Supplementary methods

Trial sites

This multicentre trial was performed in three outpatient clinics in Amsterdam, the Netherlands, belonging to respectively an academic hospital (Amsterdam University Medical Centers (Amsterdam UMC), location Academic Medical Center (AMC)), a general hospital (OLVG), and a clinic (DC Klinieken Lairesse).

Full eligibility criteria

Eligibility criteria were: HIV+ MSM of at least 18 years of age, who had a CD4 count >350 cells/µl, had biopsy-proven intra-anal HGAIN which was successfully treated in the past year with cauterisation (or other ablative treatment), cryotherapy or topical treatment with fluorouracil or imiquimod (lesions with partial remission (from HGAIN to LGAIN (AIN1)) were also eligible), had lesions which were still in remission (maximum LGAIN) at enrolment as established independently by two experienced high-resolution anoscopists at least four weeks after last treatment, had a Karnofsky performance score of ≥60 (on a scale of 0 to 100, with higher scores indicating better physical performance status)[1], and had (near-) normal pre-treatment haematology, ASAT, ALAT and creatinine levels (maximum one grade deviation according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0, 2010[2] was allowed). Exclusion criteria were: immunosuppressive medication or immunodeficiency other than HIV, a life expectancy <1 year, a history of anal carcinoma, current peri-anal HGAIN, previous HPV vaccination, allergy for qHPV vaccine (Gardasil-4®) constituents, and comorbidities not compatible with study participation.

Randomisation and masking

Randomisation was performed using an independent central web-based randomisation tool (ALEA Clinical software version 16 (FormsVision, Abcoude, The Netherlands)). To ensure equal assignment

to vaccination groups we used permuted blocks with variable sizes (ranging from two to four). After randomisation we sent the sequence directly to independent local pharmacists, who prepared the study medication according to computer-generated tables per centre. Masking was maintained by preparing both the qHPV and placebo vaccine in identical syringes wrapped in aluminium foil, which made the vaccines visually indistinguishable.

Digital anal-rectal examination and high-resolution anoscopy

Digital anal-rectal examination (DARE) was performed, followed by intra- and peri-anal inspection using high-resolution anoscopy (HRA) using a colposcope (Zeiss OPMI pico surgical microscope) after at least 1-2 minutes application of acetic acid (3-5% solution), followed by repeated application during the exam. All lesions suspect for AIN on DARE or HRA, irrespective of their HRA appearance, were photographed and biopsied after staining with Lugol's iodine when indicated.

Anal cytology

We started performing anal cytology as HRA quality measure in our clinic from September 2016 onwards. From that date, in participants that reached FU18 liquid-based anal cytology was performed before the start of the HRA. A wetted Dacron swab was inserted and retracted in 20 seconds while rotating and applying firm lateral pressure, after which the swab was transferred to a ThinPrep medium (Hologic, Bedford, MA, USA), swirled vigorously for 30 seconds to release material, followed by a pap smear and reporting according to the Bethesda system.[3] HRA was repeated within 3 months in case cytology indicated HSIL or 'Atypical Squamous Cells, cannot exclude HSIL' (ASC-H) while no biopsy was graded as HGAIN (n=1).

HPV detection and genotyping

For HPV detection and genotyping the SPF_{10} DNA enzyme immunoassay (DEIA) (Labo Biomedical Products B.V., Rijswijk, The Netherlands) assay and reverse hybridization Line Probe Assay25 (LiPA25;

version 1, Labo Biomedical Products B.V., Rijswijk, The Netherlands), detecting 25 mucosal HPV types (HPV 6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68 or 73, 70, 74) were performed. DEIA-positive samples that were negative for the 25 types on the LiPA25 strip were referred to as untypable.[4]

HPV type-specific antibody immunoassay

For vaccine types HPV16, 18, 6, 11, (and non-vaccine types 31, 33, 45, 52, 58 to investigate cross-reactivity), HPV-specific IgG antibody levels were determined using a VLP-based multiplex immunoassay performed at the National Institute for Public Health and Environment (RIVM, Bilthoven, the Netherlands), as previously described.[5] In short, sera were incubated with HPV-specific VLP-conjugated beads (Bio-Rad Laboratories, Hercules, CA, USA). HPV-specific IgG antibodies were detected using R-phycoerythrin (PE) conjugated goat anti-human IgG (Jackson Immunoresearch, West Grove, PA, USA). The 'in-house' control sera and a standard (IVIG Baxter, Utrecht, the Netherlands) were used on each Multiscreen HTS filter plate (Millipore, Burlington, MA, USA). HPV-specific antibodies were analysed using the Bioplex-system 200 with Bioplex software (Bio-Rad Laboratories, Hercules, CA, USA). Samples were assumed to be seropositive above cut-offs according to the 99% Frey-method[6] (with 99% one-sided t-values, based on concentrations measured in children of 1-10 years old (n=859) and found to be 6 Luminex units per ml (LU/ml) (HPV16), 7 LU/mL (HPV18), 11 LU/mL (HPV31), 8 LU/mL (HPV33), 19 LU/mL (HPV45), 15 LU/mL (HPV52), and 21 LU/mL (HPV58).[7] VLPs used in this study were provided by Merck Sharp & Dohme ((MSD), Kenilworth, NJ, USA).

Data management and monitoring

All study data were recorded in standardised electronic case report forms on Research Online (University Medical Center Utrecht, Utrecht, The Netherlands). Study data was independently

monitored throughout the study. The trial was considered safe and therefore was not overseen by a data and safety monitoring board.

Statistical analysis

The Statistical Analysis Plan version 1 (DOI: https://doi.org/10.21942/uva.12861026.v1) was drafted before database lock. Descriptive statistics were used to summarise participants' baseline characteristics and the secondary outcomes.

Statistical analyses were performed using IBM SPSS Statistics software (version 25; IBM Corporation, Armonk, NY, USA), Stata software packages (version 15.1 ; StataCorp LLC, College Station, TX, USA) and GraphPad Prism (version 8.2.1; GraphPad Software Inc., La Jolla, CA, USA). Reported *p*-values are two-sided, with 0.05 as the significance threshold. We adhered to the CONSORT statement and applicable extensions to report the trial.[8]

Sample size calculation

The sample size calculation was based on expected HGAIN recurrence rates of 50% within 12 months after treatment, as observed in our previous study[9], and a 50% reduced HGAIN recurrence rate (HR=0.50) after qHPV vaccination, as was previously reported.[10] The target sample size of 125 participants was determined to have 80% power to detect a 50% relative reduction or 25% absolute reduction in recurrence rate between the qHPV and placebo group using a two-group (not for continuity corrected) superiority chi-square test with a 0.05 two-sided significance level. A 5% drop-out rate was taken into account.

Incidence rates

Incidence rates (IRs) were estimated for the overall participants population and stratified by vaccination group. An incident event was defined as the first occurrence of the disease, after which

the participant stopped being at risk. We assumed an incident event occurred at the midpoint between the last visit where the participant was free of disease and the first visit where disease was diagnosed.

Logistic regression analysis

Determinants with stable estimates (i.e. standard error (SE) was lower than point estimate and 95% CI was acceptably wide) which were associated at $p<0.20$ (Wald test) were included in the multivariable model. In multivariable analysis, we forced the vaccination group into the model, as well as the interaction between the vaccination group and the three stratification factors. Backwards selection was used to obtain the model with the best fit.

Secondary outcomes

Secondary outcomes were compared between groups using independent t-tests and non-parametric Mann-Whitney-U tests for continuous variables and Pearson's Chi-square and Fischer's exact tests for differences in proportions.

Cumulative occurrence LGAIN and anogenital condylomata at FU18 was compared for participants free of disease at the baseline. To account for missing endpoints, we estimated the cumulative occurrence, ARR and RR based on a worst-case and a best-case scenario.

HPV type-specific IgG antibody concentrations were log-transformed and pre- and post-vaccination geometric mean concentrations in both groups were compared using paired t-tests.

References

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