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Efficacy of a novel high-risk HPV-16/18 therapeutic vaccine in treating cervical intraepithelial neoplasia and cervical cancer in a clinical trial: A systematic review and meta-analysis

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Abstract. Human papillomavirus (HPV) therapeutic vaccine development and ongoing clinical trials have revealed viral elimination and the waning of cervical intraepithelial neoplasia (CIN) and cervical cancer. HPV therapeutic vaccines are aimed at treating advanced cancers and pre-cancerous lesions by generating antibodies and cell-mediated immunity. The efficacy of a novel high-risk HPV-16/18 therapeutic vaccine in treating CIN and cervical cancer was systematically assessed. A total of three databases from 2012 to 2022 were systematically searched. Scopus (https://www.scopus.com/home.uri), PubMed (https://pubmed.ncbi.nlm.nih.gov/), Hinari (https://portal.research4life.org/) and Google Scholar were searched. Original articles conducted in human clinical trials were included based on a predefined inclusion criterion. The Joanna Briggs Institute (JBI) Critical Appraisal tools was utilized for use in the JBI Systematic Reviews Checklist for Systematic Reviews and Research Syntheses (http://joannabriggs.org/research/critical-appraisal-tools.html) to assess the risk of bias (RoB). Moreover, five authors worked independently during evidence searching and reached a consensus on all included studies to avoid the RoB. Data were extracted and synthesized from the included studies using an Excel spread sheet. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were used to select studies and a random model effect was also used to analyze the outcome of the review [I^2=0.00%, P=0.93, confidence interval 95%(CI)]. Only eight evidences were included out of 555 studies searched. The efficacy of the novel HR-HPV 16/18 therapeutic vaccine among 377 women suffering from high-grade CIN and cervical cancer was 88.33% (333/377). Vaccination was most efficacious (33%, 126/377) and least efficacious (23%, 87/377) in complete lesion regression and partial lesion regression, respectively. However, 12% of the women progressed to cancer. Additionally, 33% (126/377) and 23% (87/377) of women demonstrated complete viral clearance and viral load reduction, respectively. Complete lesion regression and progression of lesion to cancer have the highest and lowest effect sizes: 33% (I^2=0.00%, P=0.93, 95% CI=0.60-60.67), 22% (I^2=0.00%, P=0.93, 95% CI=11.40-55.40), and respectively. The overall effect size of the clinical trial outcome is 27.67% (I^2=0.00%, P=0.93, 95% CI=14.30-41.03). The efficacy of a novel HR-HPV 16/18 therapeutic vaccine is very promising for high-grade CIN and cervical cancer therapy. Further studies are urgently required to boost the efficacy of a novel HR-HPV 16/18 therapeutic vaccine, reduce women's health burden, and improve the quality of human life.

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Introduction

There are more than 170 types of human papillomavirus (HPV). They are divided as mucotropic and cutaneotropic according to their tropism, provided that certain HPV types may be found on both sites. Mucotropics are again classified as low-risk, potential-risk and high-risk HPVs according to their oncogenic nature (1).

HPV-induced cervical cancer causes 266,000 deaths annually. Ongoing translation of HR-HPV16/18-E6/7 oncoproteins in the cervico-vaginal epithelium transforms cervico-vaginal keratinocytes (2). Among all human cancers, 5% are caused by HR-HPV types (3). Cervical cancer due to high-risk HPV (16 or 18) is responsible for up to 70% of all cervical cancer (4-6). Persistent and relapsed HPV 16 and 18 infections are the predominant risk factors for cervical cancer (7). Of course, most lesions regress due to an immune response without treatment within a period of up to 2 years. However, cervical intraepithelial neoplasia (CIN) may occur occasionally in 10-30% of persistent infections that progress to high-grade lesions and cervical cancer (8).

The expression of HPV oncoproteins E6 and E7 is a prerequisite for the progression of steady HPV infection to CIN and, finally, to cervical cancer (9). Translation of HR HPV 16/18/E6/7 oncoproteins promotes tumorigenesis. High-risk HPVs synthesize proteins that enhance changes in the cell cycle by prompting genomic instability in healthy cells, hindering programmed cell death, and favoring the creation of mitotic abnormalities and aneuploidy. Moreover, these oncogenic proteins inhibit tumor suppressor genes and modify the defense mechanism, allowing the tumor cells to be less immunogenic. This brings immune tolerance to the malignant cells and favors HPV-16 and HPV-18-mediated oncogenicity (10,11). Despite the presence of different preventive vaccines, HR-HPV 16/18 is a substantial cause of morbidity and mortality among women. Already infected women are still suffering from CIN due to the absence of a therapeutic vaccine that triggers an immune response to the established HPV infections and malignancies.

Currently, novel high-risk HPV-16/18 therapeutic vaccines are synthesized based on live vectors such as bacterial and viral, peptides, proteins, nucleic acid (DNA or RNA-based), and whole cells [dendritic cell (DC) or tumor cell-based]. The main differences between them are their basis of preparation (aforementioned), efficacy, safety or immunogenicity, stability (to adverse conditions), durability, route of administration, ability to reach the targeted site (distribution) and cost (1). The most potent HR-HPV-16/18 therapeutic vaccine is desired to induce both cellular and humoral immunity. A candidate therapeutic vaccine needs to target-HR HPV-16/18-E6/7 oncoproteins and induce effective immunity without generating autoimmunity. The efficacy of a vaccine depends on T cells activated by antigen-presenting cells (APCs). Pre-existing T-cell immunity decreases the severity of a disease. High-risk HPV16/18-E6/7 therapeutic vaccines stimulate effector killer cells and elicit memory cells. As a result, the immune system responds vigorously, preventing HPV infection and cancer relapse (12).

Relying only on prophylactic vaccines to control HPV infection will make HPV-16/18-E6/7-associated CIN and cervical cancer a continuous and stringent global health priority. Prophylactic vaccines for HPV infection cannot avoid pre-existing infection and don't elicit a cell-mediated immune response that can clear pre-existing CIN and tumors. Furthermore, their availability is limited in low- and middle-income countries. Therefore, this gap needs to be filled urgently by a novel strategy where therapeutic vaccines are promising and can clear even established infections and malignancies by eliciting a strong cellular immune response (4,13). Therapeutic vaccines are preferable to prophylactic vaccines as they avoid established infection, prevent being re-infected, neutralize subsequent infections by the same virus, and have therapeutic properties (14).

Advancing and evaluating currently available and ongoing HR-HPV 16/18 therapeutic vaccines is one of the top global health priorities (15). Hence, therapeutic vaccines with a role in hindering low-grade lesions from scaling up, controlling metastatic cancer, resolving existing lesions, and precluding the recurrence of cancer following treatment are critically and urgently required. Additionally, they need to target oncogenic HPV types and provide cross-protection functions (16,17). Women who are already infected by HR-HPV16/18-E6/7 need therapeutic (not prophylactic) services to stay alive or to be cured. Unfortunately, there is no approved therapeutic vaccine for pre-existing infections caused by HR-HPV 16/18. The worst fate is that a number of women will be exposed to HPV-16 and HPV-18-linked morbidity and mortality due to low vaccination rates and rising incidence (18). This is bad news, as it means that a large portion of the population with an established HR-HPV 16/18 infection cannot be treated by the currently available vaccines and is excluded of curative medication (19).

Questions regarding effective HR HPV16/18 therapeutic vaccines and their demand are increasing. Unfortunately, no HR HPV16/18 therapeutic vaccine is currently available to treat HR HPV-associated CIN and cancers for general use in health facilities (20). The primary reason for developing HR-HPV 16/18 therapeutic vaccines is to treat previously established HR-HPV 16/18-associated infections, prevent reinfection, and prevent subsequent infection by the same virus by inducing a strong antitumor-specific cellular response, specifically cytotoxic T lymphocytes against HR-HPV 16/18-E6/7 oncoproteins (21). These vaccines are absolutely necessary in order to reduce society's health burden and improve people's quality of life. Among the challenges of these vaccines is their efficacy level and there are clear controversies regarding the efficacy of high-risk HPV-16/18 therapeutic vaccines. The current systematic review and meta-analysis intended to add a body of knowledge to this new insight and fill the data gap.

Rationale. The present study's interest was to fill both the data and knowledge gaps regarding the novel high-risk HPV-16/18 therapeutic vaccine (not the prophylactic vaccine) in treating CIN and cervical cancer. Because these therapeutic vaccines are designed to replace prophylactic vaccines with additional functions that cannot be covered by prophylactic vaccines, such as treating already established infections. The present study focused on HPV 16 and HPV 18 because they are the most predominant causative agents for CIN and cervical cancer globally. They are imposing social, economic and quality of life crises. Therefore, curative high-risk HPV-16/18 therapeutic vaccines are urgently required.

Current updates and adequate data on the efficacy of the novel HR-HPV16/18 therapeutic vaccine associated with CIN and cervical cancer are very important to decide on health priorities, allocate resources, plan effective treatment modality, and have a common understanding. Moreover, there is a lack of published data and information on the efficacy of the novel HR HPV16/18 therapeutic vaccine associated with CIN and cervical cancer. On that regard, the present study will have critical importance in filling the available data and information gap. The present systematic review and meta-analysis will add to the body of knowledge on the efficacy of the novel HR-HPV16/18 therapeutic vaccine associated with CIN and cervical cancer. It also fills the available data gaps by reaching stakeholders, regulatory bodies and health care providers through presentations, workshops and intervention activities. Therefore, the study can serve as a source of information for those concerned with choosing the therapeutic protocols for managing and treating CIN and cervical cancer. Moreover, it will serve as a base for further studies. The present systematic review aimed to assess the efficacy of a novel high-risk HPV-16/18 therapeutic vaccine in treating CIN and cervical cancer in a clinical trial.

Data and methods

Eligibility criteria

Inclusion criteria. All original clinical trial research articles and reviews that report the efficacy of a novel high-risk HPV-16/18 therapeutic vaccine in treating CIN and cervical cancer in a clinical trial, all countries, clinical trial study design, all females with known high-grade CIN and cervical cancer, all articles written in English language, articles studied from 2012-2022, and both published and accepted articles were included.

Exclusion criteria. All scientific reports, conference papers, books, and book chapters, studies on animals, and articles that are not fully retrieved, out of scope, inappropriate, and without full text were excluded.

Information sources. Relevant literature sources were searched and consulted to identify studies using a pre-designed automated search strategy. Three major databases, Scopus (https://www.scopus.com/home.uri), PubMed (https://pubmed.ncbi.nlm.nih.gov/), Hinari (https://portal.research4life.org/) and the search engine Google Scholar were used. All information sources were searched and consulted for the last time on December 30, 2022.

Search strategy. The primary focus of the search was drawing on all available literature to meet the objective of the review. First, to avoid duplications, databases were searched for the same systematic review and meta-analysis, and to the best of the authors' knowledge it can be confirmed that no review or meta-analysis was conducted similar to this topic. Study and review articles were searched to assess the efficacy of novel HR-HPV 16/18 therapeutic vaccines against CIN and patients with cervical cancer participating in clinical trials. HPV or 'Human papillomavirus' and 'Therapeutic vaccine' were the search terms used for all databases and Google Scholar. The existence of Medical Subject Headings (MeSH) terms and key words was checked in all databases used and Google Scholar prior to implementing the search. Boolean operators (AND, OR) were used to combine search terms. The fields searched are: Vaccinology, Human Vaccines, Immunotherapy, Cancer Immunology, Cancer Vaccines, Cancer Immunotherapy and Advanced Therapeutics. All searches were limited and filtered from 2012 to 2022. No country was excluded from the search. The search strategy for joining MeSH terms and free text words that were implemented in the databases and Google Scholar, with full filters and limits used, is demonstrated in Table SI.

Selection process. Each of the selected studies was screened and reviewed independently to decide whether a study met the inclusion criteria of a review by seven reviewers (ZH, AA, HB, BS, ST, YA and BM) using a standardized data extraction Excel spread sheet (described in the data collection process section). Each of the studies was independently retrieved by the aforementioned authors regarding the eligibility criteria.

Data collection process. After the aforementioned seven authors/reviewers screened the studies for eligibility independently, the authors decided to work independently to extract the data. The authors screened each piece of literature for title, abstract and full text. After reading the full texts independently, the authors extracted data by using a pre-designed standardized data extraction Excel spread sheet. The data extraction format included the primary author, study year, publication year, the country where the study was conducted, sample size, study design and study type, vaccine type and name, sample size, efficacy of vaccines' (partial regression, lesion progression, stable lesion, viral clearance, viral load reduction, and viral clearance), mean and standard error.

Data items. The present systematic review and meta-analysis had the following outcomes: Partial lesion regression, lesion progression, stable lesion, complete lesion regression, viral load reduction and viral clearance. Data items were sought based on the efficacy of the HR-HPV 16/18 therapeutic vaccines. The efficacy of the therapeutic HR-HPV 16/18 vaccine is defined as the potential of the vaccine to regress a tumor and/or lesion, stable disease (stop progression), or revert an established infection to a healthy state. It may result in viral clearance or viral load reduction from the tumor and/or lesion. All of the included studies' findings confirmed that all human clinical trials resulted in a virus-free normal state. All the data items were taken from patients with CIN of different stages and cervical cancer that were enrolled to take different types of HR-HPV 16/18 therapeutic vaccines. No funding was allocated throughout the development of this systematic review.

Study risk of bias assessment (RoB). Risk-of-bias assessments for each of the studies were performed independently by six authors (ZH, LG, DA, BA, WY and MB) to avoid errors in assessments and ensure that the judgment is not influenced by preconceptions of a single individual. Disagreements between authors were resolved through discussion. When independent authors failed to resolve a disagreement, another author (TD) adjudicated the final judgment. The Joanna Briggs Institute (JBI) Critical Appraisal Tools was used for Systematic Reviews Checklist for Systematic Reviews and Research Syntheses. (http://joannabriggs.org/research/critical-appraisal-tools.html). The present study was considered low-risk (favorable quality) because it scored five or more points in all quality assessment items.

Synthesis methods. A total of eight articles were extracted that were included in the data extraction phase. Permanent information was extracted from all of the included articles. An Excel spread sheet was used to synthesize reputable data that was directly related to the objective of the research question. A total of seven reviewers collected data from each report. All reviewers worked independently. The data were presented or synthesized in the form of tables and narratives. The results of individual studies were synthesized and evaluated regarding the efficacy of the therapeutic HR HPV 16/18 vaccines (tumor/lesion partial regression, tumor/lesion progression, tumor/lesion stability, complete tumor/lesion regression to a normal state, viral clearance and viral load reduction). All the data items were synthesized from women suffering from CIN and cervical cancer who took different types of HR-HPV 16/18 therapeutic vaccines. The results of individual studies are synthesized based on the efficacy of HR-HPV 16/18 therapeutic vaccines and the types of clinical outcomes.

Statistical analysis. The results of individual studies are presented by summarizing the key findings, including the main outcome (the efficacy of a novel high-risk HPV-16/18 therapeutic vaccine in treating CIN and cervical cancer), measures of effect, confidence intervals (CIs), and the overall P-value. The results of each study with summary statistics for each clinical trial and the effect estimates with their precision, along with their efficacy, weight, and overall effect size at 95% CI, are shown by using a Forest plot. The results of the individual studies show the magnitude and direction of the efficacy of a novel high-risk HPV-16/18 therapeutic vaccine in treating CIN and cervical cancer. Heterogeneity analysis for the efficacy of a novel high-risk HPV-16/18 therapeutic vaccine in treating CIN and cervical cancer was performed by using Galbraith plot. Overall, in the plot, points did not cluster around the central line, indicating that the studies have effect sizes that are inconsistent with the overall effect, indicating the presence of heterogeneity and outliers. The steepness of the slope reflects the magnitude of the overall effect. Additionally, points located in reference to the central line indicate the weight of the sample sizes. The slope of the Galbraith plot shows the overall effect size, which is generated from a weighted regression of the effect sizes and their precisions. At 95% CI, outliers are identified by confidence limits that are bare-lined parallel to the slope at ±2 standard errors. Subgroup analysis was used to understand the efficacy of a novel high-risk HPV-16/18 therapeutic vaccine in treating CIN and cervical cancer. This analysis was conducted to explore variations in treatment effects and to identify specific population sizes per year of publication. The criteria to group studies together was year of publication. The treatment effects were estimated within each subgroup via each study sample size.

Reporting biases due to publication bias, selective outcome reporting bias, location bias, language bias, time-lag bias, and citation bias were acknowledged and addressed to provide more accurate and reliable estimates of treatment effects. These biases were addressed through comprehensive search strategies and an assessment of publication bias (funnel plots). The funnel plot was analyzed and interpreted to assess the publication bias and to visualize the distribution of the studies included in this review. Its visual inspection is asymmetrical, indicating the presence of significant publication bias or small study effects. Larger and more precise studies are located at the top and close to the pooled estimate, whereas the bottom of the funnel indicates smaller and less precise studies scattered more widely at the bottom. Assessment of the risk of bias due to missing results (reporting biases) was challenging due to the limited availability of study protocols and difficulty in obtaining unpublished data. This is asymmetrical inverted funnel, indicating that larger studies (with smaller standard errors) are scattered around the true effect size while smaller studies (with larger standard errors) are scattered more widely. The plot generally shows the presence of significant publication bias, high small-study effects, unbalanced contribution of studies of different sizes, and high heterogeneity among the included studies. In an ideal situation with no publication bias, the plot should resemble a symmetrical inverted funnel. This is because smaller studies (with larger standard errors) would scatter more widely, while larger studies (with smaller standard errors) would cluster around the true effect size. Heterogeneity test (I2) and confidence intervals were calculated by importing Excel extracted (delimited csv file) into a STAT software package version 17.

Results

Study selection. A total of 555 documents were recovered from the first bibliographical search. A total of 531 articles were obtained after removing duplicates. No additional studies were taken from other sources. There was no study excluded because of the language criteria or location of the study. After removing 434 unrelated records, a cautious analysis of 111 studies was performed. Specifically, studies on animal models, conference studies, scientific studies, book chapters and books were excluded. At the end, further selection, as aforementioned, delivered a total of eight studies stating the efficacy of a novel high-risk HPV16/18-E6/7 therapeutic vaccine in treating CIN in a clinical trial. The study selection flow chart diagram is summarized in Fig. 1.

Novel therapeutic vaccine development strategies targeting HR-HPV 16/18-E6/7 oncoproteins associated with CIN and cervical cancer. Strategies of developing novel therapeutic vaccine targeting HR-HPV16/18-E6/7 oncoproteins are already established and found at different stages. There are several HR-HPV16/18 therapeutic vaccines that are completely formulated and in progress of formulation. Completely formulated HR-HPV16/18 therapeutic vaccines targeting HR-HPV16/18-E6/7 oncoproteins are found at different clinical trial phases. Strategies of developing novel therapeutic vaccine targeting HR-HPV16/18-E6/7 oncoproteins are based on cells, peptides, proteins, nucleic acids and live vectors. These strategies are preferred over others because they are easy to produce in high purity, stability at any temperature, safety, cost-effective, and easy to distribute (4). These vaccines are designed to consist of E-6 and E-7 antigens in different forms with the goal of promoting the delivery of these antigens to APCs. Delivery of these antigens to APCS initiates antigen presentation via MHC classes I and II, causing the production of $CD8^+$ cytotoxic T cells and $CD4^+$ helper T cell responses, respectively. The strategy is as follows: Proteasomes secreted by APCs digest and then convert E6 and E7 oncoproteins into smaller peptides. These peptides will then be presented to APC MHC I to stimulate $CD8^+$ responses. Surprisingly, only peptides with appropriate epitopes (not all peptides) will be effectively loaded onto the MHC fragment and accepted by antigen-specific T cells (22). An immune response will be elicited when the antigenic peptide contains a specific sequence of antigenic epitopes that allow its binding to the MHC class I receptor with strong affinity and should bind with the T cell receptor of antigen-specific T cells; that is, only some of the digested peptides have specific sequences of antigenic fragments (23).

HR-HPV 16/18 therapeutic vaccine based on a live vector. Bacteria or viruses capable of expressing and spreading HR HPV16/18-E6/7 antigens in the human body are used to prepare and administer HR HPV16/18 therapeutic vaccines. Use of live vectors to produce HR HPV16/18 therapeutic vaccines provided high immunogenicity. However, immunosuppression, difficulties with re-immunization due to the production of neutralizing antibodies specific to the vectors, and the existence of other

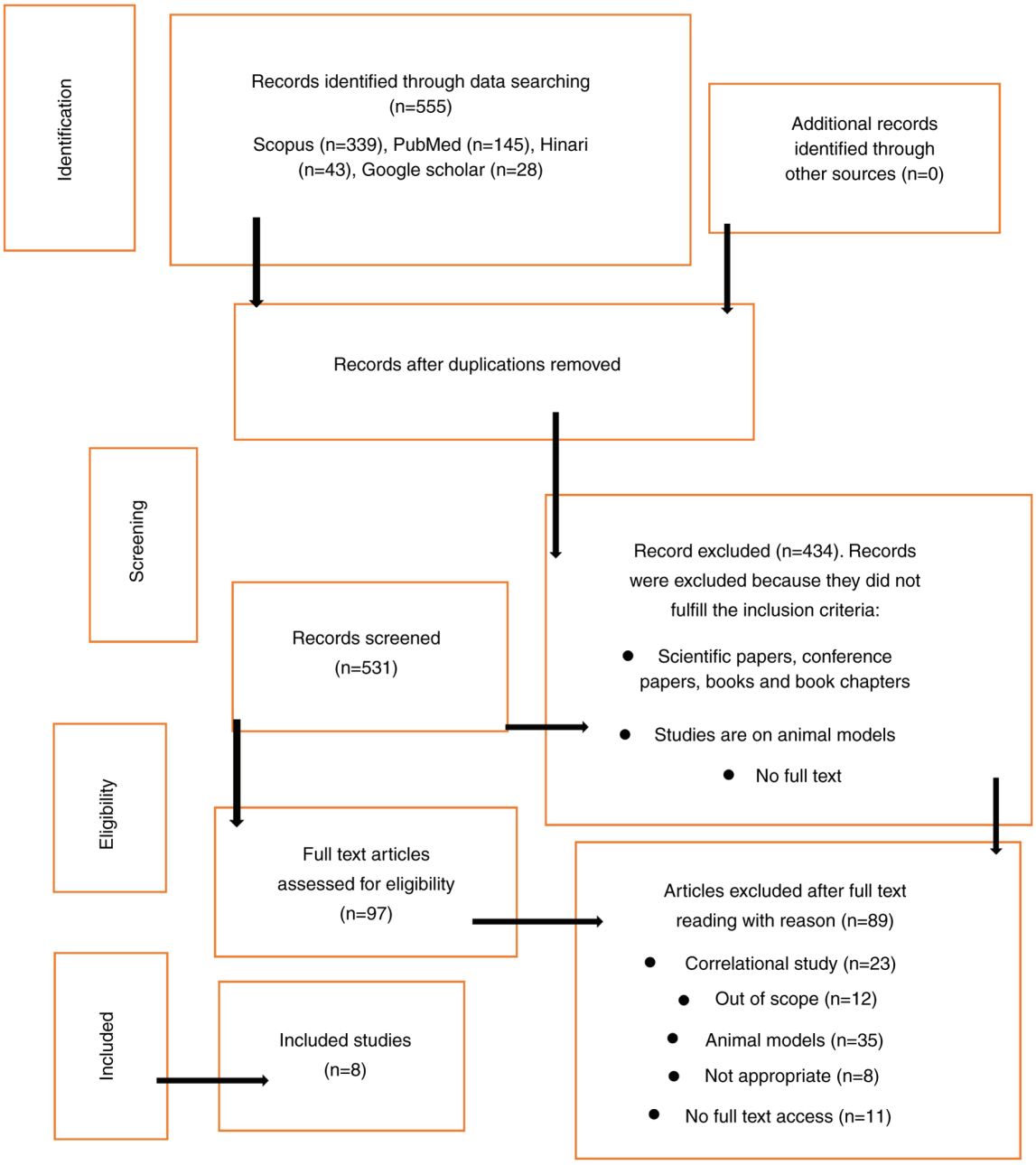

Figure 1. Flow chart diagram of study selection.

already established vector-specific immunity should be considered before implementing this strategy (24). Therefore, solving these drawbacks promotes the reliability and efficacy of live vector HR-HPV16/18 therapeutic vaccine therapy.

Bacterial vaccines. Numerous bacteria are utilized to develop HPV-16 and HPV-18 therapeutic vaccines. Among those commonly used are: *Lactobacillus lactis*, (25) *Listeria monocytogenes* (12) and *Lactobacillus casei* (26). *Listeria* is very promising live bacterial vector to produce HR-HPV16/18 therapeutic vaccine because of its natural talent to infect macrophages and release listeriolysin O (LLO). The strategy of using this bacterium to produce HR-HPV16/18 therapeutic vaccine is dependent to its natural talent and the toxin it secrets. First, the *Listeria* will be infected by HR-HPV 16/18. Then it will be allowed to infect macrophages. Of course, the bacterium is naturally talented to infect macrophages and secrets the LLO that creates a pore in the phagosome's sac. This toxin degrades the phagosome and helps the bacterium escape phagosomal lysis, which in turn enables the bacterium to replicate inside the cytoplasm, where the viral antigen is simultaneously expressed. Hence, endosomal compartments of the bacterium and the E6 and E7 peptides will be presented via MHC I as well as MHC II molecules to cytotoxic $CD8^+$ T cells and $CD4^+$ T cells, respectively (27).

The other strategy of using bacterial vector for HR HPV16/18 therapeutic vaccine is bacterial attenuation. Mutants of *Salmonella, Shigella and E. coli* are examples. First, the bacterial vector will be attenuated. Plasmids carrying E6/7 antigens will be introduced to bacterial mutants and bacterial mutants

transform genes contained in the plasmid (E6/7) to APCs. For example, *Salmonella* as a carrier delivered HPV16-E7 generated E7-specific responses, where its normal course of infection provided an advantage for oral immunization (28,29).

Viral vectors. Another strategy of developing HR-HPV16/18 therapeutic vaccine is use of viral vectors. Viral vectors are very interesting for the development of HR-HPV16/18 therapeutic vaccine because of their capacity to express encoded E6/7 antigens and successfully infect cells. Adeno-associated viruses (30), vaccinia virus (31), adenoviruses (32) and alphaviruses (33) are pronouncedly used viral vectors. Indeed, a number of viruses can deliver HR-HPV 16/18 and E6/7 antigens to APCs. The use of viral vectors as a strategy to develop a novel HR-HPV therapeutic vaccine development considers the following viral characteristics: i) A large genome; ii) naturally infectious; and iii) integration of foreign DNA into its genome is aberrantly less likely. For example, these characteristics make vaccinia virus a very promising viral vector for the development of HR HPV therapeutic vaccine (34). Initially, E7 will be allowed to fuse to a multifunctional protein in the endoplasmic reticulum calreticulin (CRT), a protein that stimulates antigenic-peptide presentation via MHC I and reduces the risk of target cell transformation. Then it will be injected into vaccinia and the virus will encode adequate amount of E7. This results in a vigorous cellular immune response (35). Additionally, vaccinia vectors can be modified to express E7 fused to LLO and E7 linked to sorting signals and lysosomal-associated membrane protein (SigE7-LAMP-1) (36). HR-HPV therapeutic vaccine produced by using SigE7-LAMP-1-encoding vaccinia virus as a vector expresses a human Fms-like tyrosine kinase-3 ligand. This vaccinia-based vaccine exhibited protective antitumor effects by enhancing antigen-specific $CD8^+$ T cell responses (37).

Another strategy of developing HR HPV therapeutic vaccine is use of lentiviruses. Lentiviruses can transduce different cell lines, such as malignant cells plus DC. Lentiviruses-based HR-HPV therapeutic vaccine can be formulated by fusing a changed form of HPV16-E7 antigen with CRT. The virus will be made to loss its integrase. Finally, changed form of HPV16-E7 antigen- IDLV complex will be administered. While applying live viral vector as a strategy of HR HPV vaccine development, the following shall be considered: the generation of antiviral immune responses during primary exposure to the vaccine and the presence of neutralizing antibodies upon primary exposure to the vaccine. Of course, administering cyclooxygenase-2 inhibitors like celecoxib can preclude the generation of neutralizing antibodies to vaccinia. This reduces frequent delivery of the vaccine from dropping infectivity (38). Indeed, the pathogenic potential of viral vectors is a huge safety alarm for immunosenescent and immunosuppressed individuals (39) implying innovative strategy of live virus vector-based HR-HPV vaccine development.

Therapeutic vaccines based on peptides and proteins. Peptides and proteins are focus areas of the strategies of a novel HR-HPV 16/18 therapeutic vaccines development. This strategy is based on peptides and proteins derived from HPV early proteins, E6 and E7. APCs process and present them to MHC I and MHC II molecules, where $CD8^+$ and $CD4^+$ T-cell immune responses will be stimulated, respectively (24,40).

A therapeutic vaccine based on peptide. This strategy of developing therapeutic vaccines is based on peptides. Since peptides are naturally poorly immunogenic, their potency is boosted by fusing peptides with adjuvants and lipids, including Toll-like receptor (TLR) ligands, cytokines and chemokines (40). These tactics boost the capacity of the specific effect of vaccines. For instance, it increases $CD8^+$ T cell responses and non-specific immunity like, cytokines. One of the challenges of a novel HR HPV 16/18, therapeutic vaccine development based on peptides is failure of small peptides to fit into the epitope required to elicit an operative immune response (41). Interestingly, peptide-based therapeutic vaccines that involve ionic peptides are MHC-specific. Therefore, CTL receptors can specifically recognize and identify all individuals' immunogenic peptides of E6 and E7 (42). Thus, these vaccines are ideal for all-encompassing treatment. One of the strategies of improving peptide-based vaccines' potential is using overlapping long peptide vaccines. Vaccines containing overlapping long peptides are effective in prompting antigen-specific T cell responses in numerous preclinical prototypes (40,42). Peptide-based HR HPV 16/18 therapeutic vaccines are easy to produce, safe and stable (40).

Therapeutic vaccines based on proteins. The other strategy of developing a novel HR-HPV 16/18 therapeutic vaccine targeting E6/7 oncoproteins is producing protein-based vaccines. These vaccines are very important because they are made to comprehensively carry the entire human leukocyte antigenic epitopes. These occasions evade lack of MHC restrictions which is not the reality in peptide-based vaccines. Challenges of this strategy of HR-HPV 16/18 therapeutic vaccine targeting E6/7 oncoproteins are proteins' weak antigenic nature and their failure to induce CTL. They elicit antibody because they are naturally exogeneous and mostly presented via MHC class II. To overcome this challenge, immuno-stimulant molecules and adjuvants are added to boost the endogenous processing of protein-based vaccines. This tactic promotes proteins' presentation by MHC class I. Hence, there will be an increment in MHC I presentation and $CD8^+$ activation because adjuvants and immuno-stimulant molecules increasing the uptake of protein by MHC class I. They are easy to produce and safe (40,42).

Therapeutic vaccines based on nucleic acid

Deoxyribonucleic acid-based vaccine. The other evolving, potentially effective and eye-catching strategy of HR-HPV 16/18 therapeutic vaccine development is the use of DNA. This strategy is attractive because DNA-based HR-HPV 16/18 therapeutic vaccines are stable, safe and easy to produce. Moreover, they are capable of enduring expression of E6/7 antigens in cells' DNA for a longer duration than protein or RNA-based vaccines. The implementation of this strategy is by introducing a plasmid DNA that encodes HPV-E6/7 antigen into the host cell (24). High risk HPV 16/18 therapeutic vaccine development strategy based on DNA may promote the expression of HPV E6 and E7 oncogenes causing cellular transformation. However, this can be alleviated by modifying the DNA that expresses E6 and E7. This will result in protein expression without the ability to transform cells (43). Production of neutralizing antibodies against a plasmid injected with E6/7 antigens is absent in DNA-based HR HPV 16/18 therapeutic vaccines. Therefore, these vaccines can be administered repeatedly (24).

The strategy of developing a novel DNA-based HR-HPV 16/18 therapeutic vaccine is by injecting HR HPV 16/18-E6/7 oncoproteins into host cell DNA. Functional mechanism of a novel DNA-based HR HPV 16/18 therapeutic vaccine is as follows: The injected DNA will be administered through intravascular injection followed by the uptake of the injected DNA by myocytes. Then DNA-transfected myocytes will express E6/7 antigens. However, myocytes cannot adequately trigger robust immune responses as it lack proficient APCs (44). Fortunately, There are two mechanisms through which DCs present antigens to naïve $CD8^+$ cytotoxic T cells (45). The first mechanism happens when transfected myocytes release exogenous antigens followed by phagocytosis of these antigens by DCs. Then, DCs will process the antigens continuously and present them on the MHC I molecule (46). Secondly, the target antigen may be directly administered to DCs. Then, they undergo endogenous expression of the target antigen and directly present it to $CD8^+$ T cells (47). The efficacy of this strategy of developing a novel HR HPV16/18 therapeutic vaccine can be maximized by i) increasing the number of DCs that express E6/7 antigens; ii) enhancing DC antigen processing and presentation; and iii) improving the communication between T cells and DCs. The disadvantage of DNA vaccines is their low inherent immunogenicity (because they are unable to multiply and transmit from transfected cells to neighboring healthy cells when compared with vaccines based on live cells) (48).

Ribonucleic acid-based vaccine. The other nucleic acid-based novel HR HPV 16/18E6/7 therapeutic vaccine development strategy is RNA-based vaccine. Naked RNA replicons of RNA viruses are predominantly important to synthesize an RNA-based novel HR-HPV 16/18 therapeutic vaccine. Venezuelan Equine Encephalitis virus (33), SVF (49) and Sindbis virus (50) are the most frequently used viruses for the strategy. The strategy of a novel HR-HPV 16/18E6/7 therapeutic vaccine development based on RNA is dependent on the ability of an RNA replicon vaccine to self-replicate (ensuing in continuous antigen expression and enhanced immunogenicity). Repeated administration of the vaccine is possible because there is no generation of neutralizing antibodies (genes that are involved in constructing viral structure are omitted from the RNA replicon vector so that viral particles do not form). Furthermore, the strategy reduces the threat of chromosomal integration and cellular growth.

Suicidal DNA (created by merging DNA vaccines and RNA replicons) is another strategy of developing a novel HR-HPV 16/18-E6/7 therapeutic vaccine. Cells will be injected by suicidal DNA. Its mechanism of action is by activating cellular apoptosis. In fact, suicide DNA for a HR HPV 16/18-E6/7 therapeutic vaccine produces HR-HPV 16/18-E6/7 antigen-specific $CD8^+$ T cell responses and anti-cellular growth effects (35). This strategy presents a poor immunogenicity since 'suicidal DNA' triggers the apoptosis of transfected cells. Integrating suicidal DNA vectors with genes encoding anti-apoptotic proteins increases the survival of transfected APCs. This evades the limitations of the aforementioned RNA replicons vaccine.

The other tactic to evade apoptosis of APCs is using the flavivirus Kunjin, a vector that delivers a naked RNA replicon because Kunjin does not induce apoptosis in transfected cells. Hence, it allows transfected DCs to directly present antigen compared with the 'suicidal DNA' vector (51). Continual clinical trials are ongoing to improve and validate the efficacy of RNA vaccines. However, RNA vaccines targeting HR HPV antigens and HPV-associated diseases have not been demonstrated in clinical settings yet.

Therapeutic vaccines based on whole-cell-based

A vaccine based on DC. The strategy of developing a novel HR HPV 16/18 DC-based therapeutic vaccines comprise DNA or RNA of transfecting DCs that express HR HPV-E6/7 oncoproteins (52-54). The strategy of using HR HPV 16/18 DC-based therapeutic vaccines takes DCs' natural function as an adjuvant as an advantage. Strategies are designed to avoid apoptosis of DCs due to T-cell mediation because DCs' life span is an important factor for vaccines based on DCs. Therefore, a new strategy is required to improve DCs' capacity to prime T cells. For example, when DCs are transfected with small interfering RNAs (siRNAs) that target pro-apoptotic molecules such as Bim, Bak, Bax and Bid proteins, they boost activation of antigen-specific $CD8^+$ T cell activation, which results in antitumor effects (55,56). This strategy applies siRNA cocktails to target immunosuppressive factors such as TGF-β and IL-10 to improve the efficacy of a DC-based vaccine. SiRNA cocktails provide vigorous antitumor effects against an immune resistant tumor cell that expresses HR-HPV16-E7 (57). DC-based vaccines have the following restrictions: i) It is technically difficult to use autologous DCs for each individual therapy. Hence, it is very challenging for large-scale production for the universal population; ii) the presence of different culturing techniques to prepare DC results in the variable quality of vaccines and the absence of standards to evaluate vaccines; iii) identifying the most appropriate route through which DC vaccines are administered. It is very important because DCs should enter lymphoid organs to generate the most effective cellular response (13).

Therapeutic vaccines based on tumor cells. This strategy of developing a novel HR-HPV 16/17 therapeutic vaccine is based on tumor cells. Firstly, tumor cells express immunomodulatory proteins that boost their immunogenic nature *in vivo* after they are isolated and operated on *ex vivo*. A tumor cell-based vaccine for HR HPV in combination with granulocyte macrophage colony stimulating factor and the cytokine genes IL-2 and IL-12 prompts transformed cells to stimulate the differentiation of naive T cells into helper T or effector cells and the generation of granulocytes by stem cells (58,59). Therapeutic vaccines based on tumor cells were clinically tried for renal cell carcinoma, melanoma, colorectal carcinoma and pancreatic cancer. However, no demonstrations regarding HPV-linked tumors have been conducted yet. Their merit is their capacity to cover more tumor genes without the need to absolutely identify tumor antigens. Basically, this has no importance for HPV-related cervical cancers, as HPV has well-known tumor-specific antigens. Their disadvantage is the possibility that they will cause new cancers in patients (13). Complications that occur during tumor cell-based vaccines vaccination, their purity and potency should be independently tailored. Generally, therapeutic tumor-based vaccines are significantly more expensive to prepare and time consuming. Due to these reasons, they have not yet been tested and utilized in clinical trials.

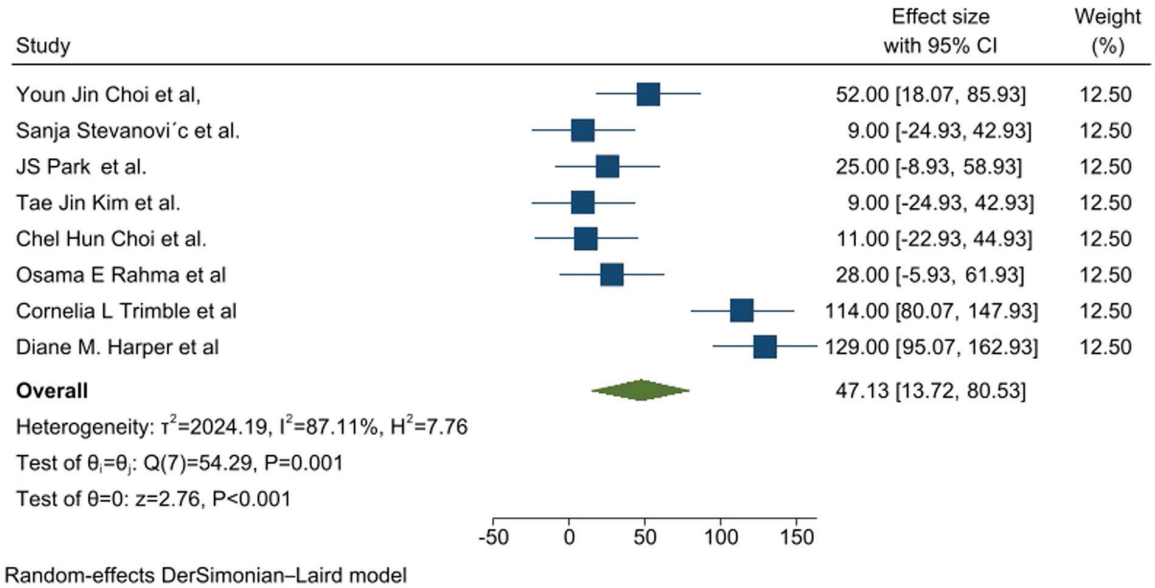

Figure 2. Effect estimates and precision (CI) of included studies. CI, confidence interval.

Immunogenicity of novel HR-HPV 16/18 therapeutic vaccines associated with CIN and cervical cancer. The therapeutic HPV16/18 vaccine is targeted to construct a robust immune system capable of inducing HPV clearance either by eliminating cells that are chronically infected or by circumventing the transmission of malignancy over the infected tissue. There are some novel therapeutic vaccine strategies that have a high potential to increase Th1 or cytotoxic cell activity. Other strategies are focused on preventing HR-HPV 16/18 oncoprotein activities, impairment of regulatory $CD4^+$, $Foxp3^+$ T cells (Treg) and $CD25^+$, and hindrance of deflection in cytokine secretion towards the Th2 profile. Mechanisms of the innate immune response are essential for prompting a specific immune response and precluding chronic infection and the development of cancer. However, HPV can circumvent these mechanisms and establish cellular transformation in the host (60-62). Studies regarding the HPV-16/18 therapeutic vaccine are mainly focused on the importance of neighboring cells to an HPV-related tumor and the entire environment surrounding it, highlighting stromal cells (for example fibroblasts), chemokines and cytokines that they harvest. Stromal cells' nature is one of the factors for HPV-related abnormal cell growth because of their robust communication with immune cells, epithelial cells, or tumor cells in the tumor microenvironment (63). Primary immune cells are currently the choice of therapeutic vaccine development strategy because they secrete cytokines, stimulate activation or differentiation of T cells and eradicate cells infected by HR-HPV (64,65). Other strategic options to develop a potent immunogenic therapeutic vaccine are focused on inflammation processes, macrophages and the use of innate responses as adjuvants. An effective HR-HPV therapeutic vaccine should be adequately immunogenic and need to comprise one or more of the following tools to prevent and treat HR-HPV-related cancer. These tools are: Cytokines, an anti-programmed death-1 receptor, anti-T cell immunoglobulin mucin 3, TLRs, ligands, an anti-PD-1 ligand and antibody-mediated blockade, such as anti-IL-10 anti-anti-CTL-related antigen 4. Interestingly, the aforementioned cited vaccines have a synergistic effect on each other and other vaccines, thereby increasing their immunogenicity and efficacy in cancer treatment. However, immune surveillance activities and mechanisms for using them as weapons against HPV-16 and HPV-18 oncogenic activities are not fully known (66,67). Immunogenicity is the main indicator of the therapeutic vaccine's potential to elicit an HPV-16/18 and E6/7-specific $CD8^+$ T cell response. The immunogenicity of different therapeutic vaccine strategies at different phases of human clinical trials is demonstrated in Table I.

Outcomes of the study. The main outcomes observed in almost all enrolled patients were partial tumor/lesion regression, tumor/lesion progression, stable disease formation and complete tumor/lesion regression to a normal state. The efficacy of the novel HR-HPV 16/18 therapeutic vaccine among 377 women suffering from high-grade CIN and cervical cancer was 88.33% (333/377). Of the total number of women enrolled in the included studies, 33% (126/377), 32% (120/377), 23% (87/377), and 12% (44/377) demonstrated complete lesion regression, stable disease, partial lesion regression and lesion progression of CIN and cervical cancer, respectively. Additionally, 33% (126/377) and 12% (44/377) of women demonstrated complete viral clearance and viral load reduction, respectively. Complete lesion regression and progression of lesion to cancer have the highest and lowest effect sizes with a percentage of 34% (I^2=99.99%, P<0.001, 95% CI=33.85-34.15), 12% [I^2=99.99%, P<0.001, 95% confidence interval (CI)=11.85-12.15], and respectively. The overall effect size of treatment outcomes in the included studies is 26.80% (I^2=99.99%, P<0.001, 95% CI=18.34-35.26). This implies that the inconsistency of treatment outcomes across studies is low (Fig. 2).

Study characteristics. The characteristics of each of the included studies is indicated in Table II.

Results of individual studies. A total of 307 women who were suffering from high-grade CIN and cervical cancer were

Table I. Immunogenicity of different therapeutic vaccine strategies at different phases of human clinical trials.

Type of vaccine	Vaccine name	Antigen	Study design	Pt' number	Clinical status	Immunogenicity	(Refs.)
Bacterial vaccine (*L. monocytogenes*)	Lm-LLO-E7	E-7	A phase I clinical trial	15	Metastatic or advanced cervical cancer	Three patients had an increase in E7-specific IFN- γ T cells	(79)
Viral vector	Modified vaccinia virus Ankara (MVA)	E6 or E7 (TA-HPV), HPV16/18	Phase I/II study	17	Progressive cervical cancer	HPV-specific CTL responses in 28% of patients with advanced cervical cancer	(34)
Peptide vaccine	A mixture of 13 overlapping SLPs covering the entire sequences of HPV-16-E6 and E7	HPV-16 E6 and E7	Phase II clinical trial	16	Advanced or recurrent cervical cancer	56% showed vaccine-induced HPV-specific T-cell proliferation, and 85% of the 13 patients tested revealed vaccine-induced immune responses. Increased lymphocyte stimulation and production of anti-tumor agents such as IFN- γ and IL	(80)
DNA vaccine	DNA vaccine GX188E (Genexine, Inc.)	HPV16/ 18-E6/7	A phase I clinical trial	9	CIN 3	HPV-16 and -18 E6 and E7 specific cell-mediated responses, including IFN-secreting $CD8^+$ and $CD4^+$ T-cell responses and HPV-specific polyfunctional $CD8^+$ T-cell responses, were elicited	(74)
Cell-based vaccines (DCs)	HPV-16 or -18 E7-loaded DCs were co-administered with IL-2.	HPV16/ 18-E6/E7	A phase I trial	4	Stage Ib or IIa cervical cancer	Specific $CD4^+$ T cell responses were detected in two out of four patients, and E7-specific $CD8^+$ responses were observed in all patients	(81)
SFV-based RNA Replicon	Vvax001	E6/E7 HPV16 and 18	Phase I trial	12	CIN 2/3	Vvax001 induced HPV16 E6 and/or E7-specific T cell responses ($CD4^+$ and $CD8^+$ T cells) and contributed to the production of IFN- γ)	(82)

TA, tissue antigen; HPV, Human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LLO, listeriolysin O.

Table II. Characteristics of each of the included studies

Authors	Publication year	Country	Sample size	Study type	Study design	Vaccine type	Efficacy						(Refs.)
							PR	PRR	SD	CR	VLR	VC	
Choi *et al*	2020	South Asia	52	Original Article	Clinical trial	GX-188E	8	5	12	27	8	27	(83)
Stevanović *et al*	2015	USA	9	Original Article	Clinical trial	HPV-TILs	1	2	5	2	1	2	(77)
Park *et al*	2019	South Korea	25	Original Article	Clinical trial	GX-188E	3	2	5	15	3	15	(68)
Kim *et al*	2014	South Korea	9	Original Article	Clinical trial	GX-188E	0	0	2	7	0	7	(74)
Choi *et al*	2020	South Korea	11	Original Article	Clinical trial	BVAC-C	3	3	5	0	0	0	(69)
Rahma *et al*	2014	USA	28	Original Article	Clinical trial	PIDC pulsed with HPV16 E6 or E7 peptide	2	25	1	0	0	0	(72)
Trimble *et al*	2015	USA	114	Original Article	Clinical trial	VGX-3100	55	7	7	45	55	45	(73)
Harper *et al*	2019	Spain, Belgium, France	129	Original Article	Clinical trial	Tipapkinogen Sovacivec	15	0	83	31	15	31	(71)

PR, partial tumor regression; PRR, tumor progression; SD, stable tumor disease; CR, tumor complete regression; VLR, viral load reduction; VC, complete viral clearance, Pre-immature dendritic cells.

Table III. Proportion of each clinical outcome of the clinical trials.

Clinical outcome	Number of lesions	Proportion	Mean	Standard deviation	Standard error
Partially regressed lesion	87	0.23	47.13	48.21	17.04
Progressed lesions	44	0.12			
Stable lesions	120	0.32			
Completely regressed lesions	126	0.33			
Lesions with reduced viral load	80	0.21			
Virus free lesions	98	0.26			

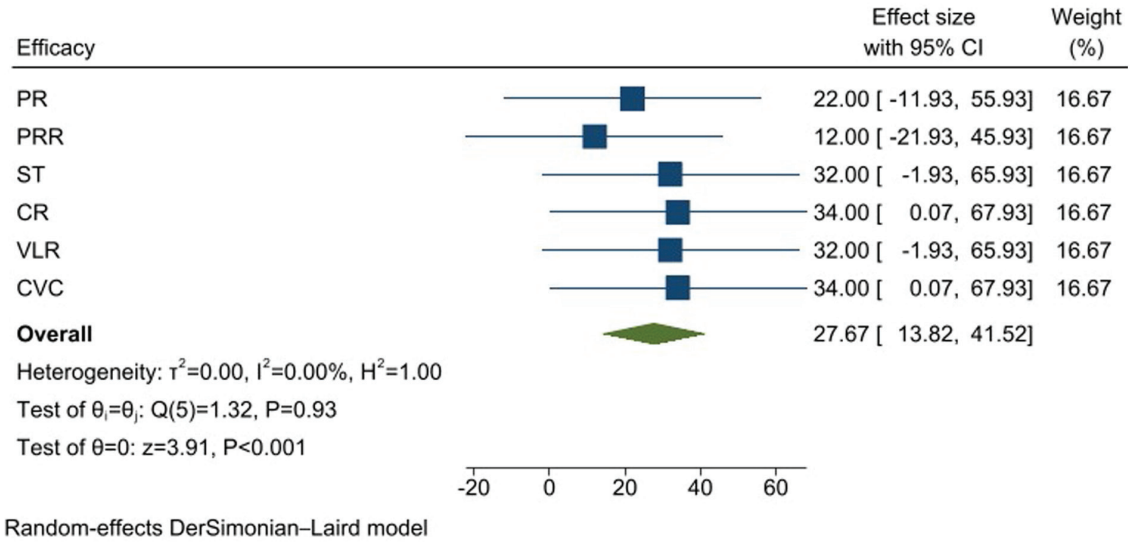

Figure 3. Effect size and CI of each clinical outcome. CI, confidence interval; PR, partial lesion regression; PRR, lesion progression to cancer; ST, stable lesion; CR, complete lesion regression; VLR, viral load reduction; CVC, complete viral load clearance.

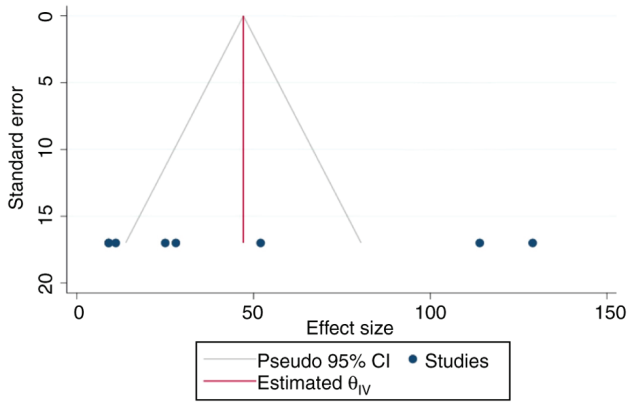

Figure 4. Funnel plot. CI, confidence interval.

included in the present review. They were allowed to take different types of novel HR-HPV 16/18 therapeutic vaccines to evaluate the efficacy of the vaccines and their efficacy is demonstrated by evidences (68-73) in Table III. Summary statistics of the outcomes for each of the studies (Table III) along with their effect estimates and precision CI are presented in plots (Fig. 1). In a random effects DerSimonian-Laird model analysis, there is heterogeneity (I^2=87.13%, P<0.001) and a non-overlapping CI regarding the size of individual studies. The percentage of heterogeneity, P-value and absence of overlap of CIs confirm the presence of statistically significant heterogeneity across the size of the studies. The overall effect size across studies is 47.13% (95% CI=13.72-80.53), which represents moderate heterogeneity (Fig. 3).

The overall efficacy of the new HR HPV 16/18 therapeutic vaccine in treating women suffering from CIN and cervical cancer is 88% (333/377). The most pronounced outcome is tumor/lesion complete regression to a normal state [34%, 126/377, 95% CI (33.85-34.15]. The least frequent outcome is lesion progression to cancer [12%, 44/377, 95% CI (11.85-12.15); Table IV].

Proportion of each clinical outcome of the clinical trials. Of the 377 women included in the study, HR-HPV 16/18 therapeutic vaccines were most efficient in establishing stable CIN and cervical cancer, followed by complete regression of the lesion with a proportion of 0.32 (120/377) and 0.33 (126/377), respectively. The proportion of tumor progression to cancer was the lowest (0.12) when compared with other clinical outcomes among women receiving HR HPV 16/18 therapeutic vaccines. Out of the included women (377) with CIN and cervical cancer, 0.26 (98/377) and 0.21 (80/377) women demonstrated viral clearance and viral load reduction, respectively. The mean and

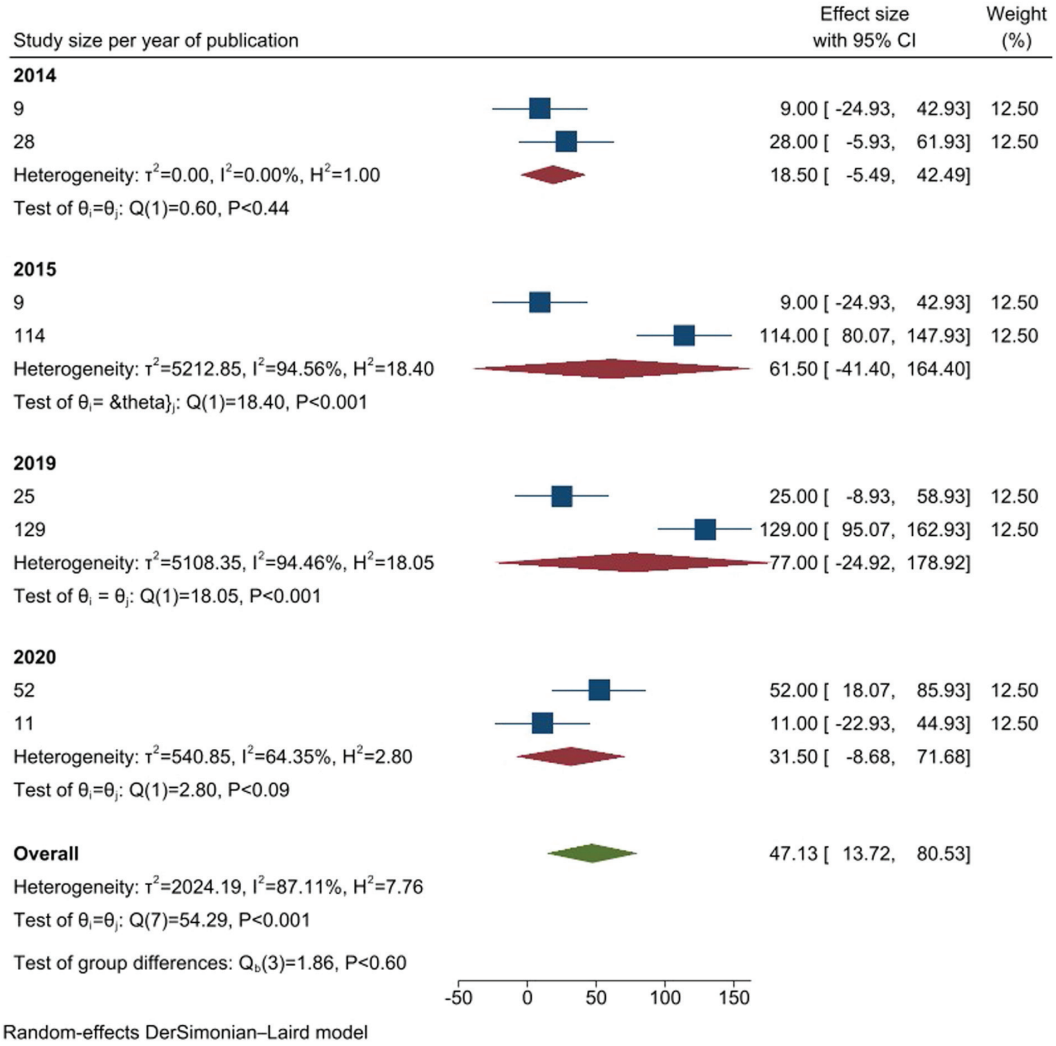

Figure 5. Subgroup analysis.

standard error of the study participants were 47.13 and 17.04, respectively (Table III).

RoB in studies. Assessments of the *RoB* across each included study are presented using a funnel plot. The variability of the individual studies (standard error) against the overall effect size is asymmetrical. At a 95% CI, the funnel plot exhibits heterogeneity among study sizes, publication bias, small study effects and true variability in effect sizes due to different underlying true effects in the populations. The plot below indicates an asymmetrical distribution of the included studies (Fig. 4). Cumulative regression was conducted by using year of publication as a variable label and individual study size effect which was stratified by country of publication to identify source of heterogeneity. The outliers are due to small sample size and substantial variation in the sample size of the included studies (Fig. 5).

Results of syntheses. The results of the synthesis for the characteristics of the studies and the RoB among contributor studies are explained briefly in the study characteristics section and the RoB assessment section, respectively. The heterogeneity test (I^2), level of significance (P-value), the absence of overlap in the Cis and general expectations (https://training.cochrane.org/handbook/current/chapter-10#section-10-10-4-1) were used to decide on the statistical model to be applied for the meta-analysis (where I^2=100.00% and P<0.001). The I^2 test, the P-value, general expectations and the absence of overlap in the CI demonstrate the presence of heterogeneity; therefore, a random model effect was applied to synthesize the results and draw statistical conclusions using Stata software (version 17; StataCorp LP).

Galbraith plot was used to assess heterogeneity across studies and publication bias. The plot revealed information regarding study-specific effect sizes, their precision, and the overall effect size. The overall effect size is explained by the slope of the regression line, the red line. At 95% CI, the scattered plot of the standardized effect size of each study on the vertical axis and its precision on the horizontal axis denotes inconsistency. The weights of individual studies account for both the sample size and the precision of the effect estimate in each study. There are no outliers (Fig. 6).

Certainty of evidence. The certainty of the evidence regarding the risk of bias (internal validity) were explained; inconsistency, imprecision, indirectness and publication bias. The

Table IV. Efficacy of a novel high-risk HPV-16/18 therapeutic vaccine in treating cervical intraepithelial neoplasia per study.

Type vaccine of clinical trial	Type	No. of patents involved	Vaccine efficacy						(Refs.)
			No. of partially regressed lesions	No. of progressed lesions	No. of stable lesions	No. of completely regressed lesions	No. of lesions with reduction in viral load	No. of virus free lesions	
A prospective, randomized, multicenter, open-label, phase II clinical trial)	DNA-based vaccine (GX-188E)	52	8	5	12	27	8	27	(68)
Cohort-clinical trial	Cell based vaccine BVAC-C	8	1	3	4	0	0	0	(84)
Multicenter, Phase I, single-arm trial	Cell based vaccine BVAC-C	11	3	3	5	0	0	0	(69)
Randomized, double blind controlled phase II trial	Protein-based vaccine (Tipapkinogen Sovacivec)	129	15	0	83	31	15	15	(71)
Clinical trial	Cell-base vaccine [Adoptive T-cell therapy (ACT)]	9	1	2	5	2	1	2	(77)
Cohort-clinical trial	DNA vaccine (GX-188E)	9	0	0	2	7	0	7	(74)
Clinical trial	Pre-mature dendritic cells PIDC)	28	2	25	1	0	0	0	(72)
A randomized, double-blind, placebo-controlled phase 2b	DNA-based vaccine (VGX-3100)	114	55	7	7	45	55	45	(73)

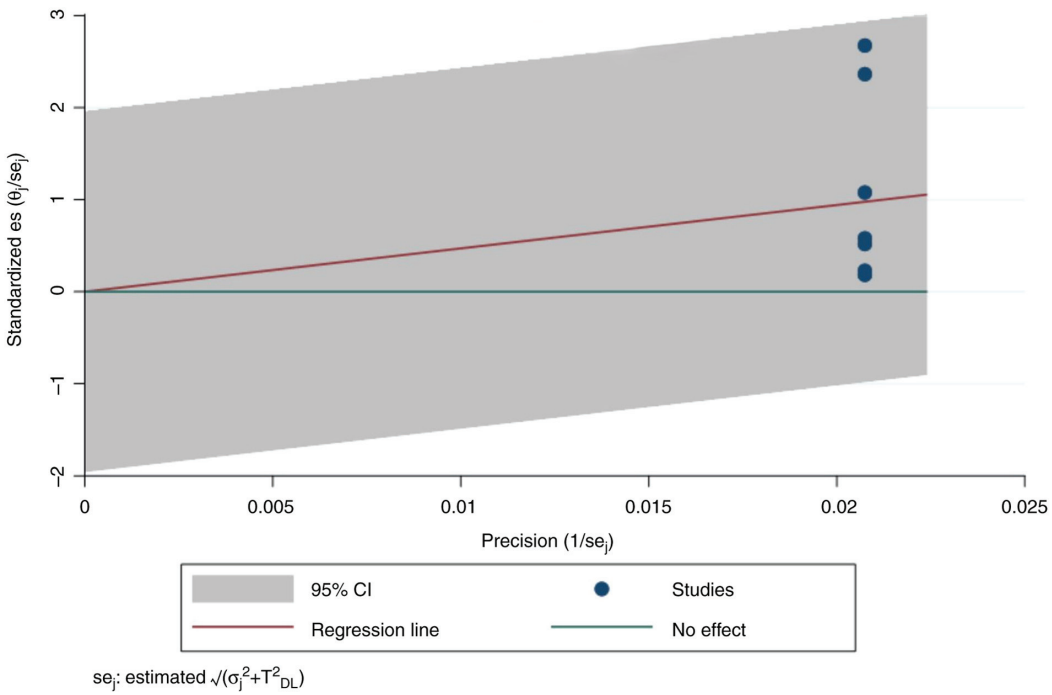

Figure 6. Galbraith plot. CI, confidence interval; SE, standard error. Standardized es (θ/SE), standardized effect size, θ, is the estimated effect size from a study.

evidence of the present study was assessed by study and across studies by considering whether they were experimental studies or observational studies. Inconsistency was assessed to determine whether studies that should show similar results indeed showed similar results or unexplained differences across the results of individual studies. There is overlapping of CIs, point estimates of effects that are widely different, formal tests and measures of statistical heterogeneity. There is no need to be concerned regarding the risk of bias. Imprecision was assessed across the studies using the results of individual studies. The CIs for each of the studies are narrow. This implies a low risk of bias due to imprecision. Indirectness was also assessed to determine how directly the research evidence applies to the question of interest. The included studies' certainty of evidence directly answers the question of interest. It addresses population, interventions and outcomes of interest. The certainty of evidence has no concern regarding indirectness. Finally, it was assessed whether evidence exists that has not been identified and may contradict the results, thus publication bias across studies was assessed where there is no concern here by the authors. Generally, there is a low risk of bias regarding the certainty of evidence (Figs. 2-5).

Discussion

All eligible studies evaluated the efficacy of therapeutic vaccines and confirmed their potential to regress high-grade CIN to low-grade, stable disease formation, complete regression of CIN and cervical tumor or lesion to normal. Additionally, HPV-16/18 load reduction and clearance were observed (Table IV). Kim *et al* (74) demonstrated clearance of cervical lesions and persistent HPV infection by using the therapeutic HPV DNA vaccine (GX-188E). The vaccine was administered to nine women with high-grade CIN who were involved in the study. Stable disease, complete regression of the lesion, and complete viral clearance were observed in 2, 7 and 2 of the women, respectively. Fortunately, none of them developed cancer (74). Another study by Daayana *et al* (75) evaluated the efficacy and immunogenicity of imiquimod, a topical immunomodulator, followed by a therapeutic HR HPV 16/18-E6 and E7 vaccine, TA-CIN (fusion protein HPV 16 E6E7L2). A total of 19 women with high-grade CIN were involved. The efficacy of the therapeutic scheme was reported to be of full histological regression [63% (12/19)] of the lesions, viral clearance [36% (5/14) and 79% (15/19] of women free of symptoms (75). A similar study reported the efficacy of a SLP vaccine among 19 women with high-grade CIN in 79% (15/19) patients, of whom 47% (9/19) patients had a complete lesion regression (76).

Choi *et al* (69) underwent a phase I clinical trial to determine the efficacy of the HR HPV 16/18 therapeutic vaccine (BVAC-C) among 11 women suffering from recurrent cervical carcinoma. Partial lesion regression and lesion progression were observed in 3 women for each outcome, and 5 women developed a stable disease. No complete lesion regression was observed in the study (69). Stevanović *et al* (77) investigated the efficacy of an HR-HPV 16/18 therapeutic vaccine, adoptive T-cell Therapy (ACT). A total of 9 women participated in the clinical trial. All women were provided with a single infusion of the ACT. Partial tumor/lesion regression, tumor/lesion regression to greater severity, stable tumor/lesion, complete tumor/lesion regression and complete viral clearance were observed in 1, 2, 5, 2 and 2 of the participating women. Viral load reduction was observed in 1 of the 9 patients (77). The probable reasons for the inconsistency of clinical outcomes among the included studies are due to the type of HR HPV 16/18 therapeutic vaccine, the immune status of the women enrolled, the combinations of therapy involved, and the duration of the study.

Current HPV vaccinations are highly effective against CIN and cervical cancer; however, they are only prophylactic and do not treat existing infections. Therapeutic vaccinations that stimulate cell-mediated immune responses are thus required to treat existing CIN and cervical cancers. The E6 and E7 early genes are attractive vaccine targets because they interrupt the cell cycle and are expressed constitutively in premalignant and malignant tissues (3). The advancement of therapeutic vaccinations is hopeful, with vaccines principally based on the tumor proteins E6/E7 to produce robust cellular immunity and, ideally, eradicate HPV-related diseases and cancer. Vaccines are frequently effective in preclinical trials; however, not in the clinical phase. The vaccine's efficacy will be improved in the future by studying more *in situ* tumor models, integrating pharmaceutical therapy, and creating other antigenic targets (such as E1, E2 and E5). The authors are hopeful that therapeutic immunizations will become available soon (78). Furthermore, the licensing, efficacy, safety, immunogenicity, stability (under adverse conditions), durability, mode of administration, capacity to reach the intended site (distribution) and cost of the HR-HPV 16/18 therapeutic vaccination are currently barriers to community use.

The main strengths of the present systematic review and meta-analysis are its potential to fill data and knowledge gaps on this research topic. All included studies are conducted in human clinical trials. Its effort to advocate this subject matter in the presence of limited records/evidence may initiate further studies by responsible bodies, institutions and stakeholders. Moreover, all included studies were conducted with a robust study design, clinical trial. The main limitations of the evidence in the included studies are the absence of control groups and the selection of study participants without randomization. Most of the studies did not report odd ratios or relative risks because the treatment groups were followed. The limitations of the review process are the failure to determine the association between HR-HPV 16/18 therapeutic vaccines' efficacy and clinical outcomes due to the absence of evidence from the included studies. Another limitation of the review process is the inclusion of small pieces of evidence due to the novelty of the research question.

The current systematic review and meta-analysis will serve as a benchmark for future studies and add a body of knowledge to the current understanding of the new HR-HPV 16/18 therapeutic vaccine efficacy against CIN and cervical cancer. More importantly, it advocates the efficacy of the new HR-HPV 16/18 therapeutic vaccine against CIN and cervical cancer in clinical trial practices. Further studies are urgently required to improve the efficacy of the new HR-HPV 16/18 therapeutic vaccine against CIN and cervical cancer and to contribute pertinent information to policymakers and the health system. Conclusion strategies to develop an efficacious novel HR-HPV 16/18 therapeutic vaccine are very promising and eye-catching. However, there are discrepancies in the efficacy of novel HR-HPV 16/18 therapeutic vaccines among clinical trials. There is very limited clinical trial evidence on the efficacy of novel HR-HPV 16/18 therapeutic vaccines in treating CIN and cancer. Further work is currently needed to boost the efficacy of the HR-HPV 16/18 therapeutic vaccine and meet the requirements for general use. Finding a mechanism to benefit from innate immune cells and adaptive immune cells may greatly increase the efficacy of these vaccines. At the same time, they are urgently required to reduce the burden of cervical cancer and high-grade CIN. The ultimate goal of the HR-HPV 16/18 therapeutic vaccine is to reduce the health burden of the disease and improve quality of life with no or minimal side effects. Future agenda A therapeutic HPV-16/18 vaccine with optimum stability, safety, durability, accessibility, affordability, efficacy and immunogenicity should be aimed at combating this deadly and growing public health threat. Completed HR-HPV 16/18 therapeutic vaccines and ongoing clinical trials should get space to be communicated and converted into meaningful activities. Furthermore, continuous clinical trials and designing strategies to develop immunogenic and effective therapeutic vaccines shall be top agenda items for the scientific community.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

ZHT contributed title conception, evidence searching, data extraction, result analysis, writing and edition, and supervision. WY, BAT, BM, MB, DA and YA designed the study, managed the project, wrote up the method, contributed data or an analysis tool, result interpretation, performed risk of bias assessment, data entry and editing. TD, AA, BS, ST and HB contributed to statistical analysis, evidence screening, evidence synthesis, result synthesis, result interpretation, revision and editing. ZHT and LGB conceived, collected data and designed the analysis, collected data, and wrote the manuscript. All authors read and approved the final manuscript. ZHT and LGB confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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