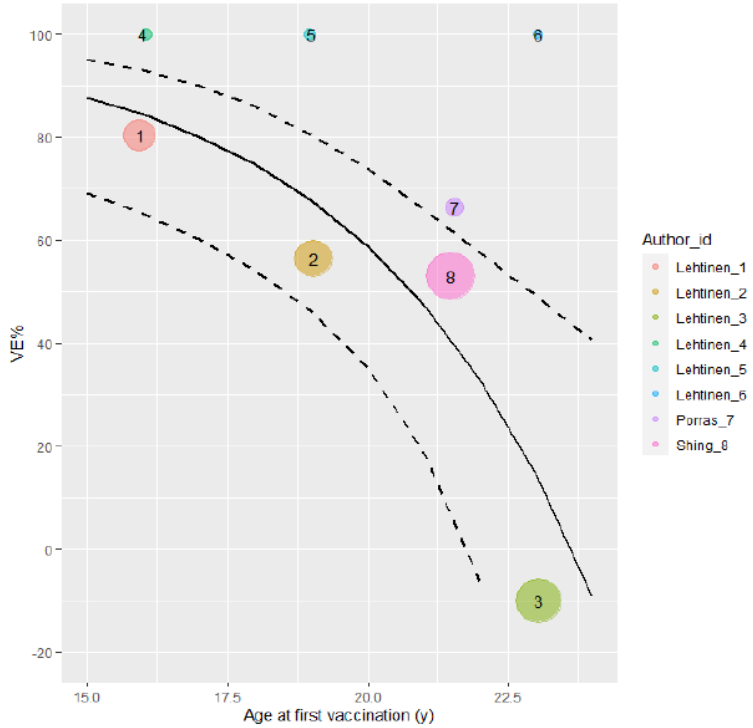
2. Univariate meta-regression analysis

Results from this analysis showed that the variables "age at first vaccination" (p=0.0136), and "analytical cohort" (TVC vs. TVC naïve, p=0.0751) presented association (even if weak for the "analytical cohort" variable) with the outcome vaccine efficacy (small p values in the univariate meta-regression analysis model). All estimates suggests that the vaccine efficacy decreases with age at first vaccination and is lower in the TVC population (irrespective of HPV baseline status of participants). Figures below show the observed and the predicted vaccine efficacy (black line with 95%CI) as a function of the univariate covariates. Size of the bubbles is proportional to the inverse of the variance (it corresponds to the weight in a classical meta-analysis).

Figure 15 Univariate effect of analytical cohort on vaccine efficacy (Analysis 3)

Figure 16 Univariate effect of age at first vaccination on vaccine efficacy (Analysis 3)

Figure 16 Univariate effect of age at first vaccination on vaccine efficacy (Analysis 3)



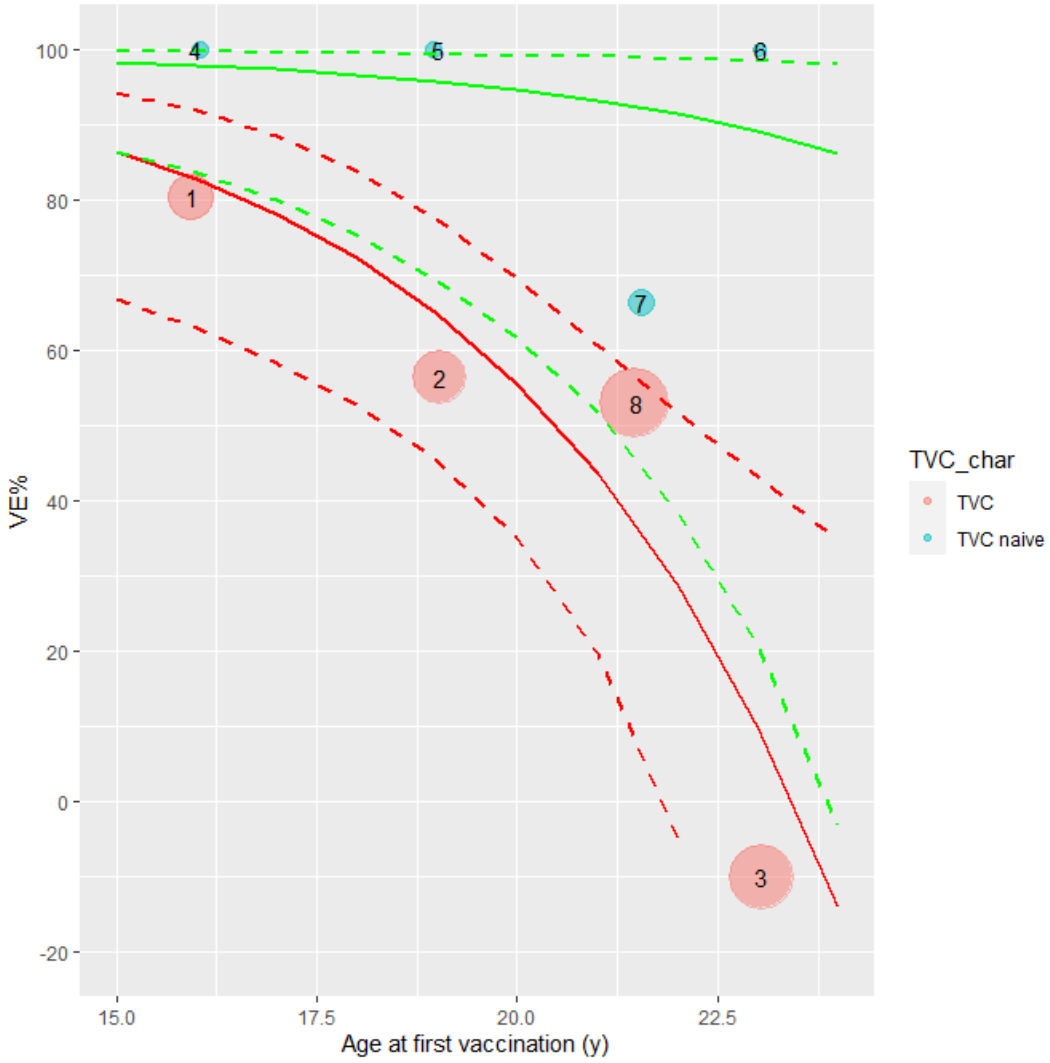
Note for interpretation of graphs:
Lehtinen 1= Lehtinen 2012, age at first vaccination 15-17 years, TVC, time since vaccination 0-4 years.
Lehtinen 2= Lehtinen 2012, age at first vaccination 18-20 years, TVC, time since vaccination 0-4 years.
Lehtinen 3= Lehtinen 2012, age at first vaccination 21-25 years, TVC, time since vaccination 0-4 years.
Lehtinen 4= Lehtinen 2012, age at first vaccination 15-17 years, TVC naïve, time since vaccination 0-4 years.
Lehtinen 5= Lehtinen 2012, age at first vaccination 18-20 years, TVC naïve, time since vaccination 0-4 years.
Lehtinen 6= Lehtinen 2012, age at first vaccination 21-25 years, TVC naïve, time since vaccination 0-4 years.
Porras 7= Porras 2020, age at first vaccination 18-25 years, TVC naïve, time since vaccination 0-4 years.
Shing 8= Shing 2022, age at first vaccination 18-25 years, TVC, time since vaccination 1-4 years.
Abbreviations: VE=Vaccine efficacy, id=identity, y=years.
Reference: [Lehtinen, 2012; Porras, 2020; Shing, 2022].

3. Multiparametric meta-regression analysis

All possible combinations of predictors were evaluated and compared using AIC (datadriven approach) to find the best model, and which predictors were the most important ones. This data-driven exploration conducted to a final model that includes the "age at first vaccination" and "analytical cohort" variables. After adjusting for the analytical cohort (TVC vs. TVC naïve), "age at first vaccination" resulted as the most impactful variable on the outcome (p=0.02). The heterogeneity explained by the selected model was R^2*= 92.95%.

The following figure shows the predictions (with 95% Confidence intervals, dotted line) from this data-driven selected model adjusting for "age at first vaccination" and "analytical cohort". Red and green curves represent the predicted vaccine efficacy as a function of age for TVC and TVC naïve populations. Red and blue bubbles represent the observed vaccine efficacies of the studies with TVC and TVC naïve population, respectively. Size of the bubbles is proportional to the inverse of the variance (it corresponds to the weight in classical meta-analysis). Observed values seem to be relatively well approximated by the multiparametric model.

Figure 17 Results of the data-driven multiparametric meta-regression analysis model (Analysis 3)



9.2.4. Analysis 4: What is the effectiveness of CERVARIX on CIN3+ caused by vaccine HPV types? (Observational studies only)

This analysis studied the combined effects of follow-up studies of observational studies ([Shing, 2022], TVC; [Lehtinen, 2017], TVC; [Rebolj, 2022], TVC) of CERVARIX on CIN3+ caused by HPV 16/18 types. The rationale behind the selection of studies for this dataset was to include observational studies with outcome results on HPV 16/18 types. Shing et al was included (instead of the observational component of Porras, 2020) to align with the other observational studies that used the TVC as analytical cohort.

1. Meta-analysis*.

Pooled VE were determined at VE=78.35 (95%CI, -123.19, 97.90) (Figure 18).

*Meta-analysis is done on the log relative risk scale assuming normality. Then results are back transformed to the vaccine effects scale. Therefore, some differences may be found in 95%CI between the pooled VE provided in the datasets and those estimated in the meta-analysis.

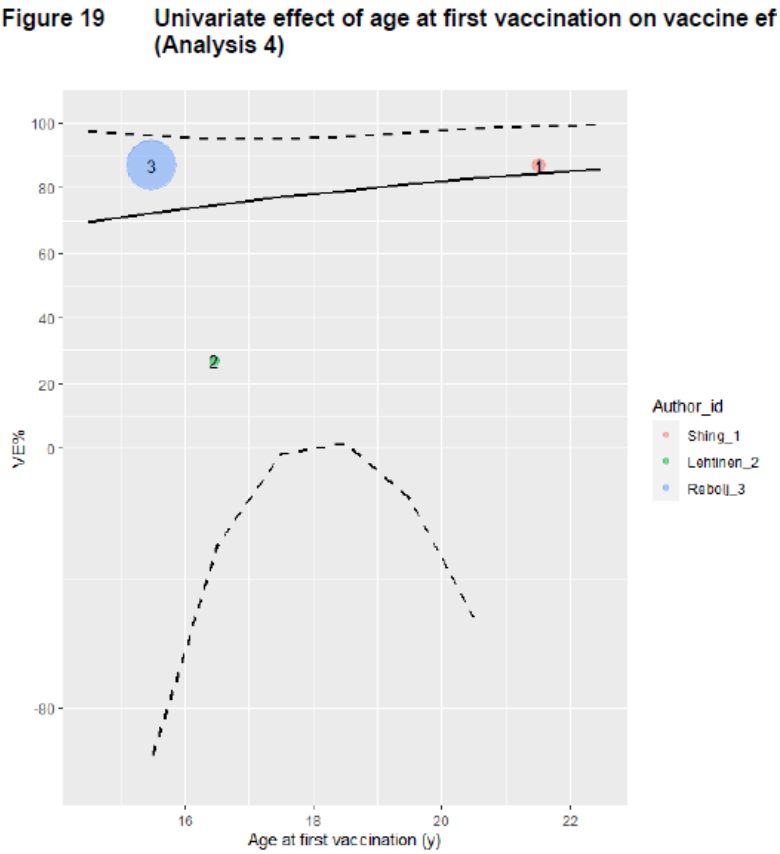
Figure 18 Pooled estimated VEs of CERVARIX on CIN3+ caused by HPV 16/18 types (Analysis 4)

The most remarkable aspect of this meta-analysis is that the lower limit of 95%CI for an individual VE estimate is negative. This is the case of the Lehtinen 2017 [Lehtinen 2] study that shows a very wide 95%CI [Lehtinen, 2017].

2. Univariate meta-regression analysis

Results from this analysis did not detect a strong univariate association between individual covariates and the outcome (VE) probably due to the small number of studies. Figures below show the observed and the predicted VE (black line with 95%CI) as a function of the univariate covariates. Size of the bubbles is proportional to the inverse of the variance (it corresponds to the weight in a classical meta-analysis).

Figure 19 Univariate effect of age at first vaccination on vaccine effectiveness (Analysis 4)



Note for interpretation of graphs:
Shing 1= Shing 2022, age at first vaccination 18-25 years, TVC, time since vaccination 7-11 years.

Figure 20 Univariate effect of time since vaccination (time of follow-up) on vaccine effectiveness (Analysis 4)

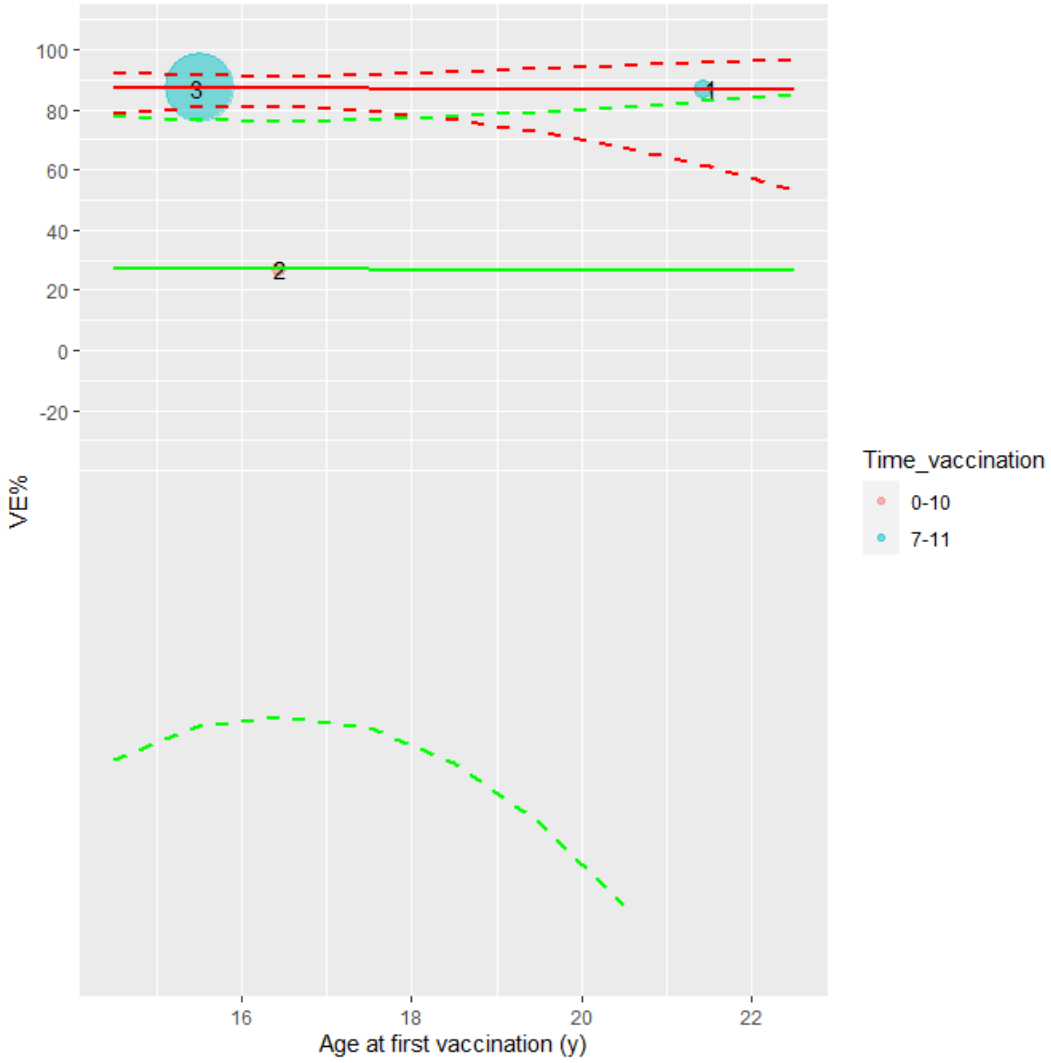
3. Multiparametric meta-regression analysis

All possible combinations of predictors were evaluated and compared using AIC (datadriven approach) to find the best model, and which predictors were the most important ones. The analysis revealed that the model including "age at first vaccination" and "time since vaccination" showed a strong correlation between the two covariates. When these two covariates are included in the model, it becomes unstable, and the variance of the random effect makes it uninterpretable. Adjusting for covariates for this specific question is not meaningful.

The heterogeneity explained by the selected model was R^2*= 100%. However, this result should be interpreted cautiously as the model was unable to properly estimate the random effect likely because of the small number of studies included in the analysis with respect to the two covariates considered.

The following figure shows the predictions (with 95% Confidence intervals, dotted line) from this data-driven selected model adjusting for "age at first vaccination" and "time since vaccination". Red and green curves represent the predicted VE as a function of age for the time since vaccination. Red and blue bubbles represent the observed VE of the studies with different time since vaccination, respectively. Size of the bubbles is proportional to the inverse of the variance (it corresponds to the weight in a classical meta-analysis).

Figure 21 Results of the data-driven multiparametric meta-regression analysis model (Analysis 4)



9.2.5. Analysis 5: What is the efficacy of CERVARIX on CIN3+ caused by any HPV type? (RCTs only)

This analysis studied the combined effects of follow-up studies of RCT ([Lehtinen, 2012], including the TVC naïve and TVC; [Konno, 2014], TVC and TVC naïve; [Shing, 2022], TVC) of CERVARIX on CIN3+ caused by any HPV type. The rationale behind the selection of studies for this dataset was to include RCTs with outcome results irrespective of the HPV types. The RCT follow-up TVC component of Shing, 2022 was included to consider the long-term follow-up of the CVT [Shing, 2022]. Since results from Konno, 2014 for the two analytical cohorts (TVC naïve and TVC) were included, the "study correlation" variable was used to account for the partial overlapping [Konno, 2014]. Overall, this approach was followed to maximize the amount of information for this analysis.

1. Meta-analysis*.

Pooled vaccine efficacy were determined at vaccine efficacy= 48.89 (95%CI, 19.84-67.41)

*Meta-analysis is done on the log relative risk scale assuming normality. Then results are back transformed to the vaccine effects scale. Therefore, some differences may be found in 95%CI between the pooled vaccine efficacy provided in the datasets and those estimated in the meta-analysis.

Figure 22 Pooled estimated vaccine efficacy of CERVARIX on CIN3+ caused by any HPV type (Analysis 5)

The most relevant feature of this Forest plot is that the lower limit of the 95%CI for some individual vaccine efficacy estimates is negative. This is especially relevant for Konno 2014 (TVC naïve cohort) (Konno 8), as already described in Section 7.8.1 [Konno, 2014]. Therefore, due to the strong weight of other studies, the combined pooled estimate

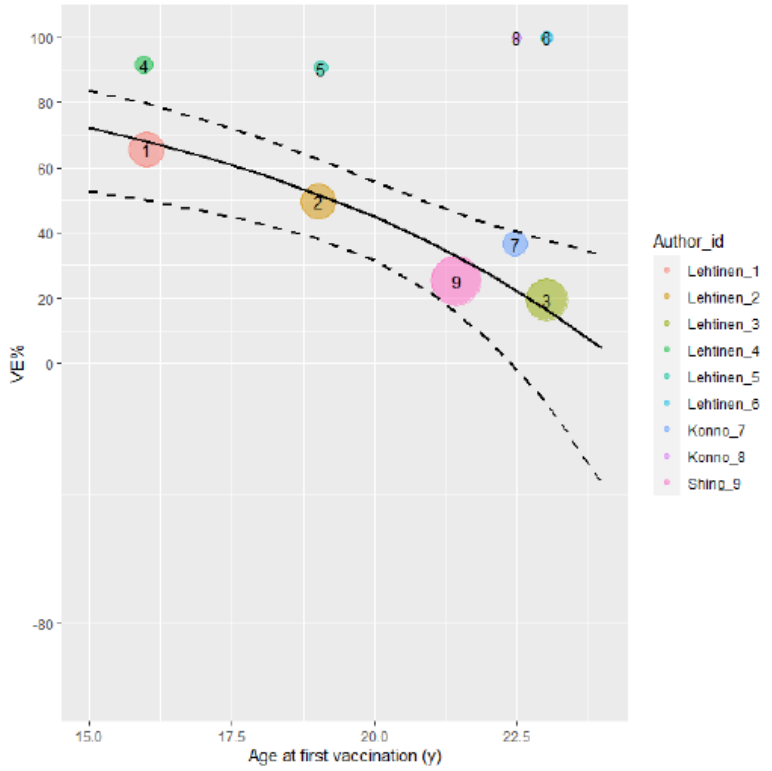
reached vaccine efficacy=48.89 (95%CI, 19.84-67.41).

2. Univariate meta-regression analysis

Results from this analysis showed that the variables "age at first vaccination" (p=0.0168), and "analytical cohort" (TVC vs. TVC naïve, p=0.0172) presented association with the outcome vaccine efficacy (small p values in the univariate meta-regression analysis model). All estimates suggests that the vaccine efficacy decreases with age at first vaccination, and is lower in the TVC population (irrespective of HPV baseline status of participants) compared to the TVC naïve (HPV negative at baseline). Figures below show the observed and the predicted vaccine efficacy (black line with 95%CI) as a function of the univariate covariates. Size of the bubbles is proportional to the inverse of the variance (it corresponds to the weight in a classical meta-analysis).

Figure 24 Univariate effect of age at first vaccination on vaccine efficacy (Analysis 5)

Figure 24 Univariate effect of age at first vaccination on vaccine efficacy (Analysis 5)



Note for interpretation of graphs:
Lehtinen 1= Lehtinen 2012, age at first vaccination 15-17 years, TVC, time since vaccination 0-4 years.
Lehtinen 2= Lehtinen 2012, age at first vaccination 18-20 years, TVC, time since vaccination 0-4 years.
Lehtinen 3= Lehtinen 2012, age at first vaccination 21-25 years, TVC, time since vaccination 0-4 years.
Lehtinen 4= Lehtinen 2012, age at first vaccination 15-17 years, TVC naïve, time since vaccination 0-4 years.
Lehtinen 5= Lehtinen 2012, age at first vaccination 18-20 years, TVC naïve, time since vaccination 0-4 years.
Lehtinen 6= Lehtinen 2012, age at first vaccination 21-25 years, TVC naïve, time since vaccination 0-4 years.
Konno 7= Konno 2014, age at first vaccination 20-25 years, TVC, time since vaccination 0-4 years.
Konno 8= Konno 2014, age at first vaccination 20-25 years, TVC naïve, time since vaccination 0-4 years.
Shing 9= Shing 2022, age at first vaccination 18-25 years, TVC, time since vaccination 1-4 years.
Abbreviations: VE=Vaccine efficacy, id=identity, y=years.
Reference: [Lehtinen, 2012; Konno, 2014; Shing, 2022].

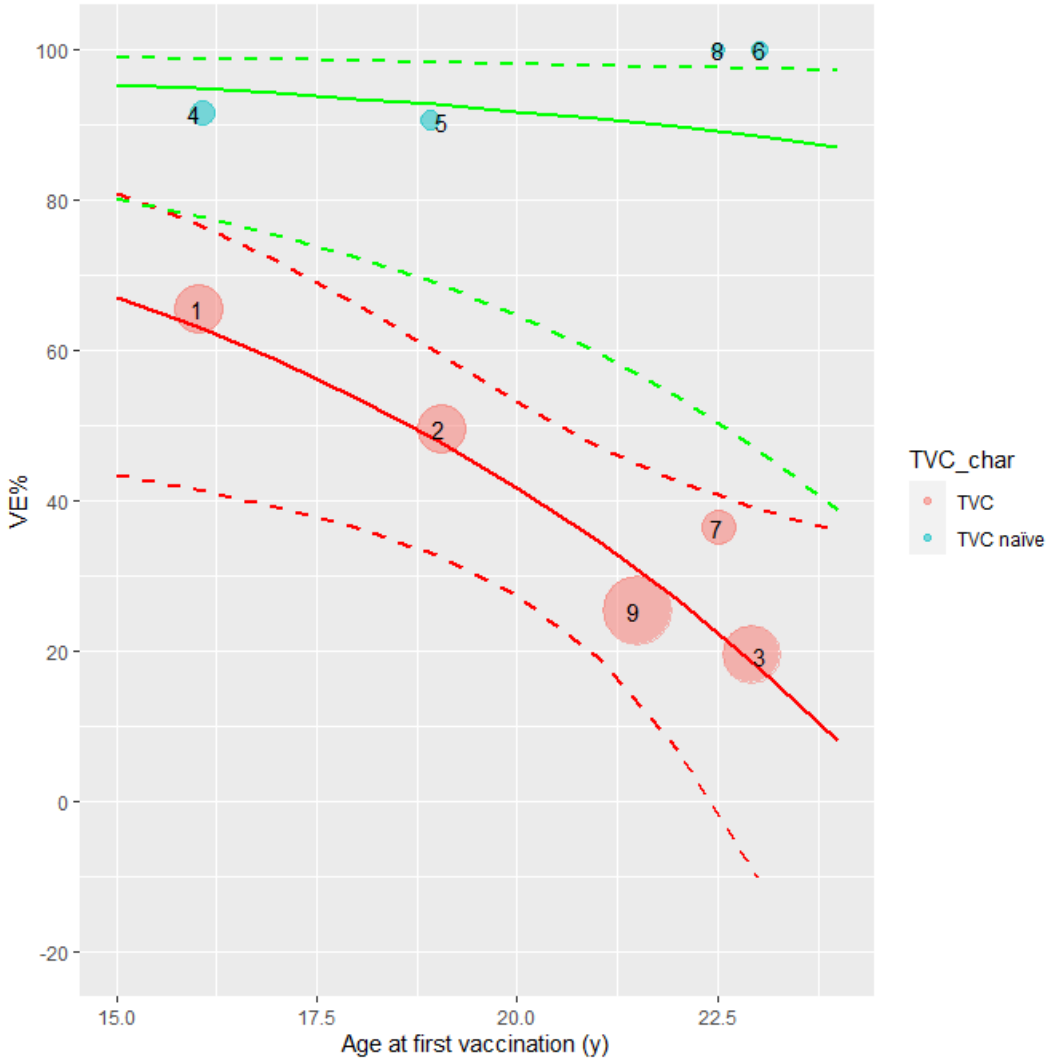
3. Multiparametric meta-regression analysis

All possible combinations of predictors were evaluated and compared using AIC (datadriven approach) to find the best model, and which predictors were the most important ones. This data-driven exploration conducted to a final model that includes the "age at first vaccination" and "analytical cohort" variables. After adjusting for the "analytical cohort", vaccine efficacy decreased with age at first vaccination.

The heterogeneity explained by the selected model was R^2*= 100%. This optimistic value is because the estimated between-trial variability is equal to "0". Therefore, the interpretation of this result should be prudent. However, as shown in Figure 25, the model is predicting the data very well. The following figure shows the predictions (with 95% Confidence intervals, dotted line) from this data-driven selected model adjusting for "age at first vaccination" and "analytical cohort". Red and green curves represent the predicted vaccine efficacy as a function of age for the time since vaccination. Red and blue bubbles represent the observed vaccine efficacies of the studies with TVC and TVC naïve population, respectively. Size of the bubbles is proportional to the inverse of the variance (it

corresponds to the weight in a classical meta-analysis). Observed values seem to be relatively well approximated by the multiparametric model.

Figure 25 Results of the data-driven multiparametric meta-regression analysis model (Analysis 5)



9.2.6. Analysis 6. What is the effectiveness of CERVARIX on CIN3+ caused by any HPV type? (Observational studies only)

This analysis studied the combined effects of observational studies ([Shing, 2022], TVC; [Lehtinen, 2017], TVC; [Palmer, 2019], TVC; [Rebolj, 2022], TVC) of CERVARIX on CIN3+ caused by any HPV type. The rationale behind the selection of studies for this dataset was to include observational studies with outcome results irrespective of the causing HPV type. The long-term follow-up TVC component of Shing, 2022 was included to consider the long-term follow-up of the CVT. Overall, this approach was followed to maximize the amount of information for this analysis.

1. Meta-analysis*.

Pooled VE were determined at VE= 65.45 (95%CI, 42.02-79.41)

*Meta-analysis is done on the log relative risk scale assuming normality. Then results are back transformed to the VE scale. Therefore, some differences may be found in 95%CI between the pooled VE provided in the datasets and those estimated in the meta-analysis.

Figure 26 Pooled estimated VEs of CERVARIX on CIN3+ caused by any HPV type (Analysis 6)

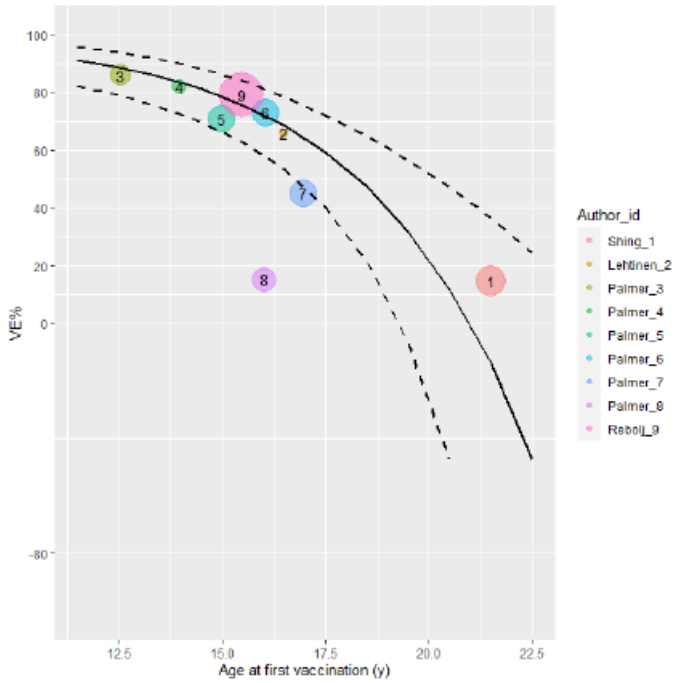
The most relevant feature of this Forest plot is that the lower limit of the 95%CI for some individual VE estimates is negative. This is especially relevant for Palmer 8 ([Palmer, 2019], age at first vaccination ≥18 years). As wide confidence intervals are not given much weight in the model the corresponding studies may not largely contribute to the pooled effect. Therefore, due to the strong contribution of large studies such as Palmer (for the younger age groups) and Rebolj [Palmer, 2019; Rebolj, 2022], the combined pooled estimate reached VE=65.45 (95%CI, 42.02-79.41).

2. Univariate meta-regression analysis

Results from this analysis showed that the variable "age at first vaccination" (p=0.0018), presented association with the outcome VE (i.e., small p values in the univariate metaregression model). The estimate is positive, suggesting that the VE decreases as age at first vaccination increases. However, "time since vaccination" (time of follow-up) is not associated with VE (p=0.9273). Figures below show the observed and the predicted VE (black line with 95%CI) as a function of the univariate covariates. Size of the bubbles is proportional to the inverse of the variance (it corresponds to the weight in a classical meta-analysis).

Figure 27 Univariate effect of age at first vaccination on vaccine effectiveness (Analysis 6)

Figure 27 Univariate effect of age at first vaccination on vaccine effec
(Analysis 6)



Note for interpretation of graphs:
Shing 1= Shing 2022, age at first vaccination 18-25 years, TVC, time since vaccination 7-11 years.
Lehtinen 2= Lehtinen 2017, age at first vaccination 16-17 years, TVC, time since vaccination 0-10 years.
Palmer 3= Palmer 2019, age at first vaccination 12-13 years, TVC, time since vaccination 0-8 years.
Palmer 4= Palmer 2019, age at first vaccination 14 years, TVC, time since vaccination 0-6 years.
Palmer 5= Palmer 2019, age at first vaccination 15 years, TVC, time since vaccination 0-5 years.
Palmer 6= Palmer 2019, age at first vaccination 16 years, TVC, time since vaccination 0-4 years.
Palmer 7= Palmer 2019, age at first vaccination 17 years, TVC, time since vaccination 0-3 years.
Palmer 8= Palmer 2019, age at first vaccination ≥18 years, TVC, time since vaccination 0-2 years.
Rebolj 9= Rebolj 2022, age at first vaccination 14-17 years, TVC, time since vaccination 7-11 years.
Abbreviations: VE=Vaccine effect, id=identity, y=years.
Reference: [Lehtinen, 2017; Palmer, 2019; Shing, 2022; Rebolj, 2022].

Figure 28 Univariate effect of time since vaccination (time of follow-up) on vaccine effectiveness (Analysis 6)

3. Multiparametric meta-regression analysis

All possible combinations of predictors were evaluated and compared using AIC (datadriven approach) to find the best model, and which predictors were the most important ones. This data-driven exploration conducted to a final model that includes the "age at first vaccination" and "time since vaccination" (time of follow-up) variables. The model fitted the data well and did not present extremely high correlation between variables. After adjusting for the "time since vaccination", VE decreased with age at first vaccination.

The heterogeneity explained by the selected model was R^2*= 82.59%. The following figure shows the predictions (with 95% Confidence intervals, dotted line) from this data-driven selected model adjusting for "age at first vaccination" and "time since vaccination" for two selected values of time. Red and green curves represent the predicted VE as a function of age for the time since vaccination (the "0-2" years of "time since vaccination" corresponds to the red curve, and the "7-11" years of "time since vaccination" is depicted by the green curve). Colors of the different bubbles represent the

observed VEs of the studies with different time since vaccination. Size of the bubbles is proportional to the inverse of the variance (it corresponds to the weight in a classical meta-analysis). As observed in the graph VE decreases with age at first vaccination and it is lower among the "7-11" years of follow-up group. Even if the time of follow-up is shorter in this age group, the "0-2" years of time since vaccination group [Palmer, 2019], represents those vaccinated at older age (≥ 18 years) whereas the "7-11" years of followup group were vaccinated at a younger age (14-17 years).

Figure 29 Results of the data-driven multiparametric meta-regression analysis model (Analysis 6)