Quadrivalent HPV vaccination after effective treatment of Anal Intraepithelial Neoplasia in HIV+ men (VACCAIN-P)

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)
AE	Adverse Event
AIN	Anal Intraepithelial Neoplasia
AR	Adverse Reaction
CA	Competent Authority
cART	Combination Antiretroviral Therapy
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
CV	Curriculum Vitae
DEIA	DNA enzyme immunoassay
DSMB	Data Safety Monitoring Board
EU	European Union
EudraCT	European drug regulatory affairs Clinical Trials
GCP	Good Clinical Practice
(HG) AIN	(High Grade) Anal Intraepithelial Neoplasia
HIV	Human Immunodeficiency Virus
HRA	High-Resolution Anoscopy
IB	Investigator's Brochure
IC	Informed Consent
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
LCM	Laser Capture Microdissection
LiPA25	HPV line probe assay, containing probes for 25 different HPV genotypes
(LG) AIN	(Low Grade) Anal Intraepithelial Neoplasia
METC	Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)
MSM	Men who have sex with men
(q)HPV	(Quadrivalent) Human Papilloma Virus
(S)AE	(Serious) Adverse Event
SPC	Summary of Product Characteristics (in Dutch: officiële productinfomatie IB1-tekst)
Sponsor	The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.
SUSAR	Suspected Unexpected Serious Adverse Reaction
Wbp	Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)
WMO	Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen)
WTS-PCR	Whole Tissue Section- Polymerase Chain Reaction

SUMMARY

Rationale: Since the introduction of combination antiretroviral therapy (cART), human immunodeficiency virus (HIV)-related morbidity and mortality have considerably decreased. However, as a result of the significantly prolonged life span, new causes of morbidity and mortality have become evident. In particular, anal cancer incidence has increased dramatically in HIV-positive men. Like cervical cancer, anal cancer is causally linked to infections with high-risk papillomaviruses, and is preceded by precursor lesions: anal intraepithelial neoplasia (AIN). Over 90% of HIV-positive MSM (men who have sex with men) have persisting anal HPV (human papilloma virus) infection, and high-grade (HG) AIN is present in 30% of all HIV+ MSM.
As in cervical intraepithelial neoplasia, early diagnosis and treatment of AIN have been advocated to prevent malignancy. Electrocoagulation/ cauterization is standard of care for intra-anal AIN, but after treatment, recurrence of lesions occurs in approx. 50% of cases. This is a major problem in an effective screening program for AIN.

In a nonconcurrent, non-blinded cohort study qHPV (quadrivalent human papilloma virus) vaccination significantly (HR 0.50) reduced HG AIN recurrence among MSM successfully treated for AIN. This is in accordance with findings in women treated for cervical intraepithelial neoplasia. Previous vaccination with quadrivalent HPV vaccine among women who had surgical treatment for HPV related disease significantly reduced the incidence of subsequent HPV related disease, including high grade disease.

Therefore, a strategy that is worth investigating is vaccination with the qHPV vaccine to prevent recurrences in HIV+ MSM who were successfully treated for HG AIN.

Objective: The primary objective of the current study is to assess the efficacy of qHPV vaccination in preventing recurrence of high-grade AIN in HIV+ MSM with CD4 counts >350 x 10E6/l who were successfully treated for high-grade intra-anal AIN in the past year.

Study population: HIV-positive MSM with a CD4 count > 350 cells/ul and intra-anal high-grade AIN (grade 2-3) that was successfully treated in the past year with conventional cauterization, cryotherapy, or other forms of local treatment.

Study design: A multicenter, randomised, double-blind clinical trial in three hospitals in the Netherlands.

Intervention: Patients are randomised for vaccination with the quadrivalent HPV vaccine (Gardasil ®) or vaccination with a matching placebo at months 0, 2 and 6.
Randomisation will be stratified for complete response versus partial response (from HG AIN to low-grade (LG) AIN) of the initial HG AIN lesion, for treatment less than 6 months ago versus treatment 6 months and longer ago, and for AMC versus other hospitals.

Main study parameters/endpoints: Screening for AIN will be performed by high-resolution anoscopy (HRA), at inclusion (first vaccination) and at last vaccination (6 months), and repeated at 6 and 12 months after the last vaccination. Safety Monitoring for adverse events and injection-site reactions will be performed one week after each vaccination and thereafter every 6 months for a total of 12 months of follow-up.

Primary end point will be the cumulative recurrence of HG AIN at 12 months after the last vaccination, as assessed by HRA (High-Resolution Anoscopy), with biopsies taken of suspect lesions.
Secondary outcome measures are toxicity/ safety, recurrence of HG AIN at last vaccination and 6 months afterwards, cumulative occurrence of LG AIN at 12 months after the last vaccination, cumulative occurrence of anogenital warts at 12 months after the last

vaccination, causative HPV type in recurrent AIN lesions, as assessed by LCM (Laser Capture Microdissection)/ PCR (polymerase chain reaction), and HPV type-specific antibody response.

The total sample size is estimated to be 125 patients based on an expected recurrence rate of 50% within 12 months. Statistical analysis will be based on the intention-to-treat principle. Both primary and secondary endpoints will be analyzed by descriptive statistics and the chi-square test with a 0,05 two-sided significance level.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness

HIV+ MSM who were successfully treated for HG AIN are still at a 50% risk for recurrences, with additional treatment sessions needed, and an ongoing risk for malignant degeneration of lesions.

Costs of 3 vaccinations are approx. € 400, but if vaccination reduces recurrence rates by 50%, this will be a highly cost-effective intervention, very likely to be introduced into regular care.

For the study, patients will be vaccinated 3 times with the quadrivalent vaccine Gardasil ® or placebo, and will undergo two extra HRAs. Clinical trial data show that the most common adverse events of Gardasil ® were mild or moderate, so few risks are associated with study participation.

1. INTRODUCTION AND RATIONALE

Since the introduction of combination antiretroviral therapy (cART), human immunodeficiency virus (HIV)-related morbidity and mortality have considerably decreased.(1) However, as a result of the significantly prolonged life span of HIV-positive patients in the cART era, new causes of morbidity and mortality have become evident. Several malignancies, in particular anal carcinoma, are observed in excess among HIV-positive patients. Between the early 1990s and 2001–2004, the incidence of anal cancer in HIV-positive individuals has risen from 11–19 per 100.000 in the pre-cART era to 40–78 per 100.000 person-years in the post-cART era.(2,3) Particularly in homosexual HIV-positive men (MSM), annual anal cancer incidences have increased dramatically, from 11–22 to 78-128 per 100.000, as compared to 5/100.000 in HIV- MSM and 1/100.000 in the general population.(4,5)

Like cervical cancer, anal cancer is causally linked to infections with high-risk oncogenic alpha-human papillomaviruses (HPV), and is preceded by cancer precursor lesions called anal intraepithelial neoplasia (AIN).(6–8) High-grade (HG) AIN (AIN 2 or 3) might progress to invasive anal carcinoma over time.(7,9,10) Progression rates to anal cancer have been reported to be 9-19% for HG AIN during a follow- up period up to 9 years.

Anal HPV infection and AIN are highly prevalent in HIV-positive men who have sex with men (MSM). Over 90% of HIV-positive MSM have persisting anal HPV infection, and in 74-88% of patients, high-risk HPV is present. AIN of any grade has been reported to be present in 63–81% of HIV-positive MSM, and high-grade disease (AIN 2 or 3) in 25–52%.(4,7,8,11) The majority (app. 80%) of HG AIN is caused by HPV type 16.(12)

As in cervical intraepithelial neoplasia (CIN), early diagnosis and treatment of AIN have been advocated to prevent malignancy. Several algorithms have been developed to treat AIN, proposing surgery, coagulation, infrared coagulation, photodynamic therapy, or topical imiquimod with or without 5-fluorouracil. Electrocoagulation/cauterization is standard of care for intra-anal AIN.(13,14)

However, after treatment, recurrence of lesions occurs frequently, in approx. 50% of cases within 12 months.(15,16) This is a major problem in an effective screening program for AIN.

By preventing HPV infection, quadrivalent HPV vaccination (qHPV) reduces the risk of precancerous lesions in young men who have sex with men (MSM) without history of precancerous lesions.(17) In a nonconcurrent cohort study qHPV vaccination also significantly (HR 0.50) reduced HGAIN recurrence among MSM successfully treated for AIN. HPV vaccination may therefore be an effective posttreatment adjuvant form of therapy.(15) This is in accordance with findings in women treated for cervical intraepithelial neoplasia. Previous vaccination with quadrivalent HPV vaccine among women who had surgical treatment for HPV related disease significantly reduced the incidence of subsequent HPV related disease, including high grade disease.(18)

Therefore, in the present proposal we want to investigate in a double-blind RCT whether qHPV vaccination indeed significantly decreases recurrence rates in HIV+ MSM who were successfully treated for intra-anal HG AIN.

The registered vaccine Gardasil ® is targeted against HPV types 6, 11, 16 and 18. A pilot study performed by our group, in which HPV-analysis of anal swabs was performed in 44 HIV+ MSM, revealed that 42 (95%) carried high-risk HPV types.(19) In case of multiple infections it was shown in CIN lesions that the LCM/PCR technology is able to assign the single responsible HPV type which is present in the lesion.(20) In a study performed by our group, using this microdissection (LCM)/PCR for anal lesions, HPV types 16 and 18 caused the majority of cases.(21) Therefore, the chosen vaccine will cover the majority of lesions.

Immune responses to the qHPV vaccine are broadly comparable in men and women, including MSM, and the observed responses were substantially higher than during natural HPV infection.(22)

A potential concern might be the immunogenicity of vaccines in HIV+ patients. Responses to several vaccines (influenza, tetanus toxoid, pneumococcal polysaccharides, hepatitis B) are lower in HIV+ patients than in controls. In general, vaccine response is satisfactory, albeit lower than in controls, in HIV patients with CD4 counts > 300 x 10E6/l.(23, 24) In HIV+ men, a HPV-16 E6E7 ISCOMATRIX vaccine induced strong and durable antibody responses and moderate interferon-gamma levels that fell to pre-vaccination levels by week 24.(25) Taken together, there is sufficient evidence that HPV vaccines are immunogenic in these patients.

Relevance

In the Netherlands, 60% of the 20.000 HIV patients receiving care in one of the 23 HIV treatment centres is MSM. Prevalence of HG AIN in HIV+ MSM is reported to vary between 25 and 52%. In our own screening program, 30% of HIV+ MSM had HG AIN, Success rate of the standard treatment is around 50%, with a considerable recurrence rate.(16) As described above, a strategy that is worth investigating is vaccination with the qHPV vaccine to prevent recurrences after successful treatment of HG AIN.

2. OBJECTIVES

Primary objective:

To assess the efficacy of qHPV vaccination in preventing recurrence of high-grade AIN in HIV+ MSM with CD4 counts > 350 x 10E6/l who were successfully treated in the past year for high-grade intra-anal AIN.

Secondary objectives:

- To assess the safety of vaccination in this patient group
- To assess the efficacy of qHPV vaccination in preventing occurrence of low-grade AIN
- To assess the efficacy of qHPV vaccination in preventing occurrence of anogenital warts
- To assess the causative HPV genotype of recurrent low-grade (LG) and HG AIN lesions
- To assess the HPV type-specific antibody response after vaccination, and relate this to the efficacy parameters

3. STUDY DESIGN

Study design:

This study is a multicenter, randomised, double-blind clinical trial in three hospitals in the Netherlands. Vaccination will take place in month 0, 2, and 6 with the qHPV vaccine or a matching placebo.

Study setting:

The study will be performed at both the Internal Medicine department as well as the Dermatology department of 3 hospitals in Amsterdam:

- Academic Medical Center (AMC)
- DC Klinieken Oud Zuid
- Onze Lieve Vrouwe Gasthuis

Study duration:

Patients will be vaccinated three times during this study. High-resolution anoscopy (HRA) will be performed at inclusion, at the moment of the last vaccination, and at 6 and 12 months afterwards.

Time schedule of the study:

Month 1-4:	Preparation of the study and obtaining ethical approval
Month 5-28:	Enrolment of patients
Month 34:	Last vaccination in last enrolled patient
Month 46:	Last follow-up visit of last enrolled patient
Month 46-48:	Analysis of results

4. STUDY POPULATION

4.1 Population

Target population are HIV-positive MSM with a CD4 count > 350 cells/ul and intra-anal high-grade AIN (grade 2-3) that was successfully treated during the past year with conventional cauterization, cryotherapy, or another form of local treatment.

Since 2007, a screening programme for AIN is in place in the AMC. Over 500 HIV-positive MSM have been screened with HRA, the gold standard to screen for AIN. High-grade AIN was found in 30% of screened men.(16) In other hospitals in Amsterdam (Slotervaart Hospital, OLVG, DC Klinieken Oud Zuid) screening programs have started more recently.

HIV+ MSM treated for high-grade AIN remain under surveillance with HRA. At the follow-up HRA, biopsies of suspect lesions are obtained. Patients without AIN lesions or Low-Grade AIN lesions can be included into this study.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Written informed consent.
- Age ≥ 18 years.
- HIV+ MSM, CD4 count > 350/ul (maximum 6 months before screening visit).
- Biopsy-proven intra-anal high-grade AIN successfully treated in the past year with cauterization, cryotherapy, Efudix, imiquimod or another form of local treatment. A maximum interval of 1 year between last treatment and first vaccination is allowed. Lesions with regression from HG to LG AIN (AIN 1) will also be eligible.
- Lesions (still) in remission.
    - Remission has to be established by 2 independent HRA anoscopists.
    - A maximum interval of 3 months is allowed between the first of these HRAs and the first vaccination, and a maximum interval of 6 weeks is allowed between the second of these HRAs and the first vaccination.
    - Biopsies of suspect lesions need to be obtained in one of the HRA sessions.
- Good performance status (a Karnofsky performance score of ≥ 60 [on a scale of 0 to 100, with higher scores indicating better performance status]).
- Pre-treatment haematology, and plasma ASAT, ALAT and creatinine levels compatible with study inclusion (maximum 6 weeks before screening visit).

4.3 Exclusion criteria

- Immunosuppressive medication or other diseases associated with immunodeficiency.
- Life expectancy less than one year.
- Previous HPV vaccination.
- History of anal cancer.
- Other diseases not compatible with study participation.
- Allergy against constituent of Gardasil ® vaccine.
- Currently peri-anal AIN2 or 3.

4.4 Sample size

The total sample size for the RCT is estimated to be 125 patients based on the following assumptions:
- Expected recurrence rate is 50% within 12 months in this study population. This recurrence rate was observed in our earlier studies (16, 19)
- A two group (not for continuity corrected) chi-square test with a 0,05 two-sided significance level will have 80% power to detect the difference between a Group 1 proportion of 0,50 and a Group 2 proportion of 0,25 when the sample size in each group is 58.
- 5% drop out in both groups.

Feasibility

Over 2000 patients are in care at the HIV Outpatient Clinic of the Academic Medical Center in Amsterdam, the Netherlands. An additional 3600 patients are in care in the DC Klinieken Oud Zuid, the Slotervaart Hospital and the Onze Lieve Vrouwe Gasthuis, all in Amsterdam. Approximately 3500 of these patients are homosexual men (MSM).
Since 2007, a screening programme for AIN is in place in the AMC. As AIN screening in HIV+ MSM is part of the regular care in the above mentioned clinics, it should not be a problem to find and enrol 125 patients meeting the inclusion criteria.

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

Patients will be vaccinated 3 times intramuscularly with the qHPV vaccine (Gardasil ®, 0.5 ml) or a matching placebo.

ARM 1: vaccinations with qHPV vaccine at months 0, 2, and 6.
ARM 2: vaccinations with placebo vaccine at months 0, 2, and 6.

5.2 Use of co-intervention (if applicable)

Not applicable.

5.3 Escape medication (if applicable)

Not applicable.

6. INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational product(s)

1. Human Papillomavirus Vaccine (Types 6, 11, 16, 18, Recombinant): Gardasil ®, suspension for injection, ATC code J07BM01.

2. Placebo: Saline 0.9%: Braun NaCl 0.9% oplossing voor injectie. RVG 55227.

6.2 Summary of findings from non-clinical studies

1. See SmPC Gardasil ®, paragraph 5.3.

2. Not applicable.

6.3 Summary of findings from clinical studies

1. See SmPC Gardasil ®, paragraph 5.1.

2. Not applicable.

6.4 Summary of known and potential risks and benefits

1. Side effects observed in >10% of the patients were erythema, swelling, and pain located on the injection site, and headache.

   More detailed information can be found in the SmPC Gardasil ®, paragraph 4.4 until 4.9.

2. Not applicable.

6.5 Description and justification of route of administration and dosage

1. The vaccine should be administered by intramuscular injection. The preferred site is the deltoid area of the upper arm or the higher anterolateral area of the thigh. One dose means 0.5 ml of the Gardasil ® vaccine.

2. The placebo should be likewise administered by intramuscular injection.

6.6 Dosages, dosage modifications and method of administration

1. The Gardasil ® vaccination series consists of 3 separate 0.5 ml doses administered according to the following schedule: 0, 2, 6 months. No doses modifications will be applied. This schedule is the registered vaccination schedule.

2. 3 separate 0.5 ml doses, administered according to the following schedule: 0, 2, 6 months.

6.7 Preparation and labelling of Investigational Medicinal Product

Gardasil vaccinations are supplied by the manufacturer. Preparation of the placebo will be done by the pharmacies of the hospitals concerned according to the relevant GMP guidelines. The pharmacy will label the vaccine and the placebo according to the relevant GMP guidelines.

6.8 Drug accountability

Drug accountability will be done according to the relevant GMP guidelines.

7. METHODS

7.1 Study parameters/endpoints

7.1.1 Main study parameter/endpoint

- Cumulative recurrence of intra-anal or peri-anal HG AIN at 12 months after the last vaccination, as assessed by HRA, with biopsies taken of suspect lesions.

7.1.2 Secondary study parameters/endpoints

- Toxicity/ safety.
- Recurrence of intra-anal or peri-anal HG AIN at the moment of last vaccination and 6 months afterwards.
- Cumulative occurrence of intra-anal or peri-anal LG AIN at 12 months after the last vaccination.
  The patients with LG AIN at inclusion are excluded from this analysis.
- Cumulative occurrence of anogenital warts at 12 months after the last vaccination, evaluated by physical examination and history taking.
- Causative HPV type in recurrent AIN lesions, as assessed by LCM/ PCR.
- HPV type-specific antibody response.

Cumulative recurrence/occurrence at 12 months after the last vaccination means any recurrence/occurrence during the 6 months' vaccination period and the 12 months following the last vaccination.

7.2 Randomisation, blinding and treatment allocation

Patients will be randomised to be vaccinated 3 times with the qHPV vaccine (Gardasil ®) or matching placebo at months 0, 2 and 6.

An independent central randomisation centre will use computer generated tables per centre to allocate treatment. Randomisation will be stratified for complete response versus partial response (from HG AIN to LG AIN) of the initial HG AIN lesion, for treatment less than 6 months ago versus treatment 6 months and longer ago, and for AMC versus other hospitals.

7.3 Study procedures

7.3.1 Safety

Before enrolment a medical history will be obtained, a physical examination will be performed, and routine haematology and chemistry will be performed.

As recommended in the SmPC, patients will be observed for 15 minutes after the vaccine administration.

Safety Monitoring for adverse events and injection-site reactions will be performed one week after each vaccination and thereafter every 6 months for a total of 12 months of follow-up. Adverse events are graded according to version 4.0 of the

Common Terminology Criteria for Adverse Events (CTCAE), which grades events on a scale of 1 to 5, with higher grades indicating greater severity.

7.3.2 Efficacy

Screening for recurrent AIN will be performed by high-resolution anoscopy (HRA), as described previously.(16,19)
HRA will be performed before the first vaccination (see also 4.2) and at last vaccination (6 months), and repeated at 6 and 12 months after the last vaccination. At the last follow up (12 months after the last vaccination), an anal papsmear (cytology) will be performed in order to rule out missing HGAIN recurrences by high-resolution anoscopy. In case the cytology indicates HSIL (high grade squamous intraepithelial lesion), high-resolution anoscopy will be repeated.
Detailed photos plus biopsies of suspect lesions will be obtained. All lesions will be monitored by means of digital photography.
At each visit patients will undergo physical examination to evaluate the presence of anogenital warts. If necessary, biopsies will be obtained to rule out HG AIN.

For histologic analysis, the specimens will be prepared in formalin and stained with hematoxylin and eosin. Grading of the lesions will be performed by experienced pathologists (CvN or AvdW) according to the criteria of the Armed Forces Institute of Pathology, with consensus review by a second pathologist. All histological analyses will be performed in a blinded fashion with respect to clinical outcome.

The chosen vaccine does not cover all HPV types. The majority of HIV+ MSM carry multiple hrHPV types in their anal canal. In case of multiple infections it was shown in CIN lesions that the LCM/ PCR technology is able to assign the single responsible HPV type which is present in the lesion.(20) The same appears to be the case in AIN.(21) Therefore, in recurrent AIN lesions, the causative HPV type will be determined in paraffin-embedded sections of biopsy specimens using a LCM/ PCR based reverse hybridization assay (SPF10 polymerase chain reaction and LiPA25 genotyping) (for further details see Assays).
In this way, we will be able to determine whether recurrent lesions in the vaccine- and placebo arm are vaccine-type HPV-induced or not.

To evaluate the HPV type-specific antibody response, venous blood samples will be drawn before the first vaccination and 3 months after the third vaccination.

ASSAYS

7.3.3 HPV-Typing of recurrent AIN lesions

This method has been described in ref. 20. It has been adapted for AIN lesions the past year by the same group. In summary:

7.3.3.1 Pathological diagnosis and grading

Diagnosis of individual areas of AIN1, 2, and 3 are made according to standard criteria on haematoxylin and eosin (H&E) stained sections. The overall diagnosis is the highest grade of AIN detected. Biopsies might also include areas of lower grade AIN and normal tissue.

After finishing the pathological analyses, including the HPV-analyses, of the biopsies, the tissues will be stored at the Department of Pathology according to the regular storage policy for clinically obtained biopsies.

7.3.3.2 Sandwich cutting

All biopsy blocks are sectioned according to the sandwich cutting procedure: a 4 µm section for diagnosis (H&E before), two sets of 3x8 µm (24 µm) sections for Whole Tissue Section (WTS)-PCR analysis stored in a tube, then two sets of 2 x 4 µm sections on positive-charged slides for p16, 2 x 4 µm sections on membrane slides for Laser Capture Microdissection (LCM) and finally a 4 µm section for pathological confirmation (H&E after). After sectioning each block the microtome is cleaned and a new knife is used. Negative control (paraffin) blocks are used after every 10 blocks sectioned to check for contamination. Cases associated with an invalid negative cutting control are excluded from this study.

7.3.3.3 DNA isolation and HPV DNA detection

Total DNA is isolated from Formalin Fixed Paraffin Enbedded material by a proteinase K procedure. For WTS-PCR, the tissue sample is added to 250 µl proteinase K lysis buffer and incubated at 70°C for 16-24 hours. Proteinase K is heat-inactivated by incubation at 95°C for 10 minutes. Specimens are tested for HPV DNA by PCR amplification/ typing using the HPV SPF10 PCR/ LiPA25 (version 1) system.(26) Each DNA isolation run and PCR run contained HPV positive and negative controls. Ten µl proteinase K-treated DNA is added to 40 µl PCR mix. The SPF10 PCR primer set amplifies a small fragment of 65 bp from the L1 region of mucosal HPV genotypes, as described earlier.(26) Amplification products are detected using the HPV SPF10 PCR (version 1) DNA enzyme immunoassay (DEIA) system. DEIA-positive SPF10 amplimers are used to identify the HPV genotype by reverse hybridization with the HPV line probe assay (LiPA25), containing probes for 25 different HPV genotypes (HPV genotypes 6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68/73, 70, and 74; SPF10 HPV LiPA25 version 1 (Labo Biomedical Products, Rijswijk, The Netherlands, based on licensed Innogenetics technology). Since the SPF10 system is a broadspectrum HPV detection system we also will perform type-specific PCR(TS-PCR) testing in case of HPV 16 and/ or HPV18 type negativity. As primer sets we will use a 92 bp HPV-16 E7 fragment (TS16) and 126 bp HPV-18 L1 fragment (TS18). Amplimers from the type-specific PCRs are detected by DEIA, similar to the method for SPF10 amplimer detection.(27). All PCR runs contain positive and negative PCR controls.

7.3.3.4 Laser Capture Micro-dissection (LCM)

When the whole section PCR of a HG AIN slides contained >1 HPV type, specific HG AIN regions within the slide will be selected for LCM.

LCM is used to obtain samples of anal epithelium from AIN and normal tissue on H-stained membrane slides. Slides are scanned using digital microscopy (Aperio Technologies Inc, Vista, Ca, USA). One or more expert pathologists select areas of each grade of AIN, normal epithelium (squamous, metaplastic and columnar epithelium) and immediately adjacent stroma for sampling. At least one LCM sample

of each AIN lesion is taken, covering at least 5% of the lesion area. Sample size is 1.200-400.000 µm2.

Selected regions are excised with the Zeiss P.A.L.M. microbeam UV laser micro-dissection system and transferred to an AdhesiveCap500 opaque tube (Zeiss). In addition, LCM is performed on a negative control tissue (for example, human placenta) for each case examined. DNA isolation and PCR for HPV DNA is performed as described above.

7.3.4 HPV type-specific antibody response

High-throughput analysis of HPV type-specific antibodies will be done by means of a VLP-based multiplex assay.(28) This analysis will be performed on serum obtained before the first vaccination and 3 months after the last vaccination. At both moments 2x 9 ml venous blood will be drawn. These venous blood samples will be stored for a maximum period of 5 years.

7.4 Withdrawal of individual subjects

A patient will leave the study:

- If the patient withdraws informed consent.
- In case of proven recurrent HG AIN.
- In the case of any illness or condition that according to the investigator is not compatible with the use of the study medication or which could interfere with the evaluations required by the study.

Subjects can leave the study at any time for any reason if they wish to do so without any consequences.

7.5 Replacement of individual subjects after withdrawal

Patients who received less than 3 vaccinations and patients who did not receive the total cycle of 3 vaccinations within 1 year after the first vaccination will be replaced. In case of a possible vaccine-related reason of vaccine discontinuation, detailed safety data will be collected.

7.6 Follow-up of subjects withdrawn from treatment

In case of recurrent high grade lesions patients are withdrawn from the study and are referred to the regular AIN care for further treatment.
In case of recurrence at the 6 month visit (third vaccination and first follow up), the venous blood samples to assess the HPV type-specific antibody response will still be drawn 3 months afterwards.

7.7 Premature termination of the study

The sponsor has the right to terminate the study prematurely if there are any relevant medical or ethical concerns, or if completing the study is no longer feasible. If such action is taken, the reasons for terminating the study must be documented. All study subjects still under treatment at the time of termination must undergo a final examination, which must be documented. The METC must be informed without delay if any investigator has ethical concerns about continuation of the trial. Premature termination of the trial will be considered if:

- The risk-benefit balance for the study subjects changes markedly;
- It is no longer ethical to continue treatment with the IMP;
- It is no longer feasible to complete the study.

8. SAFETY REPORTINGTemporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

8.2 AEs, SAEs and SUSARs

8.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the investigational product. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

8.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that at any dose:

- Results in death;
- Is life threatening (at the time of the event);
- Requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- Results in persistent or significant disability or incapacity;
- Is a congenital anomaly or birth defect;
- Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered a serious adverse experience when, based upon appropriate medical judgement, the event may jeopardize the subject or may require an intervention to prevent one of the outcomes listed above.

The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 15 days after the sponsor has first knowledge of the serious adverse reactions.

In case of any SAE occurring in one of the cooperating clinics, the principal investigator will be notified directly. The principal investigator will report the SAE as described above.

SAEs that result in death or are life threatening should be reported expedited. The expedited reporting will occur not later than 7 days after the responsible investigator has first knowledge of the adverse reaction. This is for a preliminary report with another 8 days for completion of the report.

8.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. The event must be serious (see chapter 8.2.2);
2. There must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. The adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:
   - Summary of Product Characteristics (SPC) for an authorised medicinal product;
   - Investigator's Brochure for an unauthorised medicinal product.

The sponsor will report expedited the following SUSARs to the METC:
- SUSARs that have arisen in the clinical trial that was assessed by the METC;
- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern.

The expedited reporting of SUSARs through the web portal ToetsingOnline is sufficient as notification to the competent authority.

The sponsor will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States.

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

In case of any SAE occurring in one of the cooperating clinics, the principal investigator will be notified directly. The principal investigator will report the SAE as described above.

8.3 Annual safety report

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, and competent authorities of the concerned Member States.
This safety report consists of:
- A list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- A report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

8.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/ or referral to the general physician or a medical specialist.

SAEs need to be reported till end of study within the Netherlands, as defined in the protocol.

8.5 Data Safety Monitoring Board (DSMB) / Safety Committee

Not applicable.

9. STATISTICAL ANALYSIS

Statistical analysis will be based on the intention-to-treat principle. Baseline assessments and outcome parameters will be summarized using simple descriptive statistics. Continuous variables will be summarized with standard descriptive statistics including means, standard deviations, medians, and ranges. Categorical variables will be summarized with frequencies and percentages. Ninety-five percent confidence intervals will be provided for descriptive statistics, as warranted.

Differences between the comparison groups in continuous variables will be estimated using the Student's t-test for non-skewed distributed variables and the Mann-Whitney U test for skewed variables. Differences in categorical variables will be estimated using Chi-square tests. Every effort will be made to collect all data at specified times.

9.1 Primary study parameter(s)

The main analysis will focus on the primary outcome in a comparison between the trial treatment groups. The primary outcome is the cumulative recurrence of HG AIN at 12 months after the last vaccination. After the normal checks for errors, completeness and consistency, the two groups will be compared for the primary endpoint with a chi-square test with a 0,05 two-sided significance level.

The primary outcome will be evaluated for patients who received at least one vaccination (modified intention-to-treat, mITT), and for patients who received all three vaccinations and completed the follow-up (per protocol).
In case of missing end-point data on recurrence, the two groups for the mITT analysis will be compared using Kaplan-Meier survival analysis. The difference between the fractions free of recurrence at 18 months since inclusion will be assessed for statistical significance based on the widths of the corresponding confidence intervals. Additionally, we will perform best-case and worst-case scenarios for missing data to assess what might happen in case of violation of the assumption in survival analysis that patients with missing end-points have the same probability of recurrence during the remainder of the survival period as the other non-recurrent patients at the moment of loss-to-follow-up.

Subgroup analyses will be performed for patients who had a complete response after initial treatment, versus those with regression from HG to LG AIN and for patients treated in the past 6 months versus those treated 6 months and longer ago.

Logistic regression analysis of HG AIN recurrence will be performed to account for variables predicting recurrence, up to a maximum of five variables.

9.2 Secondary study parameter(s)

- Safety

Adverse effects will be reported using descriptive statistics.

- Efficacy
  - Recurrence of HG AIN at last vaccination and 6 months afterwards.
  - Cumulative occurrence of LG AIN at 12 months after the last vaccination. The patients with LG AIN at inclusion are excluded from this analysis.
  - Cumulative occurrence of anogenital warts at 12 months after the last vaccination.

All 3 above mentioned secondary outcomes will be analysed using chi-square tests with a 0,05 two-sided significance level. This will be evaluated for patients who received at least one vaccination (modified intention-to-treat), and for patients who received all three vaccinations and completed the follow-up (per protocol).

- Causative HPV types in recurrent AIN lesions will be described.
- HPV type-specific antibody response 3 months after the last vaccination, in patients with or without recurrent lesions, will be summarized using standard descriptive statistics, and compared with the appropriate statistical tests.

9.3 Other study parameters

Not applicable

10 ETHICAL CONSIDERATIONS

10.1 Regulation statement

Full medical confidentiality will be preserved. The Declaration of Helsinki, the Note for Guidance on Good Clinical Practice (ICH GCP; CPMP/ICH/135/95, step 5 consolidated guideline) and the EU Directive for clinical trials (2001/20/EG) are followed.

10.2 Recruitment and consent

Eligible patients will be informed by the study coordinator about the study. If they are interested, they will be given the patient information letter, and an appointment will be made with the study coordinator to discuss all questions of the patient.
Patients will be given as much time as they need to consider their decision.

10.3 Objection by minors or incapacitated subjects

Not applicable.

10.4 Benefits and risks assessment, group relatedness

HIV+ MSM who were successfully treated for HG AIN are still at a 50% risk for recurrences, with additional treatment sessions needed, and an ongoing risk for malignant degeneration of lesions. Therefore, in the present proposal we want to investigate whether qHPV vaccination indeed significantly decreases recurrence rates in HIV+ MSM who were successfully treated for HG AIN.

Patients will be vaccinated 3 times with the quadrivalent vaccine Gardasil ® or placebo. Clinical trial data in over 5000 males show that the most common adverse events of Gardasil ® were mild or moderate and were most commonly injection-site reactions.(29) Costs of 3 vaccinations are approx. € 400, but if vaccination reduces recurrence rates by 50%, this will be a highly cost-effective intervention.

During the total study period, patients will need to visit the Out Patient Department seven times. In four of these visits, HRA will be performed with biopsies taken of suspect lesions. Compared with patients in the regular AIN care, patients participating in this trial will undergo two more HRAs with possibly biopsies taken. HRA may cause discomfort for the patient and afterwards a little rectal blood loss or soreness might occur.

In conclusion, this specific patient group is at high risk for recurrences of (HG) AIN whereas risks associated with study participation are low.

10.5 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7, subsection 6 of the WMO.

The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO and the Measure regarding Compulsory Insurance for Clinical Research in Humans of 23th June 2003). This insurance provides cover for damage to research subjects through injury or death caused by the study.

1. € 450.000,-- (i.e. four hundred and fifty thousand Euro) for death or injury for each subject who participates in the Research;
2. € 3.500.000,-- (i.e. three million five hundred thousand Euro) for death or injury for all subjects who participate in the Research;
3. € 5.000.000,-- (i.e. five million Euro) for the total damage incurred by the organisation for all damage disclosed by scientific research for the Sponsor as 'verrichter' in the meaning of said Act in each year of insurance coverage.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

10.6 Incentives (if applicable)

Not applicable.

11 ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

11.1 Handling and storage of data and documents

Patient data will be centralized by the Principal Investigator and kept under strict confidentiality. All patients will have access to their own information through their physician.
During the study, the anonymity and confidentiality will be guaranteed and patient identification will be coded. All subjects will be identified by initial of family name and assigned number only on all case report forms, laboratory samples or source documents forwarded to the sponsor. No subject names will be disclosed. The key to the code should be safeguarded by the principal investigator and study coordinator.
Representatives of regulatory agencies, as allowed by local regulations, may review clinical information.
The handling of personal data will comply with the Dutch Personal Data Protection Act (in Dutch: De Wet Bescherming Persoonsgegevens, Wbp).

11.2 Monitoring and Quality Assurance

The assessment of data quality, and the monitoring of compliance with the protocol, monitoring of trial conduct and monitoring of evidence for treatment harm will be performed by an independent Data monitoring team from the Clinical Research Unit, AMC, Amsterdam, the Netherlands.

11.3 Amendments

A 'substantial amendment' is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the METC and to the competent authority.

Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

11.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

11.5 End of study report

The sponsor will notify the accredited METC and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last visit.

In case the study is ended prematurely, the sponsor will notify the accredited METC and the competent authority within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC and the Competent Authority.

11.6 Public disclosure and publication policy

The principal investigators will be responsible for publication of the study results. The results of the study will be disclosed unreservedly. The principles of generally accepted specifications for authorship shall be followed in the appointing of authors and co-authors. All contributors to a specific publication (abstract, original report) will have a true opportunity for full evaluation of the final text before submission to a scientific meeting or an editor.

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Attachment 1: FLOW CHART

Visit→ **Margin[1]→** **↓ Intervention**	**Screening T0=** **Maximum 6 weeks before vaccination 1[2]**	**Vaccine 1 T1=** **0 weeks** **0 weeks**	**Vaccine 2 T2=** **2 months** 1 week ≤ **2 months** ≥ 1 week	**Vaccine 3 T3.1=** **6 months** 2 weeks ≤ **6 months** ≥ 2 weeks	**FU1 T3.2=** **6 months** 2 weeks ≤ **6 months** ≥ 2 weeks	**Serology T4=** **9 months** 2 weeks ≤ **9 months** ≥ 2 weeks	**FU2 T5=** **12 months** 2 weeks ≤ **12 months** ≥ 2 weeks	**FU3 T6=** **18 months** 2 weeks ≤ **18 months** ≥ 2 weeks	**Re-HRA (if applicable)** **T7=** **>18 months**
History taking	X								
Physical examination-General	X								
Physical examination-Genital warts	X				X		X	X	
High Resolution Anoscopy	X				X		X	X	X
Anal papsmear								X	
Routine haematology and chemistry	X								
Serology	X					X			
Vaccination		X	X	X					
History taking (interval)		X	X	X	X		X	X	X

[1] Above mentioned time schedule shows the intended margins for the study visits.
[2] Screening visit may be on the same day as vaccination 1. However, in case biopsies are taken during the last HRA before vaccination, the results must be known before randomization can take place.