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DNA Methylation Analysis to predict Regression of high-grade anal Intraepithelial Neoplasia in HIV+ men (MARINE): a cohort study protocol

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ABSTRACT

Introduction Anal cancer precursors, or high-grade anal intraepithelial neoplasia (HGAIN), are highly prevalent in HIV-seropositive (HIV+) men who have sex with men (MSM). Around 30% of lesions regress within 1 year, but current histopathological assessment is unable to distinguish between HGAIN likely to regress and HGAIN likely to persist or progress to cancer. We aim to assess if host cell DNA methylation markers can predict regression of HGAIN, thus determining the need for immediate treatment or active surveillance. This could reduce overtreatment and the associated anal and psycho-sexual morbidity.

Methods and analysis This is an active surveillance cohort study in three centres located in Amsterdam, the Netherlands, in 200 HIV+ MSM diagnosed with HGAIN. Participants will not be treated, but closely monitored during 24 months of follow-up with 6 monthly visits including cytology, and high-resolution anoscopy with biopsies. The primary study endpoint is histopathological regression of each baseline HGAIN lesion at the end of the study. Regression is defined as ≤low grade anal intraepithelial neoplasia in the exit biopsy at 24 months. Regression proportions in lesions with low versus high methylation levels (*ASCL1*, *ZNF582*), other biomarkers (HPV genotype, HPV-E4, $p16^{INK4A}$, Ki-67) and immunological markers at baseline will be compared. Main secondary endpoints are the histological and clinical outcome (ie, the number of octants affected by HGAIN) of each baseline HGAIN lesion and overall HGAIN disease (i.e., all lesions combined) after each visit. The health-related quality of life of the study group will be compared with that of a control group of 50 HIV+ MSM receiving regular HGAIN treatment.

Ethics and dissemination Ethics approval was obtained from the Institutional Review Board of the Academic Medical Center (Amsterdam, The Netherlands; reference no. 2021_099). Participants are required to provide written informed consent. Findings will be disseminated through publication in peer-reviewed scientific journals and presentations at international scientific conferences; dissemination to policy makers and the target patient group will be achieved through our (inter-)national network, professional associations and collaboration with a patient representative organisation.

Trial registration number NL9664.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ High-grade anal intraepithelial neoplasia (HGAIN) will not be treated, but closely monitored by a team of experienced anoscopists, which will enable to study the natural history of HGAIN in relation to host cell DNA methylation.
⇒ Lesional natural course may be affected by biopsy procedures.
⇒ For one of the principal challenges of this study, namely to establish lesion persistence from one visit to the next, precise documentation, photography and HPV genotyping will be used.
⇒ Due to the number of participants and 2-year follow-up period, progression to anal cancer cannot be studied.

INTRODUCTION

HIV-positive (HIV+) men who have sex with men (MSM) have a 85-fold higher risk to develop anal cancer compared with the general population.[1] Like cervical cancer, anal cancer is mainly caused by infections with high-risk (hr) human papillomaviruses (HPV).[2] Anal cancer precursor lesions, anal intraepithelial neoplasia (AIN; graded 1–3), also known as anal squamous intraepithelial lesions, are divided into low-grade (LGAIN; AIN1) and high-grade AIN (HGAIN; AIN2/3).[3] HGAIN is highly prevalent (29%) in HIV+ MSM.[4] It is therefore recommended to screen HIV+ MSM using high-resolution anoscopy (HRA) and to biopsy suspect lesions. [5] Treatment of HGAIN using electrocautery to prevent cancer is costly, burdensome for patients and has high recurrence rates (>50%) in HIV+

MSM, and is therefore under debate.[6] In addition, only a small minority of HGAIN lesions progresses to cancer. A recent large randomized controlled trial has shown a progression rate of HGAIN of 402 per 100,000 person-years (95% confidence interval, 262 to 616) in patients that were actively monitored for HGAIN, and 173 per 100,000 person-years (95% confidence interval, 90 to 332) in the group of patients that was treated for HGAIN.[7] On the other hand, 28% of HGAIN show complete and spontaneous regression within 1 year.[8–10] At present, we cannot distinguish between HGAIN likely to regress and HGAIN at risk of progression. Consequently, clinical practice in several countries is to treat all HGAIN, leading to considerable overtreatment with subsequent anal and psycho-sexual morbidity.[11] This shows the need to develop methods for risk stratification of HGAIN. Identification of HGAIN with a high probability of regression can reduce the number of unnecessary, burdensome and expensive treatments.

Host cell DNA methylation markers are promising biomarkers to guide such clinical decision-making.[11] DNA methylation markers, such as *FAM19A4/miR124-2* methylation, have been thoroughly investigated and validated for application in cervical cancer screening.[12–18] A methylation negative test demonstrated a significantly lower 14-year cervical cancer risk among HPV-positive women than a cytology negative test.[19] Likewise, for anal cancer prevention, a methylation marker panel containing markers *ASCL1* and *ZNF582* was recently shown and validated to detect anal cancer and AIN3 at high accuracy.[20 21] In HGAIN, a heterogeneous methylation pattern was observed with either low methylation levels, suggestive of HGAIN with a low progression risk towards cancer, versus high methylation levels, or 'cancer-like' methylation patterns, with a high risk of progression towards cancer. Analysis of a retrospectively collected longitudinal series of HGAIN demonstrated that high methylation levels were associated with progression towards cancer.[20 21] The next step is to determine if this marker panel can predict regression of HGAIN in a prospective study. For further characterisation of lesions and to evaluate their potential additive value for risk stratification of HGAIN HPV genotyping,[22] $p16^{INK4A}$, Ki-67 and HPV-E4 immunohistochemistry[23 24] and/or immunological markers will be assessed.

In this prospective active surveillance study, we will clinically validate if the methylation marker panel,[25] potentially combined with additional biomarkers, can predict regression or non-regression of HGAIN, thus determining the need of immediate treatment versus active surveillance. This could reduce overtreatment and the associated anal and psycho-sexual morbidity, and improve anal cancer screening efficacy and quality of life of HIV+ MSM.

METHODS AND ANALYSIS

We initiated a multicentre, active surveillance observational cohort study in which 200 HIV+ MSM with HGAIN lesions will not be treated but closely monitored during a 24-month follow-up. Individuals will be enrolled from three HIV or dermatology outpatient clinics in Amsterdam, The Netherlands, including an academic hospital (Amsterdam UMC), a general hospital (OLVG locatie Oost) and a private clinic (DC Klinieken Lairesse), respectively. Recruitment and enrolment started August 2021 and will be completed after inclusion of 200 participants. Methylation analysis, HPV testing and biomarker analysis will be performed at the Department of Pathology of Amsterdam UMC.

Eligibility criteria

Participants must meet all following inclusion criteria: HIV+ patient at least 18 years of age; cisgender man, transgender man or transgender woman who has sex with men (further referred to as MSM); HGAIN (≥1 lesion) present at the first visit and thereafter confirmed by histopathology; satisfactory HRA at baseline, that is, visualisation of the entire transformation zone with biopsies of all visible lesions. The following exclusion criteria will be applied: HGAIN covering >50% of the circumference of the anal canal (progression to cancer of these patients is estimated to be considerable, and therefore withholding treatment would be unethical)[7 26]; clinical suspicion of anal cancer; diagnosis/history of anal cancer; treatment for HGAIN in the past 6 months; previous HPV vaccination; concomitant cancer and insufficient Dutch or English language skills. Since the proportion of recurring AIN episodes after treatment in current practice is high (>50% of cases), we will include participants with and without a history of (treatment for) AIN.

To evaluate the health-related quality of life (HRQoL) during treatment, we will include a control group of 50 HIV+ MSM from the same clinics fulfilling the same eligibility criteria who undergo treatment of HGAIN in regular care.

Recruitment and informed consent procedure

Participants are recruited from screening programmes for anal cancer in the above-mentioned clinics. Potential participants will be informed about the study by phone, secured message or during regular visits to the clinic. They are invited to take part in the study when attending the clinic and will be asked to give written informed consent at the start of the HRA (online supplemental file 1). During this visit, possible anal lesions will be biopsied for histopathological review and the extent of the lesions will be assessed. Approximately 2 weeks after this initial visit, participants will be informed about the histology results of their biopsies by their HRA provider and will be allowed to continue in the study if the eligibility criteria are met. Participants are excluded from the study and replaced if HRA was unsatisfactory, no baseline HGAIN lesions are diagnosed or anal cancer is diagnosed.

Study procedures

At baseline, sociodemographic, medical, AIN, HIV and sexual history will be recorded in an electronic case report form (eCRF). The participants will undergo the following study procedures at baseline and every 6 months after that (±2 weeks), for a total of 24 months: an anal swab, digital anorectal examination (DARE) and HRA including photo documentation. HRA is performed after at least 1–2 min application of 5% acetic acid followed by repeated application during the exam, and biopsies of all suspected lesions after staining with Lugol's iodine when indicated. The HRA providers are experienced and adhere to the International Anal Neoplasia Society (IANS) guidelines.[5 27] The following lesion characteristics will be recorded: clinical appearance, number, localisation and extent (ie, recorded along eight segments (octants) of the circular transformation zone in the anal canal).

At the last follow-up visit, an exit biopsy is taken from each lesion, or if there is no visible lesion, at random in the octant where an HGAIN lesion was seen at baseline. After the last study visit, participants will return to standard care.

Anal cytology specimens will be collected using a wetted nylon-flocked swab that will be retracted after insertion while rotating and applying firm lateral pressure for 20–30 s, and then transferred and stored in ThinPrep PreservCyt Solution (Hologic, Marlborough, Massachusetts, USA). Anal cytology will be performed as a quality control measure. In case no HGAIN lesions are found, yet cytology indicates high-grade squamous intraepithelial lesions (HSIL) or atypical squamous cells cannot exclude HSIL, HRA will be repeated to make sure no HGAIN lesions were missed. Residual anal swab specimens will be stored at −20°C. The study flow chart (figure 1) shows all study procedures.

In case of a clinical suspicion of cancer during HRA and/or palpable abnormalities during DARE, MRI will be performed to rule out invasive disease. In case of MRI-proven invasion, or histologically proven anal cancer, participation of the patient in the study is stopped and they are referred for further evaluation or treatment according to the local guidelines.

Study outcomes

The primary study endpoint is regression of each individual HGAIN lesion at baseline based on the histological

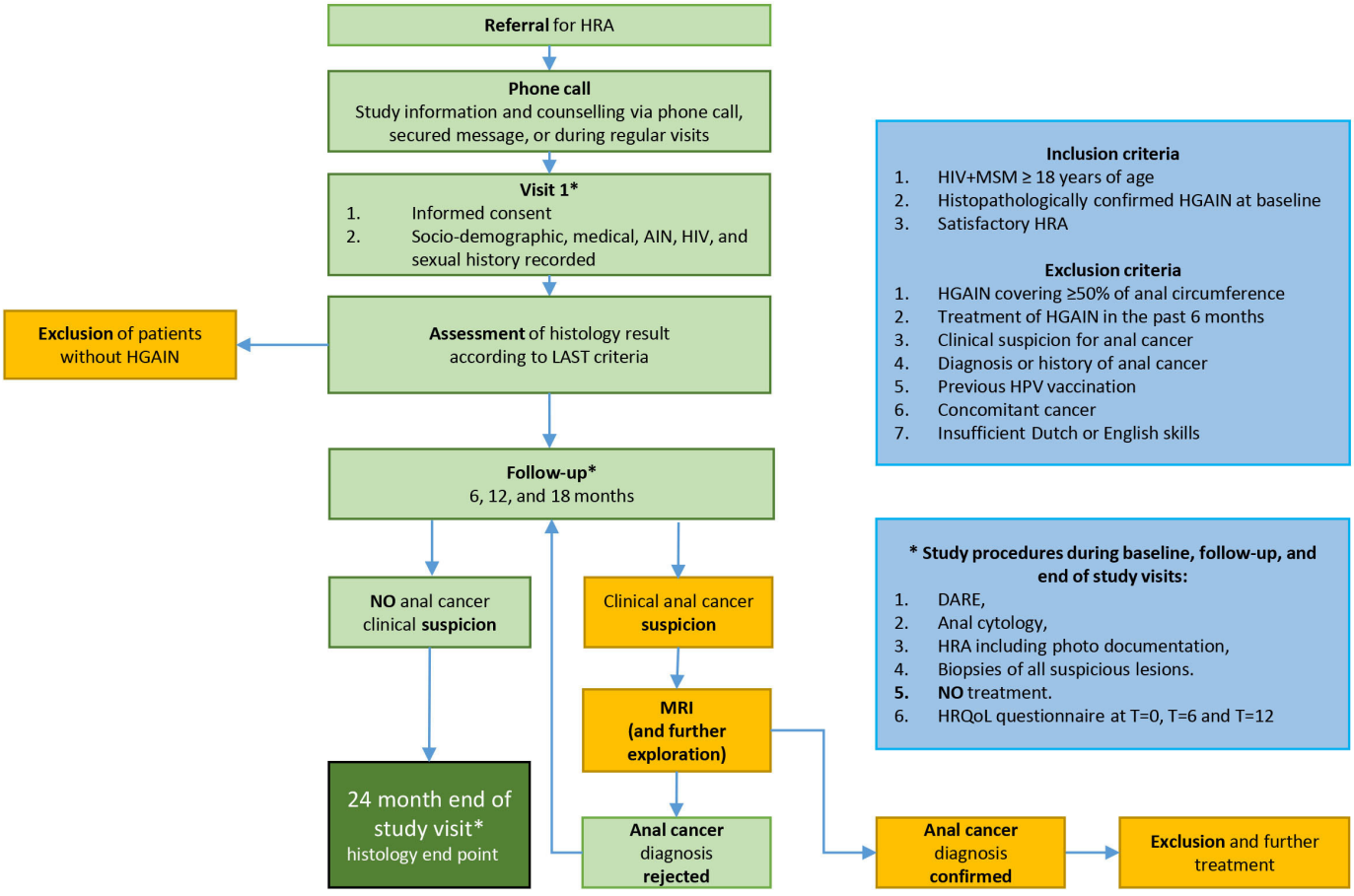

Figure 1 Flow chart of study procedures in the Methylation Analysis to predict Regression of high-grade anal Intraepithelial Neoplasia in HIV+ men study. AIN, anal intraepithelial neoplasia; DARE, digital anorectal examination; HGAIN, high-grade anal intraepithelial neoplasia; HIV+ MSM, HIV-positive men and trans persons who have sex with men; HPV: human papillomavirus; HRA, high-resolution anoscopy; HRQoL, health-related quality of life; LAST, Lower Anogenital Squamous Terminology.

outcome of the 24 months follow-up anal biopsy. The histological outcome is based on the LGAIN/HGAIN dichotomy according to the criteria, terminology and recommendations of the Lower Anogenital Squamous Terminology (LAST) project.[3] *Regression* is defined as the absence of HGAIN lesions in the octant where an HGAIN lesion was detected at baseline, and in the adjacent octants. *Non-regression* is defined as any biopsy-proven HGAIN at 24 months (or anal cancer at any moment during follow-up) in the octant of an HGAIN lesion previously seen at baseline, or in one of the adjacent octants.

Secondary outcomes are to assess:

- the histological outcome of each baseline HGAIN lesion at the 6, 12 and 18 months follow-up visits;
- the clinical outcome of each baseline HGAIN lesion at the 6, 12, 18 and 24 months follow-up visits, defined as a change in extent measured by the number of octants of the anal canal affected;
- overall histological HGAIN outcome: the highest histological outcome of all HGAIN lesions combined at the 6, 12, 18 and 24 months follow-up visits;
- overall clinical HGAIN outcome: the clinical outcome of all HGAIN lesions combined at the 6, 12, 18 and 24 months follow-up visits, defined as a change in the total extent of all HGAIN lesions, measured by the total number of octants of the anal canal affected, including incident HGAIN lesions;
- HRQoL in the study group compared with that of the HRQoL control group, at baseline and at the 6 and 24 months follow-up visits.

Study parameters

Anal biopsies will be formalin-fixed and paraffin-embedded (FFPE). First, a AIN diagnosis will be made by an experienced pathologist. Reporting of the biopsies will be performed in accordance with criteria, terminology and recommendations of the LAST project, that is, $p16^{INK4A}$ immunohistochemistry will be used to support the AIN diagnosis if indicated.[3] At the Department of Pathology of Amsterdam UMC, whole tissue sections will be cut according to the sandwich method and contamination-free DNA isolation procedures. The first and last sections (3 µm) will be H&E stained for lesion confirmation, and to render a revision AIN diagnosis by a second experienced pathologist. In case of discrepancy between the local diagnosis by the first pathologist and the revision diagnosis by the second pathologist, the two pathologists will confer to reach a consensus revision diagnosis that will be used as the study evaluation diagnosis. The highest grade of AIN in a biopsy will be considered the study diagnosis. In-between sections (5–10×10 µm) will be collected in sterile reaction tubes for DNA isolation. DNA will be isolated using the QIAamp DNA FFPE tissue kit (Qiagen, Hilden, Germany). Additional in-between sections (3 µm) will be used for immunohistochemistry staining. Participants are excluded from the analysis if no tissue remains in the biopsy block after initial histopathological grading for the local diagnosis or if the biopsy is negative for HGAIN after lesion confirmation and revision diagnosis.

Liquid-based cytology will be performed on the anal swab and graded according to the Bethesda System.[28]

Biomarker testing will be performed on the baseline biopsies blinded to the clinical or histological outcome. For DNA methylation analysis, isolated DNA will be bisulfite-converted using the EZ DNA Methylation kit (Zymo Research, Orange, California, USA) according to the manufacturer's instructions. Methylation analysis will be performed using a standardised multiplex quantitative methylation-specific PCR (qMSP) assay targeting *ASCL1*, *ZNF582* and the reference gene, β-actin (*ACTB*). H_2O is used as negative control and a calibrator based on target-specific g-blocks (IDT) will serve as internal quality control. Cycle threshold values will be measured at fixed thresholds for fluorescence. A cycle threshold (Cq) of <32 for *ACTB* indicates sufficient DNA quality and adequate bisulfite conversion. ΔΔCq ratios will be computed using the comparative Cq method by comparing the target Cq values with the Cq values of *ACTB* and of the internal quality control calibrator ($2^{-\Delta\Delta Cq}\times100$).[29]

High-risk HPV DNA detection will be performed using the clinically validated general primer GP5+/6+ PCR enzyme immunoassay (EIA), which allows detection of a broad spectrum of genital hrHPV types. Subsequent genotyping of EIA-positive samples will be conducted by a microsphere bead-based hybridisation of GP5+/6+ PCR products (Luminex xMAP, Luminex, Austin, Texas, USA).[30]

Immunohistochemistry staining for $p16^{INK4A}$, Ki-67, HPV-E4 and/or immunological markers will be performed and scored as described earlier.[24 25 31 32] Immunoscoring will be performed by an experienced pathologist blinded to the clinical outcome.

HRQoL will be assessed in the study group and HRQoL-control group at the baseline, 6 and 24 months visits using the ANCHOR Health-Related Symptom Index (A-HRSI). A-HRSI is a validated 5-point Likert scale HRQoL assessment tool for MSM undergoing screening and treatment for HGAIN, assessing physical symptoms (nine items), physical impact (seven items) and psychological symptoms (nine items) over the past 7 days.[33]

Sample size

For the sample size calculation we used the following information: the methylation marker panel for AIN3+ detection in HIV+ MSM has an area under the curve (AUC) of 0.89,[25] with 53% of HGAIN being methylation positive at 90% specificity; 30% of HGAIN show spontaneous regression[7–9]; 13% of HGAIN known to regress during follow-up are methylation positive (pilot data).

These percentages translate into a 10% regression percentage in methylation-positive lesions (13%×30%/53%≈10%) and a 50% regression percentage in methylation-negative lesions (87%×30%/47%≈50%). Given these lesion regression percentages, a sample size of 200 achieves 90% power to detect a difference of at

least 20% in regression percentage. Here, we used a one-sided superiority test with alpha=0.025, margin 20% and assumed 10% loss to follow-up. For only showing a difference between the regression percentages of methylation-positive and methylation-negative lesions (superiority margin 0%, two-sided alpha=0.025), the power is >99%. Power analysis is performed in PASS (V.15.0; NCSS, Kaysville, Utah, USA).

Statistical analysis

Methylation results for baseline biopsies will be obtained by applying a predefined multivariable logistic regression model (marker panel) and calculating predicted probabilities (PP; values ranging from 0 to 1) from ΔΔCq ratios, representing the risk for an underlying AIN3+. For samples with PP above a marker panel cut-off that will translate to a clinically acceptable detection threshold to be determined during an international expert panel meeting, methylation results will be considered methylation positive. The proportion of regressive methylation-negative HGAIN will be compared with the proportion of regressive methylation-positive HGAIN using the χ^2 test. Since participants can have multiple lesions, the unit of analysis will be a single lesion instead of a participant, except when assessing overall HGAIN disease.

Similarly, associations between regressive versus non-regressive HGAIN and lesion characteristics (extent, affected octant number), patient characteristics (eg, age and HIV parameters), history of HGAIN (treatment), HPV genotyping and immunohistochemistry results will be examined by t-tests and χ^2 tests. The effects of multiple variables, including combinations of biomarker results, on the prediction of regression will be studied by logistic regression. Multiple lesions per participant will be included as a fixed-effect co-variate in the model.

For analysis of the HRQoL, the proportions of types of answers per Likert item of the A-HRSI will be displayed in percentages and compared between the study group and the control group using the Mann-Whitney U test. The three sections of the questionnaire (physical symptoms, physical impacts and psychological symptoms) will be likewise compared between the groups using the Mann-Whitney U test.

Statistical analyses will be performed using the IBM SPSS Statistics software (V.26 or higher; IBM, Armonk, New York, USA), Stata software (V.15.1 or higher; StataCorp, College Station, Texas, USA) and/or R statistical software (V.4.0.3 or higher; Foundation for Statistical Computing, Austria) packages.

Data collection and management

The study database is built and used according to the Findability, Accessibility, Interoperability and Reusability principles. Data are collected in eCRFs, designed for this study, on a password-protected secured web-based platform. Data are coded and the participant's identity information is kept by the investigator at a secured place. The database containing all de-identifiable study information is stored at a protected server in The Netherlands.

All anal biopsies collected for histopathological assessment, methylation marker analysis, HPV testing and immunohistochemistry and swabs for cytological assessment are marked at the HRA clinic using the study participant's code and stored in the Methylation Analysis to predict Regression of high-grade anal Intraepithelial Neoplasia in HIV+ men Biobank for future research (online supplemental file 2). Handling of data and material complies with the European General Data Protection Regulation and the Dutch 'Human Tissue and Medical Research: Code of Conduct for Responsible Use' (see also https://www.federa.org/code-goed-gebruik). Data sharing and material transfer agreements are drawn up with collaborating centres to safely exchange coded study data and material. This study is conducted and reported according to the Strengthening the Reporting of Observational Studies in Epidemiology guidelines and Standard Protocol Items: Recommendations for Interventional Trials statement (online supplemental file 3).

Monitoring

The study will be monitored by an independent quality controller from the Amsterdam UMC to assess Good Clinical Practice for quality of study conduct and regulatory compliance. The frequency of monitoring will be at least twice a year. HRA training and skills of anoscopists of participating centres and adherence to the guidelines will be monitored for quality control at the start and during the study using audits.

Patient and public involvement

The patient organisation, Dutch Association of People with HIV ('HIV Vereniging Nederland'), was involved during the design of the study. The organisation evaluated the research proposal on relevance, feasibility, safety and expected willingness to participate after which the protocol has been adapted. They endorsed the clinical need for this study and expressed their support. The organisation is involved in recruitment of participants through their website, magazine and newsletter and during the study, they will oversee the participants' perspective and safety through representation in the study advisory board.

ETHICS AND DISSEMINATION

Ethics approval

The study will be conducted in compliance with the Declaration of Helsinki and the Dutch Medical Research Involving Human Subjects Act (WMO). Ethics approval was obtained at the Institutional Review Board (IRB) of the Academic Medical Center (Amsterdam, The Netherlands; reference no. 2021_099; protocol no. NL76718.018.21) on 13 July 2021). Important protocol modifications will be reported to the IRB as amendments. Participants are required to provide written informed consent before any

study procedures. The study is registered with the Netherlands Trial Register on 3 August 2021, reference number NL9664.

Safety and ethical considerations

Participants will be withheld treatment of HGAIN that potentially prevents progression to anal cancer. Efficacy of treatment is suboptimal and patients experience considerable side effects, justifying the study question. The main risk associated with participation in this study is progression to cancer. However, we consider this risk to be acceptable because: (1) the study follow-up will be 2 years, and the risk of progression to cancer will be low during that period, (2) patients at very high risk for cancer (ie, patients with >50% of anal canal affected with HGAIN, patients with clinical suspicion for cancer and with abnormalities on DARE or MRI) will not be included and (3) participants will be closely monitored during the study and referred for further examination and treatment when clinical suspicion for cancer arises or biopsies confirm progression to cancer.

Progression to anal cancer during the study will be reported to the IRB as serious adverse event. In case of an unexpected high progression rate to cancer (≥3 cases), the study will be prematurely terminated. Since this is an observational study, this premature termination threshold can be easily monitored by the study team and a data safety monitoring board was not deemed necessary by the IRB. Anal cancers that occur in octants without previous HGAIN lesions (or the adjacent octants), so-called interval carcinomas, are not preventable and will therefore not determine premature study termination.

Dissemination

Dissemination to the medical and scientific community will be achieved through publication in peer-reviewed scientific journals and presentations at international scientific conferences. Dissemination to policy makers will be achieved through our (inter-)national network and through professional associations. Dissemination to the target patient group in laymen's terms will be achieved through our collaboration with the patient representative organisation, through their website, magazine and newsletter.

Data availability

Study data and a data dictionary will be available for 5 years after publication of the primary manuscript after de-identification and at request to the corresponding author. Data will be securely transferred to researchers, who have to provide a methodologically sound research proposal and only after approval of the IRB, additional approval of study participants and signing a data sharing agreement.

DISCUSSION

Importance of this study

This study is the first to evaluate the clinical utility of methylation markers to prevent overtreatment of HGAIN in HIV+ MSM. Biomarker-based risk stratification of HGAIN will enable discrimination of men with precursor lesions likely to regress, with a negligible cancer risk when kept under close surveillance, from men with precursor lesions in need of treatment because of risk of disease persistence or progression. This study will provide important results to inform future clinical studies determining the safety of withholding treatment based on a negative methylation test. The goal is to withhold the burdensome treatment procedure in men with a negligible risk of anal cancer, improving the effectiveness of anal cancer screening and quality of life of HIV+ MSM.

Strengths of this study

This longitudinal cohort of HIV+ MSM screened by HRA-guided biopsies and in whom HGAIN will not be treated for 2 years will yield a prospectively collected and well-documented longitudinal series of anal swabs and biopsies, extensively characterised by biomarkers, together with sociodemographical and clinical data, which will enable to study the natural history of HGAIN in great detail. A team of experienced and skilled anoscopists will work according to the IANS guidelines, including regular quality control.[5 27] Moreover, the study team also consists of experienced pathologists, molecular biologists and data scientists and is well-experienced in performing clinical studies in the field of anal cancer prevention.[6 11 34–38]

Anticipated challenges

This study focusses on evaluating the prediction of *regression* of HGAIN lesions based on methylation status, while ideally the relation between methylation and *progression* to cancer is also prospectively studied. However, because progression to cancer is a long-term process and the risk of progression of an individual HGAIN lesions is still relatively low, such studies require vast numbers of participants and extensive follow-up. The principal challenges of this study relate to diagnostic uncertainty and how to determine whether or not a lesion is persistent from one visit to the next. Although HRA will be performed by skilled anoscopists, it is inevitable that lesions will be missed. Therefore, we will use anal cytology as a quality assurance measure to detect any missed lesions. To establish lesion persistence from one visit to the next, precise documentation, photography and HPV genotyping will be used. Anoscopists will study documentation and photography of previous visits to increase the likelihood of finding the same lesion again. An additional challenge is that repeated sampling through biopsies may influence the natural course of a lesion, potentially influencing the study outcome.[26] Patients who are more concerned about their HGAIN lesions might not wish to participate in this study. To minimise selection bias, the HRQoL control group, who will receive regular HGAIN treatment, will be recruited before inclusion of the study group in the same clinics and fulfilling the same eligibility criteria.

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Contributors FDGL, RPvdZ, SD, JB, MFSvdL, JMP, RDMS, and HJCdV were involved in conception and design of the study. FDGL, RPvdZ, JMP, RDMS and HJCdV drafted the protocol paper. JB and MFSvdL provided statistical expertise. All authors critically reviewed the manuscript and approved the final version. FDGL, CJMvN, HJCdV, JMP and RDMS will be involved in data acquisition. Collection, management, analysis and interpretation of data, writing of the report and the decision to submit the report for publication is the responsibility of JMP, RDMS and HJCdV, the principal investigators of the study.

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Competing interests RDMS is minority stockholder of Self-screen, a spin-off company of VUmc, which owns patents on methylation markers and HPV detection. HJCdV received financial compensation or goods for research from Medigene, Gilead and MSD; financial compensation for presentations from Abbott and Janssen and financial compensation for advice to Medigene and Novartis. MFSvdL served on advisory boards of Merck. All other authors reported no potential conflicts.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the 'Methods' section for further details.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

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