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## HPV vaccination to prevent recurrence of Anal Intraepithelial Neoplasia in HIV+ MSM: a randomised, placebo-controlled multicentre trial

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### **AIDS Guidelines**

#### **Preliminary results were previously presented at (abstracts included in submission):**

- International Anal Neoplasia Society Scientific Meeting in Amsterdam, The Netherlands, November 1-3, 2019 (oral presentation)
- Dutch HIV Treating Physicians Scientific Meeting Amsterdam, The Netherlands, January 17, 2020 (oral presentation)
- International Papillomavirus Society Conference (digital) July 20-24, 2020 (poster)

# Abstract

## Objective

Anal cancer precursor lesions high-grade anal intraepithelial neoplasia (HGAIN) are highly prevalent among HIV+ men-who-have-sex-with-men (MSM). Treatment of HGAIN is frustrated by high recurrence rates. We investigated the efficacy of the quadrivalent human papillomavirus (qHPV) vaccine as post-treatment adjuvant in preventing HGAIN recurrence in HIV+MSM.

## Design

Randomised, double-blind, placebo-controlled, multicentre trial.

## Setting

Three HIV outpatient clinics in Amsterdam, the Netherlands.

## Subjects

HIV+MSM with CD4 count >350 cells/ $\mu$ l, biopsy-proven intra-anal HGAIN successfully treated in the past year, and lesions still in remission at enrolment, as assessed by high-resolution anoscopy (HRA).

## Intervention

Participants were randomised to three doses of qHPV (Gardasil-4<sup>®</sup>, MSD) or placebo with vaccinations at 0, 2, and 6 months. HRA was repeated at 6, 12 and 18 months.

## Main outcome measure

The primary outcome was cumulative, biopsy-proven HGAIN recurrence rate at 18 months, evaluated in an intention-to-treat (received all vaccinations) and per-protocol analysis (all vaccinations and complete follow-up).

## Results

We randomised 126 participants of which 64 (50.8%) received qHPV and 62 (49.2%) placebo. All

participants received three vaccinations and in both groups for two participants follow-up was incomplete. We found no difference ( $p=0.38$ ) in cumulative HGAIN recurrence rates between the qHPV (44/64, 68.8%) and placebo group (38/62, 61.3%) in the intention-to-treat analysis (absolute risk reduction -7.5 (95%CI -24.1–9.2)). This was similar in the per-protocol analysis.

## Conclusions

Despite adequate serological responses to qHPV vaccination, short-term recurrence of HGAIN was not prevented. These findings do not support qHPV vaccination as a treatment adjuvant to prevent HGAIN recurrence in HIV+MSM.

## Keywords (5-7):

- anal intraepithelial neoplasia (AIN) / anal squamous intraepithelial lesions (aSIL);
- human immunodeficiency virus (HIV);
- human papillomavirus (HPV);
- prophylactic quadrivalent HPV vaccination (qHPV);
- treatment recurrence

## Introduction

Since the introduction of combination antiretroviral therapy (cART) for treatment of HIV infection new causes of morbidity and mortality have become evident. In people living with HIV (PLWH), particularly HIV-positive (HIV+) men who have sex with men (MSM), anal cancer is an increasing problem, with incidence rates up to 85-times higher than in the general population (85 cases per 100,000 person-years vs. 1-2 per 100,000). However, also HIV-negative MSM have a substantially increased risk of developing anal cancer (19 per 100,000).<sup>[1]</sup> Like cervical cancer, anal cancer is causally linked to infections with high-risk (hr) human papillomaviruses (HPV).<sup>[2]</sup> Anal cancer is preceded by precursor lesions called anal intraepithelial neoplasia (AIN), also known as anal squamous intraepithelial lesions (aSIL). AIN can be subdivided into high-grade (HG)AIN (or HSIL) and low-grade (LG)AIN (or LSIL).<sup>[3]</sup> Over 90% of HIV+ MSM have persistent anal HPV infections, and high-grade HGAIN (AIN2/3) is present in 29% of HIV+ MSM.<sup>[4]</sup> As in cervical intraepithelial neoplasia, early diagnosis and treatment of HGAIN have been advocated to prevent malignancy.<sup>[5]</sup> However, treatment of HGAIN in HIV+ MSM (using ablative techniques or topical creams) is frustrated by high recurrence rates, which can be over 50% after 12 months.<sup>[6-8]</sup> Moreover, treatment is costly and burdensome for patients.<sup>[9]</sup>

The prophylactic quadrivalent HPV vaccine (qHPV) is highly efficacious as primary prevention against new persistent cervical infections with hrHPV types 16 and 18 and low-risk HPV (lrHPV) types 6 and 11, and high-grade cervical intraepithelial neoplasia (HGAIN).<sup>[10]</sup> Likewise, it is efficacious in young men (16-26 years) in preventing new anal HPV infections and anogenital condylomata acuminata, caused by lrHPV.<sup>[11]</sup> Vaccination with qHPV also reduced the risk for AIN by 54.2% in young HIV-negative MSM without a history of AIN and a maximum of five sex partners.<sup>[12]</sup>

In recent years, several studies reported a secondary prevention role for the qHPV vaccine. In a non-concurrent cohort study, qHPV vaccination significantly reduced (HR=0.50) the HGAIN recurrence

rate at 2 years after qHPV vaccination in HIV-negative MSM successfully treated for HGAIN.<sup>[13]</sup> A recent meta-analysis also indicated efficacy of qHPV vaccination in preventing recurrent HGCIN lesions in women who were vaccinated around treatment for HGCIN,<sup>[14]</sup> although a recent randomised controlled trial (RCT) could not confirm this for HIV+ women.<sup>[15]</sup> The qHPV vaccine has proven to be immunogenic and safe in PLWH.<sup>[16]</sup> We therefore did a RCT to test the hypothesis that qHPV vaccination as a post-treatment adjuvant prevents recurrence of HGAIN in HIV+ MSM successfully treated for HGAIN in the year before vaccination, and assessed HPV type-specific antibody response and causative HPV types in recurrent HGAIN lesions.

## Methods

### Study design

We performed a randomised, parallel, placebo-controlled, double-blind, multicentre, phase IV trial (VACCAIN-P study) in three outpatient clinics in Amsterdam, the Netherlands (see Supplementary methods, <http://links.lww.com/QAD/C139>). This study was investigator-initiated and government-granted. The study protocol (DOI: <https://doi.org/10.21942/uva.12861044.v1>) was approved by the ethics review board at the Academic Medical Center, Amsterdam, the Netherlands. This trial is registered with ClinicalTrials.gov, number NCT02087384.

### Study participants

We recruited participants from the anal cancer screening program of three outpatient clinics for HIV and dermatology. We obtained written informed consent from all participants and screened for eligibility as described under 'Procedures'.

Main eligibility criteria were: HIV+ MSM of at least 18 years of age, who had a CD4 count >350 cells/ $\mu$ l, had biopsy-proven intra-anal HGAIN which was successfully treated in the past year (lesions with partial remission (from HGAIN to LGAIN (AIN1)) were also eligible), and had lesions still in remission (maximum LGAIN) at enrolment as established independently by two experienced high-

resolution anoscopists at least four weeks after last treatment. For full eligibility criteria see Supplementary methods, <http://links.lww.com/QAD/C139>.

### **Randomisation and masking**

If eligible after screening, we randomly assigned participants to vaccination with qHPV or placebo in a 1:1 ratio using an independent web-based randomisation tool. Randomisation was stratified for treatment centre (academic versus non-academic), timing of first vaccination after last treatment for intra-anal HGAIN (treatment  $\leq$  versus  $>6$  months before vaccination), and result of last treatment for intra-anal HGAIN (complete versus partial (from HGAIN to LGAIN) remission). For details on randomisation and masking see Supplementary methods, <http://links.lww.com/QAD/C139>.

Participants, investigators, and those assessing outcomes were masked to treatment allocation throughout the entire study until database lock for analysis.

### **Procedures**

At time of screening for eligibility a medical record review and laboratory tests were performed. In addition, socio-demographic and sexual history characteristics, smoking status, and self-reported health status were recorded. Participants underwent digital anal-rectal examination (DARE), genital inspection and high-resolution anoscopy (HRA) by two independent experienced anoscopists, with biopsies if indicated as described previously,<sup>[8]</sup> in a single or two separate screening HRA sessions (see Supplementary methods, <http://links.lww.com/QAD/C139>), adhering to the International Anal Neoplasia Society Guidelines.<sup>[17, 18]</sup>

Participants were randomised to the qHPV L1 virus-like particle (VLP) vaccine (Gardasil-4<sup>®</sup>, Merck Sharp & Dohme (MSD), Kenilworth, NJ, USA) or a placebo (0.9% saline). The first qHPV or placebo vaccine was administered within three months after the first screening HRA and six weeks after the second screening HRA, and subsequent vaccines two months ( $\pm 1$  week), and six months ( $\pm 2$  weeks) after first vaccination. Injections, 0.5 ml in the deltoid muscle, were generally given on the same side throughout the study.



DARE, genital inspection and HRA were repeated at six months (FU6; combined with last vaccination), at twelve months (FU12), and at 18 months after first vaccination (FU18; 12 months after last vaccination), allowing  $\pm 2$  weeks deviation from the interval. The focus of this trial was on HGAIN recurrence, therefore, in case of HGAIN recurrence during the study (primary endpoint reached), follow-up, also regarding secondary endpoints, was discontinued and treatment was started according to local procedures.

Biopsies were graded locally by board-certified pathologists, experienced in AIN histopathology. P16<sup>INK4A</sup> immunohistochemical staining was used, if necessary, to distinguish between LGAIN and HGAIN as recommended by the College of American Pathologists.<sup>[19]</sup> HGAIN recurrences were histopathologically confirmed by a second pathologist. Anal cytology was performed in participants that reached the last follow-up at 18 months as an additional check to rule out HGAIN recurrences not detected by HRA (see Supplementary methods, <http://links.lww.com/QAD/C139>).

To determine whether recurrent HGAIN was vaccine type-induced, the causative HPV type in recurrent HGAIN lesions was assessed by HPV detection and genotyping at DDL Diagnostic Laboratory, Rijswijk, the Netherlands, using the SPF<sub>10</sub> DEIA/LiPA 25 version 1 system (see Supplementary methods, <http://links.lww.com/QAD/C139>). In case of multiple HPV types in the whole tissue section, including at least one vaccine type, laser-capture microdissection was used to determine the causative HPV type per biopsy region, as previously described.<sup>[20]</sup> Causative HPV types were assessed on a participant level. In case of multiple lesions per participant, a participant was considered having a vaccine-type recurrent HGAIN if at least one recurrent lesion was caused by a vaccine type.

HPV type-specific antibody response was assessed in venous blood samples, obtained before first vaccination (pre) and 3 months ( $\pm 2$  weeks) after last vaccination (post). Serum HPV-specific IgG antibodies against HPV L1 VLPs for vaccine HPV types 16, 18, 6, 11 were measured using a VLP-based multiplex immunoassay performed at the National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands, as previously described (see Supplementary methods, <http://links.lww.com/QAD/C139>).<sup>[21] [22]</sup>

To assess vaccine safety, (serious) adverse event ((S)AE), including injection-site reactions (pain, erythema, swelling) and systemic AE (including fever and headache) were recorded by history taking and medical record review one week after the vaccinations and at all follow-up visits. AEs that were possibly, probably or definitely related to vaccination, as determined by the investigator, were considered vaccine-related. (S)AEs were classified and graded according to the CTCAE version 4.0, 2010 [on a scale of 1 to 5, with higher scores indicating greater severity].<sup>[23]</sup>

### **Outcomes**

The primary outcome was cumulative recurrence of biopsy-proven intra-anal or peri-anal HGAIN at 12 months after last vaccination (FU18). Secondary outcomes were: recurrence of intra- or peri-anal HGAIN at time of last vaccination (FU6) and at 6 months after last vaccination (FU12), cumulative occurrence of LGAIN at FU18, cumulative occurrence of anogenital condylomata at FU18, causative HPV genotype in recurrent HGAIN lesions, HPV type-specific antibody response after vaccination, and safety of the qHPV vaccine.

### **Statistical analyses**

For sample size calculation see Supplementary methods, <http://links.lww.com/QAD/C139>. Statistical analyses were performed as stated in the Statistical Analysis Plan (DOI:

<https://doi.org/10.21942/uva.12861026.v1>) and using software stated in the Supplementary

methods, <http://links.lww.com/QAD/C139>. The primary outcome (cumulative recurrence of HGAIN at FU18) was evaluated for all randomised participants who received all three vaccinations (intention-to-treat (ITT) analysis), and for participants who received all three vaccinations and completed the follow-up (per-protocol (PP) analysis). Absolute risk reduction (ARR), and relative risks (RR), including 95% confidence intervals (CI), were estimated for the difference in recurrence rate between the qHPV vaccine and placebo group. We constructed Kaplan Meier survival curves with corresponding log-rank tests to compare proportions free of recurrence between the qHPV and placebo group. Participants lost to follow-up were censored at their last visit. Incidence rates (IRs) including 95% CIs were calculated per 100 person-years in the ITT analysis (see Supplementary methods, <http://links.lww.com/QAD/C139>). Vaccine efficacy was calculated as  $1 - (IR_{\text{vaccine}} / IR_{\text{placebo}})$ . Determinants of HGAIN recurrence at FU18 were assessed using a univariable and multivariable logistic regression model (see Supplementary methods, <http://links.lww.com/QAD/C139>), forcing the vaccination group into the multivariable model, as well as the interactions between the vaccination group and the three stratification factors. We estimated odds ratios (OR) and adjusted OR (aOR) with their corresponding 95% CIs.

We performed sensitivity analyses using a worst-case scenario (i.e. participants who were lost to follow-up developed HGAIN if they were free of disease at their last attended visit) and a best-case scenario (i.e. all participants lost to follow-up did not develop the disease if they were free of disease at their last attended visit).

Secondary outcomes were only assessed by ITT analysis (for details see Supplementary methods, <http://links.lww.com/QAD/C139>).

## Results

Enrolment started on March 27, 2014 and was completed on June 1, 2017. A total of 207 HIV+ MSM were screened for eligibility (Figure 1). One hundred twenty-seven (61.4%) men were enrolled and

randomised. After randomisation, one participant turned out to have had his previous HGAIN treatment more than one year before vaccination, thus incorrectly enrolled. His study participation was discontinued and he was excluded from the analyses. Of 126 correctly included and randomised participants, 64 (50.8%) were vaccinated with qHPV and 62 (49.2%) with placebo. All participants received all three vaccines and were eligible for the intention-to-treat (ITT) analysis. All but four (3.2%) participants, two in each group, completed the planned follow-up (i.e. total follow up to FU18 or earlier when reaching the endpoint HGAIN recurrence at FU6 or FU12). Therefore, 122 participants were eligible for the per-protocol (PP) analysis, of whom 62 participants received qHPV and 60 placebo. The study groups were well-balanced, although the qHPV group had higher CD4 counts at enrolment (Table 1). The trial ended on February 20, 2019 after completion of the planned follow-up for all participants and was conducted in accordance with the protocol.

### **HGAIN recurrences**

In total 61.9% (78/126) of participants had recurrent HGAIN within 18 months of follow-up since first vaccination. A total of 103 recurrent HGAIN lesions were detected in these 78 participants. Median number of recurrent HGAIN lesions per participant was 1 [IQR=1-2] and 1 of 78 recurrences (1.3%) was peri-anal. Of the 78 participants with a recurrent HGAIN, 47% recurred at FU6, 24% at FU12 and 28% at FU18. No progression to anal cancer was found during follow-up.

### **Cumulative HGAIN recurrence at FU18**

At 12 months after last vaccination (FU18), cumulative HGAIN recurrence rates (primary outcome) in the ITT worst-case scenario analysis were 68.8% (44/64) for qHPV versus 61.3% (38/62) for placebo, which was not significantly different ( $p=0.38$ ; ARR= -7.5 percentage points (95%CI -24.1–9.2); RR=1.12 (95%CI 0.87–1.45)). The ITT best-case scenario and the per-protocol analyses yielded comparable outcomes. Table 2 summarises all primary and secondary outcome data (see also Supplementary results, <http://links.lww.com/QAD/C139>). Figure 2 shows Kaplan Meier curves for the

proportions free of HGAIN recurrence at FU6, FU12, and FU18 for the ITT and PP analyses.

In the ITT analysis, the incidence rate of recurrent HGAIN was 66.3 per 100 person-years (95%CI 49.0–89.7) for qHPV versus 56.5 (95%CI 40.7–78.3) for placebo (Supplementary table 1, <http://links.lww.com/QAD/C131>). Vaccine efficacy against HGAIN recurrence was -17.4% (95%CI -20.3–-14.6). The PP analysis yielded similar results.

In multivariable analysis, higher CD4 count at enrolment was associated with recurrence (aOR=1.30 per 100 cell increase (95%CI 1.05–1.61);  $p=0.02$ ; Supplementary table 2, <http://links.lww.com/QAD/C132>). Risk for cumulative HGAIN recurrence at FU18 for qHPV compared to placebo was 1.03 ((95%CI 0.32–3.36);  $p=0.96$ ; Supplementary table 2, <http://links.lww.com/QAD/C132>). There were no significant associations between the three stratification factors and the primary outcome. ITT best-case scenario and PP analyses yielded similar results (Supplementary tables 3-4, <http://links.lww.com/QAD/C133>, <http://links.lww.com/QAD/C134>). We refrained from the Cox proportional hazards regression analysis included in the statistical analysis plan because of not meeting test assumptions and the limited number of censored cases.

#### **Causative HPV genotype in recurrent HGAIN lesions**

For 83.3% (35/42) of participants with recurrent HGAIN in the qHPV group and 66.7% (24/36) in the placebo group we could identify the causative HPV type of the lesion ( $p=0.18$ ). Of qHPV recipients 40% (14/35) had recurrent lesion(s) caused by vaccine HPV types, versus 60% (21/35) caused by non-vaccine types, compared to 37.5% (9/24) versus 62.5% (15/24) for the placebo recipients ( $p=0.85$ ). More inconclusive results (i.e. non-diagnostic sample: cut through the lesion or HPV-positive but untypable) were found in the placebo group: 16.7% (7/42) for qHPV vs. 33.3% (12/36) for placebo ( $p=0.09$ ).

### **HPV type-specific antibody response**

All participants were included in the analysis to measure antibody response. In the qHPV group a significant increase in geometric mean concentrations was observed for type-specific antibodies against vaccine types HPV16, 18, 6, and 11 (for all  $p < 0.001$ ; Figure 3; Supplementary table 5, <http://links.lww.com/QAD/C135>). There was no increase in antibody levels in the placebo group (for all  $p > 0.10$ ). About two-thirds of men were already seropositive before vaccination: in the qHPV group 66% (42/64) for HPV16 and 70% (45/64) for HPV18, compared to 68% (42/62) and 69% (43/62) in the placebo group, respectively (Supplementary table 6, <http://links.lww.com/QAD/C132>). After three vaccinations with qHPV, antibody levels for HPV16 and 18 were above seropositivity thresholds in all patients. For cross-reactivity of non-vaccine types see Supplementary results, <http://links.lww.com/QAD/C139>. We refrained from analyses relating the primary outcome with magnitude of HPV-specific antibody responses or HPV serostatus at baseline, as numbers per separate vaccine type were small.

### **Safety**

One or more AE(s) were reported by 90.6% (58/64) of qHPV and 88.7% (55/62) of placebo recipients ( $p = 0.72$ ; Supplementary table 7, <http://links.lww.com/QAD/C137>). Vaccine-related AEs were reported in 67.2% (43/64) and 61.3% (38/62) of qHPV and placebo recipients ( $p = 0.49$ ). qHPV recipients reported more injection-site related AEs ( $p = 0.007$ ). All injection-site related AEs in both groups were mild (grade 1). More details on (S)AEs are presented in Supplementary table 8, <http://links.lww.com/QAD/C138>. No deaths occurred and none of nine SAEs were vaccine- or study-related.

## Discussion

In HIV+ MSM successfully treated for intra-anal HGAIN, vaccination with the prophylactic qHPV vaccine does not prevent short-term recurrence of HGAIN. This finding appeared consistent across all subgroups, accounting for the stratification factors. We could confirm that the qHPV vaccine is immunogenic and safe in HIV+ MSM.<sup>[16]</sup> Vaccination with qHPV induced a significant increase in concentrations of type-specific antibodies against all vaccine types and the non-vaccine types 31 and 45, but two-thirds of participants were already seropositive for vaccine types HPV16 and 18 at baseline. A similar proportion of recurrent HGAIN lesions in both groups were caused by vaccine types, supporting non-efficacy. Additionally, we did not observe significant effects of qHPV vaccination on the secondary outcomes prevention of LGAIN and anogenital condylomata.

Vaccination with qHPV for primary prevention of anal HPV infections and HGAIN has been proven ineffective in PLWH (aged >26 years)<sup>[24]</sup> and HIV+ MSM.<sup>[25]</sup> This in spite of the induction of adequate antibody responses by the qHPV vaccine in PLWH. Efficacy as secondary prevention, i.e. preventing new infections or lesions in patients with ongoing or previous HPV infection and/or resulting premalignant lesions, has been questioned.<sup>[26-28]</sup> A recent meta-analysis indicates efficacy (RR=0.41) of qHPV vaccination as a treatment adjuvant to prevent recurrence of HGCIN.<sup>[14]</sup> However, considerable heterogeneity between studies was noted (different age of patients (mostly younger), timing of vaccination before or after treatment and duration of follow-up), and only one included study was a randomised trial specifically designed to assess prevention of recurrent HGCIN after treatment. However, this study was neither placebo-controlled nor blinded.<sup>[29]</sup> A recent RCT could not confirm prevention of recurrent HGCIN by HPV vaccination in HIV+ women.<sup>[15]</sup> The only study in MSM evaluating qHPV as treatment adjuvant to prevent HGAIN recurrence claimed a significant reduction (HR=0.50) of HGAIN recurrence 2 years after qHPV vaccination, but this was a non-randomised, non-concurrent cohort study of HIV-negative MSM, who were on average slightly

younger than our study cohort.<sup>[13]</sup> We now provided evidence that qHPV vaccination as secondary prevention for HGAIN recurrence in HIV+ MSM is ineffective.

How the secondary prevention efficacy of qHPV as treatment adjuvant observed in aforementioned studies should be explained remains to be elucidated.<sup>[13, 14, 29]</sup> One hypothesis that has been posed in the literature suggests a 'therapeutic' effect of the vaccine.<sup>[30]</sup> This hypothesis seems somewhat counterintuitive, since a combination of innate and adaptive (including T-cell-mediated) immune responses is required to actively clear (residual) AIN lesions, whereas the mode of action of prophylactic (L1 VLP) qHPV vaccination relies predominantly on potent neutralising antibodies. Although the interplay with the immune system is not fully unravelled, vaccination does also induce a L1-specific CD8 T-cell response, however basal keratinocytes at the site of infection do not express L1 and it has been suggested that they may therefore escape the immune system.<sup>[31]</sup>

Another hypothesis in literature proposes a specific 'prophylactic' effect by preventing new lesions caused by HPV types to which patients were not previously exposed.<sup>[30]</sup> In general antibody responses adequately correlate with the efficacy of this vaccine. Our and previous observations of non-efficacy of qHPV in PLWH, in spite of adequate antibody responses, show this is not the case for PLWH.<sup>[24, 25]</sup> We hypothesise that the HIV-infection, the MSM risk group, or the combination is likely to be the cause of this non-efficacy. If this hypothesis would hold true, younger patients could still benefit, as their probability of being previously exposed to these HPV types is generally lower, especially in MSM who often harbour many hrHPV types and anal HPV prevalence does not decrease at older age.<sup>[32]</sup> We found no significant effect in our analysis for the 'younger' age group (<44 years), although our population still consisted mainly of middle-aged (median age 49 years) HIV+ MSM. At baseline two-thirds of participants were already seropositive for vaccine HPV types 16 and 18, showing previous exposure to these types. We cannot rule out that young (HIV+) MSM, possibly naïve for at least some HPV types, would benefit from post-treatment vaccination, given results in



women in which cohorts were generally younger. However, screening for anal cancer in HIV+ MSM, and thus treatment of HGAIN, is generally not started before the age of 30-35 years.

Deshmukh *et al.* modelled cost-effectiveness and concluded that post-treatment adjuvant qHPV vaccination for HIV-negative MSM aged 27 years and older is cost-effective,<sup>[33]</sup> and likewise in HIV+ MSM.<sup>[9]</sup> Based on our findings, post-treatment vaccination is unlikely to be universally cost-effective in HIV+ MSM.

This trial is, to our knowledge, the first RCT designed to investigate in HIV+ MSM the efficacy of qHPV vaccination as post-treatment adjuvant to prevent HGAIN recurrence, confirmed by determining causal HPV types in recurrent HGAIN lesions and assessing immunogenicity with HPV type-specific antibody response. We decided to investigate the clinically relevant outcome of overall HGAIN recurrence, irrespective of possible anal HPV infections at baseline, rather than HPV type-specific efficacy.

Our study has several limitations. First, although participants were thoroughly screened by two experienced high-resolution anoscopists at enrolment and all suspected lesions were biopsied, we cannot rule out that microscopical lesions remained undetected or were misdiagnosed, although this would be equally distributed by randomisation. Anal cytology in case of a negative HRA at enrolment could have slightly lowered this risk, although the diagnostic yield of additional cytology at FU18 turned out to be low. Second, as we did an ITT analysis, starting at first vaccination, a number of participants already had their recurrence at the time of third vaccination (6 months). However, also during the ensuing year we observed no difference in recurrence rates between the two study arms. We acknowledge that the follow-up of 12 months after last vaccination is short. However, in a previous study in HIV-negative MSM, the strongest significant effect was already observed within the first year after last vaccination.<sup>[13]</sup> Moreover, during our follow-up period we already observed recurrence rates that approximate current literature.<sup>[6-8]</sup> Previous follow-up studies after HGAIN

treatment have shown recurrence rates levelling off three years after treatment.<sup>[7]</sup> Hypothetically, vaccination with qHPV could have long-term effects by reaching this plateau phase earlier, lowering the total number of recurrences, and/or result in less treatment-resistant recurrent lesions. For this reason extended follow-up is planned for the study participants. Third, we were unable to determine causative HPV types in all recurrent HGAIN lesions, precluding a definite conclusion on prevention of vaccine-type recurrences. Fourth, our study does not give a decisive answer whether the nonavalent HPV vaccine, which has a wider coverage of HPV types but was not licensed yet during the preparation of this trial, would be efficacious to prevent recurrent HGAIN in HIV+ MSM, although this is not supported by the cross-reactivity we observed for HPV31 and 45. Finally, follow-up was discontinued when the primary endpoint was reached, while ideally follow-up should have been continued to assess secondary endpoints LGAIN and anogenital condylomata. Although we included a worst- and best-case scenario, the results for these endpoints should therefore be interpreted with caution, as is expressed by the wide CIs.

The anal cancer precursor HGAIN is highly prevalent in HIV+ MSM and screening for premalignant lesions is advocated for this group. However, treatment is frustrated by high recurrence rates. In search of a strategy to reduce recurrence of HGAIN, vaccination with qHPV has been suggested. We have now provided evidence from an RCT that there is insufficient scientific rationale to support qHPV vaccination as treatment adjuvant to prevent short-term HGAIN recurrences in HIV+ MSM.

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## **Author's contributions**

JMP and HJCdV are principal investigators of the study. KCMG, HJCdV, and JMP designed the study. KCMG, RPvdZ, MLSvH, IC, AvE, HJCdV, and JMP were involved in clinical data and material collection. CJMvN, HP, and WGVQ were involved in laboratory testing. KCMG and RPvdZ managed the database. RPvdZ, VWJ, and MGWD performed the data and statistical analyses. RPvdZ and VWJ created the figures and tables. RPvdZ and JMP drafted the first version of the manuscript. All authors critically reviewed the manuscript and approved the final version. All authors had full access to all of the data in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis and believe that the manuscript represents honest work. JMP affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; that any discrepancies from the study as planned have been explained; and had full responsibility for the decision to submit for publication. The opinions expressed in this paper are those of the authors and do not necessarily represent those of MSD or its affiliates. MSD was provided the opportunity to review a preliminary version of this manuscript in order to ensure the protection of its proprietary information and intellectual property, but the authors are solely responsible for final content and interpretation.

## **Data sharing statement**

*The study protocol and statistical analysis plan are readily publically available (see Methods).*

*Individual participant data that underlie the results reported in this article (text, tables, figures, and appendices) and a data dictionary will be available for 5 years after publication after de-identification and at request to the corresponding author. Data will be securely transferred to researchers, who provide a methodologically sound research proposal and only to achieve aims in the approved*

*proposal, after: approval of the ethics review board; additional approval of study participants (if applicable); and signing a data access agreement.*

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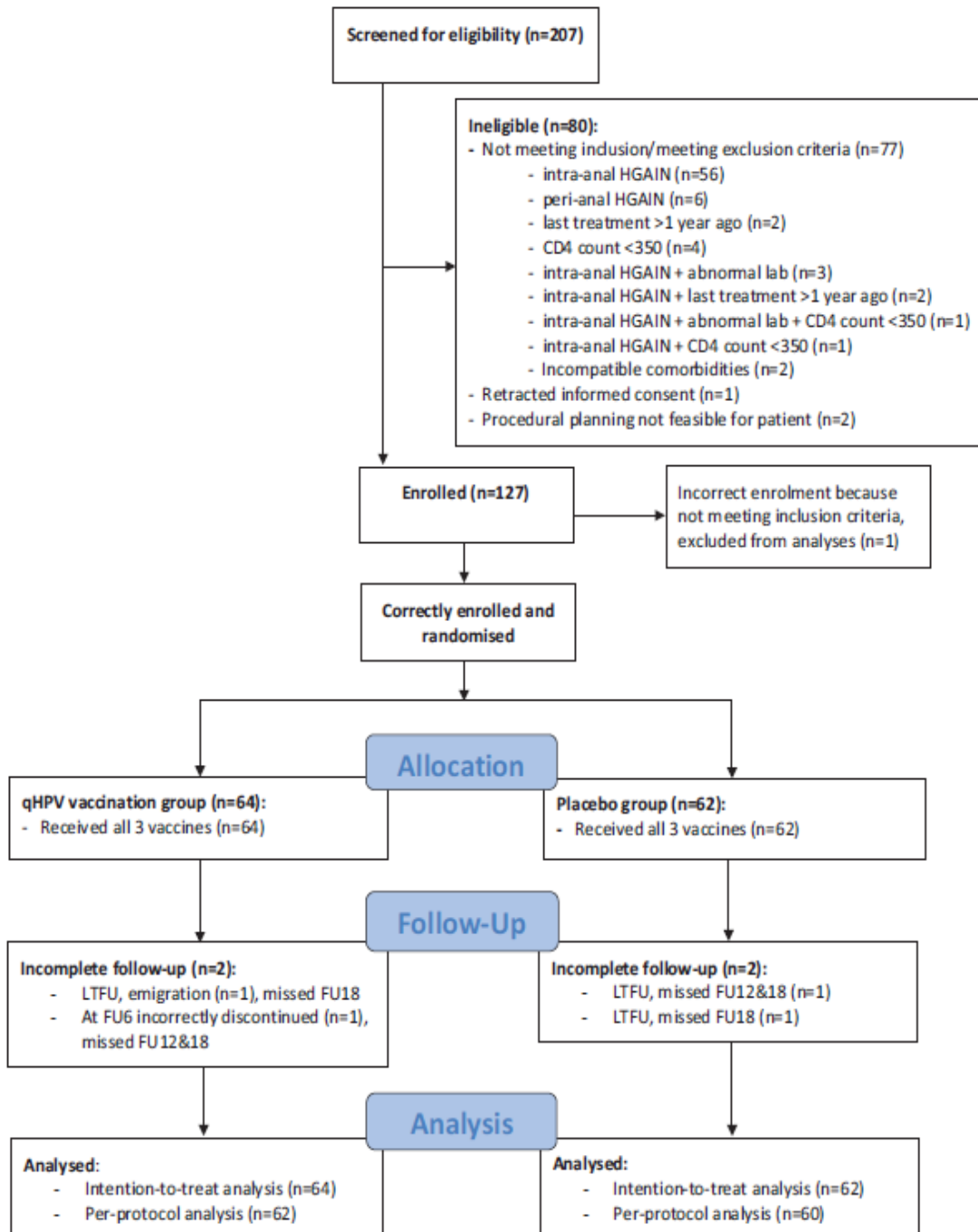
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**Figure 1: Trial profile**

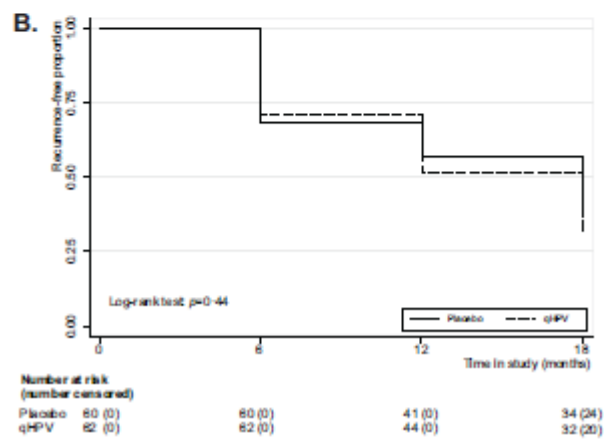
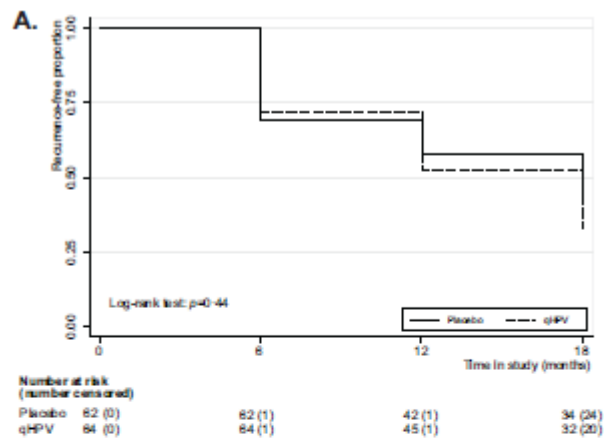
Abbreviations: FU, follow-up; HGAIN, high-grade anal intraepithelial neoplasia; LTFU, lost to follow-up; qHPV, quadrivalent human papillomavirus vaccine



**Figure 2: Proportion of participants free of recurrent HGAIN**

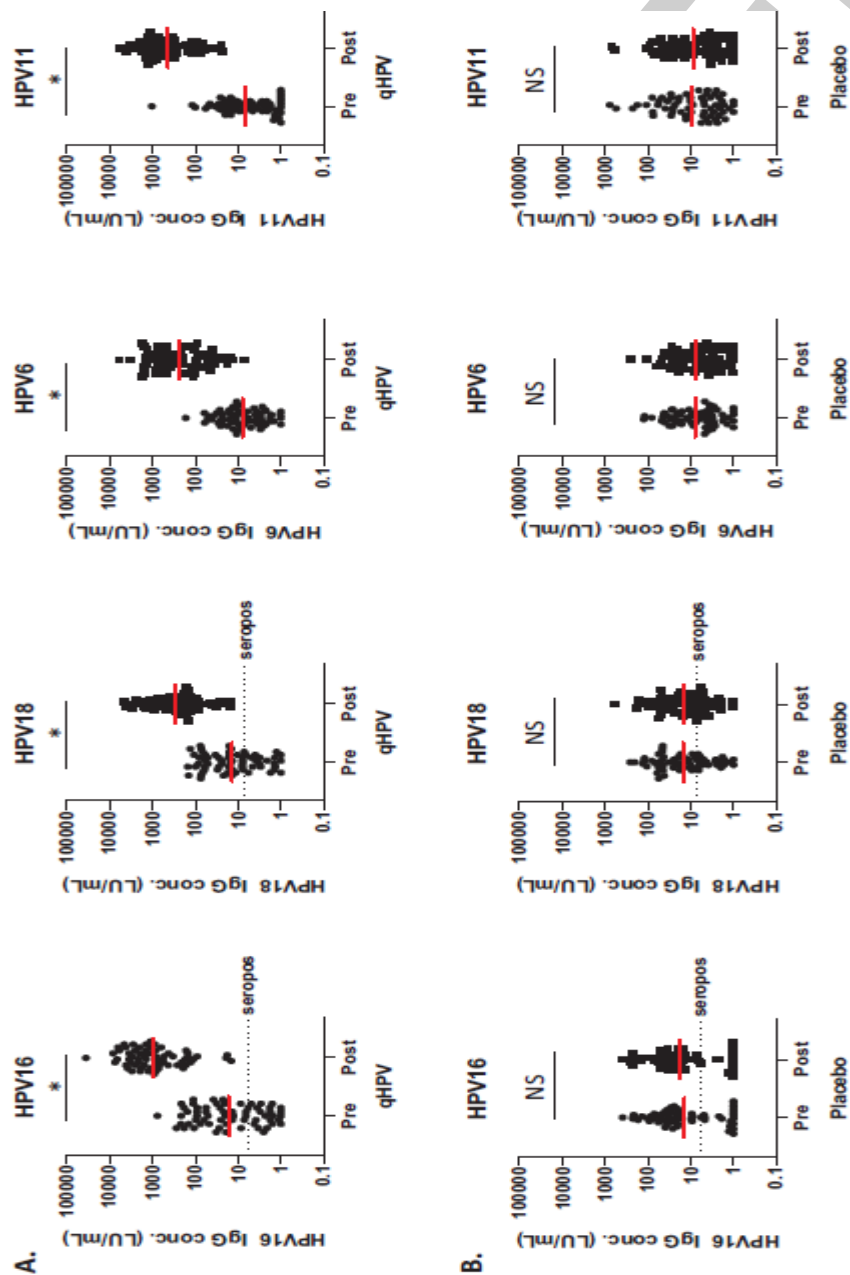
A. Intention-to-treat analysis, B. Per-protocol analysis

Abbreviations: HGAIN, high-grade anal intraepithelial neoplasia; qHPV, quadrivalent human papillomavirus vaccine



**Figure 3: HPV type-specific IgG antibody concentrations before first (pre) and 3 months after last vaccination (post)**

A. qHPV group, B. placebo group. Units: LU/mL. Red bars represent geometric mean concentrations. Dotted line/seropos: seropositivity thresholds: 6 LU/mL (HPV16), 7 LU/mL (HPV18). \* :  $p < 0.001$ ; NS : non-significant ( $p > 0.10$ ; paired t-test). Abbreviations: HPV, human papillomavirus; IgG, Immunoglobulin; qHPV, quadrivalent HPV vaccine; LU/ml, Luminex units per ml



**Table 1: Baseline characteristics of randomised participants**

	qHPV	Placebo	Total
<b>Participants</b>	64 (50.8%)	62 (49.2%)	126 (100%)
<b>Age (years), mean ± SD</b>	48.3 (±8.0)	50.3 (±10.8)	49.3 (±9.5)
<b>Smoking</b>			
- current smoker	15 (23.4%)	18 (29.0%)	33 (26.2%)
- ex-smoker	24 (37.5%)	17 (27.4%)	41 (32.5%)
- never smoked	25 (39.1%)	27 (43.6%)	52 (41.3%)
<b>Years living with HIV, median (IQR)</b>	12 [7-17]	10.5 [6-19]	12 [6-17]
<b>On cART at enrolment</b>	63 (98.4%)	61 (98.4%)	124 (98.4%)
<b>Time on cART (years), median [IQR]</b>	10 [4-15]	8 [4-17]	9.5 [4-15]
<b>Nadir CD4 count (cells/μl), median[IQR]</b>	235 [155-355]	240 [150-330]	240 [150-350]
<b>Current CD4 count (cells/μl), median [IQR]</b>	775 [605-890]	615 [500-800]	700 (560-880)
<b>Recent plasma HIV-RNA load</b>			
- undetectable	63 (98.4%)	57 (91.9%)	120 (95.2%)
- copies/ml if detectable, median [IQR]	53.0 [0] <sup>§</sup>	52.0 [44-151]	52.5 [44-151]
<b>History of any STI</b>	60 (93.8%)	58 (93.6%)	118 (93.7%)
<b>Last intra-anal HGAIN treatment mode, n (%)</b>	8 (12.5%)	4 (6.5%)	12 (9.5%)
- Cryotherapy	49 (76.6%)	52 (83.9%)	101 (80.2%)
- Electrocautery/coagulation	3 (4.7%)	1 (1.6%)	4 (3.2%)
- TCA	0 (0%)	1 (1.6%)	1 (0.8%)
- Imiquimod cream	4 (6.3%)	4 (6.5%)	8 (6.3%)
- Other <sup>&amp;</sup>			
<b>Treatment centre</b>			
- Academic	37 (57.8%)	35 (56.5%)	72 (57.1%)
- non-Academic	27 (42.2%)	27 (43.5%)	54 (42.9%)
<b>Timing first vaccination after last treatment for intra-anal HGAIN</b>			
<6 months	44 (68.8%)	45 (72.6%)	89 (70.6%)
6-12 months	20 (31.3%)	17 (27.4%)	37 (29.4%)
<b>Result of last treatment for intra-anal HGAIN</b>			
- Complete remission	47 (73.4%)	42 (67.7%)	89 (70.6%)
- Partial remission (HGAIN → LGAIN)	17 (26.6%)	20 (32.3%)	37 (29.4%)
<b>Presence of intra/peri-anal LGAIN</b>			
- any	36 (56.3%)	35 (56.5%)	71 (56.3%)
- intra-anal	34 (53.1%)	32 (51.6%)	66 (52.4%)

- peri-anal	4 (6.3%)	3 (4.8%)	7 (5.6%)
<b>Presence of anogenital condylomata</b>	26 (40.6%)	19 (30.6%)	45 (35.7%)
- <b>Presence of genital condylomata</b> <sup>~</sup>	2 (3.3%)	2 (3.2%)	4 (3.2%)
- penile	1 (1.6%)	2 (3.3%)	3 (2.4%)
- scrotal	1 (1.6%)	0 (0%)	1 (0.8%)
- os pubis	1 (1.6%)	0 (0%)	1 (0.8%)
- <b>Presence of anal condylomata</b>	25 (39.1%)	18 (29.0%)	43 (34.1%)
- intra-anal	22 (34.4%)	15 (24.2%)	37 (29.4%)
- peri-anal	6 (9.4%)	4 (6.5%)	10 (7.9%)

Data are n (%), median [IQR], mean ( $\pm$ SD).

Abbreviations: AIN, anal intraepithelial neoplasia; cART, combination antiretroviral therapy; CI, confidence interval; IQR, interquartile range; LGAIN, low-grade anal intraepithelial neoplasia; HIV, human immunodeficiency virus; HGAIN, high-grade anal intraepithelial neoplasia; ml, milliliter;  $\mu$ l, microliter; SD, standard deviation; STI, sexually transmitted infection; TCA, trichloroacetic acid; qHPV, quadrivalent human papillomavirus vaccine

\* 1 missing

<sup>§</sup> only one participant

<sup>&</sup> therapeutic biopsy (n=2); cryotherapy (6 sessions) combined with 5-fluoro-uracil cream(34 weeks) (n=1); electrocautery combined with 5-fluoro-uracil cream (n=5)

<sup>@</sup> could be more than one per participant

<sup>†</sup>Therapeutic biopsy

<sup>~</sup> 2 missing

**Table 2: Summary of primary and secondary outcomes in the different analyses**

	Total	qHPV	Placebo		Absolute risk reduction	Relative risk
	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	<i>p</i> -value	%-point (95% CI)	(95% CI)
<b>Cumulative HGAIN recurrence 12 months after last vaccination (FU18; ITT worst-case)</b>				0.38		
No	44 (34.9%)	20 (31.3%)	24 (38.7%)		-7.5% (-24.1%–9.2%)	1.12 (0.87–1.45)
Yes	82 (65.1%)	44 (68.8%)	38 (61.3%)			
<b>Cumulative HGAIN recurrence 12 months after last vaccination (FU18; ITT best-case)</b>				0.38		
No	48 (38.1%)	22 (34.4%)	26 (41.9%)		-7.6% (-24.5%–9.4%)	1.13 (0.86–1.49)
Yes	78 (61.9%)	42 (65.6%)	36 (58.1%)			
<b>Cumulative HGAIN recurrence 12 months after last vaccination (FU18; PP)</b>				0.37		
No	44 (36.1%)	20 (32.3%)	24 (40.0%)		-7.7% (-24.7%–9.3%)	1.13 (0.86–1.48)
Yes	78 (63.9%)	42 (67.7%)	36 (60.0%)			
<b>Cumulative occurrence of LGAIN 12 months after last vaccination (FU18; ITT worst-case)<sup>§</sup></b>				0.50		
No	16 (29.1%)	7 (25.0%)	9 (33.3%)		-8.3 (-32.3%–15.6%)	1.13 (0.80–1.58)
Yes	39 (70.9%)	21 (75.0%)	18 (66.7%)			
<b>Cumulative occurrence of LGAIN 12 months after last vaccination (FU18; ITT best-case)<sup>§</sup></b>				0.23		
No	33 (60.0%)	19 (67.9%)	14 (51.9%)		16.0 (-9.6%–41.6%)	0.67 (0.34–1.30)
Yes	22 (40.0%)	9 (32.1%)	13 (48.2%)			
<b>Cumulative occurrence of anogenital condylomata 12 months after last vaccination (FU18; ITT worst-case)<sup>&amp;</sup></b>				0.32		
No	41 (50.6%)	17 (44.7%)	24 (55.8%)		-11.1 (-32.8%–10.6%)	1.25 (0.80–1.94)
Yes	40 (49.4%)	21 (55.3%)	19 (44.2%)			
<b>Cumulative occurrence of anogenital condylomata 12 months after last vaccination (FU18; ITT best-case)<sup>&amp;</sup></b>				0.18		
No	76 (93.8)	34 (89.5%)	42 (97.7%)		-8.2 (-19.0%–2.6%)	1.53 (0.53–38.76)

Yes	5 (6.2%)	4 (10.5%)	1 (2.3%)
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Data are n (%). Abbreviations: CI, confidence interval; FU, follow-up; ITT, intention-to-treat; LGAIN, low-grade anal intraepithelial neoplasia; HGAIN, high-grade anal intraepithelial neoplasia; PP, per-protocol; qHPV, quadrivalent human papillomavirus vaccine

<sup>§</sup> Among participant without LGAIN at baseline (n=55)

<sup>&</sup> Among participants without anogenital condyloma at baseline (n=81)

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