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SAFETY, EFFICACY AND IMMUNOGENICITY OF THERAPEUTIC VACCINES IN THE TREATMENT OF PATIENTS WITH HIGH-GRADE CERVICAL INTRAEPITHELIAL NEOPLASIA ASSOCIATED WITH HUMAN PAPILLOMAVIRUS: A SYSTEMATIC REVIEW PROTOCOL

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Author Contributions: CAG, LCLJ, and GPS conceptualized and designed the protocol, drafted the initial manuscript, and reviewed the manuscript. CAG and LCLJ defined the concepts and search items, data extraction process as well as methodological appraisal of the studies. FKN and AZ planned the data extraction and statistical analysis. PCMM and GPS, provided critical insights. All authors have approved and contributed to the final written manuscript.

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ABSTRACT

Introduction: Eighty percent of the sexually active population will get human papillomavirus (HPV) infection, which is the most prevalent sexually transmitted disease worldwide. Persistence of high-grade HPV (CIN 2/3) infection may evolve to a cervical intraepithelial neoplasia and these lesions may be precursors of cervical cancer. However, this progression can be prevented by the administration of therapeutic vaccines which use the main oncoproteins responsible for cancer development, in an attempt to trigger a more specific and effective immunological response against this disorder. We aim to evaluate the safety, efficacy and immunogenicity of therapeutic vaccines in the treatment of patients with high-grade CIN 2/3 associated with HPV.

Methods and analysis: A systematic review of randomized controlled trials (RCTs) will be undertaken. MEDLINE, Embase, CENTRAL Cochrane, Web of Science, LILACS, SciELO and Scopus will be searched, with no restriction regarding publication date. Primary outcomes will include measures related to safety, efficacy and the immunogenicity of the therapeutic vaccines used in these patients. Study selection will follow the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. Methodological appraisal of the studies will be assessed by the Jadad Scale and Cochrane Risk-of-Bias Tool for RCTs. A narrative synthesis will be done for all included studies. Outcomes will be analyzed according to the subgroups of HPV type, CIN grade, route of vaccine administration and vaccine type. Also, if sufficient data are available, a meta-analysis will be conducted. The effect sizes will be generated using Hedges' g score, for both fixed and random effect models. I^2 statistics will be used to assess heterogeneity and identify their potential sources.

Ethics and dissemination: Ethical approval is not required as primary data will not be collected. Findings will be disseminated widely via peer-reviewed publication and in different media, e.g. conferences, congresses or symposia.

PROSPERO registration number: CRD42017077428.

Keywords: Cervical Intraepithelial Neoplasia; Papillomavirus Infections; Uterine Cervical Neoplasms; Vacinnes.

Strengths and limitations of this study:

- This protocol reduces the possibility of duplication, gives transparency to the methods and processes that will be used, reduces possible biases and allows peer review.
- Will offer highest level of evidence for informed clinical decisions from this systematic review of randomised controlled trials.
- This systematic review will be the first to evaluate the safety, efficacy and immunogenicity of therapeutic vaccines in the treatment of patients with high-grade cervical intraepithelial neoplasia (CIN 2/3) associated with human papillomavirus (HPV).
- The scarcity of of randomised controlled trials undertaken with therapeutic vaccines in the treatment of patients with CIN 2/3 associated with HPV, the publication bias and the methodological quality of the grey literature found may be the main limitations of the study.

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INTRODUCTION

In recent decades, sociocultural changes have influenced human behavior leading to the emergence of various sexually transmitted diseases, including those caused by human papillomavirus (HPV).[1] HPV is a non-encapsulated DNA virus with approximately 8000 base pairs belonging to the family Papillomaviridae[2] which affects approximately 105 million women at least once in their lives.[3] HPV is present in 99.7% of cervical intraepithelial neoplasia (CIN)[4] and is closely related to the onset of cervical cancer, and these pathologies are considered to be a public health global problem.[1]

Approximately 80% of the sexually active population is infected with any subtype of HPV.[3] Most lesions regress without treatment within a period of up to 24 months as a result of the immune response, however, occasionally 10 to 30% of infections persist and may progress to high-grade lesions (CIN 2/3).[5]

There are approximately 200 HPV genotypes and these may be related to low (CIN 1) or high grade (CIN 2/3). The main risk factor for the development of CIN is the persistence or relapse of high-risk HPV, especially subtypes 16 and 18 that are present in up to 75% of lesions.[6] These viruses express proteins that promote cell cycle alteration inducing genomic instability in normal cells, inhibiting apoptosis, favoring the formation of mitotic defects and aneuploidy. In addition, they inhibit tumor suppressor genes and modulate the immune system making the tumor cells low immunogenic, which results in immunological tolerance to the tumor and favors the HPV-mediated oncogenicity.[7,8]

When the virus is detected, the therapy of choice is the physical removal of the lesion, which is able to eliminate more than 80% of initial lesions. However, viral DNA often remains[9] and may lead to a recurrence of the lesion that may progress to cervical cancer[10] requiring more aggressive treatments, such as chemotherapy and radiotherapy, resulting in the death of 50% of patients.[11] On the other hand, treatments that stimulate the immune response have been shown to eliminate up to 90% of CIN 2 lesions upon 24 months.[13] Therefore, new therapeutic strategies that effectively and permanently eliminate the HPV virus are currently needed.[12]

The production of therapeutic vaccines focuses on the effectiveness of specific immunological responses against antigens[14,15] in order to eliminate the established pathology

or prevent the patient from being reinfected, neutralizing subsequent infections by the same virus. Because of this characteristic, therapeutic vaccines differ significantly from the available prophylactic vaccines because these later are ineffective in treating established lesions and therefore have no therapeutic properties.[16] Moreover, because the risk population continues to be exposed to the virus without having an associated protective factor, therapeutic vaccines have low adherence rates and therefore the picture of HPV infections that can progress to aggressive pathologies remains unchanged.[17]

Hence, based on the fact that HPV infections are frequent and associated with significant public health morbidity and mortality, it is necessary to develop effective and safe therapeutic vaccines against already established HPV-associated lesions. Following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P) checklist as guidance,[18] we propose a systematic and reproducible strategy to query the literature about the safety, efficacy and immunogenicity of therapeutic vaccines in the treatment of patients with high-grade cervical intraepithelial neoplasia (CIN 2/3) associated with human papillomavirus (HPV).

RESEARCH AIMS

The main objectives of this systematic review are: (1) To evaluate the efficacy of therapeutic vaccines in patients with high-grade cervical intraepithelial neoplasia, evaluated through histopathological regression of the lesion as well as regression of lesion size or other parameters that the authors considered relevant to assess this variable; (2) To assess the safety of therapeutic vaccines in patients with high-grade cervical intraepithelial neoplasia, reporting possible adverse effects to its administration; (3) To assess the immunogenicity of therapeutic vaccines in patients with high-grade cervical intraepithelial neoplasia by evaluating changes in the immunological profile of individuals who received the treatment compared to those who did not receive it.

METHODS AND ANALYSIS

Search Strategy

The search strategy will be carried out using resources that enhance methodological transparency and improve the reproducibility of the results and evidence synthesis. The search strategy will be elaborated and implemented prior to study selection, according to the PRISMA-P checklist as guidance.[18] In addition, using the PICOS acronym[19] we elaborated the

guiding question of this review, in order to ensure the systematic search of available literature: *" What are the scientific evidences on the safety, efficacy and immunogenicity of therapeutic vaccines in the treatment of patients with high-grade cervical intraepithelial neoplasia (CIN 2/3) associated with HPV?"* The PROSPERO – International Prospective Register of Systematic Reviews – registration number is: CRD42017077428 (https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=77428).

Studies will be retrieved using seven databases: MEDLINE - Medical Literature Analysis and Retrieval System Online (via PubMed), Embase (Excerpta Medica Database), Cochrane Central Register of Controlled Trials (CENTRAL), Web of Science, LILACS (Latin American and Caribbean Health Sciences Literature), SciELO (Scientific Electronic Library Online) and Scopus. There will be no restriction regarding publication date. Language restrictions will be applied and only articles in English will be included. Additionally, secondary searches in other sources, such as, Google Scholar and registration sites of clinical trials (e.g. ClinicalTrials.gov) will be also carried out. Also, the reference section of the included studies will be hand searched for additional relevant studies. It is noteworthy that two researchers (CAG and LCLJ) will perform the search strategy independently. In addition, the bibliographic software EndNote (https://www.myendnoteweb.com/) will be used to store, organize, and manage all the references and ensure a systematic and comprehensive search.

Initially, the existence of controlled descriptors (such as MeSH terms, Emtree terms, and DeCS-Health Science Descriptors) and their synonyms (key words) was verified in each database. The search terms were combined using the Boolean operators "AND" and "OR".[20]

Subsequently, the search strategy combining MeSH terms and free-text words that will be used in MEDLINE (via PubMed) and adjusted to the other electronic databases will be as follows in Table 1.

Table 1 Concepts and search items

Databases	Search items
MEDLINE Embase CENTRAL Cochrane Web of Science Scopus LILACS SciELO	#1 (Cervical Intraepithelial Neoplasia) OR (Neoplasia, Cervical Intraepithelial) OR (Cervical Intraepithelial Neoplasms) OR (Cervical Intraepithelial Neoplasm) OR (Intraepithelial Neoplasm, Cervical) OR (Intraepithelial Neoplasms, Cervical) OR (Neoplasm, Cervical Intraepithelial) OR (Neoplasms, Cervical Intraepithelial) OR (Intraepithelial Neoplasia, Cervical) OR (Cervical Intraepithelial Neoplasia, Grade III) OR (Cervical Intraepithelial Neoplasia Grade II) OR (High Grade Cervical Intraepithelial Neoplasia) OR (CIN) OR (High-grade Cervical Intraepithelial Neoplasia) OR (Cervical Intraepithelial Neoplasia) OR (Precancerous Conditions)

	OR (Preneoplastic Condition*)
	#2 (Papillomaviridae) OR (Human papilomavírus) OR (Human Papilloma Viruses) OR (Papilloma Virus, Human) OR (Papilloma Viruses, Human) OR (Virus, Human Papilloma) OR (Viruses, Human Papilloma) OR (HPV, Human Papillomavirus Viruses) OR (Human Papillomavirus Viruses) OR (Human Papillomavirus Virus) OR (Papillomavirus Virus, Human) OR (Papillomavirus Viruses, Human) OR (Virus, Human Papillomavirus) OR (Viruses, Human Papillomavirus)
	#3 #1 AND #2
	#4 (Vaccine) OR (Immunomodulatory Therapy) OR (Therapies, Immunomodulatory) OR (Therapy, Immunomodulatory) OR (Vaccines, Neoplasm) OR (Injection, Therapeutic Vaccine) OR (Vaccinotherapy) OR (Therapeutic vaccine) OR (Vaccinotherapy) OR (Vaccine Immunogenicity) OR (Antigenicity, Vaccine) OR (Adjuvant) OR (Vaccination)
	#5 #3 AND #4
	#6 (Randomized Controlled Trial) OR (Controlled Clinical Trial) OR (Randomized Controlled Trials) OR (Random Allocation) OR (Clinical Trial) OR (Clinical Trials) OR (Random*) OR (Prospective Studies) OR (Control) OR (Prospective*)
	#7 #5 AND #6

Abbreviations: MEDLINE, Medical Literature Analysis and Retrieval System Online; Embase, Excerpta Medica Database; CENTRAL, Cochrane Central Register of Controlled Trials; LILACS, Latin American and Caribbean Health Sciences Literature; SciELO, Scientific Electronic Library Online).

Study selection criteria

A summary of the population (P), interventions (I), comparators (C) and outcomes (O) considered, as well as studies designs (S) included according to PICOS acronym, is provided in Table 2.

Table 2 Inclusion and exclusion criteria

PICOS Acronym[19]	Inclusion criteria	Exclusion criteria
P – Population	Patients with high-grade cervical intraepithelial neoplasia (CIN 2 and 3) associated with HPV.	Patients with other immunosuppression associated conditions.
I – Intervention	Use of therapeutic vaccines for the treatment of high-grade cervical intraepithelial neoplasia associated with HPV.	
C – Comparison	Usual standard of care without receiving the therapeutic vaccine.	
O – Outcome	The safety, the efficacy and the immunogenicity of the therapeutic vaccines used in patients with high-grade cervical intraepithelial neoplasia associated with HPV	Studies that do not report safety, the efficacy and the immunogenicity of the therapeutic vaccines as primary outcome
S – Study design	Randomized controlled trial.	All the non-primary literature, such as reviews, dissertations, theses, editorials, protocol studies and clinical guidelines.

Screening and data extraction

Initially the screening of studies will be based on the information contained in their titles and abstracts and will be conducted by two independent investigators (CAG and LCLJ). When the reviewers disagree, the article will be reevaluated and, if the disagreement persisted, a third reviewer (GPS) will make a final decision. Full-paper screening will be conducted by the same independent investigators. Cohen's kappa will be used to measure inter-coder agreement in each screening phase.

Data will be extracted using previously proposed tools[21–23], including four domains: i) identification of the study (article title; journal title; impact factor of the journal; authors; country of the study; language; publication year; host institution of the study [hospital; university; research center; single institution; multicenter study]); ii) methodological characteristics (study design; study objective or research question or hypothesis; sample characteristics, e.g. sample size, age, race, baseline characteristics; groups and controls; recruitment methods and study completion rates; stated length of follow-up; validated measures; statistical analyses, adjustments; iii) main findings and implications for clinical practice; and iv) conclusions.

In the event that the information in any specific article is unclear or data are missing, the review author will contact the correspondent author of the study. For data extraction two independent Microsoft Excel spreadsheets will be elaborated by two reviewers (CAG and LCLJ) to summarize the data from the included studies. Then, the spreadsheets will be combined into one. Disagreements will be resolved by a third investigator (GPS).

Quality assessment

Methodological quality of the RCTs will be assessed using the Jadad scale,[24] a widely used tool for classification of the quality of the evidence from RCTs. The Jadad scale scores range from 0 to 5, with studies scoring < 3 considered as low quality, and studies that score ≥ 3 classified as high quality.[24] The internal validity and risk of bias for RCTs will be assessed with the appraisal tool from the Cochrane Handbook for Systematic Reviews of Interventions 5.1.0,[25] which assesses the following study-level aspects: (1) randomization sequence allocation; (2) allocation concealment; (3) blinding; (4) completeness of outcome data and (5) selective outcome reporting; and classifies studies into low, high or unclear risk of bias.

The same two independent reviewers (CAG and LCLJ) will assess the methodological quality of eligible trials as well as will score the selected studies. Disagreements will be resolved by a third reviewer (GPS). The risk of bias for each outcome across individual studies will be summarized as a narrative statement, and supported by a risk of bias table. A review-level narrative summary of the risk of bias will also be provided.

Descriptive analysis and meta-analysis

For studies with a high or unclear risk of bias, defined as high or nuclear risk in 50% or more of the quality assessment outcomes, a narrative description of the risk of bias will be provided. Risk of Bias assessments will be incorporated into synthesis by performing sensitivity analysis (i.e., limiting to studies at lowest risk of bias in a secondary analysis).

A narrative synthesis will be conducted for all the selected studies, including: i) characteristics related to the quality of the selected studies as number of drop-outs per follow-up, early withdrawal by benefit, intention-to-treat analysis, blindness scheme, allocation secrecy and randomization; ii) characteristics of the protocol used in studies such as type of intervention and control group, sample size, treatment time, dose and interval of the vaccine administration; iii) study population characteristics, such as, age, staging of disease, association of treatments or surgeries and other relevants information; iv) outcomes, for instance, the changes in immunological parameters, signs of local and systemic toxicity, histopathological regression of the lesion, regression of lesion size or reduction of viral load.

Furthermore, whenever possible, continuous and dichotomous outcomes will be pooled together for meta-analysis purposes. All effect sizes will be transformed into a common metric, in order to make them comparable across studies – the bias-corrected standardised difference in means (Hedges' g) – classified as positive when in favour of the intervention and negative when in favour of the control. Heterogeneity will be assessed using

I^2.[26] The presence of publication bias will be evaluated by using a funnel plot and the Duval and Tweedie's trim and fill method.[27]

Patient and public involvement, ethics and dissemination

Patients were not directly involved in the design of this study. Because this is a protocol for a systematic review and no participant recruitment will take place, their involvement on the recruitment and dissemination of findings to participants was not applicable. Additionally, any amendments to this protocol will be documented with reference to saved searches and analysis methods, which will be recorded in bibliographic databases (Ovid), EndNote and Excel templates for data collection and synthesis.

The results of the review will be disseminated via peer-reviewed publication as well as in different media, e.g. conferences, congresses or symposia.

DISCUSSION

One of the strengths of the proposed study is to apply a reproducible and transparent procedure for systematic review of the literature. In this protocol, we clearly describe the types of studies, participants, interventions and outcomes that will be included, as well as the data sources, search strategy, data extraction methods (including quality assessment) and methods of combining data.[28] By publishing the research protocol, we reinforce the clarity of the strategy and minimize the risk of bias, namely selective outcome reporting.[25] Second, we will focus solely on the impact of the safety, efficacy and immunogenicity of therapeutic vaccines in the treatment of patients with high-grade cervical intraepithelial neoplasia (CIN 2/3) associated with human papillomavirus (HPV). These results shall provide high-level information to inform, support and customize decisions from the oncology clinicians.

Potential limitations of this study include the heterogeneity of measures and outcomes evaluated and the potentially reduced number of studies in subgroup analyses, which may negatively influence the statistical power in data synthesis.

It is noteworthy that although prophylactic vaccines against HPV are safe and provide protective immunity against viruses that cause high-grade cancers[3,29,30], the adherence to these vaccines is low, impairing an effective prevention against the development of this disease as well as cervical cancer. Low adherence to the vaccination also allows the spread of Sexually Transmitted Diseases associated with this pathogen, constituting a serious global problem for public health. Once the disease is already in activity, prophylactic vaccines are no longer effective, and therefore effective and safe therapeutic vaccines that also activate a memory

immune response by promoting the regression of pre-cancerous lesions are needed, thus reducing mortality, morbidity, time and cost of treatment in these patients. In this sense, the present study will provide relevant evidence on the efficacy, safety and immunogenicity of therapeutic vaccines used in the treatment of patients with high-grade cervical intraepithelial neoplasia in order to address the gap in the literature on this new therapy to women's health.

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Reporting checklist for protocol of a systematic review.

Based on the PRISMA-P guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

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		Reporting Item	Page Number
Identification	#1a	Identify the report as a protocol of a systematic review	1
Update	#1b	If the protocol is for an update of a previous systematic review, identify as such	n/a
	#2	If registered, provide the name of the registry (such as PROSPERO) and registration number	2
Contact	#3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contribution	#3b	Describe contributions of protocol authors and identify the guarantor of the review	1
	#4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important	n/a

		protocol amendments	
Sources	#5a	Indicate sources of financial or other support for the review	1
Sponsor	#5b	Provide name for the review funder and / or sponsor	1
Role of sponsor or funder	#5c	Describe roles of funder(s), sponsor(s), and / or institution(s), if any, in developing the protocol	1
Rationale	#6	Describe the rationale for the review in the context of what is already known	3 and 4
Objectives	#7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	4 and 5
Eligibility criteria	#8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	5, 6 and 7
Information sources	#9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	5
Search strategy	#10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	5 and 6
Study records - data management	#11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	5
Study records - selection process	#11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	5
Study records - data collection process	#11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	7
Data items	#12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	7

Outcomes and prioritization	#13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	7
Risk of bias in individual studies	#14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	8
Data synthesis	#15a	Describe criteria under which study data will be quantitatively synthesised	8 and 9
	#15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I2, Kendall's τ)	8 and 9
	#15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	8 and 9
	#15d	If quantitative synthesis is not appropriate, describe the type of summary planned	8 and 9
Meta-bias(es)	#16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	n/a
Confidence in cumulative evidence	#17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	8 and 9

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SAFETY, EFFICACY AND IMMUNOGENICITY OF THERAPEUTIC VACCINES IN THE TREATMENT OF PATIENTS WITH HIGH-GRADE CERVICAL INTRAEPITHELIAL NEOPLASIA ASSOCIATED WITH HUMAN PAPILLOMAVIRUS: A SYSTEMATIC REVIEW PROTOCOL

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***Title Page** – BMJ Open*

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ABSTRACT

Introduction: Eighty percent of the sexually active population will get human papillomavirus (HPV) infection, which is the most prevalent sexually transmitted disease worldwide. Persistence of high-grade HPV (CIN 2/3) infection may evolve to a cervical intraepithelial neoplasia and these lesions may be precursors of cervical cancer. However, this progression can be prevented by the administration of therapeutic vaccines which use the main oncoproteins responsible for cancer development, in an attempt to trigger a more specific and effective immunological response against this disorder. We aim to evaluate the safety, efficacy and immunogenicity of therapeutic vaccines in the treatment of patients with high-grade CIN 2/3 associated with HPV.

Methods and analysis: A systematic review of clinical trials will be undertaken. MEDLINE, Embase, CENTRAL Cochrane, Web of Science, LILACS, SciELO and Scopus will be searched, with no restriction regarding publication date. Primary outcomes will include measures related to safety, efficacy and the immunogenicity of the therapeutic vaccines used in these patients. Study selection will follow the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. Methodological appraisal of the studies will be assessed by the Cochrane Risk-of-Bias Tool for randomized controlled trials (RCTs) and the quality evidence of the risk of bias in single studies, will be evaluated by GRADE. A narrative synthesis will be done for all included studies. Outcomes will be analyzed according to the subgroups of HPV type, CIN grade, route of vaccine administration and vaccine type. Also, if sufficient data are available, a meta-analysis will be conducted. The effect sizes will be generated using Hedges' g score, for both fixed and random effect models. I^2 statistics will be used to assess heterogeneity and identify their potential sources.

Ethics and dissemination: Ethical approval is not required as primary data will not be collected. Findings will be disseminated widely via peer-reviewed publication and in different media, e.g. conferences, congresses or symposia.

PROSPERO registration number: CRD42017077428.

Keywords: Cervical Intraepithelial Neoplasia; Papillomavirus Infections; Uterine Cervical Neoplasms; Vacinnes.

Strengths and limitations of this study:

- This protocol reduces the possibility of duplication, gives transparency to the methods and processes that will be used, reduces possible biases and allows peer review.
- Will offer highest level of evidence for informed clinical decisions from this systematic review of clinical trials.
- This systematic review will be the first to evaluate the safety, efficacy and immunogenicity of therapeutic vaccines in the treatment of patients with high-grade cervical intraepithelial neoplasia (CIN 2/3) associated with human papillomavirus (HPV).
- The scarcity of of randomized controlled trials undertaken with therapeutic vaccines in the treatment of patients with CIN 2/3 associated with HPV, the publication bias and the methodological quality of the grey literature found may be the main limitations of the study.

SAFETY, EFFICACY AND IMMUNOGENICITY OF THERAPEUTIC VACCINES IN THE TREATMENT OF PATIENTS WITH HIGH-GRADE CERVICAL INTRAEPITHELIAL NEOPLASIA ASSOCIATED WITH HUMAN PAPILLOMAVIRUS: A SYSTEMATIC REVIEW PROTOCOL

INTRODUCTION

In recent decades, sociocultural changes have influenced human behavior leading to the emergence of various sexually transmitted diseases, including those caused by human papillomavirus (HPV).[1] HPV is a non-encapsulated DNA virus with approximately 8000 base pairs belonging to the family Papillomaviridae[2] which affects approximately 105 million women at least once in their lives.[3] HPV is present in 99.7% of cervical intraepithelial neoplasia (CIN)[4] and is closely related to the onset of cervical cancer, and these pathologies are considered to be a public health global problem.[1]

Approximately 80% of the sexually active population is infected with any subtype of HPV.[3] Most lesions regress without treatment within a period of up to 24 months as a result of the immune response, however, occasionally 10 to 30% of infections persist and may progress to high-grade lesions (CIN 2/3).[5]

There are approximately 200 HPV genotypes and these may be related to low (CIN 1) or high grade (CIN 2/3). The main risk factor for the development of CIN is the persistence or relapse of high-risk HPV, especially subtypes 16 and 18 that are present in up to 75% of lesions.[6] These viruses express proteins that promote cell cycle alteration inducing genomic instability in normal cells, inhibiting apoptosis, favoring the formation of mitotic defects and aneuploidy. In addition, they inhibit tumor suppressor genes and modulate the immune system making the tumor cells low immunogenic, which results in immunological tolerance to the tumor and favors the HPV-mediated oncogenicity.[7,8]

When the virus is detected, the therapy of choice is the physical removal of the lesion, which is able to eliminate more than 80% of initial lesions. However, viral DNA often remains[9] and may lead to a recurrence of the lesion that may progress to cervical cancer[10] requiring more aggressive treatments, such as chemotherapy and radiotherapy, resulting in the death of 50% of patients.[11] On the other hand, treatments that stimulate the immune response have been shown to eliminate up to 90% of CIN 2 lesions upon 24 months.[12] Therefore, new therapeutic strategies that effectively and permanently eliminate the HPV virus are currently needed.[12,13]

The production of therapeutic vaccines focuses on the effectiveness of specific immunological responses against antigens[14,15] in order to eliminate the established pathology

or prevent the patient from being reinfected, neutralizing subsequent infections by the same virus. Because of this characteristic, therapeutic vaccines differ significantly from the available prophylactic vaccines because these later are ineffective in treating established lesions and therefore have no therapeutic properties.[16] Moreover, because the risk population continues to be exposed to the virus without having an associated protective factor, therapeutic vaccines have low adherence rates and therefore the picture of HPV infections that can progress to aggressive pathologies remains unchanged.[17]

Hence, based on the fact that HPV infections are frequent and associated with significant public health morbidity and mortality, it is necessary to develop effective and safe therapeutic vaccines against already established HPV-associated lesions. Following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P) checklist as guidance,[18] we propose a systematic and reproducible strategy to query the literature about the safety, efficacy and immunogenicity of therapeutic vaccines in the treatment of patients with high-grade cervical intraepithelial neoplasia (CIN 2/3) associated with human papillomavirus (HPV).

RESEARCH AIMS

The main objectives of this systematic review are: (1) To evaluate the efficacy of therapeutic vaccines in patients with high-grade cervical intraepithelial neoplasia, evaluated through histopathological regression of the lesion as well as regression of lesion size or other parameters that the authors considered relevant to assess this variable; (2) To assess the safety of therapeutic vaccines in patients with high-grade cervical intraepithelial neoplasia, reporting possible adverse effects to its administration; (3) To assess the immunogenicity of therapeutic vaccines in patients with high-grade cervical intraepithelial neoplasia by evaluating changes in the immunological profile of individuals who received the treatment compared to those who did not receive it.

METHODS AND ANALYSIS

Search Strategy

The search strategy will be carried out using resources that enhance methodological transparency and improve the reproducibility of the results and evidence synthesis. The search strategy will be elaborated and implemented prior to study selection, according to the PRISMA-P checklist as guidance.[18] In addition, using the PICOS acronym[19] we elaborated the

guiding question of this review, in order to ensure the systematic search of available literature: *" What are the scientific evidences on the safety, efficacy and immunogenicity of therapeutic vaccines in the treatment of patients with high-grade cervical intraepithelial neoplasia (CIN 2/3) associated with HPV?"* The PROSPERO – International Prospective Register of Systematic Reviews – registration number is: CRD42017077428 (https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=77428).

Studies will be retrieved using seven databases: MEDLINE - Medical Literature Analysis and Retrieval System Online (via PubMed), Embase (Excerpta Medica Database), Cochrane Central Register of Controlled Trials (CENTRAL), Web of Science, LILACS (Latin American and Caribbean Health Sciences Literature), SciELO (Scientific Electronic Library Online) and Scopus. There will be no restriction regarding publication date. Language restrictions will be applied and only articles in English will be included. Additionally, secondary searches in other sources, such as, Google Scholar and registration sites of clinical trials (e.g. ClinicalTrials.gov) will be also carried out. Also, the reference section of the included studies will be hand searched for additional relevant studies. It is noteworthy that two researchers (CAG and LCLJ) will perform the search strategy independently. In addition, the bibliographic software EndNote (https://www.myendnoteweb.com/) will be used to store, organize, and manage all the references and ensure a systematic and comprehensive search.

Initially, the existence of controlled descriptors (such as MeSH terms, Emtree terms, and DeCS-Health Science Descriptors) and their synonyms (key words) was verified in each database. The search terms were combined using the Boolean operators "AND" and "OR".[20]

Subsequently, the search strategy combining MeSH terms and free-text words that will be used in MEDLINE (via PubMed) and adjusted to the other electronic databases will be as follows in Table 1.

Table 1 Concepts and search items

Databases	Search items
MEDLINE Embase CENTRAL Cochrane Web of Science Scopus LILACS SciELO	#1 (Cervical Intraepithelial Neoplasia) OR (Neoplasia, Cervical Intraepithelial) OR (Cervical Intraepithelial Neoplasms) OR (Cervical Intraepithelial Neoplasm) OR (Intraepithelial Neoplasm, Cervical) OR (Intraepithelial Neoplasms, Cervical) OR (Neoplasm, Cervical Intraepithelial) OR (Neoplasms, Cervical Intraepithelial) OR (Intraepithelial Neoplasia, Cervical) OR (Cervical Intraepithelial Neoplasia, Grade III) OR (Cervical Intraepithelial Neoplasia Grade II) OR (High Grade Cervical Intraepithelial Neoplasia) OR (CIN) OR (High-grade Cervical Intraepithelial Neoplasia) OR (Cervical Intraepithelial Neoplasia) OR (Precancerous Conditions)

	OR (Preneoplastic Condition*)
	#2 (Papillomaviridae) OR (Human papilomavírus) OR (Human Papilloma Viruses) OR (Papilloma Virus, Human) OR (Papilloma Viruses, Human) OR (Virus, Human Papilloma) OR (Viruses, Human Papilloma) OR (HPV, Human Papillomavirus Viruses) OR (Human Papillomavirus Viruses) OR (Human Papillomavirus Virus) OR (Papillomavirus Virus, Human) OR (Papillomavirus Viruses, Human) OR (Virus, Human Papillomavirus) OR (Viruses, Human Papillomavirus)
	#3 #1 AND #2
	#4 (Vaccine) OR (Immunomodulatory Therapy) OR (Therapies, Immunomodulatory) OR (Therapy, Immunomodulatory) OR (Vaccines, Neoplasm) OR (Injection, Therapeutic Vaccine) OR (Vaccinotherapy) OR (Therapeutic vaccine) OR (Vaccinotherapy) OR (Vaccine Immunogenicity) OR (Antigenicity, Vaccine) OR (Adjuvant) OR (Vaccination)
	#5 #3 AND #4
	#6 (Randomized Controlled Trial) OR (Controlled Clinical Trial) OR (Randomized Controlled Trials) OR (Random Allocation) OR (Clinical Trial) OR (Clinical Trials) OR (Random*) OR (Prospective Studies) OR (Control) OR (Prospective*)
	#7 #5 AND #6

Abbreviations: MEDLINE, Medical Literature Analysis and Retrieval System Online; Embase, Excerpta Medica Database; CENTRAL, Cochrane Central Register of Controlled Trials; LILACS, Latin American and Caribbean Health Sciences Literature; SciELO, Scientific Electronic Library Online).

Study selection criteria

A summary of the population (P), interventions (I), comparators (C) and outcomes (O) considered, as well as studies designs (S) included according to PICOS acronym, is provided in Table 2.

Table 2 Inclusion and exclusion criteria

PICOS Acronym[19]	Inclusion criteria	Exclusion criteria
P – Population	Patients with high-grade cervical intraepithelial neoplasia (CIN 2 and 3) associated with HPV.	Patients with other immunosuppression associated conditions.
I – Intervention	Use of therapeutic vaccines for the treatment of high-grade cervical intraepithelial neoplasia (CIN 2 and 3) associated with HPV.	
C – Comparison	Usual standard of care without receiving the therapeutic vaccine.	
O – Outcome	The safety, the efficacy and the immunogenicity of the therapeutic vaccines used in patients with high-grade cervical intraepithelial neoplasia (CIN 2 and 3) associated with HPV	Studies that do not report safety, the efficacy for CIN 2 and 3 and the immunogenicity* of the therapeutic vaccines as primary outcome
S – Study design	Clinical trial	All the non-primary literature, such as reviews, dissertations, theses, editorials, protocol studies and clinical guidelines.

*Immunogenicity will be evaluated across the various studies in exploratory way in the blood and in the target tissue (including immune response to vaccine antigen assessment of HPV-specific CD8 and CD4 immune response; or also, via systemic induction of HPV E6- and E7- specific T-cell immune responses and changes of involved lesions and HPV infection status at the uterine cervix), among other parameters (e.g. generation of antibodies and release of cytokines).

Screening and data extraction

Initially the screening of studies will be based on the information contained in their titles and abstracts and will be conducted by two independent investigators (CAG and LCLJ). When the reviewers disagree, the article will be reevaluated and, if the disagreement persisted, a third reviewer (GPS) will make a final decision. Full-paper screening will be conducted by the same independent investigators. Cohen's kappa will be used to measure inter-coder agreement in each screening phase.

Data will be extracted using previously proposed tools[21–23], including four domains: i) identification of the study (article title; journal title; impact factor of the journal; authors; country of the study; language; publication year; host institution of the study [hospital; university; research center; single institution; multicenter study]; conflict of interest and study sponsorship); ii) methodological characteristics (study design; study objective or research question or hypothesis; sample characteristics, e.g. sample size, age, race, baseline characteristics; groups and controls; recruitment methods and study completion rates; stated length of follow-up; validated measures; statistical analyses, adjustments; iii) main findings and implications for clinical practice; and iv) conclusions.

In the event that the information in any specific article is unclear or data are missing, the review author will contact the correspondent author of the study. For data extraction two independent Microsoft Excel spreadsheets will be elaborated by two reviewers (CAG and LCLJ) to summarize the data from the included studies. Then, the spreadsheets will be combined into one. Disagreements will be resolved by a third investigator (GPS).

Quality assessment

The internal validity and risk of bias for RCTs will be assessed with the appraisal tool from the Cochrane Handbook for Systematic Reviews of Interventions 5.1.0,[24] which assesses the following study-level aspects: (1) randomization sequence allocation; (2) allocation concealment; (3) blinding; (4) completeness of outcome data and (5) selective outcome reporting; and classifies studies into low, high or unclear risk of bias. In addition, the quality evidence of the risk of bias in single studies, will be evaluated by the Grades of Recommendation, Assessment, Development and Evaluation (GRADE)[25].

The same two independent reviewers (CAG and LCLJ) will assess the methodological quality of eligible trials as well as will score the selected studies. Disagreements will be resolved by a third reviewer (GPS). The risk of bias for each outcome across individual studies will be summarized as a narrative statement, and supported by a risk of bias table. A review-level narrative summary of the risk of bias will also be provided.

Descriptive analysis and meta-analysis

For studies with a high or unclear risk of bias, defined as high or nuclear risk in 50% or more of the quality assessment outcomes, a narrative description of the risk of bias will be provided. Risk of Bias assessments will be incorporated into synthesis by performing sensitivity analysis (i.e., limiting to studies at lowest risk of bias in a secondary analysis).

A narrative synthesis will be conducted for all the selected studies, including: i) characteristics related to the quality of the selected studies as number of drop-outs per follow-up, early withdrawal by benefit, intention-to-treat analysis, blindness scheme, allocation secrecy and randomization; ii) characteristics of the protocol used in studies such as type of intervention and control group, sample size, treatment time, dose and interval of the vaccine administration; iii) study population characteristics, such as, age, staging of disease, association of treatments or surgeries and other relevant information; iv) outcomes, for instance, the changes in immunological parameters, signs of local and systemic toxicity, histopathological regression of the lesion, regression of lesion size or reduction of viral load.

Furthermore, whenever possible, continuous and dichotomous outcomes will be pooled together for meta-analysis purposes. All effect sizes will be transformed into a common metric, in order to make them comparable across studies – the bias-corrected standardised difference in means (Hedges' g) – classified as positive when in favour of the intervention and negative when in favour of the control. Heterogeneity will be assessed using

I^2.[26] The presence of publication bias will be evaluated by using a funnel plot and the Duval and Tweedie's trim and fill method.[27] Therefore, we will assess the publication bias if enough studies per outcome are identified.

Patient and public involvement, ethics and dissemination

Patients were not directly involved in the design of this study. Because this is a protocol for a systematic review and no participant recruitment will take place, their involvement on the recruitment and dissemination of findings to participants was not applicable. Additionally, any amendments to this protocol will be documented with reference to saved searches and analysis methods, which will be recorded in bibliographic databases (Ovid), EndNote and Excel templates for data collection and synthesis.

The results of the review will be disseminated via peer-reviewed publication as well as in different media, e.g. conferences, congresses or symposia.

DISCUSSION

One of the strengths of the proposed study is to apply a reproducible and transparent procedure for systematic review of the literature. In this protocol, we clearly describe the types of studies, participants, interventions and outcomes that will be included, as well as the data sources, search strategy, data extraction methods (including quality assessment) and methods of combining data.[28] By publishing the research protocol, we reinforce the clarity of the strategy and minimize the risk of bias, namely selective outcome reporting.[25] Second, we will focus solely on the impact of the safety, efficacy and immunogenicity of therapeutic vaccines in the treatment of patients with high-grade cervical intraepithelial neoplasia (CIN 2/3) associated with human papillomavirus (HPV). These results shall provide high-level information to inform, support and customize decisions from the oncology clinicians.

Potential limitations of this study include the heterogeneity of measures and outcomes evaluated and the potentially reduced number of studies in subgroup analyses, which may negatively influence the statistical power in data synthesis.

It is noteworthy that although prophylactic vaccines against HPV are safe and provide protective immunity against viruses that cause high-grade cancers[3,29,30], the adherence to these vaccines is low, impairing an effective prevention against the development of this disease as well as cervical cancer. Low adherence to the vaccination also allows the spread of Sexually Transmitted Diseases associated with this pathogen, constituting a serious global problem for public health. Once the disease is already in activity, prophylactic vaccines are no longer

effective, and therefore effective and safe therapeutic vaccines that also activate a memory immune response by promoting the regression of pre-cancerous lesions are needed, thus reducing mortality, morbidity, time and cost of treatment in these patients. In this sense, the present study will provide relevant evidence on the efficacy, safety and immunogenicity of therapeutic vaccines used in the treatment of patients with high-grade cervical intraepithelial neoplasia in order to address the gap in the literature on this new therapy to women's health.

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Reporting checklist for protocol of a systematic review.

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In your methods section, say that you used the PRISMA-P reporting guidelines, and cite them as:

Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart LA. Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) 2015 statement. Syst Rev. 2015;4(1):1.

		Reporting Item	Page Number
Identification	#1a	Identify the report as a protocol of a systematic review	1
Update	#1b	If the protocol is for an update of a previous systematic review, identify as such	n/a
	#2	If registered, provide the name of the registry (such as PROSPERO) and registration number	2
Contact	#3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contribution	#3b	Describe contributions of protocol authors and identify the guarantor of the review	1
	#4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important	n/a

		protocol amendments	
Sources	#5a	Indicate sources of financial or other support for the review	1
Sponsor	#5b	Provide name for the review funder and / or sponsor	1
Role of sponsor or funder	#5c	Describe roles of funder(s), sponsor(s), and / or institution(s), if any, in developing the protocol	1
Rationale	#6	Describe the rationale for the review in the context of what is already known	3 and 4
Objectives	#7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	4 and 5
Eligibility criteria	#8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	5, 6 and 7
Information sources	#9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	5
Search strategy	#10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	5 and 6
Study records - data management	#11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	5
Study records - selection process	#11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	5
Study records - data collection process	#11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	7
Data items	#12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	7

Outcomes and prioritization	#13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	7
Risk of bias in individual studies	#14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	8
Data synthesis	#15a	Describe criteria under which study data will be quantitatively synthesised	8 and 9
	#15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I2, Kendall's τ)	8 and 9
	#15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	8 and 9
	#15d	If quantitative synthesis is not appropriate, describe the type of summary planned	8 and 9
Meta-bias(es)	#16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	n/a
Confidence in cumulative evidence	#17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	8 and 9