SUMMARY OF CHANGES

ANCHOR Study: Anal Cancer Prevention Study
Anal Cancer/HSIL Outcomes Research Study
Version 15.0

NCI Protocol #: AMC-A01
Local Protocol #: ANCHOR

NCI Version Date: 22JAN2021
Protocol Date: 22JAN2021

I. Comments Requiring a Response – Major issues from the 22 December 2020 disapproval letter for protocol version 14:

#	Section	Comments
1.	8.5.11, 8.6.13	Response to comment 2 is unclear: the protocol should make it clear whether missed follow-up evaluation visits are considered a protocol violation. Please note: CTEP does not approve open-ended missed follow up and endpoint re-evaluation visits that can affect the primary endpoint analysis. Guidelines and time limits must be made clear, including when to consider successive missed visits a protocol deviation. It would appear that the investigators wish to follow patients regardless of potential impact on the primary endpoint. If this is the case, then a definition of when a patient can no longer viably contribute to the primary endpoint must be included, along with a sensitivity and feasibility analysis for when the protocol itself can no longer accomplish it's primary goal. **PI Response: There are no stated exemptions from protocol deviations from missed 6-month visits. We stated in our prior response that these are unavoidable deviations. If a site performs a telehealth visit because the 6-month visit cannot be performed, it will still require reporting as a missed visit and is still considered a protocol deviation. This has been communicated previously to sites per CTEP guidance regarding management of the effects COVID-19 on research visits. At CTEP's request, this is now repeated in the protocol.** **Participants are evaluable for the primary endpoint until they develop a confirmed anal cancer diagnosis or expire, or withdraw informed consent for participation. This is described in section 4.5 and the investigators find no other reason to omit a participant from the primary analysis (ITT). As stated in the prior response, telehealth visits also do not supplant in-office visits in determining person-years of follow-up completed. The approach**

#	Section	Comments
		to determining the amount of follow-up for all cases involves calculating the duration between the latest date HRA was performed and the randomization date, and to compare visit completion rates to the expected visit completion rates based on the participant's visit schedule. The DSMB reviews this information during its regular reviews of the protocol, in conjunction with the interim analyses and the dropout rate. While visits have been missed due to necessary COVID-19 -related restrictions, the protocol is not at risk of failing to accomplish its primary analysis (last reviewed by the DSMB November 2020, and to be reviewed at least annually for the remainder of trial duration).

II. Recommendations from the 22 December 2020 disapproval letter for protocol version 14:

#	Section	Comments
2.	8.1.12	The protocol indicates that collection tubes containing oropharyngeal swabs from ARAS-CoV-2 testing must be stored at -70C or colder until shipment to the biorepository (e.g., see page 89). The investigators should confirm that all sites that will participate in the ancillary SARS-CoV-2 study have the facilities to store the collection tubes at this temperature. **PI Response: The study team qualified the freezers available at each site before site initiation. If the clinical site does not have a -70C (or colder) refrigerator, the manual of procedures specifies appropriate alternate procedures (shipping specimens on the same day as collection, or, if collected on a Friday or before a holiday, storing specimens frozen at -20C and then shipping on dry ice on the next available business day). The study specimens may also be stored at -20C and the protocol has been revised accordingly. This level of detail for these special cases is not required for the protocol document and is appropriately managed in a manual of procedures.**
3.	10.6	Under Aim 1 in section 10.6 the protocol states: "With a sample size of 400 for each swab type, the 95% confidence interval for the SARS-CoV-2 prevalence will be no greater than +/- 0.3%." The 0.3% figure appears incorrect. Under the worst-case scenario of a prevalence of 0.5 (50%), the 95% CI width would be 5.0%. Possibly the investigators are computing the width under the hypothesized prevalence of 10%, but in that case the width would be 3.0% and not 0.03%. Assumptions should be clarified, and corrections made as needed. **PI Response: A key sentence was omitted in this section. We postulated that the prevalence of SARS-CoV-2 would be 10%; thus, the width of the 95% confidence interval would be ± 3.0%.**

III. Comments Requiring a Response – Major issues from the 24 November 2020 disapproval letter for protocol version 13:

#	Section	Comments
1.	2.3.11	This section states, "These results provide evidence of A-HRSI clinical responsiveness that will be further explored as part of the ANCHOR trial through refined assessment windows". Please clarify what additional psychometric work on the tool is needed. **PI Response: Please refer to the subsequent sentences that describe how the windows for these evaluations were refined for the QoL objective. This sentence introduces this subsequent description for the QoL objective. Minor phrasing changes were applied so that the reader appropriately identifies the context of this paragraph.**
2.	8.5.11, 8.6.13	Amended sections 8.5.11 and 8.6.13 allow replacing follow-up evaluation visits with a virtual visit. This may compromise evaluation of the primary endpoint and hurt study ability to address its primary goal. Every effort should be made to maintain the follow up visit schedule (missed visits should be rescheduled, and if a visit cannot be rescheduled it should be considered protocol non-compliance). **PI Response: As stated in our prior response, the study team's intent is that every effort must be made to maintain the follow-up visit scheduled. No waiver for the 6-month visit is provided with these changes. Missed visits due to COVID-19 may be unavoidable deviations due to local site closures or restrictions. The intent of this provision is to provide a provision for telehealth when in-person visits are not possible or the participant is unwilling to attend a visit, for the purposes of collecting follow up information for safety, study arm adherence, supporting participant retention, and determining whether the participant has any anal symptoms that may warrant an immediate visit for the participant's care. Clinic staff report that this process supports retention by maintaining communication with the participant when the 6-month visit cannot be scheduled. Sites are still instructed to conduct a makeup 6-month visit once this is feasible again. This statement has been added to the protocol to emphasize this intent.** **The study team believes this process minimizes the effect of COVID-19 on the primary endpoint by allowing clinicians to remain in contact with participants when visits are not feasible and to identify participants who require immediate care due to concern for potential cancer, on the basis of reported symptoms. The study team recognizes this information cannot supplant evaluation for the primary endpoint by HRA with biopsy, but in the presence of pandemic-related restrictions preventing visit conduct, this is the best available approach to weigh when the potential benefit of conducting a visit or providing care outweighs the risk of SARS-CoV-2**

#	Section	Comments
		transmission in the clinic. The information from these follow-up calls are collected in a separate case report form in the database that will not affect the follow-up calculation for the primary analysis; this will be based on the time elapsed between the participant's randomization date and the date of the last HRA performed.
3.	10.1.2	It is not clear why this is an secondary objective: To assess the responsiveness (sensitivity to change) and clinical significance of the A-HRSI subscales by comparing change scores within groups of participants as defined by participant responses to the PGIC item (n=500). Hasn't this work been completed in February with a sample size of 100 patients? **PI Response: This was a copy/paste error that has been deleted. See section 10.1.4 for the QoL objectives, which are also stated in section 1.5.**
4.	10.5	There is no explanation of how missing data will be handled. **PI Response: The sample sizes for the QoL substudy and QoL objective account for rates of non-response and dropout, based on the substudy. In addition to the specified analyses that assume data are missing at random, multiple imputation methods will be applied as a sensitivity analysis to assess the impact of missing data that may not be missing at random.**

IV. Comments Requiring a Response – Major Issues from the 25 June 2020 Disapproval Letter for protocol version 12:

#	Section	Comments
5.	2.3.11	Aims of the A-HRSI substudy were to validate the HR-QoL questionnaire-final data collection was completed in February 2020 and final results are still being analyzed. Based on the interim results, the questionnaire is now being implemented in the ANCHOR study. However, the results of the psychometric work are not reported in the protocol. Please provide the results, scoring, responsiveness over time and the change in score that is considered clinically meaningful. There is no information regarding clinical significance of calculated non-normalized ES for the A-HRSI subscales. **PI Response: Results from the A-HRSI substudy have been added to the background section (section 2.3.11). In section 10.5, we comment on the minimum differences we can detect based on our sample size calculations for the final implementation of A-HRSI as compared to those seen in the responsiveness substudy when comparing those with worse ECOG PS scores versus those with no change. The A-HRSI substudy provided a preliminary indication of change that would be considered clinically meaningful.**

#	Section	Comments
6.	4.1 8.5.10, 8.6.13	Section 4.1 states patients will undergo HRA every 6 months, and biopsy of HSIL every 12 months. New sections 8.5.1 and 8.6.13 provide for visit deferrals with the possibility of telemedicine consultation instead. There seems to me limit on length of deferrals on these follow-up exams, or provisions for scheduling a deferred follow-up visit. Please reconcile section 4.1 with the appropriate sections in 8, and provide more direction for how much deferral can occur before requiring patients to come off study. **PI Response: Per the guidance of the ANCHOR DSMB during its second visit in December 2015, participants should not be taken off study unless they expire or their disease satisfies the primary endpoint (progression to cancer). As this is a time to event study, if a participant remains willing and able to return to the trial, a visit should be scheduled to perform HRA with biopsy regardless of whether prior visits were missed or performed as a telemedicine follow-up visit. Telemedicine visits during the COVID pandemic are a means to continue to collect data on participant safety and study arm compliance if an in-person visit is not feasible due to COVID-related restrictions only.**
7.	8.2, 8.7 2.3.11	There is not justification provided for the 9 timepoints of measurement. **PI Response: As discussed during a teleconference with representatives from CTEP on 25AUG2020, time 2 (T2) will be revised to occur 2-7 days after randomization, to correspond with the period during which most participants are symptomatic after the initial treatment or monitoring arm HRA, and time 3 (T3) has been revised to occur at 4 weeks after randomization, at which point the investigators would not expect participants to be symptomatic. Subsequent time points 4-8 are now scheduled to occur around annual visits through the fifth year of study participation, to evaluate any long-term psychological effects of each study arm's strategy. Time 1 (baseline) remains within 2 weeks before randomization. Time points 4-8 will be used in an exploratory analysis in order to characterize prospective changes in HR-QoL.**
8.	8.4.7	The value of completing the HR-QoL questionnaire 2 weeks post-randomization is unclear. Is there an expected change from the baseline measurement in such a short period of time? **PI Response: Please see our responses to items #3 and 6 for the rationale for time point 2 and its role as the expected timeframe in which physical effects of the monitoring and treatment strategies will be measured.**
9.	8.6.10.1	Please explain the new provision to allow treatment of HSIL if it was biopsied 6 months prior.

#	Section	Comments
		PI Response: This provision permits empirical treatment of clinically suspected HSIL lesions in the treatment only arm only if the biopsies are collected at the same visit as they are treated and not the prior six month visit. The new provision allows clinicians to treat these lesions to avoid having to biopsy the lesion, wait for the result and bring the participant back at a separate visit for treatment, as long as their suspicion of cancer is low. To confirm they that did not unintentionally treat a cancer, we do however require that they biopsy the lesion before treating it.
10.	10.5.2	There is no stated hypothesis for the HR-QoL sub-study. **PI Response: The primary endpoint for the QoL objective is changes in physical symptoms and impacts from T2 to T3, adjusting for T1. It is anticipated that participants assigned to the treatment arm will experience significant reduction in physical symptoms and impacts from T2 to T3 versus those in the active monitoring arm. The secondary endpoint for the QoL objective is changes in psychological symptoms from T3 to T4, adjusting for T1. There is uncertainty of the timing of psychological symptoms related to lack of treatment among those in the active monitoring arm, but it is anticipated that participants assigned to the active monitoring arm will experience significant increases in psychological symptoms from T3 to T4, a later time point compared with those in the treatment arm. The exploratory endpoint is HR-QoL changes in physical symptoms/impacts and psychological symptoms from T4 through the subsequent T5-T8 follow-ups.**
11.	10.5.2	The HR-QoL study endpoint is not clear. The investigators state they will perform mixed model repeated measures ANOVA with change at baseline calculated at each follow up visit for each subscale. Do the investigators intend to do a longitudinal analysis? What comparison and timepoint(s) are of interest? **PI Response: Please see our response to item #6. The analyses for these endpoints are detailed in section 10.5.2.**
12.	10.5.2	It is not appropriate to use 5% two-sided alpha per sub-scale, because of multiplicity of analyses. The overall type one error should be controlled for at 5% level. **PI Response: For the primary endpoint, we are testing two subscales and anticipate that participants assigned to the treatment arm will experience significant reduction in physical symptoms and impacts from T2 to T3 versus those in the active monitoring arm. Therefore, we will conduct two tests at the one-sided alpha level of 0.025, based on applying a Bonferroni correction to maintain an overall one-sided alpha level of 0.05.**
13.	10.5.2	For responsiveness, the investigators consider the possibility of a scenario leading to type one error +0.31. If this scenario is realistic, analysis does not

#	Section	Comments
		make sense. **PI Response: This language was not a part of the current amendment for the main QoL objective but was related to the responsiveness substudy. It was intended to show increased Type I error if effect sizes were smaller than what the substudy was designed around.**

V. Scientific and Substantive Changes

#	Section	Description of Change
14.	Synopsis 1.6 2.6 3.5 3.6 3.7 8.1.12 8.2 8.5 10.1 10.6 13.0 Appendix I	The protocol has been revised throughout to add a supplemental study to describe detection of SARS-CoV-2 in anal swab samples from PLWH being screened for the ANCHOR study; examine its relationship to prevalent anal HPV infection and HSIL in the screening population; and determine its effect on the natural history of anal HPV infection and HSIL by examining its relationship to regression of HSIL and clearance of HPV infection in the subset of enrolled participants randomized to the active monitoring arm. Accordingly, our specific aims are outlined in Section 1.6.
15.	Synopsis Schema 1.5 2.3.11 2.4 2.4.1 2.4.2 3.3 3.4 3.6.1 3.7 8.3 8.4 8.5 8.6 8.7 8.8 8.9 10.1 10.2	The protocol was revised to report completion of the A-HRSI scale responsiveness substudy and to implement the A-HRSI instrument in the protocol as a formal QoL aim. The objectives, eligibility criteria, study procedures, statistical section, and appendices were revised accordingly. The QoL aim will also include collection of data using the Spanish language version of the A-HRSI as validated in the AMC-A04 protocol, *Development of a Health-Related Symptom Index for Spanish-Speaking Persons Diagnosed with and either Treated or Monitored for Anal High-Grade Squamous Intraepithelial Lesions (HSIL).*

#	Section	Description of Change
	10.4 Appendix I Appendix X Appendix XI Appendix XII Appendix XIII	
16.	2.3	A definition for an anal cancer case that qualifies for the primary endpoint was added to the protocol, based on current National Comprehensive Cancer Network (NCCN) guidelines for the extent of disease that is considered anal squamous cell carcinoma.
17.	4.3	The exclusion criterion prohibiting recent receipt of other investigational drugs (excepting investigational ART agents, treatments for Hepatitis C, and treatments/vaccinations for SARS-CoV-2) has been extended as prohibited therapy during study participation.
18.	6.4	Data on COVID-19 diagnoses (suspected and confirmed) will now be collected as routine adverse events, for the purpose of identifying cases in the future as needed for ancillary research proposals in development.
19.	8.5.11 8.6.13	Telephone follow-up procedures were added to the protocol for 6-month visits unable to be conducted due to COVID-19, to formalize the study's procedures for maintaining contact with participants and evaluating patient safety and adherence to the assigned study arm to the extent feasible during the SARS-CoV-2 pandemic.
20.	Appendix III	Guidance regarding insufficient or inconclusive cytology at screening was added, requiring repeat testing if it occurs. Valid results must be available prior to randomization (may occur up to 12 weeks prior to randomization).

VI. Administrative and Editorial Changes

#	Section	Description of Change
21.	Global	The protocol version and version date were updated to version 15.0, dated 22JAN2021.
22.	Global	Spelling, grammar, and editorial changes (abbreviations, company names) were applied throughout the document.
23.	Global	The manufacturer name for topical medications was updated from Valeant Pharmaceuticals to Bausch Health.

#	Section	Description of Change
24.	List of Tables List of Figures	Lists of tables and figures were added to the protocol, and each table and figure renumbered accordingly.
25.	2.1.1	The background section was updated with current anal HSIL incidence estimates.
26.	8.5.9.1 8.6.10.1	Statements regarding empiric treatment of HSIL in the treatment arm following biopsy at a given visit were repeated in the evaluations section at visits after randomization.
27.	Appendix VI	The AMC Data and Safety Monitoring Plan, which applies to all AMC protocols, was updated from version 6.0 to version 9.0. Key revisions include: the addition of an introduction to address the variety of systems the AMC uses for individual trials; changes to the data entry systems used by domestic AMC trials (OPEN/Rave) activated September 1, 2020 and later; participation with a single IRB for new domestic AMC protocols; updated procedures for data reporting, determination of requirements for DSMB review, and safety review/pharmacovigilance; and administrative changes (updates to document organization, external links, and group terminology).

the ANCHOR study

ANCHOR Study: Anal Cancer/HSIL Outcomes Research Study (AMC Protocol #A01)

A Collaboration of the
AIDS Malignancy Consortium
Human Papillomavirus Working Group
and the University of California, San Francisco

Sponsored by:	National Cancer Institute Office of HIV and AIDS Malignancy (OHAM)
NCT Registration Number:	NCT02135419
Regulatory Status:	IND exempt drugs IDE exempt study devices
Commercially Available Agents:	5-Fluorouracil (5-FU) (NSC 19893) Topical Imiquimod Cream (5%) (NSC 741062)
Protocol Chair:	Joel Palefsky, MD, CM, F.R.C.P.(C)
Protocol Co-Chair:	J. Michael Berry, MD

AMC-A01 Version 15.0, 22 January 2021

NCI Version Date 22 January 2021

PROTOCOL SIGNATURE PAGE

I, _____, Principal Investigator at site _____, agree to conduct and follow this protocol: **ANCHOR Study (AMC-A01, Version 15.0, 22JAN2021),** as written according to NCI, OHRP, and FDA guidelines, and group policies. I understand that no deviations from the protocol eligibility criteria or waivers for protocol deviations will be permitted.

_____ _____

PI Signature Date (mm/dd/yyyy)

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PROTOCOL ROSTER

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Anal Cancer/HSIL Outcomes Research Study

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PROTOCOL SYNOPSIS

Title:	ANCHOR Anal Cancer Prevention Study
Phase of Study:	Phase III
Participating Institutions:	This protocol will be open to sites that have been approved for participation by the ANCHOR Coordinating Committee and have at least 2 practitioners certified in high resolution anoscopy (HRA) and 1 practitioner certified in HSIL treatment (infrared coagulation (IRC), hyfrecation/electrocautery, or laser ablation) by the ANCHOR HRA Training and Certification Committee. At least fifteen sites will be targeted for participation.
Accrual Target:	5,058 participants
Population:	Men and women age 35 and older with HIV infection and biopsy-proven high-grade squamous intraepithelial lesions (HSIL) at baseline. Eligible participants will have no history of anal cancer, or treatment or removal of HSIL. Approximately 17,385 participants who provide informed consent for study participation will be screened to identify 5,058 eligible participants with previously untreated HSIL.
Regimen:	Eligible participants will be randomized to treatment or active monitoring at baseline. Participants will be followed every six months for HSIL outcomes for up to five years after the last participant's date of randomization. Throughout the study, the incidence of invasive cancer in both arms will be monitored, and biospecimens and associated participant data will be collected for correlative science studies. Effects of the treatment and monitoring strategies on participants' quality of life will be assessed using the ANCHOR Health Related Symptom Index (A-HRSI) in a subset of consenting participants (n=500) before randomization and at 7 time points through 5 years after randomization).
Ancillary Studies:	Anal shedding of SARS-CoV-2: relationship to anal HPV infection and high-grade squamous intraepithelial lesions in people living with HIV (Availability of Urgent Competitive Revision and Administrative Supplements on Coronavirus Disease 2019, PA-18-59), Section 2.6
Duration:	Minimum of 5 years of follow-up for each participant

Primary Objective:

To determine whether treating anal high-grade squamous intraepithelial lesions (HSIL) is effective in reducing the incidence of anal cancer in HIV-infected men and women.

Secondary Objectives:

- To determine the safety of infrared coagulation (IRC), electrocautery, imiquimod, laser and 5- fluorouracil treatments for anal HSIL.
- To assess the responsiveness (sensitivity to change) and clinical significance of the ANCHOR Health-Related Symptom Index (A-HRSI) subscales by comparing change scores within groups of participants as defined by participant responses to the participant global impression of change (PGIC) item.

Exploratory Objectives:

Collect clinical specimens and data to create a bank of well-annotated specimens that will enable correlative science:

- Identification of viral factors in HSIL progression to cancer;
- Identification of host factors in HSIL progression to cancer;
- Identify host and viral biomarkers of progression from HSIL to cancer;
- Identify medical history and behavioral risk factors for HSIL progression to cancer.

Quality of Life Objectives:

Primary QOL Objective: To compare arms in terms of changes in physical symptoms and impacts from T2 to T3, adjusting for T1.

Secondary QOL Objective: To compare arms in terms of changes in psychological symptoms from T3 to T4, adjusting for T1.

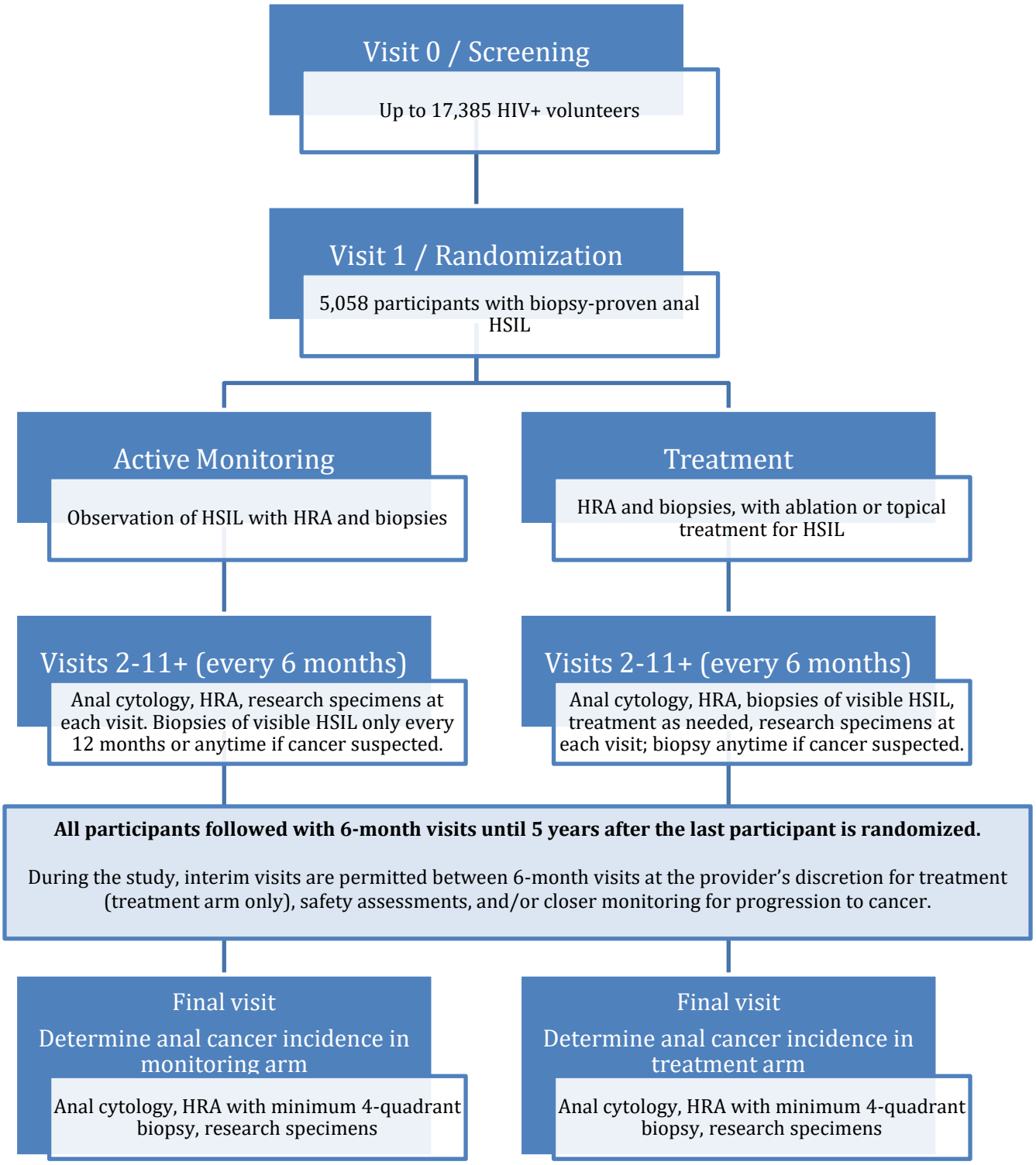
Exploratory QOL Objective: To assess of long-term HR-QoL changes in physical symptoms/impacts and psychological symptoms from T4 through the subsequent T5-T8 follow-ups overall and by arm.

Ancillary Study Objectives:

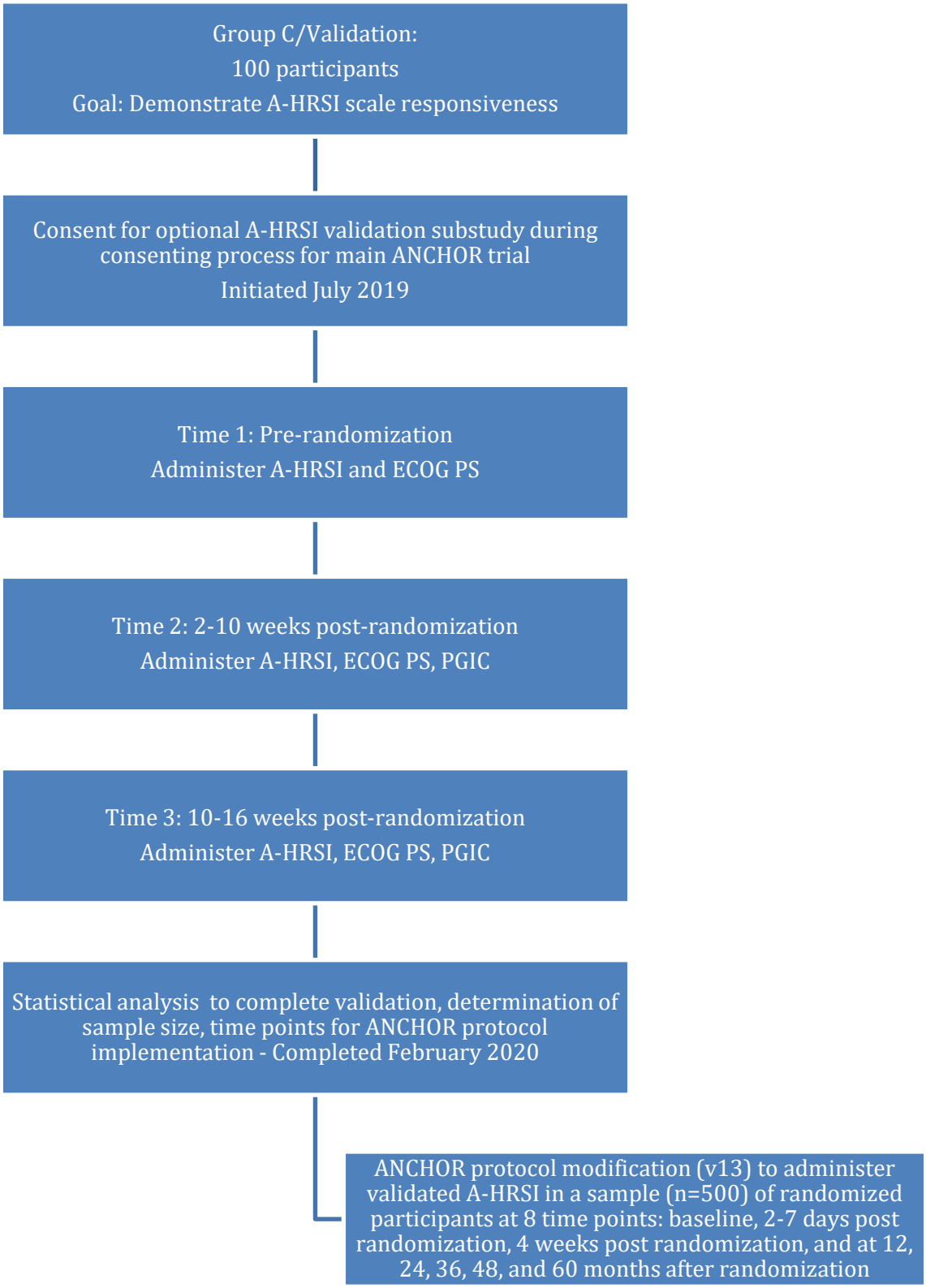
1. Determine the prevalence of SARS-CoV-2 detection in anal and oropharyngeal swabs among people living with HIV (PLWH) being screened for the ANCHOR study
2. Determine the relationship in the ANCHOR screening population between prevalent anal SARSCoV-2 positivity, anal HPV infection, and anal high-grade squamous intraepithelial lesions (HSIL)
3. Determine the 6-month incidence of SARS-CoV-2 detection in anal and oropharyngeal swabs among participants with anal HSIL newly enrolled into the ANCHOR study

4. Determine the relationship between prevalent or incident SARS-CoV-2 detection and regression of anal HPV infection or HSIL among participants newly enrolled into the ANCHOR study and who are randomized to the monitoring arm

PROTOCOL SCHEMA



ANCHOR Health-Related Symptom Index (A-HRSI) Validation and Implementation Schema



LIST OF ABBREVIATIONS

5-FU	5-fluorouracil
A-HRSI	ANCHOR Health-Related Symptom Index
ACSR	AIDS and Cancer Specimen Resource
ADL	Activities of daily living
AE	Adverse event
AIDS	Acquired immunodeficiency syndrome
AIN	Anal intraepithelial neoplasia
AM	Active monitoring
AMC	AIDS Malignancy Consortium
AML	Acute myelocytic leukemia
AMC	AIDS Malignancy Consortium
ANC	Absolute neutrophil count
ANCHOR	Anal Cancer/HSIL Outcomes Research Study
APOT	Amplification of Papillomavirus Oncogene Transcript
ART	Antiretroviral therapy
ASCCP	American Society for Colposcopy and Cervical Pathology
ASIL	Anal squamous intraepithelial lesions
AWE	Acetowhite epithelium
BID	Bis in die (twice a day)
cART	Combination antiretroviral therapy
CBC	Complete blood count
CDC	Centers for Disease Control and Prevention
CDUS	Clinical Data Update System
CLIA	Clinical Laboratory Improvement Amendments
CR	Complete response
CRT	Chemoradiation therapy
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
CTEP-AERS	Cancer Therapy Evaluation Program Adverse Event Reporting System
CTMS	Clinical Trials Monitoring Service

DAB	Diaminobenzidine
DARE	Digital anorectal examination
DARF	Drug accountability record form
DE	Differentially expressed
DHHS	Department of Health and Human Services
DMC	Data management center
DMR	Differentially methylated region
DNA	Deoxyribonucleic acid
DSMB	Data and Safety Monitoring Board
ECOG	Eastern Cooperative Oncology Group
ELISA	enzyme-linked immunosorbent assay
EMT	Epithelial-mesenchymal transition
ePRO	Electronic patient-reported outcomes
FCBP	Female of childbearing potential
FDA	Food and Drug Administration
FDR	False discovery rate
FFPE	Formalin-fixed paraffin-embedded
FU	Follow-up
GO	Gene Ontology
HIER	Heat-induced epitope retrieval
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
HRA	High resolution anoscopy
HRQoL	Health-related quality of life
HSIL	High grade squamous intraepithelial lesions
IDB	Investigational Drug Branch
IDE	Investigational device exemption
IHC	Immunohistochemistry
IND	Investigational new drug
IRB	Institutional review board
IRC	Infrared coagulation

ITT	Intent-to-treat
LAST	Lower anogenital squamous terminology
LCM	Laser capture microdissection
LSIL	Low grade squamous intraepithelial lesions
KPS	Karnofsky Performance Score
MDS	Myelodysplastic syndrome
MOP	Manual of Procedures
MSM	Men who have sex with men
NCBI	National Centre for Biotechnology Information
NCI	National Cancer Institute
NCTN	NCI Clinical Trials Network
ODMC	Operations and Data Management Center
OHAM	Office of HIV AIDS Malignancy
PCR	Polymerase chain reaction
PGIC	Participant global impression of change
PIO	Protocol Information Office
PR	Partial response
PS	Performance status
RACE	Rapid amplification of cDNA ends
RCT	Randomized controlled trial
RLU	Relative light units
RMA	Robust Multi-Array
RNA	Ribonucleic acid
SAE	Serious adverse event
SCC	Squamous cell carcinoma
SCJ	Squamocolumnar junction
SIL	Squamous intraepithelial lesion
SIR	Standardized incidence ratio
SISCCA	Superficially invasive squamous cell carcinoma
SOC	System organ class
SOP	Standard Operating Procedure

STD	Sexually transmitted disease
TNF	Tumor necrosis factor
TUA	Treatment under anesthesia
TZ	Transformation zone
UADE	Unanticipated Adverse Device Effect
UCSC	University of California, Santa Cruz
UCSF	University of California, San Francisco

1.0 OBJECTIVES

1.1 Hypothesis

Treatment of anal high-grade squamous intraepithelial lesions (HSIL) will lead to a reduction of 75% of incident anal cancer compared with a population with anal HSIL that is observed without treatment.

1.2 Primary Objective

To determine whether treating anal HSIL is effective in reducing the incidence of anal cancer in HIV-infected men and women.

1.3 Secondary Objectives

1.3.1 To determine the safety of infrared coagulation, electrocautery, imiquimod, laser, and 5-fluorouracil treatments for anal HSIL.

1.3.2 To assess the responsiveness (sensitivity to change) and clinical significance of the A-HRSI subscales by comparing change scores within groups of participants as defined by participant responses to the PGIC item.

1.4 Exploratory Objectives

1.4.1 Collect clinical specimens and data to create a bank of well-annotated specimens that will enable:

a) Identification of viral factors in HSIL progression to cancer

1.4.1.a.1 Determine the HPV type in cancer and compare to that of overlying HSIL and HSIL biopsies collected concurrently that did not progress to cancer.

1.4.1.a.2 Determine the strain variant of HPV 16 in participants who progressed to anal cancer and compare to participants with HSIL biopsies who did not progress to cancer.

1.4.1.a.3 Determine the HPV integration site in overlying anal cancer to that of HSIL overlying the cancer and HSIL biopsies collected concurrently that did not progress to cancer.

b) Identification of host factors in HSIL progression to cancer

1.4.1.b.1 Perform gene expression array analysis comparing expression in anal cancer with HSIL overlying the cancer. Perform gene expression array analysis comparing expression in HSIL biopsies that progressed to cancer with non-progressing HSIL biopsies at other locations. Perform similar analyses comparing expression in HSIL biopsies that progressed to cancer with the same lesion at earlier time points prior to progression.

1.4.1.b.2 Characterize genetic changes in anal cancers compared with HSIL overlying the cancer. Characterize genetic changes in HSIL biopsies that progressed to cancer compared with non-progressing HSIL biopsies at other locations. Characterize genetic changes in HSIL

biopsies that progressed to cancer with the same lesion at earlier time points prior to progression.

c) Identify host and viral biomarkers of progression from HSIL to cancer

1.4.2 Evaluate medical history and behavioral risk factors for HSIL progression to cancer.

1.5 Quality of Life Objectives

1.5.1 Primary QOL Objective: To compare arms in terms of changes in physical symptoms and impacts from T2 to T3, adjusting for T1.

Hypothesis: Participants assigned to the treatment arm will experience significant reductions in physical symptoms and impacts from T2 to T3 as compared to those in the active monitoring arm.

1.5.2 Secondary QOL Objective: To compare arms in terms of changes in psychological symptoms from T3 to T4, adjusting for T1.

Hypothesis: Due to potential uncertainty related to lack of treatment for those in the active monitoring arm, participants assigned to the active monitoring arm will experience significant increases in psychological symptoms from T3 to T4 as compared to those in the treatment arm.

1.5.3 Exploratory QOL Objective: To assess of long-term HR-QoL changes in physical symptoms/impacts and psychological symptoms from T4 through the subsequent T5-T8 follow-ups overall and by arm.

1.6 Ancillary Study Objectives

1.6.1 Determine the prevalence of SARS-CoV-2 detection in anal and oropharyngeal swabs among people living with HIV (PLWH) being screened for the ANCHOR study.

Hypothesis: 10% of PLWH will have prevalent anal swab SARS-CoV-2 positivity, and will have concurrent oropharyngeal positivity.

1.6.2 Determine the relationship in the ANCHOR screening population between prevalent anal SARSCoV-2 positivity, anal HPV infection, and anal high-grade squamous intraepithelial lesions (HSIL).

Hypothesis: Anal SARS-CoV-2 positivity will be associated with higher quantities of anal HPV DNA and higher prevalence of anal HSIL among those who are high-risk HPV-positive.

1.6.3 Determine the 6-month incidence of SARS-CoV-2 detection in anal and oropharyngeal swabs among participants with anal HSIL newly enrolled into the ANCHOR study.

Hypothesis: Among PLWH enrolled into the ANCHOR study who were SARS-CoV-2-negative at screening, 10% will have incident anal and oropharyngeal positivity at 6 months.

1.6.4 Determine the relationship between prevalent or incident SARS-CoV-2 detection and regression of anal HPV infection or HSIL among participants newly enrolled into the

ANCHOR study and who are randomized to the monitoring arm.Hypothesis: In the monitoring arm of the ANCHOR Study, prevalent or incident anal SARS-CoV-2 detection will be associated with a lower rate of HSIL regression and clearance of anal HPV DNA than those who remain SARS-CoV-2-negative for 6 months.

2.0 BACKGROUND

2.1 Anal Cancer

2.1.1 Incidence of anal cancer in the general population and in high-risk groups

Anal cancer is a growing problem in the United States. In the U.S. general population, the incidence of anal cancer from 2006-2010 was 1.5/100,000 among men and 1.9/100,000 among women (1). Updated estimates for the period 2011-2015 report the incidence at 1.15/100,000 among men and 1.93/100,000 among women (131). Compared with the general population, the standardized incidence ratio (SIR) of anal cancer is increased more than 100-fold among some risk groups of HIV-infected persons who are successfully treated with combination antiretroviral therapy (cART) (2). Among men in the general population, the incidence is highest among Black men, at 1.9/100,000 (3). The American Cancer Society projected that there would be 7090 cases and 880 deaths from anal cancer in 2013 (4). At the time the protocol was initiated, the most recent data with actual number of cases were available from 2009. There were 2,210 cases of cancer of the anus, anal canal, and anorectum among males and 3,624 cases among females. There were 302 deaths among males and 516 among females from these cancers. As of 2019, the rates of anal cancer were reported to be increasing at a rate of 2.7% per year from the period 2001-2015, with the number of cases from the period 2011-2015 at 9,598 among males and 18,683 among females; for the period from 2001-2016, there were 4,720 deaths among males and 7,391 among females (131).

The incidence of anal cancer is largely concentrated among several groups well-known to be at increased risk: men who have sex with men (MSM) (5), HIV-infected men and women (6), men and women immunosuppressed for reasons other than HIV infection, including solid organ transplant (7)(8), women with a history of HPV-related cancer or high-grade squamous intraepithelial lesions (HSIL) elsewhere in the anogenital tract (9), and those with a history of genital warts (10). Of all the groups listed above the highest incidence of anal cancer is among HIV-infected men and women, particularly MSM (2)(6).

Overall it is estimated that the proportion of individuals with anal cancer who are also infected with HIV increased from 1980-1984 to 2001-2005, rising from 1.1% to 28.4% among males, and from 0% to 1.2% among females (11). From 1980-2005, the HIV epidemic had little impact on the trends in anal cancer among females, among whom the incidence has been increasing annually by 3.3%. However, the HIV epidemic has had a strong impact on the trends in anal cancer incidence among males, with an annual increase of 1.7% among men without HIV infection and 3.4% among men with HIV infection. The risk of anal cancer was elevated 52-fold in HIV-infected men who have sex with men (MSM), 32-fold in HIV-infected men, and 24-fold in HIV-infected women compared with the general population (11).

Recent studies indicate an incidence of 131/100,000 among HIV-infected MSM in the U.S. from NA-ACCORD (6). The incidence of anal cancer was 46/100,000 among HIV-infected men other than MSM, and 30/100,000 among HIV-infected women. The incidence of anal cancer among HIV-infected individuals had also

been reported outside the U.S. In the period from 2005-2008, compared with the general population, HIV-infected MSM from the French Hospital Database on HIV had a SIR for anal cancer of 109.8 (2). Other HIV-infected men had an SIR of 49.2 and HIV-infected women had an SIR of 13.1 (2). Among the four most common non-AIDS-defining malignancies, the largest increase has been in anal cancer (12).

Several studies show an increase in the incidence of anal cancer since cART became available (11)(13)(14)(15). The relationship between risk of anal cancer, CD4 level, and complete versus incomplete HIV suppression is unclear. In the French study cited above, among patients who maintained a CD4 count above 500/mm^3 for at least 2 years, a CD4 nadir below 200/mm^3 was associated with a higher SIR for anal cancer than a CD4 nadir above 200/mm^3 (2). Another report showed that being on cART with an undetectable HIV viral load 60% or more of the time was associated with reduced incidence of anal cancer compared with those who had an undetectable HIV viral load for less than 60% of the time (13). However, in that study the incidence of anal cancer was high, even among those receiving cART, and similar to the data reported in NA-ACCORD. Overall a cART-associated reduction in the incidence of anal cancer has not materialized (2).

While it is possible that better control of HIV infection with earlier initiation of cART may moderate the increased risk of anal cancer, the long-term effect of cART on anal cancer risk has not yet been studied. Moreover, among HIV-infected individuals, the great majority of people currently living with HIV were begun on cART later in the course of their HIV infection than is currently recommended. A recent meta-analysis estimated that 1 in 377 HIV-infected MSM with anal HSIL progress every year to anal cancer since the introduction of cART (16), and since current cART has extended the life spans of HIV-infected individuals, this may result in a 10% lifetime risk of anal cancer or higher among HIV-infected MSM if nothing is done (17).

Taken together, these data highlight two important points: HIV-infected men and women are disproportionately affected by anal cancer, and the impact of HIV infection on anal cancer will likely continue to increase. Second, most of the cases of anal cancer in terms of absolute numbers occur among HIV-uninfected men and women, and thus the results of the ANCHOR study will impact on both HIV-infected and HIV-uninfected populations. In the absence of active intervention, the number of cases of anal cancer will likely continue to grow in the general population in the U.S. with varying degrees of contribution by the at-risk groups described above. Data on the global incidence of anal cancer are not as reliable but it is certain that there are many thousands more cases and deaths from anal cancer each year world-wide. Combined with the possibility that anal cancer is preventable, the incidence of anal cancer is unacceptably high and calls for urgent intervention.

2.1.2 Anal cancer may be preventable

There are two approaches that may be used to prevent anal cancer. Primary prevention, in the form of vaccination against HPV infection may be useful to reduce infection with HPV, the underlying causative agent of anal cancer. Although

there is great potential for HPV vaccination to reduce anal cancer in the long term, there are several limitations to this approach as described below. Secondary prevention, which consists of screening for, and treating HSIL prior to progression to cancer, is the approach needed for the many individuals who have already been exposed to HPV and who have developed HPV-associated precancerous lesions.

Current cervical cancer prevention programs rely on both approaches, and in the long-term, it is likely that both will be needed for anal cancer prevention.

2.1.3 A study of anal HSIL treatment in prevention of anal cancer is needed despite the availability of an HPV vaccine

Like cervical cancer, most cases of anal cancer are caused by HPV 16 and HPV 18 (18). Vaccination with the quadrivalent and bivalent vaccine has been shown to reduce anal infection with HPV 16 and HPV 18 in both males and females naïve to those types (19)(20). Vaccination has also been shown to reduce the risk of anal HSIL in males (20). In the long-term, HPV vaccination may represent an excellent tool for reduction of anal cancer. Vaccination is currently routinely recommended for males (quadrivalent) and females (bivalent or quadrivalent) with a target of 11-12 years of age, and up to 26 years of age for all women, and for men who are MSM, or HIV-infected/immunocompromised (21). Routine vaccination is recommended for other men up to age 21 years. However, the great majority of HIV-infected individuals currently at risk for anal cancer are older than 26 years and do not qualify for HPV vaccination. Even if the vaccine is made available to them, a high proportion will already have been exposed to HPV 16 and 18.

Even among those who might benefit from vaccination, the impact of the vaccine has been reduced by poor uptake. Uptake among young women is limited at this time (22) with only 32% of eligible women receiving all three doses of the vaccine in 2010, and uptake among young men is even more limited. While herd immunity due to vaccination of females may contribute to protection against HPV even among those who have not been vaccinated, it is likely to be very limited among MSM. There are several reasons for the poor rates of HPV vaccination in the U.S. including varying levels of access, fear of HPV vaccine side effects, limited understanding of the benefits of HPV vaccination, and fear of vaccination in general. Globally the cost of the vaccine and lack of the necessary infrastructure to administer the vaccine are also major reasons. Finally, given the long period of time required for progression from cervical or anal HSIL to invasive cancer, it is expected that it will be decades before any reduction in cancer incidence is realized., even among those who have been vaccinated, it will likely be several decades before a vaccine-related reduction in anal cancer is observed and the full potential of the vaccine to reduce cancer incidence will most likely not be realized.

In summary, the combination of low vaccine uptake among vaccine-eligible men and women means that the full potential of the vaccine to reduce cancer incidence will likely not be realized unless rates of uptake improve. Combined with the fact that most HIV-infected men and women are too old for vaccination or were exposed to HPV 16 and 18 before vaccination became available, millions of men and women remain susceptible to HPV 16- and HPV 18-related HSIL and cancer. For these

individuals, secondary prevention in the form of identifying and treating HSIL may be the only option to reduce the risk of anal cancer. Determination of the efficacy of HSIL treatment to prevent anal cancer is therefore a current and public health concern for the foreseeable future for this target population.

2.1.4 Similarities between anal and cervical cancer inform secondary prevention approaches to anal cancer prevention

Cervical and anal cancer share many biological features. As described above, like cervical cancer, most cases of anal cancer are associated with HPV 16 or 18 infection (18). For both the cervix and the anus, the transformation zone is the primary target area for HPV due to the conjunction of two types of epithelium. In the cervix, these are the squamous epithelium of the exocervix and columnar epithelium of the endocervical canal. In the anus, the anorectal junction contains a transformation zone where the squamous epithelium of the anus is juxtaposed to the columnar epithelium of the rectum. HPV-associated changes in the cervix may be manifest as a spectrum of changes ranging from cervical low-grade squamous intraepithelial lesions (LSIL) to cervical HSIL. It is now well known that the precursor lesion to cervical cancer is cervical HSIL and the identification and removal of cervical HSIL before progression to cancer occurs is the basis for the success of the cervical cytology screening program. Similarly, anal squamous intraepithelial lesions (ASIL) span the same spectrum of changes as cervical lesions, and there is evidence that anal HSIL is the precursor to invasive anal cancer (23)(24) (25). In recognition of the similarity of HPV-associated anal and cervical disease, the American Society for Colposcopy and Cervical Pathology (ASCCP) and College of American Pathologists recently recommended standardization of terminology across all anogenital sites where HPV-associated cancers occur (LAST) (26).

Prior to the introduction of cervical cytology screening programs, the incidence of cervical cancer in the U.S. was 40-50/100,000. Largely due to the success of cervical cytology screening, in which the cervical cancer precursor lesion, cervical HSIL, is sought and treated before progression to cancer occurs, the incidence of cervical cancer has declined to approximately 8/100,000 (27). Typically, an abnormal cervical cytology leads to referral for colposcopy, in which the lesions are visualized directly under magnification and with the aid of 5% acetic acid. The lesions are biopsied to establish the grade of the lesion, and HSIL is ablated using one or more different methods, most often loop electroexcision procedure in the U.S.

Due to its success in reducing the incidence of cervical cancer, the cervical cytology screening program is considered to be one of the most successful cancer prevention programs ever implemented. Notably, however, the program was introduced prior to the era of evidence-based medicine, and its effect on reduction of cervical cancer only became apparent after following millions of women for several decades. While there are similarities between cervical and anal cancer, evidence must be developed as to whether anal cancer prevention is effective, to inform public health policy.

2.1.5 A study of anal HSIL treatment in prevention of anal cancer is needed even though it is known that treatment of cervical HSIL is effective in prevention of cervical cancer

Although anal cancer and cervical cancer are very similar, it cannot be assumed that treatment of anal HSIL will be effective in preventing anal cancer like treatment of cervical HSIL is effective in preventing cervical cancer. Compared with the cervix, a high proportion of anal lesions are large and multifocal, especially in HIV-infected individuals. The lesion recurrence rate and incidence of new lesions may be higher than is seen in the cervix in these groups. Compared with removal of cervical lesions using loop electroexcision procedure, complete removal of anal lesions can be comparatively difficult and may lead to more post-treatment discomfort given the need for regular bowel movements. Complete eradication of HSIL may therefore be more difficult in the anus than in the cervix. In addition, data on the efficacy of individual treatments for anal HSIL are far more limited than for treatment of cervical HSIL. While cervical cancer screening was adopted as standard of care prior to the era of evidence-based medicine, adoption of new medical practices such as screening and treatment of anal HSIL is increasingly requiring rigorous demonstration of the value of these practices.

Although a growing number of groups around the U.S. have begun anal screening programs, but at this time only a small fraction of individuals at risk for anal cancer have had access to any form of anal screening and treatment for anal HSIL. With the exception of the State of New York, there are no formal public health guidelines recommending anal screening and treatment of anal HSIL. This is because no studies have yet been done to demonstrate that treatment of HSIL reduces the incidence of anal cancer. The American Society of Colorectal Surgery recently issued guidelines recommending that HSIL be treated, but based on low-quality evidence (28). The United States Public Health Service has indicated that evidence of the efficacy of HSIL treatment is needed before treatment of anal HSIL can be recommended (29). CDC STD guidelines for 2014 are being updated, and will state that “Some centers perform anal cytology to screen for anal cancer among high-risk populations (e.g. HIV-infected persons, MSM), followed by high-resolution anoscopy for abnormal cytologic results (e.g. ASC-US or worse). More evidence is needed concerning the natural history of anal intraepithelial neoplasia, the best screening methods and target populations, the safety and response to treatments, and other programmatic considerations before screening can be routinely recommended” (Park I, personal communication). Many insurance companies will not cover the cost of screening for anal HSIL or for treating it in the absence of guidelines establishing these services as standard of care. At the same time, standard of care guidelines require evidence that treatment of HSIL reduces the incidence of anal cancer. Conversely, if efficacy is not demonstrated, it is important that individuals with HSIL not be subjected to ineffective treatments that can confer medical harm and unnecessary cost to the healthcare system.

2.2 Study Agents and Treatments

2.2.1 Overview of treatment of anal HSIL

As noted previously, treatment of anal HSIL in HIV-infected men and women is more challenging than in HIV-uninfected individuals. This is because HSIL tends to be larger in size and number in HIV-infected individuals. They may also recur more often at the site of prior treatment, and incident lesions may develop more often at sites that were not previously shown to have HSIL (metachronous lesions).

All of the treatments described below are used routinely by clinicians involved in treating HSIL. With the exception of treatment under anesthesia (surgical excision), which we expect to be necessary for only a very small percentage of patients with HSIL, each is readily available for use in the office setting by a wide variety of medical professionals, including MDs, nurse practitioners, and physician assistants. Thus, if the ANCHOR study shows that treatment of HSIL reduces the incidence of anal cancer, it will be possible to immediately implement the study findings.

2.2.2 Infrared coagulation

Infrared coagulation (IRC) is an office-based procedure that uses the Redfield IRC 1900 or 2100, a therapeutic device to treat anal HSIL. It delivers short pulses of a narrow beam of visible and infrared light through a small contact tip applicator that is applied directly to the target tissue, transmitted down the rigid quartz glass of the light guide. The tungsten-halogen lamp (150 watts of power) is the light source. This light causes thermal coagulation that results in tissue necrosis. The depth of coagulation is determined by the total amount of energy delivered, which is adjustable via pulse duration setting (0.5 to 3.0 seconds in 0.1-second intervals) in repeated applications. The depth of tissue destruction is directly proportional to the duration of infrared impulses. The device is cleared by the Food and Drug Administration for use in the treatment of hemorrhoids, tattoo removal, chronic rhinitis, and anal condyloma.

The procedure typically takes about 30 minutes to perform. It is generally well tolerated, and it is not uncommon for patients to go to work after the procedure. Overall the procedure is safe and can be performed by a wide variety of non-surgeon medical professionals. It requires only local anesthetic injected directly into or around the lesions to be treated, and does not require smoke evacuation apparatus or protection.

There may be occasional mild intra- and post-procedural pain, and bleeding for up to 2 weeks. There may also be mild textural changes of anal canal mucosa for several weeks post-procedure. The sequence of events is similar to that seen with cryosurgery: hemorrhagic blistering and necrosis of the treatment site followed by a shallow erosion and ulcer, and then healing over several weeks. There is a small risk of infection from the procedure. The risk of serious bleeding prompting emergency room evaluation is <1%. The PI of this protocol and his colleagues have performed thousands of IRC procedures, and most patients easily tolerate multiple procedures.

Most published data show that on an individual lesion basis, the "cure" rate is high, defined as absence of HSIL in the treated area (30)(31)(32)(33) as well as when combined with excision under anesthesia for the patients with the most extensive disease (34). In these studies a therapeutic response is defined as absence of HSIL, and the remaining tissue may include normal tissue or LSIL. We expect that even if LSIL is present after IRC (or any treatment), that the main goal of treatment will have been attained, since there is no evidence that LSIL is a cancer precursor.

In the largest study reported to date, the probability of curing an individual lesion after the first IRC ablation was 67% in HIV-infected men (30) and increased with additional ablations. Most recurrence was due to the development of metachronous lesions occurring in 82% of HIV-infected men after their first infrared coagulator treatment. At their last visit, 82% of HIV-infected MSM were free of HSIL. A similar result was obtained in AMC, in which the safety of IRC was studied in a multisite prospective cohort study performed by the AMC HPV Working Group (32).

2.2.3 Electrocautery and surgical lasers

Electrocautery and laser ablation are ablative techniques with a long history of use in the operating room and the office for the treatment of HPV-associated anal lesions. A recent publication showed no statistically significant differences between electrocautery and IRC in their efficacy and safety profile (33). In the ANCHOR study, clinicians may use IRC, hyfrecator/electrocautery, and surgical lasers interchangeably for in office removal of anal HSIL. Legally marketed electrosurgical units and carbon dioxide lasers that are indicated for excision, coagulation, and/or ablation of tissue, and that are appropriate for use in dermatologic, general, and/or gastrointestinal surgical procedures may be used for in-office anal HSIL treatment on this protocol. The choice of therapy typically depends on the prior training of the clinician and the technique with which they have the most experience. Non-surgeons in the U.S. who treat anal HSIL were primarily trained by Dr. Palefsky and his group to use IRC, but those with a surgical background may prefer electrocautery. The main advantages of electrocautery are that it is faster than IRC to perform and the electrocautery probe tip can be more precisely applied to the lesion than the IRC probe tip, which is larger in diameter. The main disadvantage of electrocautery is that it generates a smoke plume, requiring protection from smoke for providers and patients in the room.

2.2.4 Treatment under anesthesia

On this protocol, the term treatment under anesthesia (TUA) will be used to designate excision of HSIL lesions, performed by a surgeon on participants who are given spinal or general anesthesia. Procedures may be performed in an operating room as a surgical referral or in an office setting on participants with conscious sedation. The procedure will generally be performed using electrocautery devices or surgical lasers indicated for the excision, ablation, or coagulation of tissues, as described above. For TUA, selection of the medical devices to be used will be left to the discretion of the treating clinician. Combined with post-procedural IRC, it has been shown to be effective in treatment of HSIL for patients with disease too

widespread to treat with targeted ablation therapy (34). TUA is usually required for only a small percentage of patients being treated for anal HSIL. These include 1) disease that is so extensive that it cannot be treated by any of the other methods described in this section, or 2) if a lesion is so large that it cannot be sufficiently biopsied in the office to establish or exclude a diagnosis of cancer to the satisfaction of the clinician. The disadvantage of TUA is that it is expensive compared with the other methods. In addition, by definition, TUA is only done when more extensive

excision is required and patients typically experience more post-procedure side effects, including pain, bleeding, and infection after the procedure when compared with less aggressive treatment modalities. The costs of TUA will only be covered by the study with the QA committee's approval.

2.2.5 Topical 5% fluorouracil cream (5-FU)

Topical treatment with 5-FU was first reported in 1962 for treatment of skin cancers following a report that systemic 5-FU induced regression of keratosis (35). It inhibits DNA synthesis by blocking the conversion of uracil deoxyribonucleotide to thymine deoxyribonucleotide, resulting in thymine deficiency and inhibition of cell division. Its most marked effect is on rapidly proliferating tissues where it can cause erythema and edema followed by erosion, ulceration, and necrosis. It has been used since the 1970s to treat HPV-related lower genital tract disease in women (36)(37). In one study of HIV-infected women, topical 5-FU was shown to be effective in preventing recurrence of cervical HSIL following loop electroexcision procedure (36). Most patients who use 5-FU exhibit local side effects in the form of pain or discomfort associated with epithelial ulceration. Moderate to severe side effects were reported by 48% of patients (38). In a subsequent randomized controlled trial, 27% of patients treated with 5-FU had grade 3 or 4 side effects (39).

Topical 5-FU is typically used when disease is too extensive for IRC or electrocautery. It is given with the intent to clear as much of the lesion as possible, and while it is unusual for a large lesion to clear completely, it may be particularly useful to "debulk" a lesion to the point where it can them be treated with a targeted ablative approach such as IRC or electrocautery. Among 20 patients with extensive HSIL who were treated with topical 5-FU at UCSF, complete response was seen in only 3 and no response was seen in one, but 16 had a significant decrease in volume of disease to 25-50% that allowed for in-office IRC. At UCSF the protocol for use of topical 5-FU is for the patient to apply intra-anal or perianal 5-FU twice daily for 5 days, followed by a 9-day rest period (Jay N and Palefsky J, personal communication). This cycle can be repeated eight times.

There are limited reports of use of topical 5-FU specifically for treatment of anal HSIL. 7 of 8 patients treated with topical 5-FU cream for perianal Bowen's disease applied twice weekly for 16 weeks had no evidence of Bowen's disease on biopsy at 12 months post-treatment (40). In another study using intra-anal 5-FU complete responses were seen in 12 of 34 patients with anal HSIL and partial responses with regression to LSIL in 8 of 34 patients (38).

In a recently reported randomized controlled trial of 5-FU, imiquimod or electrocautery among 148 HIV-infected MSM with AIN (57% with HSIL) the

response rate was 29, 39, and 48% respectively (39). Severe side effects were reported as 27, 43, and 18% respectively, and recurrence rates were 57, 25, and 17% at 6 months post-treatment. Of note the treatment regimens in this study were different from what we typically do and what is proposed for the ANCHOR study. The investigators used lower doses of 5-FU than we typically use, but their study participants also appeared to have more side effects than we have experienced with our patients.

2.2.6 Topical 5% imiquimod cream

Like 5-FU, imiquimod is a topical agent that has typically been used when the extent of disease has been too large to allow for use of a targeted ablative modality such as IRC or electrocautery. Imiquimod has been used for treatment of external genital condyloma since 1997. It is a synthetic compound that exhibits antiviral activity by up-regulating the immune response, at least in part through toll-like receptors. It may lead to a Th1 cytokine response that activates HPV-specific cell-mediated immunity and clearance of lesions. Imiquimod has been used for treatment of HPV-associated mucosal disease including vulvar (41) (42), penile (43)(44) and anal SIL (45).

The advantage of imiquimod is that, like topical 5-FU cream it can be used to treat extensive disease that is too large for targeted ablation. It has a long safety record for treatment of condyloma, including in the setting of HIV infection. The disadvantages of imiquimod are side effects may be severe, particularly when patients are experiencing a robust clinical response. These may be local irritation or pain, or may be systemic flu-like symptoms. Another disadvantage is that there are relatively few randomized study data on the efficacy of imiquimod in treatment of perianal or anal disease. A randomized, controlled trial of 100 patients with anogenital warts assigned to 5% imiquimod cream or placebo for 16 weeks showed a low rate of complete response (11% drug vs. 6% placebo). 47% of patients in the imiquimod group reported greater than 50% reduction in total wart area vs. 20% in the placebo arm. Response rates were higher in HIV-uninfected patients compared with HIV-infected patients (62% and 31% respectively) (46).

There are limited reports that demonstrate efficacy using imiquimod specifically for treatment of anal HSIL in cohort studies and case reports (47). In one study, 14 of 19 (74%) HIV-infected MSM had complete regressions after treatment with imiquimod (48). In a randomized, double-blind placebo controlled study of imiquimod for intra-anal HSIL, 4 of 28 participants on active drug resolved and 8 downgraded to LSIL, compared with only 1 of 25 in the placebo arm who had a spontaneous regression (49). Participants were treated with open-label imiquimod after completing the study, and overall 29 of 47 (61%) had sustained regression of anal HSIL.

Our clinical impression over the years is that imiquimod has relatively limited value for treatment of anal HSIL, particularly in the setting of HIV infection where induction of immune response may be attenuated. However, we have not studied it formally in the U.S., and in light of the RCT data described above, the AMC HPV Working Group was recently approved by CTEP to perform a randomized trial of

imiquimod vs. 5-FU vs. observation for extensive HSIL in HIV-infected men and women. We have also elected to leave imiquimod on the list of treatment modalities available to ANCHOR study clinicians but may change that pending the outcome of the AMC study described above or other study data as they become available.

2.3 Study Design and Rationale

In this trial we will screen HIV-infected men and women for HSIL and if they meet the enrollment criteria for randomization, we will randomize them to treatment vs. active monitoring (AM) and follow them for up to five years after the last participant's date of randomization. Throughout the study we will be monitoring the incidence of invasive cancer in both arms and reporting regularly to a Data and Safety Monitoring Board (DSMB).

We will also be collecting samples and clinical data that will allow us to perform the correlative science studies described in Section 9.0, which are focused on exploiting a unique opportunity to understand progression from HSIL to cancer.

Definition of anal cancer

The anus is composed of the anal canal and the perianus. Proximally, the anal canal is bordered by the squamocolumnar junction (SCJ). This border is not static and anal squamous cell carcinoma (SCC) may occasionally be diagnosed proximal to the SCJ. These SCCs, diagnosed in the distal rectum, proximal to the SCJ, will be classified as anal cancers.

Distally, the anus includes the perianus, defined as a circumferential area with a 5 cm radius from the anal verge, (after the buttocks have been retracted gently), and includes the squamous mucocutaneous junction (151). For a perianal SCC to be classified as an anal cancer, it must be part of a lesion (LSIL/HSIL/cancer) that is located wholly or partially within the perianus. If located outside the perianus, it must be contiguous with a LSIL or HSIL lesion that resides as least partially within the perianal region. An exception may be in the situation where an anal cancer is diagnosed outside the perianus, that was previously documented to be part of a lesion that was at least partially within the perianus, but at the time of cancer diagnosis a lesion within the boundaries of the perianus is no longer present due to prior treatment. Among women, the anterior border of the perianus is defined as the posterior half of the perineum. SCC located on the posterior half of the perineum are considered anal cancers even if they extend to the anterior, vulvar region.

2.3.1 Rationale for selected approach and trial design

To have an impact on standard of care guidelines, it is critical that a study of the value of anal HSIL treatment in prevention of anal cancer be designed to provide the highest quality of evidence. The highest quality evidence will be obtained in a randomized controlled trial (RCT). Such a trial would require HIV-infected men and women with biopsy-proven HSIL, randomized to a treatment or active monitoring arm, and followed for up to five years.

Careful consideration has been given to alternative, less costly options for study designs that will yield the data needed to definitively define standard of care for anal cancer prevention. Least informative are small, non-randomized treatment

studies of anal HSIL. There are few of these published to date. Two such studies showed that anal cancers occurred in patients whose anal HSIL was not treated, in contrast to patients whose HSIL was treated and who did not develop cancer (50) (52). Although encouraging, these small studies are subject to many potential biases and do not constitute definitive evidence of the value of anal HSIL treatment.

A more robust study design would be an ecologic study, in which the rates of anal cancer at locations where HSIL is sought and treated are compared with locations where there is no such screening and treatment.

There are two recent reports comparing the incidence of anal cancer in the San Francisco region, where the UCSF Anal Neoplasia Clinic has been treating patients since the early 1990s, with incidence reported from registries outside California. Individuals with anal cancer in the San Francisco region had a 39% lower mortality risk than those reported from registries outside California but there was no decrease in the incidence of invasive cancer in the San Francisco region (53). There are also data showing that while rates of anal cancer may not have decreased in the San Francisco area during this time, they may have increased more outside of California (54).

Unfortunately, there are several limitations to these kinds of analyses with regard to inferences about the efficacy of treating anal HSIL in prevention of anal cancer. Methodologically-sound ecologic studies of anal HSIL treatment are not truly feasible for several reasons. First, screening and treatment for anal HSIL have begun on a small scale on an ad hoc basis in many centers with large populations of at-risk men and women. There is little standardization of clinical screening and treatment practices, and it is not possible to collect reliable data on treatment outcomes. Second, even in those locations where screening and treatment are performed, we estimate that only a small percentage of at-risk individuals have been screened to date and the effect of screening on reducing anal cancer incidence at these locations will be limited at best. This is true even in San Francisco, where we estimate that fewer than 10% of individuals with anal HSIL will have been treated, despite having one of the most active treatment programs in the country. Taken together, the data described above hint at the potential benefits of treating HSIL, but do not obviate the need for a RCT.

2.3.2. Prevention of anal cancer is highly desirable

One option to reduce mortality from anal cancer would be to focus efforts on finding cancers as early as possible, instead of trying to prevent them by treating HSIL. In the absence of screening programs to identify anal cancer or anal HSIL in at-risk populations, most anal cancers are currently diagnosed when individuals present with anal pain, bleeding, or a mass. Occasionally asymptomatic cancers are detected on digital anorectal examination (DARE) or incidentally at procedures such as hemorrhoidectomy. Treating cancers when they occur, which is the current approach, has the advantage of not over-treating many individuals whose HSIL would never have progressed to cancer if left untreated.

However, there are several reasons why focusing on diagnosis after cancer develops is a poor strategy. Many cancers found incidentally or because of symptoms will

be diagnosed at more advanced stages. The survival rate declines substantially when the cancers are diagnosed at a more advanced stage (55). Even at stage 1, the most common stage at which anal cancer is diagnosed, nearly 30% of individuals die within 5 years. At more advanced stages, the mortality increases further, and when metastatic cancer is diagnosed (Stage IV) the 5-year survival rate is only 21%.

Like cervical cancer, anal cancer may be preventable. An important reason to focus on cancer prevention rather than treatment of existing cancer is that while the standard anal cancer treatment, chemoradiation therapy (CRT), is often successful at the earlier stages of disease, it also leads to substantial morbidity (56). Acute toxicity is a major issue, particularly in HIV-infected patients, with one study reporting Grade 3 or 4 toxicity in nearly half of the patients (57). Chronic toxicity is also a major issue. Radiation therapy leads to radiation proctitis in a substantial proportion of patients, with anal pain and bleeding for years after successful therapy. Some require a colostomy for adverse late effects of CRT. The late effects likely result more from the radiation therapy rather than the chemotherapy. The symptoms related to treatment generally peak within the first 2 years, but radiation injury can develop after an interval of 5 to 10 years. Anal ulcers, fistulae, severe fibrosis, skin ulceration, and rectal stenosis may occur. There may be major issues with sexual function after CRT and there may be adverse psychosocial effects including depression. Radiation-induced pelvic and hip insufficiency fractures are also increasingly being recognized and reported, and are more common in women. Although HIV-infected individuals on cART are now commonly able to complete a full course of CRT, a study from the United Kingdom showed that CRT was associated with a reduction in CD4 level. The reduced CD4 level persisted after completion of therapy and may have contributed to the deaths of patients in remission (58).

Currently we have no way to distinguish HSIL that will progress to cancer from HSIL that will never progress. This is a problem, since treatment of HSIL, while well tolerated, does have consequences in terms of acute morbidity and cost to the healthcare system. Further, HSIL is common among HIV-infected individuals, and a high proportion of HIV-infected men and women will be diagnosed with anal HSIL at some point if screening programs are implemented. Of note, however, even with the need to "over-treat" anal HSIL, screening for and treating anal HSIL is likely to be cost-effective compared with treating anal cancer. Several years ago Drs. Sue Goldie and Palefsky published formal cost-effectiveness studies of screening for and treating ASIL in HIV-infected and HIV-uninfected MSM (59)(60). Both of these studies showed these procedures to be highly cost-effective. Since that time, we believe that our treatment approaches have improved further, and that computation of cost-effectiveness today would yield even better numbers. Although the treatment of anal HSIL is not inexpensive, and may require multiple attempts and visits, anal screening and treatment is highly cost-effective because the risk of anal cancer is not evenly distributed throughout the population and we know who the high-risk groups are. A more recent study from the United Kingdom concluded that anal screening was not cost-effective for MSM, but the assumptions that went into their model were very different from those used in the Goldie papers, stemming from paucity of data and perhaps the very different cost structures of the

U.K. and U.S. health care systems (61). Unlike cervical screening, which is recommended for all sexually active women, we only need to screen a subset of the population to have a substantial impact on the incidence of anal cancer. It is also possible that biomarkers of progression from HSIL to cancer can be identified, and this might reduce the number of HSIL lesions that need to be treated. This is one of the major aims of the correlative science component of the ANCHOR study and if successful, it will allow clinicians to target their therapy to only those HSIL at highest risk of progression. This would reduce unnecessary treatment and likely improve cost-effectiveness even further.

2.3.3 Impact of preventing anal cancer through secondary prevention

In considering the value of preventing anal cancer, it is critical to consider both prevention of deaths and impact on quality of life among those who survive treatment of anal cancer. Simple treatments such as those that will be evaluated in the ANCHOR study to prevent development of cancer are far less morbid than treating invasive cancer (56). Clearly prevention of anal cancer through identification and treatment of HSIL would be preferable to waiting until cancers develop. If we can prevent most anal cancers, we will reduce the need for CRT in thousands of patients per year in the U.S., and prevent about 800 deaths per year. Worldwide we will reduce the need for CRT in many more thousands of patients, and likely prevent several thousand deaths per year. To address the issue of unnecessary treatment of anal HSILs that would not have progressed if left untreated, ANCHOR study has a robust correlative science component designed to identify biomarkers that have the potential to distinguish anal HSIL at high risk of imminent progression to cancer from anal HSIL that can be safely observed. Given the biological similarity to other HPV-related cancers, these data may be of value in prevention of cervical and HPV-related oral cancer, and may contribute to prevention of many hundreds of thousands, if not millions, of cases of cancer and deaths. The ANCHOR study correlative science program also has the potential to lead to new therapies for anal HSIL and cancer that would simplify treatment, and these too may potentially of value in prevention and treatment of cervical and oral cancer.

2.3. Implementation of the ANCHOR study findings if the data support treating anal HSIL to reduce the risk of anal cancer

If the ANCHOR study shows that treatment of anal HSIL significantly reduces the incidence of anal cancer, it is likely that these results will quickly lead to standard of care guidelines. The ANCHOR study is a “strategy” trial, in which the strategy of treating HSIL is being tested for its ability to reduce the incidence of anal cancer. It is not a treatment trial of any one specific treatment modality. Thus, if the ANCHOR study shows that treatment of anal HSIL is effective in reducing anal cancer, standard of care guidelines will be adopted that use the most current information on how to treat anal HSIL, including the methods used in this study. If better methods become available in the future, the demonstration that treatment of anal HSIL reduces the risk of cancer will likely allow those newer treatments to be adopted as standard of care by showing their efficacy in treating HSIL. This is much easier and less expensive than needing to show efficacy in reducing the incidence

of cancer, as is proposed for the ANCHOR study.

Conversely, if the data from the ANCHOR study show that treating HSIL is not effective in reducing the incidence of cancer, we expect that dissemination of these findings will lead to a reduction and probably cessation of anal HSIL screening and treatment activities. If so, data from the ANCHOR study may still lead to an overall improvement in patient outcomes. Here it is possible that new progression biomarkers and therapeutic approaches identified in the ANCHOR study correlative science program may allow us to identify and treat anal cancers much earlier than is the case currently. This in turn may lead to successful treatment of these cancers with much less morbidity than CRT. It is very possible that the data will also be of value for prevention and treatment of cervical and oral cancer.

If treating HSIL is effective in reducing the incidence of anal cancer, then methods to identify those with anal HSIL through screening of at-risk individuals will become standard of care as well. As with treatment, there are several approaches that are currently available, but newer screening methods are being tested and others will be tested in the future. Correlative science studies enabled by the ANCHOR study as well as several others currently in progress will likely lead to optimization of screening strategies for anal HSIL, to the identification of those patients with HSIL who are at increased risk of progression to anal cancer, and potentially to new treatment approaches to HSIL and cancer.

2.3.5 The impact of the ANCHOR study for setting public health policy extends well beyond HIV-infected men and women

In addition to patients with HIV infection, those with a history of genital warts, or signs of perianal HPV-related lesions, whether warts or HSIL, may also potentially benefit from screening. With the growing incidence of anal cancer among the general population of women, we may reach a point where screening larger numbers of women who do not fit into any of these groups (e.g., women reporting a history of anal intercourse), may also become useful, but this will require further study. Although the non-HIV-infected groups listed above are not eligible for the ANCHOR study for several reasons outlined in Section 2.3.7, we also expect that the standard of care guidelines adopted for HIV-infected men and women will apply to the non-HIV-infected groups as well. Anal HSIL in HIV-infected men and women is harder to treat than in HIV-uninfected men and women for a number of reasons, including multifocal lesions, large lesion area, and high risk of HSIL recurrence after treatment. We believe that if the ANCHOR study shows that treatment reduces the risk of anal cancer in HIV-infected individuals, then the results will be generalizable to other groups in whom treatment of HSIL will be less challenging. Since most cases of anal cancer in the general population are occurring in HIV-uninfected individuals, it is clear that the data from ANCHOR study will have an impact on populations at risk for anal cancer that extend well beyond those with HIV infection.

2.3.6 The impact of the ANCHOR study extends beyond anal cancer

The ANCHOR study is also designed to have wide impact through its correlative science component. Multiple samples will be collected from patients during the

trial, and we plan to analyze them through a series of separately funded correlative studies. The ANCHOR study is unique because it focuses on prevention of progression from HSIL to cancer. A study such as this is not currently possible at any other anatomic site at which HPV-associated cancer develops. A study of progression to cervical cancer is not possible because it is currently standard of care to treat cervical HSIL. A study of progression to oral cancer is not possible because it is difficult to reliably identify oral cancer precursor lesions for treatment. The ANCHOR study not only offers the opportunity to determine the efficacy of HSIL treatment to prevent cancer, but careful follow-up and sample collection from those in the active monitoring arm will allow us to better understand the molecular pathogenesis of progression to cancer. This may lead to new approaches to treating HSIL to prevent progression to cancer and potentially cancer itself. The study also provides the opportunity to identify biomarkers of progression to cancer. Given the strong biological similarities between anal, cervical and oral cancer it is very possible that biomarkers and treatments identified in the ANCHOR study will be applicable to cervical and HPV-associated oral cancer. Biomarkers of progression from cervical HSIL to cervical cancer would be of great value in resource-limited settings where the cost of treatment could be reduced if there were ways to identify those at highest need of intervention. This is often the case in developing countries that lack organized cervical cancer screening programs. In developed countries, this information could be valuable to identify those at highest need of close follow-up after treatment of cervical HSIL. Recurrence after treatment of cervical HSIL is particularly common in HIV-infected women (62) and this is just one group that might benefit from use of these biomarkers. Development of better treatments to reduce the incidence of HPV-related cancers regardless of the anatomic site at which they occur, or better biomarkers to identify those at risk, and who most need treatment of HSIL, has the potential to save hundreds of thousands, if not millions of lives.

In summary, the ANCHOR study will have a major impact in several ways: 1) If treatment of HSIL is shown to be effective in reducing the incidence of anal cancer, it will establish standard of care guidelines for treatment of HSIL in at-risk populations. The results will be applicable to both HIV-infected and HIV-uninfected at-risk populations; 2) Information obtained on molecular pathogenesis of progression from anal HSIL to anal cancer, and development of biomarkers of progression may lead to an ability to target those with anal HSIL most in need of treatment, and better methods treating anal HSIL to prevent anal cancer, and 3) the results of ANCHOR will likely extend to cervical and HPV-associated oral cancer.

2.3.7 Rationale for eligibility criteria

The ANCHOR study includes only HIV-infected men and women for several reasons. First, this is the group with the highest incidence of anal cancer. Second, since the incidence of cancer is highest in this group, we expect to obtain the necessary number of cases of anal cancer in the active monitoring arm with the smallest number of participants possible, and over the shortest amount of time possible. Third, given the challenges associated with treating HSIL in this group (multifocal lesions, risk of HSIL recurrence after treatment) we believe that if the

study shows that treatment reduces the risk of anal cancer in HIV-infected individuals, then the results will be generalizable to other groups in whom treatment of HSIL will be less challenging. In addition, performing a RCT like ANCHOR study in HIV-uninfected individuals would not be practical since such a study would require many more participants who would need to be followed for a substantially longer period of time. It would also be difficult to generalize the results of any study in HIV-uninfected individuals to HIV-infected individuals.

We also have elected to study only HIV-infected men and women 35 years or older. This is meant to enrich the study population at risk for cancer since anal cancer occurs only rarely under this age even among HIV-infected individuals. Fewer than 1% of anal cancers occur under the age of 35 years (1).

Finally, our goal is to recruit to this study at locations around the U.S. that will allow us to enroll participants whose racial/ethnic background mirror the current demographics of the HIV epidemic, to ensure the generalizability of the results to the HIV-infected population into the future.

We have considered other potential methods to enrich the study population for risk of cancer such as HPV 16 or 18 positivity as an enrollment criterion but other HPV types may also play a role in anal cancer, particularly in the HIV setting, and we want to ensure that our results are as generalizable as possible. Although HPV 16 is the predominant HPV type in anal cancers overall (18), little is known about the distribution of HPV in anal cancers among HIV-infected men and women. Recent data suggest that HPV 16 may play a less prominent role in cervical cancers of HIV-infected women compared with HIV-uninfected women (63)(64), and it will be important to know whether this is also true of anal cancers. In one study from Germany, all five squamous cell carcinomas (SCCs) of the anal canal contained HPV 16 compared with only one of four anal margin SCCs. HPV 31, HPV 33 and HPV 68 were found in the other three anal margin SCCs) (52).

We do not plan to use CD4 level, HIV viral load, history of cART, and nadir CD4 level as entrance criteria, since our own data show that individuals on cART have a wide range of CD4 levels at the time of anal cancer diagnosis (25). A high proportion of HIV-infected individuals are on ART and thus use or lack of use of cART also would not be a useful entrance criterion. Other than the recent publication using VA data suggesting that having more time with an undetectable HIV viral load was protective against developing anal cancer (13), there are insufficient data to use HIV viral load as an entrance criterion. It would be difficult to reliably quantify a prospective participant's past viral load control, and regardless of what it was at the time of study entry, we would feel ethically obliged to work with the participant and their HIV care provider to maximize the likelihood that they have the best viral load control possible.

2.3.8 Equipoise in the treatment and active monitoring arms

We have had many discussions with specialists in medical ethics and with many members of the HIV community. We believe that there is equipoise in the treatment and active monitoring arms of the ANCHOR study. Participants in the active monitoring arm are at risk of progressing to anal cancer. However, we do not know

that treatment of anal HSIL is effective to reduce anal cancer, and individuals in the treatment arm may be at similar risk, hence the need for the ANCHOR study. If HSIL treatment is shown to be ineffective in prevention of incident HSIL, then participants in the active monitoring arm are being spared treatments that may be causing unnecessary morbidity.

We have designed the study to minimize the risk of harmful outcomes associated with developing invasive cancer for ethical reasons and to maximize the likelihood of study retention. For this reason, we are following participants in both arms very closely, i.e., at 6-month intervals or shorter. With close follow-up, it is possible (if not probable) that if a cancer does develop, it will be an early cancer. Detecting cancers early is likely to be advantageous since treatment is usually successful at the early stages, and is less successful at later stages. Cancers that occur while still defined as superficially invasive squamous cell carcinoma (SISCCA) (26) may be treatable with local excision. Local excision is not standard of care for intra-anal SISCCA but this is a research question that will be addressed in a study under investigation by the AMC HPV Working Group (participants in the ANCHOR study who are diagnosed with SISCCA may be referred to that study if it is still recruiting). Local removal could potentially spare participants the side effects associated with CRT. However, in the informed consent process, we will be explicit with the participants that despite the frequent monitoring in the study, those who develop cancer may need CRT with its associated morbidity, and they may also die of their anal cancer.

It is very possible that in the absence of the ANCHOR study, many individuals who agree to participate and who are randomized to the active monitoring arm would not have undergone any form of screening- screening is not currently standard of care and is not available to most at-risk individuals. If they were going to develop cancer, they would have done so anyway, outside the context of any study and these individuals would eventually present with symptoms from their cancer. The cancers may be diagnosed at a later stage than if they were being monitored in the ANCHOR study, would more likely require CRT and they may have increased risk of death. Being in the active monitoring arm therefore offers the possibility of earlier diagnosis of cancer than if they were not in the study at all, a better chance of survival and very possibly fewer side effects of cancer treatment.

Being in the treatment arm offers the possibility of reduced risk of developing anal cancer compared with being in the active monitoring arm. However, as noted above, we do not yet know if this is the case, and it is possible that individuals in the treatment arm will be undergoing a series of procedures with little or no clinical benefit. Many of the treatments described above lead to a high clearance rate per lesion, but patients may still have a focus of anal HSIL somewhere even after successful treatment of a given lesion because of metachronous disease. We will therefore allow ongoing treatment of HSIL throughout the course of the study. Although the HSIL treatment procedures that we will use in the ANCHOR study are generally well tolerated, participants in the treatment arm may experience treatment-associated side effects, including post-procedure pain or discomfort, bleeding and although rare, infection or stenosis.

The DSMB will review data including progression rates to cancer in both arms on a regular basis (e.g., 6 months, and not less than once per year) and will discontinue the study if the data indicate that it is no longer ethical to continue. The DSMB will also monitor study data relevant to the assumptions so that necessary changes can be made to accrual strategies, eligibility criteria, sample size, or other study parameters in a timely manner. Interim analyses are described in Section 10.5.2.

In summary, participants in both arms who develop anal cancer may need CRT, and may be at risk of death due to anal cancer. It is not known if this risk is different between the two arms. Participants in both arms may also benefit from early diagnosis of anal cancer and may be spared the side effects of CRT. Some might benefit from being in the study compared with not being followed at all, which is currently the case for most at-risk individuals. Finally, if the study hypothesis is rejected, i.e., that treatment of HSIL is not effective to reduce the incidence of anal cancer, then the participants in the active monitoring arm will have been spared the morbidity associated with treatments of those participating in the treatment arm.

2.3.9 Feasibility of recruitment to the ANCHOR study

In preparation for the ANCHOR study, we performed a series of studies examining willingness to enroll in the study. Funded by NIH, we performed focus groups in ten different U.S. cities comprised of HIV-infected men and women. In each city we obtained data from several groups, individually composed of HIV-infected White, Black and Hispanic men or women. Our goal was to assess the feasibility of performing the ANCHOR study among the demographic groups that currently comprise the HIV epidemic. Given that we expect to recruit to the ANCHOR study in 10 sites spread around the United States where the HIV epidemic is prominent, we sought to assess willingness to participate across a wide geographic and demographic spectrum of potential participants. We assessed willingness to participate in a trial with the same design as ANCHOR in an ethnically diverse sample of HIV-infected men (n=202) and women (n=39) in 10 US cities. Focus group participants were screened for eligibility (35 or more years of age and HIV-infected) by telephone and assigned to one of four focus group types based on ethnicity and gender (Black, Hispanic or Latino, and White men, and a multi-ethnic group of women). Two moderators traveled to each of the ten cities to conduct the focus groups following a semi-structured guide. The Hispanic groups were conducted primarily in Spanish, primarily in English or both, depending on participant preference.

We also performed an internet-based survey of 257 HIV-infected men and women in 20 cities. The online survey targeted the same population living in the same 10 cities used for the focus group portion of the study, plus 9 additional US cities (Boston MA, Minneapolis MN, Omaha NE, Philadelphia PA, Sacramento CA, San Antonio TX, San Diego CA, Tampa FL, and Washington DC) and one Canadian city (Vancouver BC). The study was conducted using SurveyGizmo and participants were recruited through flyers mailed to AIDS service organizations and HIV clinics. We also directly surveyed HIV-infected men to determine their willingness to participate in study with the ANCHOR study design after being given a diagnosis of anal HSIL.

In summary, most HIV-infected individuals surveyed expressed an interest in participating in the ANCHOR study even with a 50% chance of being randomized to either arm. In so doing they would be supported by their HIV primary care provider. Survey respondents mostly preferred being in the treatment arm, but with appropriate support, would likely stay in the active monitoring arm. Among the 20 participants who were surveyed after being given a diagnosis of HSIL, most expressed a willingness to be randomized even after having undergone procedures very similar to the ANCHOR study screening protocol and after having come to the clinic fully expecting to be treated for their HSIL. In the worst-case scenario, individuals may choose not to continue in the study after their screening visit, and if that is the case, we will screen additional individuals to reach our recruitment target of 5058 randomized men and women. Taken together, these data give us confidence that we will be successful in recruiting to the ANCHOR study as described in the schema, and that we will be able to successfully retain participants in both arms with sufficient ongoing education and support.

2.3.10 Rationale for health-related quality of life assessment

The ANCHOR study presents a unique opportunity for the assessment of health-related quality of life (HRQoL) among those diagnosed with anal HSIL who are either treated or observed. At the time of protocol implementation, no measure existed that captures the specific symptoms and related experiences of living with or being treated or monitored for anal HSIL. Although definitions of HRQoL vary, there is general agreement that it is a "multi-domain concept that represents the patient's general perception of the effect of illness and treatment on physical, psychological, and social aspects of life." (125). The original funding application for the ANCHOR trial included a HRQoL measurement component using existing symptom measures not specific to anal HSIL. During the NCI's Cancer Therapy Evaluation Program (CTEP) review of the initial protocol draft, CTEP reviewers strongly recommended that the PI work to develop a HRQoL measure that was specific to anal HSIL with the goal of implementing such a measure within the ANCHOR trial so as to provide important secondary outcomes. To this effect, the AMC began development (protocols AMC-A02, AMC-A03, and AMC-A04, described further below), with the ultimate aim of validating and implementing the new measure within the ANCHOR trial.

There is a paucity of data on symptoms and concerns of persons diagnosed and treated for anal HSIL. Studies examining aspects of HRQoL in persons being screened for and treated for anal HSIL used diverse measures to assess HRQoL, ranging from possibly study-specific ad hoc items (126), symptom measures designed for HIV-positive persons but not specific to anal HSIL (127), and generic HRQoL measures (128), supplemented with validated sexual functioning items (128) or psychological distress measures (128). Although many of these measures showed sensitivity to the impact of anal cancer screening or treatment, the variety of measures used and lack of specificity for anal HSIL diagnosis, monitoring, and treatment support the need for a rigorously developed and validated measure to provide reliable, comparable data across anal HSIL populations and treatments. In addition, within the ANCHOR trial, different treatment modalities are permitted for

those participants assigned to the treatment study arm: Infrared coagulation, electrocautery, laser, treatment under anesthesia (vs. local anesthesia), topical 5% fluorouracil cream (5-FU), and topical 5% imiquimod cream. Thus, it is important to develop a HRQoL measure that can capture the array and severity of symptoms.

2.3.11 Development of the ANCHOR Study Health-Related Symptom Index and study implementation for QoL assessment

Initial development of the ANCHOR Study Health-Related Symptom Index (ANCHOR HRSI, or A-HRSI) was conducted under protocol AMC-A02, and included a four-phase process to establish content validity. This measure was specifically created for use in the ANCHOR trial using state-of-the-art instrument development methodology. Expert consultation was used to inform concept elicitation interviews with 41 participants who were eligible for ANCHOR. Based on these interviews, a 23-item measure was drafted and then tested in a second cohort of 45 participants eligible for ANCHOR in a process of cognitive interviewing. This resulted in a 25-item content-valid measure of physical symptoms, physical impacts and psychological symptoms (129). Reliability was then assessed in 100 ANCHOR participants across a 7-10 day timeframe, with Cronbach's α in the fair to good range across the three domains (i.e., 0.79 – 0.82), indicating adequate internal consistency for the measure (130). The test-retest reliability was good across each of the three domains (intraclass correlation coefficients = 0.80 – 0.84). Construct validity of the measure was then established in a fourth cohort of individuals enrolled in ANCHOR (n=303) who completed A-HRSI at a single time point, with the three domain model (i.e., physical symptoms, physical impacts, psychological symptoms) confirmed via confirmatory factor analysis, with these three domains strongly associated (Pearson's *r*) with corresponding domains from the well-established MD Anderson Symptom Inventory and Functional Assessment of Cancer Therapy – General tools.

The final version of the A-HRSI (both English and Spanish) includes 25-items that asks participants to rate the degree of prevalence or impact of their physical symptoms (10 items), physical impacts (6 items), or psychological symptoms (9 items) on a 0 (not at all) to 4 (very much) scale. Items within each domain are averaged, with higher scores indicative of worse HRQoL. The measure takes approximately 6-10 minutes to complete either via telephone-facilitated interview or electronic patient-reported outcomes (ePRO) platform in the data entry system for the trial, AdvantageEDC.

The ePRO system is a separate module of AdvantageEDC, the data entry system for this trial, that allows the participant to complete questionnaires directly in the data entry system. This eliminates any need to administer a paper questionnaire and to transcribe the data into the system. The ePRO data capture system is user-friendly and compatible with major internet browsers and a variety of electronic devices. In an effort to evaluate participant behavior in using the ePRO, the amount of time spent on each ePRO item screen will be captured.

The completed substudy also made use of the patient version of the Eastern Cooperative Oncology Group Performance Status (Patient ECOG) and at time of

follow-up assessment, the Patient Global Impression of Change (PGIC) scale. The Patient ECOG was adapted from clinician to patient language through focus groups, interviews, and comparisons of clinician and patient responses. This measure consists of a single item that asks participants to rate their current performance status from 0-4. This item takes approximately 1 minute to complete. The PGIC allows participants to consider, using a 7-point scale ranging from "very much worse" to "very much better," whether their overall quality of life has changed since the last time they were assessed. This item takes approximately 1 minute to complete.

To adapt A-HRSI for ANCHOR participants who prefer Spanish as their primary language for healthcare delivery, content validation of A-HRSI was completed in Spanish under AMC-A04. A-HRSI was translated into Spanish by the MSKCC Patient-Reported Outcomes, Community-Engagement, and Language (PRO-CEL) Core Facility. Using similar qualitative methodology as AMC-A02, cognitive interviews were completed across two rounds with a total of 17 participants who were eligible for ANCHOR. Through this process, changes to item and response translations were made based on suggestions from participants, but no substantive changes were implemented to alter item content. As such, the Spanish version of A-HRSI has been adequately content validated and is appropriate for use in the ANCHOR trial.

Final stage of A-HRSI development and substudy results

The aim of the A-HRSI substudy wass to assess the responsiveness (sensitivity to change) and clinical significance of the A-HRSI subscales by comparing change scores within groups of participants as defined by participant responses to the PGIC item. Responsiveness posits that a psychometric scale is able to detect the difference between patients who experience changes in symptoms and patients who experience no change. Thus, we used the PGIC item to document change between follow-up 1 (time 2) and FU 2 (time 3) time points ("Since the last time you completed a questionnaire, how would you rate your OVERALL QUALITY OF LIFE?", marked on a 7-point scale from "Very much worse" to "Very much better" with a mid-point of "No Change"). Each participant was be categorized into one of 3 groups: change for the worse (a response of "Very much worse", "Moderately worse", or "A little worse"), no change ("About the Same"), and change for the better ("A little better", "Moderately better", and "Very much better"). Changes in the A-HRSI scores were also be calculated (FU1 scores minus baseline scores). Responsiveness is supported if changes in the A-HRSI scores correspond reliably with the anticipated changes between the three groups. This can be addressed by a one-way ANOVA on the changes in the A-HRSI scores across the 3 groups ("worse", "no change", and "better").

The PGIC item has been used with recall periods up to 6 weeks. It may or may not work for recall periods exceeding 6 weeks. We collected the dates of assessments and thus can assess for trends in PGIC item.

For this final stage of the A-HRSI development, up to 100 eligible participants who consented to participate to the ANCHOR study and to the optional A-HRSI

substudy to demonstrate measurement scale responsiveness (Section 1.3.2) were consented and enrolled between July- and October 2019, and were administered the A-HRSI and several legacy measures before randomization and at two time points post-randomization and treatment. Final data collection occurred in February 2020.

This fifth cohort of individuals enrolled in ANCHOR (n=103) completed A-HRSI at three time points in order to establish clinical responsiveness (i.e., sensitivity to change) of the tool. A-HRSI and self-reported Patient Global Impression of Change (PGIC) scale and ECOG Performance Status (ECOG PS) item were administered either via ePRO tool or via telephone facilitated interview at time of enrollment up until time of trial randomization (T1), 14-70 days post-randomization (T2), and 71-112 days post-randomization (T3). Participants at follow-up timepoints were categorized into two sets of three groups based on PGIC and ECOG PS responses ("worse," "no change," "better"), with the primary responsiveness analysis using these three groups in a one-way analysis of variance (ANOVA). Below we show the results for change at T3 vs. T2. The change from T3 to T2 in subscales showed those who had changed for the worse on the PGIC or ECOG PS had worsening A-HRSI subscales as compared to those who changed for the better or had no change.

Table 2-A: Descriptive statistics for change in subscales (T3 minus T2) according to change in PGIC and ECOG PS, mean (SD)

	PGIC			ECOG PS		
A-HRSI Subscale	Change for better N=34	No Change N=38	Change for the worse N=12	Change for better N=18	No Change N=43	Change for Worse N=24
Physical Symptoms	-0.12 (0.86)	0.05 (0.74)	0.27 (0.65)	-0.23 (0.61)	-0.06 (0.80)	0.37 (0.78)
Physical Impacts	0.01 (1.14)	-0.06 (0.99)	0.65 (1.25)	-0.76 (1.18)	0.10 (0.74)	0.63 (1.22)
Psychological Symptoms	-0.22 (1.39)	0.05 (1.19)	0.90 (1.50)	-0.34 (1.42)	-0.08 (1.11)	0.64 (1.55)

Table 2-B: Responsiveness comparison of changes (T3 minus T2), mean difference (95% confidence interval)

	PGIC		ECOG PS	
A-HRSI Subscale	Change for worse vs. no change	Change for worse vs. better	Change for worse vs. no change	Change for worse vs. better
Physical Symptoms	0.22 (-0.30 to 0.73) P=0.401	0.39 (-0.13 to 0.90) P=0.144	0.43 (0.05 to 0.82) P=0.027	0.60 (0.13 to 1.07) P=0.013
Physical Impacts	0.71 (-0.01 to 1.43) P=0.052	0.65 (-0.08 to 1.38) P=0.081	0.53 (0.02 to 1.03) P=0.041	1.39 (0.77 to 2.01) P<0.001
Psychological Symptoms	0.86 (-0.02 to 1.73) P=0.055	1.12 (0.23 to 2.01) P=0.014	0.72 (0.04 to 1.39) P=0.037	0.98 (0.14 to 1.82) P=0.022

Note: P-values are two-sided and from post-hoc pairwise comparison of means in an analysis of variance. The overall F-statistic was significant for PGIC group for psychological symptoms (p=0.047) and for ECOG PS group for physical symptoms (p=0.026), physical impacts (p<0.001), and psychological symptoms (p=0.042).

There was a significant moderate relationship (i.e., standardized response means = 0.52 and 0.60 for physical impacts and psychological symptoms, respectively) between changes in A-HRSI subscales and changes in ECOG PS from T2 to T3. These results provide evidence of A-HRSI clinical responsiveness that will be

further explored as part of the ANCHOR trial through the refined assessment windows for the formal QoL objective: T1 will remain the same (enrollment up until time of trial randomization); however, T2 will now be revised to occur 2-7 days post-randomization to correspond with the period during which most participants are symptomatic after their initial treatment or assignment to active monitoring. T3 will be revised to occur 4-weeks after randomization, at which time it is expected that participant symptoms or impacts due to treatment or assignment to active monitoring will be lessened. Additional assessment time points T4-T8 will then occur at annual visits through the fifth year of study participation in order to evaluate any long-term health-related symptom impacts related to treatment or assignment to active monitoring.

A-HRSI implementation for QoL aim

After validating the A-HRSI, the A-HRSI is being implemented with an amendment (protocol version 13.0), and administered to a sample of 500 ANCHOR participants who consent to participation for these additional surveys. The questionnaire will be administered before randomization and at seven time points thereafter (2-7 days, 4 weeks and 12, 24, 36, 48, and 60 months),

2.4 Overview of Study Design

2.4.1 Guiding principles to the study design

1) To address our primary objective, clinicians will make their best attempt to treat HSIL throughout the course of the study for those in the treatment arm. The approaches used in the ANCHOR study to treat anal HSIL are essentially identical to those used in current clinical practice in the U.S. The proposed treatment approach parallels real-world clinical practice, in which patients may respond well to a given treatment, but experience a recurrence or development of a new lesion. The treating clinician may then embark upon another course of treatment with the same modality or a new one depending on a number of factors. Our goal in this study is to ensure that the results are as generalizable as possible while also ensuring that clinical standard operating procedures are rigorously followed. Standardized protocols will be used to treat HSIL in which the choice of therapy will depend on the size, number and location of lesions but clinicians will be allowed some latitude in their choice of therapy within these well-defined parameters.

   Participant safety is our first priority and if a clinician or a participant feels uncomfortable about continuing in either arm, they will be withdrawn from the study. Within those parameters, we will emphasize ongoing participant support and education to maximize study retention within each arm. Participants who are shown to have anal cancer during screening will be immediately referred for appropriate therapy. For all participants diagnosed with cancer during the study, stage of cancer at diagnosis will be recorded at the time of study discontinuation.

2) We have designed the study to make the procedures, including biopsies as similar as possible between the observation and treatment arms, with of course,

the exception of the treatment procedures in the treatment arm. We have included annual biopsies in both arms as a means of ongoing monitoring of disease status and to collect samples for correlative studies. However, biopsies may occur in either arm at additional visits. In both arms, this may occur if the clinician suspects progression to cancer at any visit. In the treatment arm, this may occur if the protocol for the treatment modality being used calls for documentation of disease status to enable a decision about the need for additional treatment.

3) We will have regular meetings among all study investigators (every 6 months) and with study investigators at their sites that include ongoing and rigorous quality assurance monitoring.

4) We will continuously monitor developments in the HPV/SIL/cancer field and if a new therapy emerges that should be implemented, we will modify the study design to allow it; this will not compromise the integrity of the study, since our goal is to do our best to eradicate HSIL. For example, a study will soon be initiated in the AMC comparing 5-fluorouracil cream to imiquimod for treatment of anal HSIL. If one of these is shown to be superior, we will modify the protocol to recommend prioritizing the use of the superior medication.

5) Although we believe that most HSIL lesions can be treated successfully, there is still the possibility that some will progress to cancer after treatment, or that cancer may develop from a focus of HSIL that was clinically unrecognized and therefore not treated. For this reason, we have powered the study to detect a 75% reduction, not 100% reduction, in the incidence of anal cancer in the treatment arm compared with the active monitoring arm. We have shown that a 75% reduction in incident anal cancer in the treatment arm would be cost-effective (56). It would likely be viewed in the community as a worthwhile clinical outcome and would likely result in implementation of screening for HSIL and treatment in at-risk populations.

6) Management of HIV infection will not be part of the ANCHOR study per se. Rather, the ANCHOR study staff will work closely with the participant and their HIV care provider to ensure that the participant is receiving optimal HIV care.

2.4.2 Schema

Information Visit: This is a recommended information session and may be done with potential participants individually or in groups. The study will be explained in detail, and knowledge will be assessed. Participants will be given a copy of the informed consent to take with them and encouraged to discuss participation in the study with their health care provider, or may give consent for participation at the information visit. Potential participants should be asked to return for a screening visit within 4 weeks; this is done to ensure that the he or she is truly committed to being in the study if qualified, and to answer additional questions that may have arisen since the information visit. By explaining the study in detail in advance, it is also expected that some potential participants may inform the study staff of issues that may preclude them from enrolling, such as prior history of HSIL or known abnormalities in blood tests such as platelets, etc. In so doing, we will maximize the efficiency of the screening visit that follows. If the participant gives informed consent at the information visit, screening procedures may be provided at that visit at the investigator's discretion.

Visit 0 (targeted for within 4 weeks of the information visit): This is the screening visit. Prior to performing any of the study procedures, the participants will be asked to sign the informed consent (if not signed previously) that gives us permission to screen them for HSIL and other eligibility criteria. The informed consent also gives us permission to randomize them if they meet all eligibility criteria after the screening visit. Participants who do not return for their screening visit (Visit 0) or baseline visit (Visit 1), or who are determined to be ineligible based on the results of the screening visit, will not be enrolled in the study.

We will collect three anal swabs, the first for cytology and correlative research studies, the second for DNA-based research testing, and the third for RNA/protein analyses (please see Section 9.0). The clinician will perform a digital anorectal exam (DARE), HRA, and HRA-guided biopsies. Blood will be collected for complete blood count and serum storage.

Anal biopsy and cytology samples will be read by the local pathologist with these results used to guide enrollment and ongoing decisions in the course of follow-up. Slides will be sent to a central laboratory for interpretation by a central study pathologist. Interpretations generated by the central pathologist will be used for the purposes of study data analysis. Readings that differ between the local and central pathologist with respect to presence or absence of HSIL or cancer will be adjudicated by a third pathologist. For scheduling/planning purposes, once the cytology and biopsy results are available as assessed by the local pathologist, the HRA clinician will indicate the treatment that would be done if one or more biopsies are positive for HSIL and the participant is randomized to the treatment arm.

If the participant is determined to be eligible based on the results of testing done at the screening visit, he or she will be contacted and asked to come in for Visit 1. Eligible ANCHOR participants were also made aware of the optional health-related quality of life (HRQoL) questionnaire (substudy to validate the A-HRSI completed

enrollment in October 2019) to this protocol and provided informed consent to this study if they are interested in participating. Participants who were eligible and enrolled for the HRQoL objective (or the preceding substudy to validate the instrument) completed their initial HRQoL questionnaires via telephone-based or electronic administration up until the time of Visit 1 (within 2 weeks before randomization).

Participants who are not eligible for randomization to the ANCHOR study will not be followed any further. At that time, they may potentially be eligible for other studies funded and performed outside the context of the ANCHOR study, e.g., a prospective study could be done (funded separately) of people who had LSIL but not HSIL to determine the natural history of LSIL. If the participant in the LSIL study is later diagnosed with HSIL, they could potentially be re-screened for the ANCHOR study if screening is still in progress and they did not receive any treatment for their HSIL within six months before randomization.

Visit 1 (baseline visit with randomization): Within 1-12 (targeted for 6) weeks of the screening visit (Visit 0) we will see participants who were determined to be eligible for the study. A questionnaire detailing medical history and behaviors will be administered to all randomized participants to determine potential risk factors for progression to cancer among those who were enrolled into the study.

At this point, the visit procedures will depend on the randomization arm. If the participant is randomized to the active monitoring arm, we will perform HRA to confirm the lesions being followed, counsel the participant, and discharge from the clinic with a follow-up appointment. If randomized to the treatment arm, the participant will undergo their first treatment at that visit (delay of up to two weeks is permitted), unless the plan is for the participant to have TUA. For participants randomized to the treatment arm and for whom TUA is planned, planning for the surgery will begin at Visit 1. If participants are randomized to the treatment arm, and the clinician opts to treat the lesions with patient-applied modalities, such as imiquimod or 5-fluouracil cream, the participant will be counseled regarding their use. Treatment will begin at that visit (or in no more than two weeks if a delay is required). Clinic staff will show the participant how to use the treatment at randomization. The participant will continue treatment on his or her own thereafter per protocol. Staff will check in regularly with the participant after initiation of treatment to determine if there are any problems. A staff member will contact the participant between visits in an effort to identify issues that may pose challenges for ongoing study participation. This will allow the study staff to intervene as necessary to ensure maximal participant retention.

Participants who consented to the optional A-HRSI scale responsiveness substudy (n=100, completed in February 2020) completed questionnaires at 2 additional timepoints, targeted for 2 weeks after completing the initial treatment (time 2 window: 10-70 days after randomization) and after completing all treatment for the first six-month block (time 3 window: 71-116 days after randomization) via facilitated interview with MSKCC staff or self-administered ePRO questionnaire in AdvantageEDC.

Participants who consent to the HRQoL objective (n=500, added with protocol version 13.0) will complete questionnaires at randomization (discussed above) and 7 additional timepoints (see Appendix I, HrQOL Objective, for the windows for target completion and accepted forms:

Table 2-C: A-HRSI completion time points for QoL objective

Time Point	Target Date
T1	Visit 1, before randomization
T2	2-7 days after randomization
T3	4 weeks after randomization
T4 – T8	12, 24, 36, 48, and 60 months

Participants will be encouraged to complete questionnaires as self-administered via ePRO in AdvantageEDC; the option to complete the questionnaires via facilitated interview with clinical site staff (during visits or via telephone) will be available. A separate form collecting contact information updates and the participant's preferences on the next questionnaire completion will also be administered at each time point.

Treatment cycles consist of 6-month blocks. The schedule of interim visits during these blocks varies according to the treatment, as specified in the algorithms of Figure 4-A and Figure 4-B..

Visit 2 and Subsequent Visits: If randomized to the active monitoring arm, the participant will be seen every 6 months. At each of these visits, the clinician will collect three anal swabs: the first for local cytology and correlative studies, the second for DNA, and a third swab for RNA and protein studies. A DARE will be performed. HRA will be performed. At HRA the clinician will be asked to determine whether a lesion at a given visit is a recurrence in an area previously noted, or an incident lesion in a new area. At each visit the clinician will carefully map the location of each lesion and where they did their biopsies. Sites will also be requested to photograph each lesion at every visit using Second Opinion or other software that allows for easy sharing of images between study sites. The purpose of the cytology is to serve as quality control measure for the presence of HSIL. HSIL on cytology has high predictive value for detection of a HSIL on biopsy (64) (65). If the clinician is performing biopsies and the cytology shows HSIL but the biopsies do not, then the clinician will ask the participant to return for a repeat HRA at 3 months after the visit (± 4 weeks) to try to locate the HSIL.

If the participant has been randomized to the treatment arm, he or she will follow the protocol specific to the treatment modality selected by the clinician. Once the participant has been cleared of HSIL, he or she will then be followed every 6 months as described for those in the active monitoring arm. Further details of the procedures in the treatment and active monitoring arms are provided in Section 8.0.

Participants will be asked to donate samples of blood and anal swabs for research

at every 6-month HRA visit. An additional blood sample (processed for plasma and whole blood fraction) will be stored for future studies at Visit 2, and serum will be stored from each 6-month visit. Anal biopsy tissue will be collected at each 6-month visit for those in the treatment arm who are suspected of having HSIL at HRA and potentially more often depending on the study arm and treatment modality. HSIL lesions will be biopsied annually for those in the active monitoring arm. The cytology swabs and formalin-fixed biopsy tissues will be processed as described in Section 9.0. Additionally, participants treated with ablative therapies will have one additional biopsy taken from the lesion with the most severe appearance of disease prior to treatment, no more than once every six months. This biopsy will be placed in RNALater for banking only. If a participant is diagnosed with cancer during screening or following randomization, an optional cancer biopsy (placed in RNALater) may be collected if the participant is willing to return to the clinic for biopsy before referral to treatment and study discontinuation.

2.5 Correlative Studies

The ANCHOR study offers many scientific opportunities. We will be screening 17,385 HIV-infected men and women, to identify and enroll 5,058 with anal HSIL. Approximately 12,000 individuals will not be enrolled in the study, many of whom because they had LSIL or no disease at screening. Studying these individuals may be of great interest scientifically for the natural history of incident HSIL, but this is beyond the focus of the ANCHOR study. The correlative science studies described in this section were instead selected to focus on the issues that make this trial truly unique, i.e., progression of HSIL to cancer. Using the data and samples collected in the ANCHOR study, many questions can be asked about the molecular mechanisms of progression of HSIL to cancer. This correlative science section therefore focuses on three kinds of studies: 1) studies that will elucidate viral and host molecular mechanisms of progression from HSIL to cancer, 2) studies that focus on identification of biomarkers that will identify individuals at particularly high risk of progression from HSIL to cancer, and 3) studies that identify medical and behavioral risk factors for progression of HSIL to cancer.

Any use of biospecimens collected as part of this trial requires specific review and approval by NCI CTEP or other mechanisms as will be set up for the NCI Clinical Trials Network (NCTN) or by OHAM, and by the ANCHOR Correlative Science Committee. For a request for use of specimens to be considered, a protocol for use of banked specimens (or an amendment to the protocol if the trial is still active) must be submitted to NCI CTEP; the protocol submitted for review must contain a clear statement of the scientific objectives and hypotheses, a statistical section that provides a brief description of the statistical design and analysis strategy along with sample size/power justification, a description of the assay methodology, and identification of the laboratory/individuals that will perform the assays. For non-standard assays, information about the assay's analytical performance (e.g., sensitivity, specificity, bias, linearity, reproducibility, as applicable) also may be requested by the reviewers.

The studies described below are not meant in any way to be a complete list; in each category there are several other questions that could be addressed, and we fully expect that many new questions will arise in the course of the study for each category. We therefore plan to ensure that specimen collection will be adequate to address the questions below, as

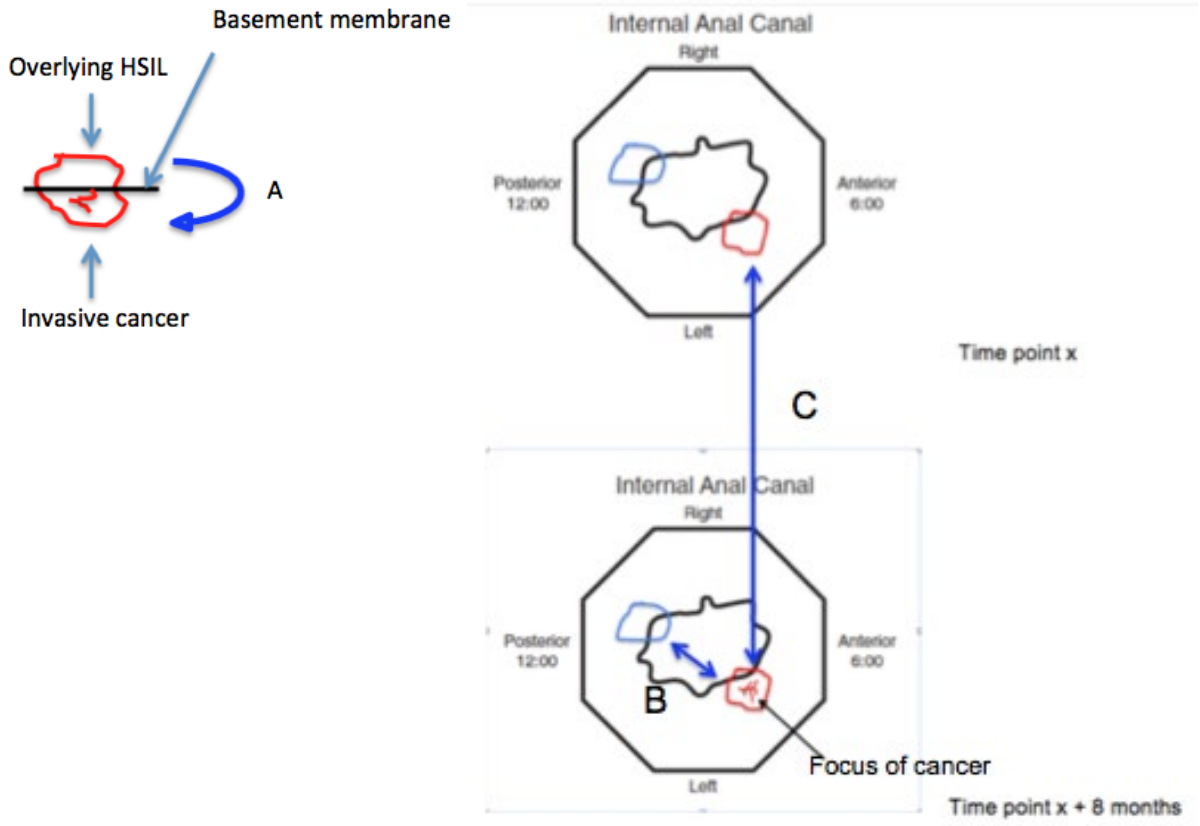
well as provide the opportunity to address new questions in the future as new data become available. These studies will be funded by mechanisms external to the ANCHOR study, mostly R21 and R01 grants. As such they will also undergo additional peer review prior to implementation, as described above.

The studies described below that involve analysis of HSIL and cancer biopsies include three parallel experiments. Please see Figure 2-D on the next page. It is expected that most participants with anal cancer will have overlying HSIL, as well as several, anatomically discrete areas of HSIL that did not progress to cancer. Tissues shown to have invasive cancer will be microdissected to allow for analysis of the invasive cancer specifically, and compared with overlying HSIL. By identifying differences between the cancer and overlying HSIL, we will elucidate some of the steps involved in progression from HSIL to cancer.

The biopsy that is microdissected for the invasive cancer part of the tissue will allow us to analyze the microdissected HSIL portion of the tissue that had not invaded. We will therefore have the opportunity to compare HSIL that progressed to cancer with concurrent HSIL that did not progress from the same participant, since most participants will have multiple foci of HSIL. We will also have the opportunity to compare the HSIL that progressed to cancer at a given visit to the same HSIL at one or more visits prior to the cancer progression.

Comparing cancers to overlying HSIL offers different information from comparing HSIL that progressed with HSIL that did not progress. Comparing cancers and overlying HSIL may identify some of the last steps in progression to cancer. Similar comparisons between HSIL that progressed and HSIL that did not progress will provide insight into some of the penultimate steps in cancer progression. Steps that are even earlier in the process may be identified by comparing HSIL that progressed to cancer to the same lesion at earlier time points prior to progression to cancer. Analysis of host and viral factors in these distinct subsets of HSIL and in the cancer portion of the tissue will elucidate steps in the progression to cancer and will be useful to develop biomarkers that identify individuals at high risk of incipient progression to cancer.

Figure 2-D Paired Lesion Comparisons



The left side of the figure shows Comparison A, depicted in the blue arrow, between an invasive cancer invading through the basement membrane, and the overlying HSIL. The tissue is viewed as it would be cut for routine histopathology assessment. The right side of the figure depicts two other sets of comparisons. The view is through an anoscope via high resolution anoscopy. The participant is on the examining table in the left lateral decubitus position, and left, right, posterior, and anterior positions are indicated. Two HSIL lesions are seen. One is at the right posterolateral octant (depicted in blue) at the squamocolumnar junction, and the other is at the left anterolateral octant at the squamocolumnar junction (depicted in red). If the cancer is detected for the first time in the left anterolateral lesion at a given study visit, it will be possible to compare the overlying HSIL from that lesion to the right posterolateral lesion, which has not changed and which has not progressed to cancer (Comparison B). It will also be possible to compare the overlying HSIL at the time the cancer is diagnosed at the left anterolateral lesion with HSIL at the same location at an earlier time point (Comparison C). For the purpose of this figure we have illustrated a time point 8 months before the cancer was first diagnosed, but any study visit prior to the diagnosis of cancer at which the HSIL was visualized at that location could be chosen.

Anal biopsy samples will be collected as described previously. It is presumed that most if not all participants who are diagnosed with anal cancer after enrollment will by definition have an early cancer since they did not have cancer at baseline and they will have been followed closely every 6 months. Many of these cancers will be diagnosed as "at least superficially invasive squamous cell carcinoma (SISCCA), and further surgical excision

(TUA) is needed to determine if they meet the full definition of SISCCA. This is clinically important since they may be treatable with surgical excision instead of CRT. TUA also offers the opportunity to collect additional biopsies for research, including frozen biopsies if the size of the lesions permits.

Individuals whose cancer is shown to be beyond the point of SISCCA at the time of initial diagnosis will not undergo TUA because there is no clinical indication to do so; they will instead be referred immediately for CRT. In this case, there will not be additional biopsies obtained for research but the area of invasive cancer will by definition be larger than what is available for research in a SISCCA, and it is likely that these fully invasive tumors will contain sufficient material for the proposed analyses.

The first two sets of correlative science studies described below focus on identifying viral and host factors important in the pathogenesis of progression from HSIL to cancer, respectively. The focus of the third correlative study is to study viral and host proteins, genetic changes, etc., already shown in the literature to be of interest in predicting prevalent or incident HSIL (primarily cervical) and determining whether these are of value as potential clinical biomarkers to predict progression from HSIL to cancer.

2.5.1 Overview of sample collection

1) Participants who sign the informed consent and agree to be screened (N= 17,385) at Visit 0 will provide a sample of blood and three anal swabs for anal cytology, DNA and RNA/protein testing. We will create extra monolayer cytology slides from the swab used for anal cytology. These specimens will be donated to the ANCHOR Biorepository if the participant does not qualify for the study and if the participant consents to future testing of his/her specimens. These samples will be used for studies that wish to explore risk factors for having prevalent anal LSIL or HSIL and HPV infection, as well as studies that recruit from among those who did not qualify for the ANCHOR study but who will be recruited for other prospective studies.

2) We will collect serum (N= 5,058) from each participant every 6 months during study participation (Visits 2-11). An additional blood sample (processed for plasma and whole blood fraction) will be stored for future studies at Visit 2. At each 6-month HRA visit, we will collect an anal swab for cytology, a second anal swab for DNA studies, and a third anal swab for RNA and protein studies. We will create extra monolayer cytology slides from the swab used for anal cytology. A sample of blood from each participant (N= 5,058) will also be stored for each participant at Visit 2. Serum, blood, and swab samples will be stored centrally at the ANCHOR Biorepository but will belong to the ANCHOR study until released to outside investigators after completion of the primary ANCHOR analyses as described in this section.

3) Formalin-fixed, paraffin-embedded anal biopsy specimens will be processed for routine H and E histopathologic assessment. The remaining tissue block will be stored locally if the local institution requires it, as long as they agree to send specific blocks to the central ANCHOR study laboratory upon request. Alternatively, institutions may choose to send all of their remaining blocks to the central laboratory after the initial diagnostic slides are read. Each participant

will have at least one biopsy per year, and these will likely span the range from normal to LSIL, HSIL and cancer. Biopsy slides will be sent to the Central Pathology Laboratory at UCSF upon request for central review. Additionally, participants treated with ablative therapies will have one additional biopsy taken from the lesion with the most severe appearance of disease prior to treatment, no more than once every six months. This biopsy will be placed in RNAlater for banking only. Lastly, participants diagnosed with cancer during screening or following randomization, may provide an optional cancer biopsy (placed in RNALater) if willing to return to the clinic for biopsy before referral to treatment and study discontinuation.

4) Formalin-fixed, paraffin-embedded biopsies shown to have invasive anal cancer will be sectioned for laser capture microdissection (LCM). Areas of the tissue containing invasive cancer will be removed and processed for the studies described below. The HSIL portion of the tissue containing the cancer will be similarly processed, as will biopsies of HSIL taken at the same visit that did not progress to cancer. HSIL biopsies collected at visits prior to the visit at which that HSIL lesion was shown to have progressed to cancer will also be available. We can use these samples to study expression of genes and proteins of interest at different time points prior to the development of anal cancer, since we will have a set of preceding HSIL biopsies obtained at least annually prior to the time of cancer diagnosis. A portion of each tissue will be placed on tissue microarrays for immunohistochemical analysis, and the remaining tissue will be processed for DNA studies and RNA microarray gene expression analysis. As described in Section 4.4.2, if a woman participating in the ANCHOR study is diagnosed with cervical cancer during the study, we will ask her for permission with a separate informed consent form to retrieve tissue blocks of the cancer. These may be of value for correlative science and comparison with the anal specimens collected as part of the ANCHOR study.

5) We will maintain an inventory of all of the tissues collected in the study and stored at the ANCHOR Biorepository. For any requests to use banked samples for other correlative science studies, a full protocol will be submitted for approval in accordance with NCI, CTEP policies. No correlative study using the specimens collected in this trial will be conducted unless it is explicitly approved as part of the main trial protocol or it is approved through mechanisms that will be established for the ANCHOR Study. Top priority will be given to using the samples to address the correlative science studies that have been separately peer-reviewed and funded. Additional studies consistent with the ANCHOR study agenda may be proposed by outside investigators, and reviewed by ANCHOR study investigators using a letter of intent mechanism. Upon approval of the ANCHOR study committee and CTEP, the tissues will be released to the outside investigator. Tissues and samples that remain after all of the ANCHOR study scientific questions have been addressed will be released to the ANCHOR Biorepository. These are available to investigators around the world who submit applications to use these specimens for ACSR scientific review.

2.6 Ancillary Study: SARS-CoV-2 and Anal HPV Infection

2.6.1 Summary and aims

The SARS-CoV-2 pandemic has had a dramatic effect impact on the conduct of the study, prompting all ANCHOR study sites around the country to cease screening, enrollment and follow-up of randomized participants from March 20, 2020 until May 18, 2020, with sites resuming normal activity over time through September 2020. However, apart from affecting normal study activities, SARS-CoV-2 may have additional relevance by affecting the natural history of anal HPV infection, development and persistence of anal HSIL, and progression from HSIL to anal cancer. This is because of the growing body of evidence that SARS-CoV-2 is shed in stool and may infect the gastrointestinal tract. Nothing is known at present as to whether SARS-CoV-2 can infect the anal epithelium specifically, whether it affects the biology of anal HPV infection, how it affects the local immune response, and whether it affects the natural history of anal HSIL. Furthermore, nothing is known about anal SARS-CoV-2 infection in the group at highest risk of anal cancer, PLWH. The ANCHOR study offers an ideal opportunity to begin to address these issues, particularly since a high proportion of participants in the ANCHOR Study are from medically underserved minority populations who are also at very high risk of SARS-CoV-2 infection.

The focus of this supplemental study is to describe detection of SARS-CoV-2 in anal swab samples from PLWH being screened for the ANCHOR study; examine its relationship to prevalent anal HPV infection and HSIL in the screening population; and determine its effect on the natural history of anal HPV infection and HSIL by examining its relationship to regression of HSIL and clearance of HPV infection in the subset of enrolled participants randomized to the active monitoring arm. Accordingly, our specific aims are outlined in Section 1.6.

2.6.2 Background

In early 2020, the global pandemic of SARS-CoV-2 virus spread to the United States and as of late October 2020, has led to over 8.7 million cases and over 225,000 deaths (132). While the main cause of mortality from SARS-CoV-2 is respiratory failure, there is growing evidence that SARS-CoV-2 can infect the gastrointestinal tract and be associated with gastrointestinal symptoms such as diarrhea (133-136). Several studies have demonstrated that SARS-CoV-2 is shed in stool samples (133-142). Multiple studies also show that stool shedding may persist after oropharyngeal shedding is no longer detectable (134, 138, 140, 142, 143). In one study from Hong Kong, SARS-CoV-2 RNA was detected in stool samples from 48% of patients after respiratory samples tested negative (139). In another study from Wuhan, 18 (64%) patients remained positive for viral RNA in feces after pharyngeal swabs turned negative. The duration of viral shedding from the feces after negative conversion in pharyngeal swabs was 7 (6-10) days, regardless of SARS-CoV-2 severity (136).

Consistent with stool shedding of SARS-CoV-2, a recent study showed that the virus can productively infect intestinal enterocytes (144). Another study using electron microscopy showed what appeared to be intact viral particles in stool

specimens (145) consistent with the possibility that virus shed from the gastrointestinal tract is infectious. Furthermore, there are also data showing that SARS-CoV-2 may remain viable in environmental conditions that could facilitate fecal-oral transmission (146). Thus, there are multiple implications of detection of SARS-CoV-2 in the stool. First, if stool shedding persists after oropharyngeal shedding, diagnosis of SARS-CoV-2 infection, contact tracing and pandemic control may be facilitated by routine anal swab detection of SARS-CoV-2 in addition to oropharyngeal sampling. Second, if SARS-CoV-2 infects the gastrointestinal tract and viable virus is shed in stool, there is the possibility of sexual transmission, particularly through oro-anal contact.

This supplement focuses on the third important implication of stool shedding, i.e., if SARS-CoV-2 infects the anal epithelium as it does in other parts of the gastrointestinal tract, there is the possibility that it may affect the natural history of anal HPV infection and HPV-associated pre-cancerous lesions, high-grade squamous intraepithelial lesions (HSIL). SARS-CoV-2 is known to lead to a large inflammatory response in the lungs, but its effect on the gastrointestinal tract is less well understood.

Infection with SARS-CoV-2 of enterocytes elicited a broad signature of cytokines and interferon -stimulated genes (144). These data suggest that SARS-CoV-2 may affect local immune response. Based on its effect on pulmonary inflammation, it is plausible that SARS-CoV-2 has the potential to lead to a pro-inflammatory anal environment with exacerbation of existing infection with HPV, impaired clearance of HSIL, and increased likelihood of progression to cancer.

The gastrointestinal tract is a highly diverse organ with different parts exhibiting distinct biology and microenvironments. The anal epithelium is the most distal part of the gastrointestinal tract and is probably the most under-studied section. It is biologically quite distinct from the rectum, colon and small intestine, being comprised of stratified squamous epithelium. It has a distinct vascular supply and lymphatic drainage from the colon and rectum. We recently showed that the anal microbiome is distinct from that of stool specimens, which reflect the entirety of the colon and rectum (C. Brickman and J. Palefsky, submitted for publication). HPV infects only the anus, but not the rectum or colon.

Nothing is known at present as to whether SARS-CoV-2 can infect the anal epithelium and/or mediate local inflammatory changes that might affect the natural history of HPV-related disease. SARS-CoV-2 uses the receptor ACE-2 for entry (147). A recent study has shown that, consistent with their susceptibility to SARS-CoV-2 infection and replication, high levels of ACE-2 mRNA and protein are found in the small intestinal enterocytes but not in the goblet cells or intestinal immune cells. High expression of ACE-2 on the surface cells may lead to gastrointestinal symptoms, mediating SARS-CoV-2 invasion and amplification of the virus and activation of gastrointestinal inflammation (148).

In addition to binding of SARS-CoV-2 spike proteins to ACE-2, entry of coronaviruses depends on S protein priming by host cell proteases. In a recent study it was shown that SARS-CoV-2 uses the serine protease TMPRSS2 for S protein

priming (147). In another recent study, expression of two mucosa-specific serine proteases, TMPRSS2 and TMPRSS4, were shown to facilitate SARS-CoV-2 spike fusogenic activity, promoting virus entry into host cells (149). In the Preliminary Results section we show that anal tissues express both ACE-2 and TMPRSS2.

2.6.3 Preliminary results

Studying anal SARS-CoV-2 infection in the ANCHOR study: There are many unanswered questions about the impact of SARS-CoV-2, infection on the risk of developing anal cancer in the group at highest risk, people living with HIV (PLWH) and there are several reasons to perform this study as a supplement to the ANCHOR Study. First, the ANCHOR Study is continuing to screen participants, with enrollment currently at 4033 out of a target of 5058. Second, the protocol includes collection of all of the specimens and information that we would want to collect for this supplement, with the exception of the oropharyngeal swab for SARS-CoV-2 detection. This includes anal swabs for HPV testing and SARS-CoV-2 testing, and high resolution anoscopy (HRA)-guided biopsies to determine the presence or absence of anal HSIL. Third, the demographic groups typically enrolled in ANCHOR are also known to be among the groups at highest risk for SARS-CoV-2 infection, with African American and Hispanic men and women comprising well over half of our screened and enrolled population. Fourth, all PLWH enrolled into ANCHOR have anal HSIL and are followed every 6 months or more often, giving us the opportunity to determine the incidence of SARS-CoV-2 infection in this high-risk population. Fifth, half of the enrolled participants will be randomized to the active monitoring arm, which will allow us to determine if prevalence or incidence of SARS-CoV-2 affects persistence of HPV or anal HSIL.

Impact: Our results could have important implications for development of anal cancer in PLWH. SARS-CoV-2 is likely to be circulating for several years, and there is a possibility of latency or reinfection. This is not likely to be an acute infection like influenza, and if SARS-CoV-2 can infect the anal epithelium an effect on the natural history of anal HPV infection and progression from HSIL to cancer is very plausible. We need to understand this relationship. Further studies will be needed to explore ACE-2 expression, TMPRSS2 expression and SARS-CoV-2 in anal epithelium, how expression varies in lesions of different histologic severity, the effect of SARS-CoV-2 on local inflammatory response and interaction between HPV and SARS-CoV-2 at the molecular level. If we find anal shedding in the absence of oropharyngeal positivity, this also has implications for case finding and control.

Studies of anal epithelium and susceptibility to SARS-CoV-2: Nothing has been published about ACE-2 or TMPRSS2 expression in the anal epithelium. We previously created RNA microarray libraries from a set of anal biopsies, and reviewed those data for expression of ACE-2 or TMPRSS2. Our data showed that there were detectable levels of expression of both of these genes in the tissues. Nothing is known at this time as to where ACE-2 is found in anal epithelium, nor its relationship to HPV-related lesions. Given the wide organ tropism of SARS-CoV-2, evidence for intestinal ACE-2 expression and intestinal SARS-CoV-2 infection and replication, and our preliminary evidence of ACE-2 and TMPRSS2

expression in anal epithelium, it is plausible that SARS-CoV-2 also infects anal epithelium.

We have now had the opportunity to study the natural history of anal HSIL among those randomized to the monitoring arm for up to 3 years. Among 390 participants examined at the 3-year timepoint, 39% had no HSIL. At the one-year timepoint among 1250 participants, 27% had no HSIL. These data indicate that a substantial number of PLWH undergo regression of HSIL after entry into the study. Some of this may reflect biopsy-associated physical removal of the lesion among those who entered the study with small lesions, and some may have missed disease that will be diagnosed again at future visits. However, some of these data almost certainly reflect true regression. Those with persistent HSIL are the ones who are probably the likeliest to progress to cancer over time.

SARS-CoV-2 testing in anal specimens: The test that we propose to use for SARS-CoV-2 testing is the Atila AmpFire system, the same system that we will use for HPV testing for this study. This is a system that we have installed in our laboratory and which we have used for HPV testing and specific genotyping. We have also installed the Atila system in Rwanda, Zimbabwe, Mexico and Puerto Rico. We have validated the assay against the WHO panel in our laboratory and the test works very well. In a recent study, the AmpFire assay detected HPV in clinical samples with positive percent agreements of 100.0% for HPV16, 100.0% for HPV18, and 94.7% for non-16/18 HR HPV, and 100% negative percent agreements for HPV16, HPV18, and non-16/18 HR HPV (150). This same technology had received FDA approval for SARS-CoV-2 testing of oropharyngeal swabs. It has not been tested yet in anal swab specimens, but based on our experience with HPV testing, we expect that this system will work well for this purpose.

2.6.4 Research plan

Study summary: To address Aim 1 we will enroll the first 400 PLWH who are being screened for the ANCHOR Study. Participants will be recruited at five ANCHOR centers: UCSF, Laser Surgery Care (New York), Anal Dysplasia Clinic MidWest (Chicago), University of Miami, and Emory University (Atlanta). The only additional procedure that participants will need to undergo to participate is an oropharyngeal swab for SARS-CoV-2 testing. Each participant in the ANCHOR Study has 3 anal swabs collected. The first and second swabs are placed in Thinprep, vigorously swished to remove the cellular material, and the swab is discarded. The third swab will be stored in RNALater. The anal specimens that will be tested this supplement will be retrieved from the ANCHOR Biorepository. An aliquot of Anal Swab 2 will be used for HPV testing and SARS-CoV-2 testing.

To address Aim 2, we will quantify the HPV data in Swab 2 based on the Ct value, and categorize the quantitative results into tertiles. The presence or absence of HPV, quantity of HPV among those who are HPV-positive, and presence or absence of anal HSIL will be correlated with the presence of SARS-CoV-2 in the anal swab.

To address Aim 3, we will repeat these procedures at 6 months among those who were enrolled into the active monitoring arm of the study. As at baseline, the only

additional procedure that participants would need to undergo to participate is an oropharyngeal swab for SARS-CoV-2 testing. Participants will undergo standard study procedures including collection of the anal swabs and HRA, and the presence of absence of visible HSIL will be noted at that visit. If we screen 400 PLWH we expect approximately 160 to be enrolled into either the treatment arm or the active monitoring arm and will be re-sampled at 6 months. Anal swab 1 will be studied for SARS-CoV-2 and for HPV DNA.

To address Aim 4, we will focus the analysis on the half of the 160 enrolled participants who were randomized to the monitoring arm (N=80). These individuals do not undergo treatment of their lesion. The results of the SARS-CoV-2 testing in the anal swab will be correlated with the HPV results and presence or absence of HSIL. Detection of HPV and SARS-CoV-2 in anal and oropharyngeal specimens. To detect HPV, we will use the Atila AmpFire system. This is a real-time reverse transcription fluorescent isothermal amplification test, based on isothermal amplification technology termed OMEGA amplification (20). The test individually detects the 15 most common high-risk HPV genotypes with the quantity measurable by determining the threshold cycle (Ct) at which the DNA is detected.

Like the HPV detection test that we will be using for this study, the iAMP COVID-19 Detection Kit is a real-time reverse transcription fluorescent isothermal amplification test. OMEGA primer sets are designed to specifically detect RNA and later cDNA from the N and ORF-1ab genes of the SARS CoV-2 virus in nasal, oropharyngeal and/or oropharyngeal swabs from patients with signs and symptoms of infection who are suspected of SARS-CoV-2. The test detects 2000 copies of viral RNA per swab.

One of the advantages of the iAMP SARS-CoV-2 assay is that it can detect SARS-CoV-2 RNA directly from samples without a prior RNA extraction process. To detect SARS-CoV-2 from Thinprep solution, we will transfer 1ml Thinprep sample from the first swab to a 2 ml Eppendorf tube. We will then centrifuge and pellet the cells, discard the supernatant, then lyse the cells to detect SARS-CoV-2 RNA. Results are available in approximately one hour.

We will first determine the analytic sensitivity of the SARS-CoV-2 test in Thinprep by spiking known quantities of SARS-CoV-2 RNA into Thinprep specimens. We will then extract RNA from the specimens using the Qiagen AllPrep PowerViral DNA/RNA Kit**.** This kit is designed for efficient purification of viral and bacterial total nucleic acids from samples high in PCR inhibitors, including stool. Viral nucleic acids eluted in RNase-free water are ready to use with the iAMP-CoV-2 assay.

If we are able to detect 50 viral copies in material eluted from 1 mL of Thinprep material with the RNA extraction step, we will begin to analyze real anal swab Thinprep specimens from non-ANCHOR participants to determine if this results in loss of analytic sensitivity. If our results show acceptable sensitivity and specificity, we will proceed to analyze the specimens obtained from ANCHOR participants in this study. If we are not satisfied with the results, we also have the opportunity to use Anal Swab 3, which was placed in RNALater. We are confident that this swab

in RNALater will work well but have chosen to use Swab 2 in Thinprep because it does not require a RNA extraction step and because it is a more abundant specimen than the RNALater specimen.

Oropharyngeal swabs will be collected from participants and placed in a dry tube per the protocol approved by the FDA, stored at -20C at the ANCHOR Biorepository and shipped on dry ice to UCSF. Once ready for testing 1 ml of SARS-CoV-2 sample buffer will be added and incubated at room temperature for 15 minutes. 10 uL of SARS-CoV-2 master mix are added and the SARS-CoV-2 isothermal reaction is performed. Results are expressed as positive or negative, but we will also have the opportunity to examine quantity of viral RNA by determining the Ct at which it is detected.

3.0 PATIENT SELECTION

All protocol participants must meet all stated eligibility criteria. Before any participant is randomized, a clinician who is certified in HRA by the ANCHOR HRA Committee must document that the participant satisfies each eligibility requirement. In compliance with CTEP policy, no exceptions to eligibility criteria will be granted under any circumstance.

3.1 Eligibility Criteria

3.1.1 HIV positive. Documentation of HIV-1 infection by means of any one of the following:

- Documentation of HIV diagnosis in the medical record by a licensed health care provider;
- Documentation of receipt of ART by a licensed health care provider (Documentation may be a record of an ART prescription in the participant's medical record, a written prescription in the name of the participant for ART, or pill bottles for ART with a label showing the participant's name. Receipt of at least two agents is required; each component agent of a multi-class combination ART regimen will be counted toward the 2-agent requirement, excepting receipt of a PrEP regimen alone [e.g., Truvada], which is exclusionary);
- HIV-1 RNA detection by a licensed HIV-1 RNA assay demonstrating >1000 RNA copies/mL;
- Any licensed HIV screening antibody and/or HIV antibody/antigen combination assay confirmed by a second licensed HIV assay such as a HIV-1 Western blot confirmation or HIV rapid multispot antibody differentiation assay.

NOTE: A "licensed" assay refers to a U.S. FDA-approved assay, which is required for all IND studies.

3.1.2 Age 35 years or older. This age restriction is intended to enrich the study population at risk for cancer since anal cancer occurs only rarely under this age even among HIV-infected individuals. Fewer than 1% of anal cancers occur under the age of 35 years (1).

3.1.3 Biopsy-proven anal HSIL at baseline (AIN2 with a positive p16 stain, AIN2-3, or AIN3).

3.1.4 At least one focus of HSIL must be identified that is not within a condyloma that may be treated after enrollment into the study. This requirement is to ensure that there will still be at least one focus of HSIL among participants in the Active Monitoring Arm even if they undergo treatment for condyloma.

3.1.5 For females, documentation that the participant is being followed with cervical cytology and/or HPV testing per current "Guidelines for the Prevention and Treatment of Opportunistic Infections in HIV-Infected Adults and Adolescents" and American Society for Colposcopy and Cervical Pathology (ASCCP)

guidelines.[1,2] Cervical cytology must be performed prior to enrollment for women who are overdue for screening per the guidelines. Women should also have confirmation of absence of cancer or suspected cancer upon visual examination of the vulva, vagina, and cervix within 12 months prior to enrollment.

3.1.6 ECOG performance status ≤ 1 (Karnofsky ≥ 70%, see Appendix II)

3.1.7 Life expectancy of greater than 5 years

3.1.8 Participants must meet the following parameters within 90 days before enrollment

- Absolute neutrophil count: ≥750/mm^3
- Platelets: ≥75,000/mm^3
- Hemoglobin ≥ 9.0 g/dL

3.1.9 Women of childbearing potential (FCBP)† must have a negative urine pregnancy test within 7 days prior to randomization enrollment. Female participants enrolled in the treatment arm are advised to not become pregnant during study participation due to the risks of the study treatments. All women of childbearing potential must agree to either commit to continued abstinence from heterosexual intercourse or to use a reliable birth control method during heterosexual intercourse (oral contraceptive pills, intrauterine device, Nexplanon, Depo-Provera, or bilateral tubal ligation, etc., or another acceptable method as determined by the investigator) during the entire period of the trial (5 years or more), and must not intend to become pregnant during study participation and for 3 months after treatment is discontinued if the participant is enrolled in the treatment arm. Female participants, if engaging in heterosexual intercourse, must be willing to comply with an acceptable birth control regimen as determined by the Investigator.

† A female of childbearing potential is a sexually mature woman who: 1) has not undergone a hysterectomy or bilateral oophorectomy; 2) has not been naturally postmenopausal for at least 24 consecutive months (i.e., has had menses at any time in the preceding 24 consecutive months).

3.1.10 Men randomized to the treatment arm should not father a baby while receiving topical treatment during this study. Men who could father a child must agree to use at least one form of birth control during or continued abstinence from heterosexual intercourse if receiving topical treatment during the study, and for 2 weeks after stopping topical treatment.

3.1.11 Participant is willing to be randomized and able to comply with the protocol

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[1] Panel on Opportunistic Infections in HIV-Infected Adults and Adolescents. Guidelines for the prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from the Centers for Disease Control and Prevention, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. Available at http://aidsinfo.nih.gov/contentfiles/lvguidelines/adult_oi.pdf. Accessed March 2, 2016.

[2] Massad LS, Einstein MH, Huh WK, Katki HA, Kinney WK, Schiffman M, Solomon D, Wentzensen N, Lawson HW; 2012 ASCCP Consensus Guidelines Conference. 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors. Obstet Gynecol. 2013 Apr;121(4):829-46.

3.1.12 Clinician is comfortable that cancer has adequately been ruled out and is willing to follow the participant for up to 5 years without treatment of the HSIL.

3.2 Exclusion Criteria

Patients who do not fulfill the criteria as listed in Section 3.1 above, are ineligible. Additionally**,** the presence of any of the following conditions will exclude a participant from study enrollment:

3.2.1 Participant is unable to provide informed consent

3.2.2 Participants who received any other chronic (defined as more than 50% of the time in the last 6 months) systemic immunomodulatory agents (replacement doses of steroids for adrenal insufficiency are permitted or treatment with prednisone ≤5 mg/day). Receipt of investigational agents within the 4 weeks before randomization enrollment, other than investigational antiretroviral agents for HIV and investigational or approved agents for Hepatitis C, are also exclusionary.

3.2.3 History of anal cancer, penile, vulvar, vaginal, or cervical cancer, or signs of any of these malignancies at baseline. Participants with prior carcinoma in situ will not be considered to have prior cancer for eligibility purposes.

3.2.4 Treatment or removal of HSIL less than 6 months prior to randomization.

3.2.5 Participant has symptoms related to HSIL and would benefit more from immediate treatment than from entry into the study and potential for randomization to active monitoring arm

3.2.6 Current systemic chemotherapy or radiation therapy that potentially causes bone marrow suppression that would preclude safe treatment of HSIL

3.2.7 Participants who only have a single HSIL lesion that is likely to be removed entirely with the initial screening biopsy

3.2.8 Warts so extensive that they preclude the clinician from determining the extent and location of HSIL

3.2.9 Participant plans to relocate away from the study site to a location that does not have an ANCHOR study site during study participation

3.3 Inclusion Criteria for the HRQoL Substudy

The HRQoL substudy was completed in February 2020.

3.3.1 Participant has consented for the ANCHOR Study and the A-HRSI scale responsiveness substudy

3.3.2 Fluent in English

3.3.3 The participant is willing to conduct a phone interview with MSK if questionnaires are not completed at the site or as self-administered

3.3.4 Participant is in screening for ANCHOR and the investigator can report a target randomization date for the participant

3.4 Inclusion Criteria for the QoL Objective

3.4.1 Participant has consented for the ANCHOR Study and A-HRSI questionnaire completion

3.4.2 Fluent in English or Spanish

3.4.3 The participant is willing to conduct a phone interview with site staff if questionnaires are not completed at the site or as self-administered

3.4.4 Participant is in screening for ANCHOR and the investigator plans to randomize the participant based on screening anal biopsy results

3.5 Inclusion Criteria for the SARS-CoV-2 Ancillary Study

3.5.1 Participant has consented for the ANCHOR Study and SARS-CoV-2 ancillary study, and is being enrolled at one of the participating centers for this study

3.6 Number of Participants to be Enrolled

3.6.1 Sample size

This study will enroll 5,058 participants.

The optional HRQoL substudy (completed February 2020) enrolled 100 eligible participants (50 from the active monitoring arm and 50 from the treatment arm) who were enrolled for screening and were randomized to ANCHOR.

For the QoL objective, up to 500 randomized participants will be enrolled for QoL questionnaire completion.

For the SARS-CoV-2 ancillary study, a total of 400 participants will be enrolled.

3.6.2 Accrual rate

Approximately 141 participants per month.

3.7 Participant Enrollment Procedures

Sites must have this protocol approved by their Institutional Review Boards (IRB) and be registered for study participation with the ANCHOR Data Management Center (DMC) before they may enroll participants.

3.7.1 Registration for screening

After an informed consent form has been signed by the participant, the participant must be registered for screening on the ANCHOR Protocol, Segment A (on-line via AdvantageEDC) no more than one day after signing consent. After successful registration into screening, the participant will receive an alphanumeric participant ID and will then enter the screening process (screening visit).

Registration for optional A-HRSI substudy or QoL objective

Participants will also be advised of the optional HRQoL substudy or QoL objective (as available for enrollment) during screening for the ANCHOR protocol and will be asked to provide informed consent to the substudy if interested. After the participant agrees to participate in the optional HRQoL substudy or QoL objective and after enrollment into the ANCHOR protocol screening segment in

AdvantageEDC has been completed, the participant must also be registered for the optional A-HRSI substudy on-line via AdvantageEDC no more than one day after the participant is determined to be eligible for the applicable optional study.

Registration for optional SARS-CoV-2 ancillary study

Participants will also be advised of the optional ancillary SARS-CoV-2 study (as available for enrollment) during screening for the ANCHOR protocol and will be asked to provide informed consent to the study if interested. After the participant agrees to participate in the study, the participant will be enrolled at the same time as completing the ANCHOR protocol screening segment in AdvantageEDC.

3.7.2 Enrollment

After the screening evaluations have been obtained and the participant is determined to be eligible, the participating site will complete the protocol-specific eligibility checklist and enroll the participant into the ANCHOR Protocol, Segment B (on-line via AdvantageEDC). Enrollment should occur on the day of randomization and before administration of the first dose of the protocol agent(s). Once the eligibility checklist is submitted, a system generated confirmation email will be sent to the enroller upon successful completion of the participant enrollment. If the on-line system is inaccessible, the site should notify the ANCHOR DMC (via email at anchordmc@emmes.com or via phone at 301-251-1161) for further instructions.

Participants must be enrolled into ANCHOR Protocol Segment B to be randomized to a study arm. This enrollment must occur before receiving the first dose of the protocol agent or treatment.

4.0 TREATMENT PLAN

4.1 Group 1: Active Monitoring Arm

Participants in the active monitoring arm will be assessed with HRA every 6 months, but will have HSIL only biopsied every 12 months unless the clinician chooses to biopsy a lesion sooner because of concern for possible progression to cancer. Biopsies at each annual visit may be deferred if the participant was biopsied at a preceding interim visit that occurred no more than 3 months before the annual visit target date and the lesion appearance has not changed since the prior visit as assessed during the annual visit HRA. Every 12 months, all visible lesions suspicious for HSIL, including index and metachronous HSIL, will be biopsied. At the end of study, all visible lesions and any prior areas of HSIL that appear to have regressed will be biopsied. If this totals less than 4 biopsies, normal-appearing tissue in remaining quadrants will be biopsied so that a minimum of 4 areas are sampled at the final study visit.

Participants will have anal cytology, additional swabs, and blood obtained at each 6-month visit for correlative science studies.

Participants in both arms will also be biopsied any time there is suspicion of progression to cancer. A diagnosis of cancer will lead to immediate referral for treatment. A diagnosis of "suspicious for invasion" or "cannot rule out invasion" will require repeat biopsy. If the diagnosis remains inconclusive after repeat biopsy the case will be submitted to a Central Pathology Review Committee for adjudication. Surgeons performing excisional biopsies in participants in the active monitoring arm to rule out cancer should be asked to remove as much tissue as needed in their clinical judgment to rule out cancer, but not treat or remove any more HSIL than clinically required. If cancer is ruled out, the participant will remain on the study. If the diagnosis is inconclusive, the participant will undergo surgical excision of the lesion to rule out cancer. If cancer is not diagnosed after surgical excision, follow-up will continue. If cancer is diagnosed, the participant will be immediately referred for further evaluation and the stage will be recorded.

4.1.1 Definition and management of drop-in participants

Participants on the active monitoring arm who have anal HSIL treated at any time after randomization will be reported as drop-ins to treatment. Any anal HSIL treatment given to active monitoring arm participants will occur as medical care outside of the study. Any anal HSIL treatment given to active monitoring arm participants will be reported in the HSIL therapy form in AdvantageEDC for each relevant 6-month study period in which treatment was administered. Drop-in participants shall remain on the active monitoring arm and will receive all active monitoring arm study procedures as planned for the duration of the study.

4.2 Group 2: Treatment Arm

Participants in the treatment arm will be assessed with HRA every 6 months and biopsied if persistent or metachronous HSIL is suspected, or if visible changes can be seen in areas of prior treatment. Areas of prior treatment that appear completely normal (e.g., not acetowhite epithelium (AWE), no vascular changes, and strongly Lugol's positive) do not need to be biopsied to confirm the success of treatment. Biopsies at each 6-month visit may be deferred if the participant was biopsied at a preceding interim visit that occurred no

more than 3 months before the 6-month visit target date and the lesion appearance has not changed since the prior visit as assessed during the 6-month visit HRA. At the last study visit, all visible lesions will be biopsied and additional biopsies should be done of normal-appearing areas that were previously treated. If this totals less than 4 biopsies, normal-appearing tissue in remaining quadrants will be biopsied so that a minimum of 4 areas are sampled at the final study visit.

Treatment will be provided from among a list of protocol-approved therapeutic modalities at the discretion of the clinician with the goal of eradicating HSIL as completely as possible. If a participant has persistent HSIL, then a protocol-approved treatment should be continued as clinically indicated or a new protocol treatment should be considered. **Clinicians may choose to use more than one treatment in a given 6-month block, concurrently or sequentially.** If a participant misses a treatment visit for the prior 6-month block and HSIL remains present at the next 6-month visit, the clinician has the option to biopsy, then treat the lesion at the same visit.

If HSIL is not present, the participant will be re-examined every 6 months. Lesions seen on HRA will be biopsied to confirm the success of treatment and to determine the need for additional therapy going forward. Lesions suspicious for HSIL will be biopsied. Participants will have cytology, additional anal swabs, and blood obtained at every 6-month HRA visit. Clinicians also have the option of seeing participants in between 6-month visits if they are concerned for imminent progression to cancer.

Participants will also be biopsied any time there is suspicion of progression to cancer. A diagnosis of cancer will lead to immediate referral for treatment. A diagnosis of "suspicious for invasion" or "cannot rule out invasion" will require repeat biopsy. If the diagnosis remains inconclusive after repeat biopsy the case will be submitted to a Central Pathology Review Committee for adjudication. If cancer is ruled out, the participant will remain on the study. If the diagnosis is inconclusive, the participant will undergo surgical excision of the lesion to rule out cancer. If cancer is not diagnosed after surgical excision, follow-up will continue. If cancer is diagnosed, the participant will be immediately referred for further evaluation and the stage will be recorded.

<u>Participants with persistent HSIL not responsive to multiple treatment courses:</u> Participants with persistent HSIL have the option of being monitored without treatment at the discretion of the treating provider in discussion with the participant. Generally, this should be considered after at least 3 successive 6-month treatment courses and should be for less than one year at a time.

Clinicians may change therapeutic modalities as needed but are asked to make every effort to complete the maximally-allowed number of treatments before concluding that the treatment modality needs to be changed. If a participant is unable to tolerate a given therapy, that will be sufficient reason to change modality regardless of the number of treatments.

Incident lesions will be considered "not previously treated" and may be treated de novo using the approaches described below. In general, study clinicians will be encouraged to use only one treatment modality at a time when treating recurrent and metachronous lesions. It is expected that these lesions will in general be limited in size if the participant is being followed carefully every 6 months or more often if currently under active treatment

for pre-existing HSIL.

The following treatment modalities may be used in the treatment arm of the ANCHOR study:

4.2.1 Patient-applied topical treatments

For imiquimod and 5% 5-fluorouracil (5-FU) cream, we will re-examine the participant at 8 weeks and 16 weeks (± 2 weeks; 16 week visit is optional) after initiation of therapy. The primary goal of the 8-week visit is to examine and interview the participant for clinical toxicity and treatment tolerability. If the lesion appears to be resolved at 8 weeks, the clinician may elect to stop the therapy at that time. If lesions are still seen, treatment may continue for up to 16 weeks. The clinician may perform a treatment follow-up visit at week 16 at his/her discretion. Participants will then be biopsied at the next 6-month visit. This eight-week window between 16 and the 6-month visit is built in to allow inflammation to subside and healing to occur, since this will facilitate assessment of treatment efficacy and biopsy. An algorithm showing the use of imiquimod and 5-FU cream is shown in Figure 4-A.

a) Imiquimod. 5% imiquimod cream will be used. Participants will be instructed to insert the cream intra-anally, peri-anally or both, depending on the location of the lesion. Imiquimod is applied three times per week on alternate days. Each of these weekly cycles may be repeated up to 16 weeks. The imiquimod dose will be 750 mg of cream per week, administered as one single use sachet (250 mg) applied 3 times per week. Participants will be instructed to apply half the contents of a sachet onto a right hand glove or a finger cot and insert the cream approximately one inch into the anus on the right side. They will then place the remaining cream on the left finger, and insert the cream one inch into the anus on the left side. Similar instructions apply for perianal application, but without insertion of the cream into the anal canal. Participants who are being treated for both intra-anal and perianal disease will be given twice the number of sachets as those being treated for intra-anal or perianal disease only. In these cases, double the maximum dose will be administered to treat both areas.

b) 5% fluorouracil cream. The UCSF protocol will be used, in which the patient is instructed on how to apply the cream and where. The cream is applied for 5 consecutive days twice per day (i.e., in the morning and at bedtime), and then there is a break of 9 days. We will allow up to 8 two-week cycles. Participants will be instructed to apply 1 ml of topical 5-fluorouracil 5% cream in the anus. One gram of topical 5-fluorouracil is the equivalent of 1 ml. The total daily dose will be 1 gram of cream, and the weekly dose is 5 grams of cream. 5% 5-fluororacil contains 5 grams of 5-fluororacil per 100 grams. The daily dose is therefore 50 mg of 5-fluororacil delivered in divided doses of 25 mg BID, and the total weekly dose is 250 mg delivered in 5 grams of cream. Participants will be given a 40 gram tube of 5-FU cream and instructed to squeeze out the cream to the marked line on the applicator (0.5 mL of cream), or onto a right hand glove or finger cot. Using a glove or a finger cot they will then squeeze one half the amount of cream (0.25 mL) from the syringe (if using an applicator) onto

their right index finger and insert the cream approximately one inch into the anus on the right side. The participant will then place the remaining cream (0.25 mL) on the left finger, and insert the cream one inch into the anus on the left side. Similar instructions apply for perianal application, but without insertion of the cream into the anal canal. Participants who will be treated for perianal HSIL will be dispensed an additional tube. In these cases, double the maximum dose will be administered to treat both areas.

4.2.2 Ablative treatments

The ablative treatment may be repeated if persistent or metachronous HSIL is documented. An algorithm showing the use of ablative treatments is shown in Figure 4-B.

1) Infrared coagulation, hyfrecation/electrocautery, and surgical lasers can be used interchangeably for in-office ablation. These devices will be used for excision or ablation of lesions with anesthetic using HRA (e.g., magnification with a colposcope or operating microscope, along with 5% acetic acid and Lugol's solutions) at the discretion of the clinician. Staged ablations are permitted for extensive lesions. For large lesions investigators may treat the participant with ablative therapies in a staged manner within the 6-month treatment block. For example, a large lesion may be partially treated at the beginning of the 6-month block, followed by a second procedure to treat the remainder of the lesion within 8 weeks. The number of such treatments and timing will be at the discretion of the investigator.

2) Treatment under anesthesia (TUA): Procedures may be performed in an operating room as a surgical referral or in an office setting on patients with conscious sedation. Referral for surgical excision in an operating room will be performed only as a last resort, if the clinician believes that none of the treatment approaches described above have the potential to resolve the HSIL, or if the participant refuses office ablation. The costs of TUA will only be covered by the study with the QA committee's approval. Patients treated in the operating room for extensive lesions will be re-evaluated 8 to 12 weeks after surgery and then every 6 months if HSIL is not documented. Clinicians may initiate a new 6-month block of therapy if HSIL is still documented at the 8-12 week visit. We expect TUA to be used in only a small percentage of ANCHOR participants, and for these few participants, it is expected that there will be only one procedure during the course of the study. However, there may be exceptions where more than one procedure is required. The procedure will be done either by study clinicians who are surgeons, or by surgeons guided by study clinicians in the operating room.

4.2.3 Recommended treatment algorithms

As indicated previously the ANCHOR study is a "strategy" study, not a study of individual treatment modalities. As such we are examining the effect of making a best effort to treat HSIL on the incidence of anal cancer. We therefore leave the final choice of treatment to the treating clinician, as would be the case in the real world. However, we will provide guidance to the clinicians regarding choice of

treatment approaches, and will require that the modalities that are chosen are administered per protocol using protocol-specified dosing. The choice of therapy will vary depending on the size of lesions, number of lesions, location of lesions, clinician preference, and patient preference. Within these parameters, guidance given to clinicians will be as follows:

1) Lesions larger than 1 cm will generally be treated with an ablative method, with the choice of method depending on the expertise and preference of the clinician
2) If there is a limited amount of residual disease after completion of the first treatment modality, in terms of number or size of lesions these may be treated with patient-applied 5-FU or imiquimod creams.
3) For lesions larger than 1 cm, the clinician may also opt to start with 5-FU or imiquimod, and use targeted ablation of residual disease depending on the number and volume of residual lesions.
4) Metachronous lesions may be treated de novo following the guidelines for lesions found at baseline.
5) In the event that the participant refuses the initial treatment selected for a 6-month block, the clinician may select an alternative treatment as clinically indicated.
6) As described above, TUA will be performed only as a last resort, if the clinician believes that none of the treatment approaches described above have the potential to resolve the HSIL. We expect TUA to be used in only a small percentage of ANCHOR participants.

Figure 4-A: Participant initiates imiquimod/5-FU at visit 1 (randomization visit)

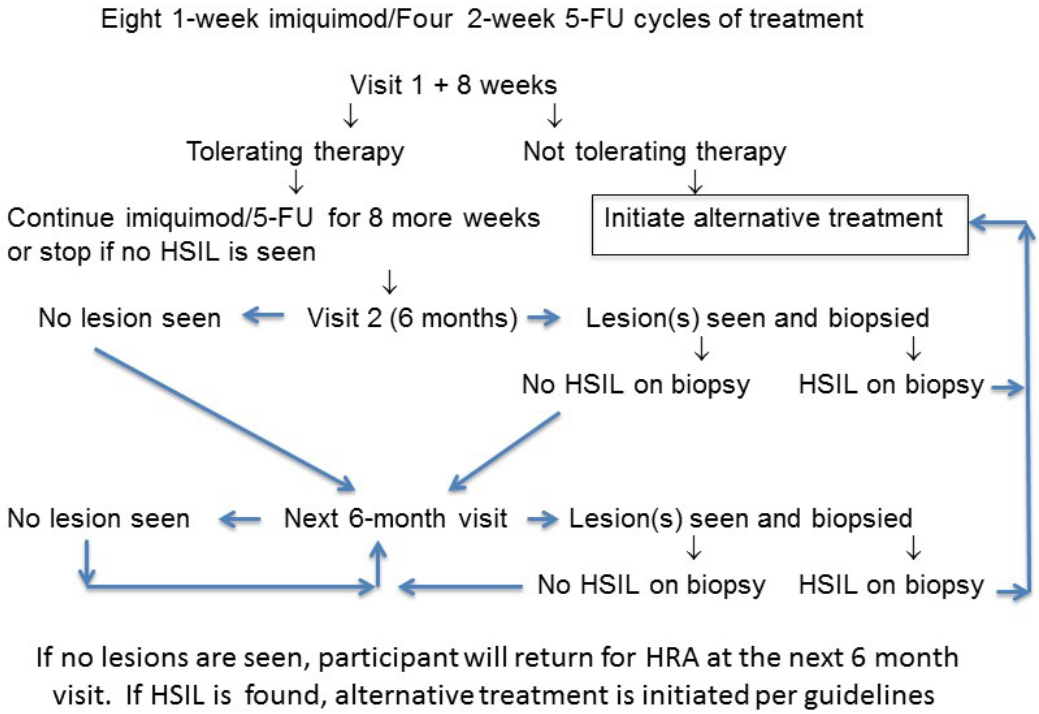
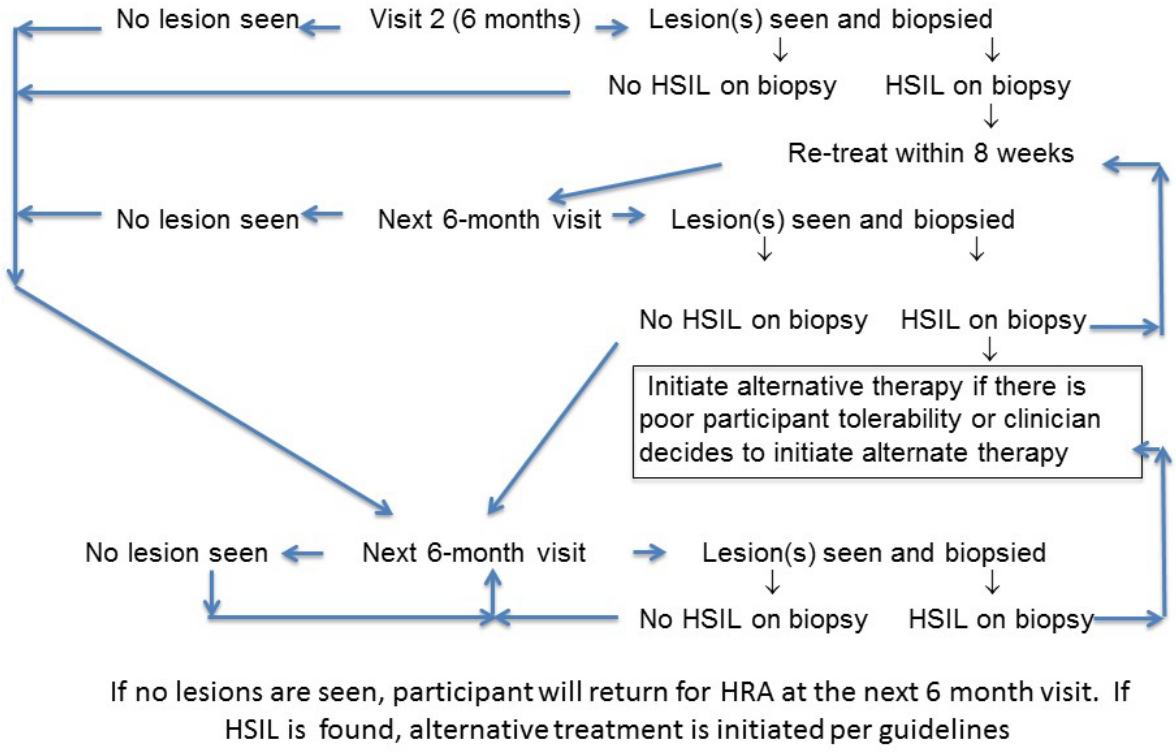


Figure 4-B: IRC, hyfrecation, or electrocautery performed at visit 1 (randomization visit)



4.2.4 Duration of therapy

In the absence of treatment delays due to adverse event(s), treatment of participants in the treatment arm may continue at the discretion of the clinician until one of the following criteria applies:

- HSIL progression to cancer,
- Intercurrent illness that prevents further administration of treatment,
- Other unacceptable adverse event(s),
- Participant decides to withdraw from the study, or
- General or specific changes in the participant's condition render the participant unsuitable for further treatment in the judgment of the investigator.

The reason for discontinuing study treatment will be reported in the HSIL Therapy form in AdvantageEDC for the 6-month period in which the discontinuation occurred. Any adverse events will be reported as required in Section 6.0.

4.3 General Concomitant Medication and Supportive Care Guidelines

4.3.1 Prohibited medications

Therapeutic HPV vaccinations and any other treatment for anal HSIL outside of this protocol are prohibited. Participants are prohibited from participating in other clinical trials for the treatment of HPV-related disease. Prophylactic HPV vaccines were removed from the exclusion criteria with version 8.0 of this protocol. Receipt of other investigational agents during this protocol (except for investigational antiretroviral therapy, Hepatitis C treatments, or for SARS-CoV-2 vaccination or treatment) is prohibited.

4.3.2 Concomitant medication documentation

During screening, study staff must obtain a listing of all current prescription medications taken within the last 45 days. Actual or estimated start and stop dates, indication, dosage, and schedule will be collected for the following classes of agents below to assess eligibility. These agents must be recorded in AdvantageEDC once the participant is randomized:

- Anticoagulants
- Antiretroviral therapy
- Immunomodulatory agents (if receiving, evaluate duration of receipt for 6 months prior to randomization per 3.2.2)
- Investigational agents
- HPV vaccines

After randomization, all prescription medication received during the study must be listed in the source only. Changes to or new administration of agents of the following classes will be recorded in the source, documenting actual or estimated start and stop dates, indication, dosage, and schedule, and reported in the

Concomitant Medications Form as changes occur: anticoagulants, immunomodulatory agents, HPV vaccines, and investigational agents. Medications that the investigator determines to have a causal relationship to serious adverse events must be recorded similarly in the source and in the Concomitant Medications Form after screening enrollment. HPV vaccinations should be deferred until study participation is completed. If administered while on study, then this must be recorded in the source and the Concomitant Medications Form.

The participant's antiretroviral medications will be managed by the participant's usual healthcare provider; participants should be referred for care for their HIV as medically indicated. Clinicians may opt to defer biopsies or treatment if he/she believes that biopsy or treatment may put the participant at unacceptable risk of complications due to concomitant medications for any reason. The reason for deferral must be listed in the source documents.

4.4 Concurrent HPV-related Disease Treatment

4.4.1 Anal warts and vulvar HSIL in study participants

Anal warts are commonly found in the populations that will be screened for and enrolled in the ANCHOR study. Some will have warts found at the time of screening, and incident warts are also expected among some of those who will be enrolled. Treatment of warts may be important recruitment and retention tools, as some participants may not be willing to enter or remain in the study if their warts are not treated. Targeted treatment modalities are the only modalities permitted for the warts among participants in the active monitoring arm, including IRC, electrocautery, laser, and surgical excision. An additional targeted approach, cryotherapy, may also be used to treat concurrent warts in study participants on either arm at the discretion of the treating physician. Cryotherapy may not be used for the treatment of HSIL on this protocol. Imiquimod, 5-fluorouracil cream or other topical treatments, which could "inadvertently" treat HSIL that is physically separate from the wart(s) being treated are not permitted for those in the active monitoring arm, but may be allowable for those in the treatment arm if these are chosen by the clinician to treat the HSIL. FDA-approved topical treatments for condyloma may be used to treat perianal warts in treatment arm participants, but may not be used in the anal canal. Study-sponsored supplies of 5-fluorouracil 5% and topical imiquimod 5% may be used for condyloma treatment of the perianus for treatment arm participants, and will only be used in the anal canal when administered for concurrent HSIL treatment, adhering to the dose administration, modification, and accountability instructions in this protocol if used for condyloma treatment. It is recognized that some participants in the observation who are having warts treated may also be having small foci of HSIL within the wart being treated as well and this may reduce the overall rate of progression from HSIL to cancer in the active monitoring arm.

At screening, participants may report that they have a past or current history of internal and/or external warts. If they agree to be screened for the study, they will be told that if warts are seen by the clinician, they will need to be biopsied before treatment. Of note, this practice will be recommended as standard of care for HIV-

infected individuals in the upcoming 2014 CDC guidelines for treatment of warts (Park, I, personal communication). Participants will be informed that if they qualify for the study and are randomized to the treatment arm, all areas of HSIL and warts will be treated. Participants will also be informed that if they are randomized to the active monitoring arm, warts may be treated at the discretion of the participant and the clinician. Potential participants with warts so extensive that they preclude the clinician from determining the extent and location of HSIL will not be enrolled in the study.

After enrollment, participants in both study arms with incident warts will have their warts treated to the greatest extent possible, at the discretion of the participant and the clinician.

Vulvar HSIL may be commonly found in HIV-infected women who have perianal HSIL. Women with vulvar HSIL may be enrolled and vulvar HSIL may be treated with targeted ablation. If they are randomized to the treatment arm, both the vulvar and perianal HSIL may be treated. If they are randomized to the active monitoring arm, vulvar HSIL will be treated per standard of care but HSIL within the boundary of the perianal region may not be treated. For the purpose of determining how far toward the perianus vulvar HSIL can be treated among those in the Active Monitoring arm, the perianus will be defined as extending up to 5 cm from the anal verge, but in females not farther than the midway point between the fourchette and the anal verge (after the buttocks have been retracted gently).

4.4.2 Cervical HSIL or cervical cancer in study participants

All women who participate in the ANCHOR study will undergo screening and treatment for cervical HSIL per current standard of care guidelines for HIV-infected women. This care will be provided outside the context of the ANCHOR study although in some cases, the women may be receiving cervical care by the same clinician who is seeing them for their anal HSIL. ANCHOR study staff will work closely with the participant and her primary HIV care provider to ensure that she is being screened and treated as required.

Should a female participant be found to develop cervical cancer during the course of the ANCHOR study, attempts will be made to ascertain a tissue sample to use comparatively in future molecular and/or viral correlative analyses planned. A separate consent form for these samples will be given to the participant for her agreement before obtaining these samples.

4.5 Duration of Follow-Up

All participants will be followed for up to 5 years after **the last participant's date of randomization**, diagnosis of invasive anal cancer, or until death, whichever occurs first. Participants who develop malignancies other than anal cancer, who are on the monitoring arm and drop in to treatment, who are on the treatment arm and discontinue treatment, or who develop unacceptable adverse events will remain in follow-up for anal cancer outcomes. Participants who cannot attend study visits should be followed for anal cancer outcomes via telephone contact, as defined in the protocol Manual of Procedures. Medical records will be requested from the participant's provider in the event that cancer is

diagnosed. Participants removed from study for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

4.6 Criteria for Removal from the Study (Drop-Out)

Participants will be removed from the study and reported as drop-outs if one of the following events occurs:

- Participant decides to withdraw from the study
- Death

Participants who discontinue the study due to an anal cancer diagnosis will be removed from the study but will not be considered drop-outs.

The reason for study removal and the date the participant was removed from the trial must be documented in the Off Study Form in AdvantageEDC.

5.0 DOSING DELAYS/DOSE MODIFICATIONS

5.1 Dose Modifications Guidelines

Guidelines for ablative therapy are provided in Section 4.2.2. When administering topical treatments, clinicians must follow the algorithms for each treatment modality as shown in Figure 4-A and Figure 4-B. Participants experiencing grade 3 adverse events that are at least possibly related to treatment should hold the product until the AE resolves or reduces in severity to grade 1. The study product should be restarted at the discretion of the site investigator using a reduced dose (described in the table below). If the AE continues to be resolved or is grade 1 at the reduced dose, then the dose can be escalated again at the discretion of the site investigator.

Participants experiencing grade 2 adverse events that are at least possibly related to treatment can either continue the product at the standard dose (preferred) or dose-reduce one level. If the participant tolerates the reduced dose after 2 weeks, then the dose can be escalated again at the discretion of the site investigator.

Participants experiencing grade 1 adverse events that are at least possibly related to treatment can continue the product at the standard dose. A dose reduction should only be done if necessary for continued adherence to study follow-up.

Table 5-A:1. Dose modifications

	Topical 5-fluorouracil 5%	Topical Imiquimod 5%
Standard dose	Twice daily for 5 days followed by 9 days off	1 single use sachet applied 3 times per week prior to normal sleeping hours and left on the skin for 6–10 hours.
Reduced dose	Twice daily for 3 days followed by 11 days off	1 single use sachet applied twice per week prior to normal sleeping hours and left on the skin for 6–10 hours.
Minimal dose	Once daily for 3 days followed by 11 days off	1 single use sachet applied once per week prior to normal sleeping hours and left on the skin for 6–10 hours.

6.0 ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs (Section 6.1) and the characteristics of an observed AE (Section 6.2) will determine whether the event requires expedited reporting **in addition** to routine reporting. All adverse event reporting will be conducted via AdvantageEDC for this protocol.

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting beginning April 1, 2018. The CTEP Version 5.0 of the CTCAE is identified and located on the CTEP website at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm. All appropriate treatment areas should have access to a copy of the CTEP Version 5.0 of CTCAE.

6.1 Potential Risks Lists

6.1.1 Expected adverse events related to the diagnostic procedures

Anal cytology collection, high resolution anoscopy (HRA), and anal biopsy.

Participants will likely experience pressure and urgency to defecate during the cytology collection and HRA. Anal bleeding may occur up to one week after the biopsy is taken.

The risk of infection is less than 1%.

6.1.2 Adverse event list for topical 5-FU

Most expected adverse events are local effects where the cream is applied. These include pain, burning, irritation, pruritus, erythema, edema, ulceration, bleeding, flaking and crusting of the skin, and allergic reactions. Scarring of treated areas has also been reported. Participants receiving 5-fluorouracil 5% have also experienced emotional upset, medicinal taste, thrombocytopenia, leukocytosis, hair loss, rash at other sites, pain/erythema/ulceration at sites adjacent to treated sites (e.g. scrotum), eye and nasal irritation, and herpes simplex reactivation. Women who received 5-fluorouracil 5% have reported miscarriages, and one birth defect (ventriculoseptal defect) occurred in an infant born to a woman who was exposed to 5-fluorouracil 5% during pregnancy.

6.1.3 Adverse event list for imiquimod

Most expected adverse events are local effects where the cream is applied. These include pain, burning, irritation, pruritus, erythema, edema, ulceration, bleeding, flaking, and crusting of the skin, and allergic reactions. Participants receiving imiquimod have also experienced headache, rash at other sites, back pain, pain/erythema/ulceration at adjacent sites (e.g., scrotum). Sinus infection, nausea, fever, and flu-like illness have also been reported.

6.1.4 Adverse event list for infrared coagulation, hyfrecation/electrocautery, and laser ablation

Most expected adverse events are local effects where the lesions have been ablated. Potential adverse events include minor bleeding (common), textural changes at the treatment site, and mild to moderate pain for up to two weeks post-procedure and occasionally longer, that is usually well-controlled with pain or anti-inflammatory medicines (common). These side effects are generally mild and self-resolve in short order. Self-limited bleeding not requiring intervention may occur up to 7-10 days post-procedure. Reactivation of herpes simplex has been reported following treatment, but a causal relationship to treatment has not been established. Rare adverse events (occurring <1% of the time) include heavy bleeding 7-10 days post-procedure requiring transfusion or operative intervention, severe pain interfering with self-care activities of daily living (ADL), stenosis, fissure, fistula, and abscess.

The rare adverse events described above will be considered unexpected due to increased severity if occurring at grade 3 or greater severity.

6.1.5 Adverse events for surgical excision (TUA)

Most expected adverse events are local effects where the surgical procedure was performed to excise the lesions. Adverse events include minor bleeding (common) and pain for up to one to two weeks post-procedure (common). Rare adverse events include sustained pain with bowel movements, heavy bleeding, stenosis, fissure, fistula, and abscess. In addition, there are side effects related to the anesthesia that may be administered for the procedure. These vary with the anesthetic agents used and are rare but may be severe including stroke, myocardial infection, and death.

6.2 Adverse Event Characteristics and Definitions

6.2.1 Adverse Event: Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure (attribution of unrelated, unlikely, possible, probable, or definite).

This includes the following:

- AEs not previously observed in the participant that emerge during the protocol-specified AE reporting period, including signs or symptoms associated with anal HSIL that were not present prior to study entry.
- Complications that occur as a result of protocol interventions.
- Preexisting medical conditions (other than the condition being studied) judged by the investigator to have worsened in severity or frequency, or changed in character during the protocol-specified AE reporting period.

The investigator is responsible for ensuring that adequate medical care is provided to a participant for all adverse events that occur during the trial, including clinically significant laboratory values, related to the trial. All AEs reported in the study database will be followed for the participant's medical care until resolved to the

baseline condition or protocol completion; for chronic conditions, resolution may be documented when the AE is stable with appropriate medical management. Any adverse events that do not require reporting in the study database will be managed and followed as appropriate for the participant's medical care.

6.2.2 Life-threatening Adverse Event: Any AE that places the participant or participant, in view of the Investigator, at immediate risk of death from the reaction.

6.2.3 Serious Adverse Event (SAE): Any AE occurring at any dose that results in any of the following outcomes: Death, a life-threatening AE, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/ incapacity, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

6.2.4 Hospitalization: hospitalization for expedited AE reporting purposes is defined as an inpatient hospital stay equal to or greater than 24 hours. Hospitalization is used as an indicator of the seriousness of the AE and should **ONLY** be used for situations where the AE truly fits this definition and **NOT** for hospitalizations associated with less serious events. (*e.g.*, a hospital visit where a patient is admitted for observation or minor treatment such as, hydration and released in less than 24 hours).

Prolongation of hospitalization is defined as an extension of current hospitalization equal to or greater than 24 hours.

Please note for hospitalization – All hospitalizations (or prolongation of existing hospitalization) for medical events equivalent to CTCAE Grade 3, 4, 5 must be reported regardless of the requirements for Phase of study, expected or unexpected, and attribution. For example, do not report an admission for pharmacokinetic sampling, but do report an admission for a myocardial infarction.

6.2.5 Toxicity: Toxicity is a term NOT clearly defined by regulatory organizations. Toxicity has been described as an AE that has an attribution of possibly, probably or definitely related to investigational treatment. To minimize confusion the NCI would recommend that the term toxicity NOT be utilized for AE reporting purposes. The CTCAE continues to use the term 'toxicity' because of familiarity.

6.2.6 Unexpected Adverse Event: Any AE that is not listed in available sources including the package insert, the Investigator's Brochure, or the protocol, or is not consistent with the severity or specificity of the risk information described in the available sources.

6.2.7 CTEP Adverse Event Reporting System (CTEP-AERS): An electronic system for expedited submission of AE reports. A SAE reporting form in AdvantageEDC will be used in lieu of CTEP-AERS for this trial.

6.2.8 Attribution: An assessment of the relationship between the AE and the medical intervention. The CTCAE does not define an AE as necessarily "*caused by a therapeutic intervention."* After naming and grading the event, the clinical

investigator must assign an attribution to the AE using the following attribution categories:

Table 6-A: Mapping adverse event attribution assignments to causal relationship designations

RELATIONSHIP	ATTRIBUTION	DESCRIPTION
Unrelated to investigational agent/intervention	Unrelated	The AE *is clearly* ***NOT*** *related* to the intervention
	Unlikely	The AE *is* ***doubtfully related*** to the intervention
Related to investigational agent/intervention	Possible	The AE ***may be related*** to the intervention
	Probable	The AE ***is likely related*** to the intervention
	Definite	The AE ***is clearly related*** to the intervention

NOTE: AEs listed as 'possibly, probably, or definitely' related to the investigational agent/intervention are considered to have a suspected 'reasonable causal relationship' to the investigational agent/intervention (ICH E2A). For routine adverse event reporting purposes on this protocol, "attribution" defines the relationship between the adverse event and the investigational agent(s)/intervention.

6.2.9 Unanticipated adverse device effect: Any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of participants.

6.3 Expedited Adverse Event Reporting

6.3.1 Expedited AE reporting for this study must use the SAE form in AdvantageEDC. As a commercial agent study, CTEP-AERS will not be used for this protocol. The reporting procedures to be followed in AdvantageEDC are provided in this section and will align with the principles for SAE reporting in the "CTEP, NCI Guidelines: Adverse Event Reporting Requirements," which can be downloaded from the CTEP home page (http://ctep.cancer.gov). These requirements are briefly outlined below (Section 6.3.3).

A 24-hour notification is to be made to the ANCHOR DMC by telephone at 301-251-1161, only when Internet connectivity is disrupted. Once Internet connectivity is restored, a 24-hour notification phoned in must be entered electronically into AdvantageEDC by the original submitter at the site.

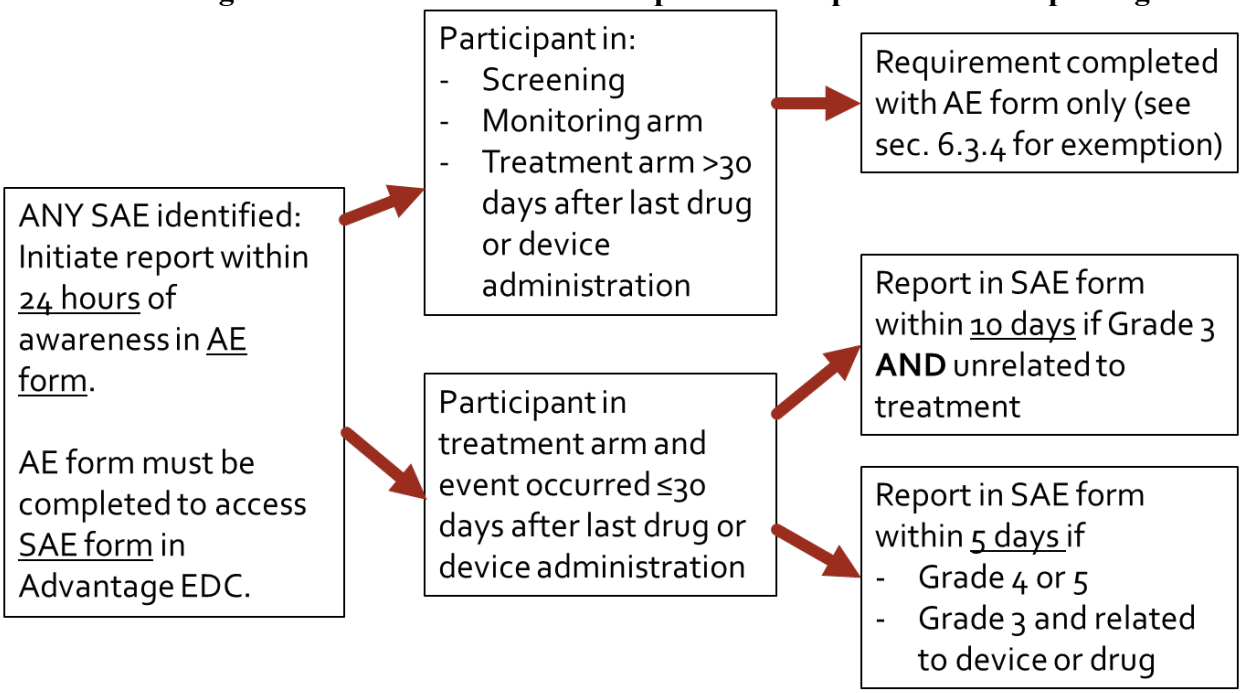
6.3.2 AdvantageEDC is programmed for automatic electronic distribution of SAE reports to the following individuals: the ANCHOR DMC, the ANCHOR Medical Monitor, Protocol Chairs, and the Principal Investigator at the institution. The ANCHOR

DMC is responsible for distribution of SAE reports to other parties on behalf of the sponsor.

6.3.3 Expedited reporting guidelines

Investigators must report ALL SAEs (as defined in Section 6.2.3) to the ANCHOR DMC and IRBs as required by the protocol and local IRB policy. The investigator's initial expedited report to the ANCHOR DMC for the SAE will be made using the Adverse Event form in AdvantageEDC within 24 hours of awareness of the event (routine report), and unless an exception from expedited reporting applies per Section 6.3.4, will be followed by a completed SAE form in AdvantageEDC as soon as possible, and in no more than 10 days of awareness. If the event is a grade 4 or 5 SAE, or determined by the investigator to be an unanticipated adverse device effect or grade 3 SAE that is at least possibly attributed to protocol treatment, the completed SAE form will be submitted within 5 calendar days of awareness. Timeframes for reporting SAEs at any time during trial participation are outlined in the flowchart provided below, with exceptions from SAE form reporting noted.

Figure 6-B: Timeframes and Exceptions for Expedited SAE Reporting



6.3.4 Exceptions to expedited adverse event reporting

Expedited reports (SAE form in AdvantageEDC) are not required for serious adverse events that occur in participants in screening and participants on the Active Monitoring arm. On the treatment arm, an expedited report is not required if the SAE occurred more than 30 days after administration of the last protocol treatment (last dose of topical therapy, ablative treatment, or TUA, whichever occurred last) and has an attribution of unrelated or unlikely to the protocol agent(s). SAEs that occur in these participants will be submitted as routine adverse event reports only (AE form in AdvantageEDC).

6.3.5 Reporting to regulatory authorities

As all agents used on this trial are exempt from the requirements for an IND or an IDE, respectively, no serious adverse event reporting to FDA is required. In the event that any unexpected and serious adverse events reported on this trial are determined to have a reasonable possibility of a causal relationship to the study drugs or devices, the SAE or UADE will be reported to FDA using its voluntary reporting mechanism (MedWatch Online Voluntary Reporting Form). Voluntary SAE/UADE reporting will be performed by the ANCHOR DMC following the medical monitor's determination that the AE meets these criteria.

6.4 Routine Adverse Event Reporting

The following AEs must be reported in an Adverse Event form in AdvantageEDC for routine study data submissions:

- All serious adverse events regardless of relationship to study treatment or study procedures
- All grade 3 events that are at least possibly related to study treatment or study procedures
- All grade 1 or 2 adverse events leading to stopping or changing protocol treatment; all other adverse events must be recorded in the source documents only
- Suspected or confirmed COVID-19 diagnoses (any grade), with information on the type of testing performed to confirm the test, if done

6.4.1 Timeline for routine adverse event reporting

All adverse events meeting the routine reporting criteria that occur from the time of enrollment for screening through the final study visit must be reported in AdvantageEDC. Adverse events that are ongoing at the time of protocol discontinuation and that are attributed to one of the protocol agents or procedures must be followed until resolution or until the investigator deems the event to be clinically stable.

6.4.2 Clinical laboratory abnormalities

Clinical laboratory abnormalities will be considered AEs if determined to be clinically significant by the investigator. In assessing laboratory results, an abnormal laboratory value will be considered clinically significant if it is characterized by one or more of the following criteria:

- Is judged by the investigator to have a causal relationship to the investigational agent
- Requires clinical intervention or monitoring, such as: close observation, more frequent follow-up assessments, further diagnostic intervention, treatment/therapeutic intervention, or protocol therapy dose modification
- Is associated with clinical signs or symptoms, which may suggest a disease and/or organ toxicity, or may represent a new condition or worsening of a baseline condition

- Is associated with a serious adverse event, or is otherwise judged by the Investigator to be of significant clinical impact

Laboratory results that are proven erroneous by repeat testing will not be considered clinically significant.

In general, a laboratory abnormality that is not clinically significant will be consistent with CTCAE grade 1 (mild) or 2 (moderate) severity, as categorized by the relevant severity description in the Investigations System Organ Class (SOC) or Metabolism and Nutrition Disorders SOC. Investigators may not designate laboratory abnormalities that are consistent with grade 3 or greater severity as not clinically significant.

6.5 Secondary Malignancy

A secondary malignancy is a cancer caused by treatment for a previous malignancy (e.g., treatment with investigational agent/intervention, radiation, or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm. Three options are available in the CTCAE to describe secondary malignancies:

- Leukemia secondary to oncology chemotherapy (e.g., acute myelocytic leukemia [AML])
- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

As prior anal cancer is exclusionary and no cancer therapies are provided for this protocol, any malignancy possibly related to a cancer treatment preceding protocol participation (including AML/MDS) will be reported via the routine reporting mechanisms outlined in this protocol.

6.6 Second Malignancy

A second malignancy is one unrelated to the treatment of a prior malignancy (and is **NOT** a metastasis from the initial malignancy). Second malignancies require **ONLY** routine adverse event reporting.

7.0 PHARMACEUTICAL AND STUDY DEVICE INFORMATION

A list of the adverse events and potential risks associated with the investigational or commercial agents administered in this study can be found in Section 6.1.

7.1 5-Fluorouracil 5% Cream

NOTE: Please refer to the commercial package insert for more information.

7.1.1 Other names

Efudex Cream

7.1.2 Classification

Topical 5% fluorouracil cream is an antineoplastic antimetabolite. It is a topical preparation, available in a 40 gm tube, containing the fluorinated pyrimidine 5-fluorouracil, an antineoplastic antimetabolite. Five percent (5%) 5-fluororacil cream contains 5 g of 5-fluororacil per 100 g of cream. The cream contains 5% fluorouracil in a vanishing cream base consisting of white petrolatum, stearyl alcohol, propylene glycol, polysorbate 60 and parabens (methyl and propyl). Chemically, fluorouracil is 5-fluoro-2,4(1H,3H)-pyrimidinedione. It is a white to practically white, crystalline powder that is sparingly soluble in water and slightly soluble in alcohol. One gram of fluorouracil is soluble in 100 mL of propylene glycol. The molecular weight of fluorouracil is 130.08 and the molecular formula is $C_4H_3FN_2O_2$.

7.1.3 Mode of action

There is evidence that the metabolism of fluorouracil in the anabolic pathway blocks the methylation reaction of deoxyuridylic acid to thymidylic acid. In this manner fluorouracil interferes with the synthesis of deoxyribonucleic acid (DNA) and to a lesser extent inhibits the formation of ribonucleic acid (RNA). Since DNA and RNA are essential for cell division and growth, the effect of fluorouracil may be to create a thymine deficiency which provokes unbalanced growth and death of the cell. The effects of DNA and RNA deprivation are most marked on those cells which grow more rapidly and take up fluorouracil at a more rapid rate. The catabolic metabolism of fluorouracil results in degradation products (e.g., CO_2, urea, α-fluoro-β-alanine) which are inactive.

7.1.4 Storage and stability

Store at 25°C (77°F); excursions permitted to 15°C-30°C (59°F-86°F).

7.1.5 Dose specifics and administration

Participants will be instructed to apply 1 ml of topical 5-fluorouracil 5% cream. One gram of topical 5-fluorouracil is the equivalent of 1 ml. The total daily dose will be 1 gram of cream, and the weekly dose is 5 grams of cream. 5% 5-fluororacil contains 5 grams of 5-fluororacil per 100 grams. The daily dose is therefore 50 mg of 5-fluororacil delivered in divided doses of 25 mg BID, and the total weekly dose is 250 mg delivered in 5 grams of cream. Participants will be given a 40 gram tube of 5-FU cream and instructed to squeeze out the cream to the marked line on the applicator (0.5 mL of cream), or a right hand glove or finger cot. Using a glove or

a finger cot they will then squeeze one half the amount of cream (0.25 mL) from the syringe (if using an applicator) onto their right index finger and insert the cream approximately one inch into the anus on the right side. The participant will then place the remaining cream (0.25 mL) on the left finger, and insert the cream one inch into the anus on the left side. Similar instructions apply for perianal application, but without insertion of the cream into the anal canal. Participants who will be treated for perianal HSIL will be dispensed an additional tube to treat the perianus (dose doubled).

7.1.6 Preparation

Efudex Cream (5-FU) is available in 40-gm tubes containing 5% fluorouracil (NDC 0187-3204-47) in a vanishing cream base consisting of white petrolatum, stearyl alcohol, propylene glycol, polysorbate 60 and parabens (methyl and propyl). It is distributed by Bausch Health. FDA-approved generic versions of 5% fluorouracil are also permitted on this protocol.

7.1.7 Side effects

See Section 6.1.2. Refer to the approved package insert for complete prescribing and toxicity information.

7.2 Topical Imiquimod Cream (5%)

NOTE: Please refer to the commercial package insert for more information.

7.2.1 Other names

Aldara.

7.2.2 Classification

Imiquimod belongs to the chemical class of substances known as imidazoquinolinamines. Five percent (5%) imiquimod cream contains 50 mg of imiquimod per 1 g of cream, supplied in single-use packets that contain 250 mg of cream.

7.2.3 Mode of action

Imiquimod works through stimulation of local immune responses, in part activation of toll-like receptors.

7.2.4 Storage and stability

Store at less than 25ºC (77ºF). Avoid freezing.

7.2.5 Dose specifics and administration

The imiquimod dose will be 750 mg of cream per week, administered as one single use sachet (250 mg) in cartons of 12 or 24 sachets, applied 3 times per week for up to 16 weeks (two 8-week courses). Contents of one packet are applied to the affected area at bedtime, three times per week. The cream is left on for 6-10 hours and washed off in the morning with soap and water. Twenty-four packets will be dispensed for each 8-week course of intra-anal treatment; if perianal HSIL is to be treated, an additional 24 packets will be dispensed to the participant for each 8-week course of perianal treatment (dose doubled to treat the perianus).

7.2.6 Preparation

Chemically, imiquimod is 1-(2-methylpropyl)-1H-imidazo[4,5-c]quinolin-4-amine. Imiquimod has a molecular formula of C14H16N4 and a molecular weight of 240.3. Imiquimod is available as a white to faintly yellow oil-in-water cream base consisting of isostearic acid, cetyl alcohol, stearyl alcohol, white petrolatum, polysorbate 60, sorbitan monostearate, glycerin, xanthan gum, purified water, benzyl alcohol, methylparaben, and propylparaben. It is marketed by Bausch Health. FDA-approved generic versions of 5% imiquimod are also permitted on this protocol.

7.2.7 Side effects

See Section 6.1.3.

7.3 Study Devices

All study devices will be used in a final form as obtained from the manufacturer.

7.3.1 Infrared Coagulator

The Infrared Coagulator 1900 and 2100 are supplied by the Redfield Corporation. See Section 2.2.2 for details of IRC treatment.

7.3.2 Hyfrecation

The Hyfrecator 2000 is a mobile unit manufactured and serviced by Conmed Corporation of Utica, New York. See Section 2.2.3 for details of hyfrecation treatment.

7.3.3 Other ablative devices

The following classes of devices are permitted for in-office removal of anal HSIL. All devices in this category must be reported to the protocol chairs before use to confirm these criteria are met.

- Legally marketed electrocautery devices regulated under 21 CFR 878.4400, that are indicated for cutting or coagulating tissue, and that are appropriate for use in dermatologic, general, and gastrointestinal surgical procedures.
- Legally marketed carbon-dioxide lasers regulated under 21 CFR 878.4810, that are indicated for the excision, coagulation, or ablation of tissue, and that are appropriate for use in dermatologic, general, and gastrointestinal surgical procedures.

Cryotherapy will not be permitted for HSIL treatment on this trial. Concurrent wart treatment using targeted cryotherapy may occur at the discretion of the treating physician for the participant's medical care.

7.4 Drug Supply and Accountability

Participating institutions may source topical imiquimod cream (5%) and 5-fluorouracil 5% for this protocol through commercial pharmacy supply channels for the institution. For all agents sourced by the institution, the institution will maintain records of the inventory and disposition of all drugs according to local pharmacy standards of procedure.

A supply of Efudex (5-fluorouracil 5%) will be available for use on this protocol for no charge (while supplies last). Participating institutions are to refer to the study drug request form located on the ANCHOR DMC website (www.anchordmc.com) to order study-supplied 5-fluorouracil 5% cream.

For all study-supplied topical agents, the Investigator, or a responsible party designated by the Investigator, must maintain a careful record of the inventory and disposition of all drugs received or reimbursed by the study using the NCI Investigational Agent Accountability Record Form (DARF), available on the CTEP home page (http://ctep.cancer.gov) or by calling the Pharmaceutical Management Branch at 240-276-6575. The DARF documents the drug delivery date to the site, inventory at the site, use by each study participant, and disposal of the drug (if applicable). A site-specific accountability record, either manual or electronic, may be used if it includes all the information required on the DARF and if the paper printout is identical to the DARF. A separate DARF is required for each protocol using the same agent. The investigator will ensure that the drugs are used only in accordance with this protocol for the treatment of HSIL or concurrent condyloma, as outlined in Section 4.2 and 4.4.

A tube of study-supplied agent may be designated on the DARF for clinic use for demonstrating application of the cream at the randomization visit (Section 8.3.9).

After ordering, study-supplied topical agents may not be transferred to other institutions. Expired drug or drug returned by study participants must be destroyed in accordance with the institution's standards of procedure.

8.0 CLINICAL AND LABORATORY EVALUATIONS

Schedules shown in the study calendar below are provided in Appendix I.

Treatment cycles are generally organized into 6-month blocks.

8.1 Special Instructions and Definitions of Evaluations

All clinical and laboratory information required by this protocol is to be present in the source documents. All data requested by the study should be recorded in the source documents. A subset of the data points stated here will be entered on the appropriate CRF via the AdvantageEDC^{SM} Internet Data Entry System, as specified in the ANCHOR Study Forms Instructions.

8.1.1 Medical history

- History of receptive anal intercourse ever
- History of genital or anal condyloma
- History of prior anal cytologies or anal biopsies
- History of CDC HIV risk group and AIDS defining illnesses
- History of systemic cytotoxic chemotherapy and/or radiation
- History of hemophilia
- Other aspects of the medical history including allergies to any medications (iodine, lidocaine, or contrast agents) should be in the source documents only

8.1.2 Medication history

A medication history must be present in source documents per Section 4.3.2, including, including actual or estimated start and stop dates, indication, dosage, and schedule. All other prescription medications will be listed in the source.

- HIV treatment history in the 45 days prior to study entry.
- Any history of HPV vaccination
- Anticoagulants
- Systemic immunomodulatory agents. If receiving any immunomodulatory agents at screening, evaluate duration of receipt for 6 months prior to randomization.
- Investigational agents
- Medications that the investigator determines to have a causal relationship to serious adverse events that occur after screening enrollment

8.1.3 Concomitant medications/antiretroviral medication modifications

Refer to Section 4.3.2.

8.1.4 Study treatment record

All modifications to study treatment initiated by the investigator, including initial doses, dose reductions, and permanent discontinuation of treatment.

8.1.5 Nadir CD4+ cell count

The participant's prior nadir CD4+ cell count (absolute value, and if available, date) should be documented, with a copy of the nadir CD4+ cell count report when possible, and will be entered on the CRF. If this documentation is not available, then participant recollection will suffice. For participants who do not know the exact nadir value and for whom there is no source documentation, then recall of the categorical nadir (e.g., <50, <100, <200 cells/mm^3) will suffice.

8.1.6 Complete physical exam (at screening visit)

This examination includes at a minimum an examination of the skin, head, mouth, and neck; auscultation of the chest; cardiac exam; abdominal exam; examination of the lower extremities for edema; and Karnofsky performance test. The complete physical exam will also include signs and symptoms, height, weight, diagnoses, and vital signs (temperature, pulse, respiration rate, and blood pressure).

8.1.7 Targeted physical exam (subsequent visits)

A targeted physical examination is to include pulse, blood pressure, and review of any previously identified or new signs or symptoms, including diagnoses that the participant has experienced since the last visit. Staff should inquire about symptoms and examine the treatment areas for any adverse events.

8.1.8 Digital anorectal examination and examination of inguinal nodes

A digital anorectal examination and an examination of inguinal nodes are to be performed to note the presence or absence of any abnormalities.

8.1.9 Adverse event evaluation

All signs and symptoms that begin during or after the entry visit must be recorded in the source regardless of grade. Any signs and symptoms that began prior to the screening visit should be recorded, and will only be considered an adverse event if there is a significant worsening of the sign or symptom during the study. All signs and symptoms that begin during or after the last dose of protocol treatment must be recorded in the source regardless of grade or attribution to treatment.

All adverse events that require routine reporting per Section 6.4 must be recorded by clinical term and/or CTCAE categorization, severity (grade), seriousness, attribution to protocol interventions, and event status. AEs that are not serious (as that term is defined in Section 6.2), are of grade 1 or 2 severity, and that do not require modification to protocol treatment do not require routine AE reporting, and may be recorded in the source only by denoting the clinical term, severity, and seriousness.

If known at the time of reporting, a diagnosis should be reported as a single adverse event rather than individual signs and symptoms. However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or

syndrome at the time of reporting, it is acceptable to report the information that is currently available. If a diagnosis is subsequently established, it should be reported as follow-up information.

8.1.10 Laboratory evaluations

- Hematology (CBC with differential) may be performed by the enrolling site or the results from prior testing may be obtained, provided that the results were issued within 90 days prior to randomization from a CLIA-certified laboratory.
- CD4 count and HIV viral load, to be performed by the enrolling site at baseline unless a copy of the results are available from clinical care within 6 weeks prior to randomization. The result from routine clinical care may be collected at all subsequent visits.
- Urine pregnancy test for females of childbearing potential (FCBP). After the randomization visit, pregnancy tests will only be required for FCBP in the treatment arm before treatment procedures, and will not be required at these time points if the participant is receiving standard, long-acting contraception (e.g., IUDs).
- For females, a record (from a licensed healthcare provider) of the cervical cytology result and documentation of visual examination of the vulva, vagina, and cervix to rule out lesions suspicious for cancer within 12 months prior to randomization, per Section 3.1.5.
- Anal cytology for local cytology result, and residual cytology media for correlative studies (See Appendix III, Anal Cytology Sampling Procedures). A second anal swab will be collected in Thinprep for DNA studies, and a third anal swab will be collected in RNAProtect for RNA/protein studies. All three swabs will be collected in this order at each visit where required. If a local cytology result exists to satisfy protocol requirements at the time of the visit, the first anal swab will be recollected with the second and third swabs, and the first swab sent to the ANCHOR Biorepository without any local processing.
- High resolution anoscopy and biopsies (See Appendix IV).

8.1.11 Blood samples for correlative studies

A blood sample (one red top tube) will be drawn for storage at the ANCHOR Biorepository for future testing. Whole blood (one lavender top tube) will be collected at Visit 2 only. Please refer to the ANCHOR Study Manual of Procedures for information regarding serum sample collection, preparation, and shipment.

8.1.12 Oropharyngeal swab collection for ancillary SARS-CoV-2 study

The swab for oropharyngeal SARS-CoV-2 testing will be collected on the same day as the anal swabs. A dry, scored swab will be inserted into the posterior pharynx and tonsillar areas. The swab will be rubbed over both tonsillar pillars and posterior oropharynx and the clinician collecting the sample will avoid touching the tongue, teeth, and gums. He/she will place the swab immediately into the labeled sample collection tube and will break the applicator stick off at the score line to permit

tightening of the cap. The tube will be stored at -20C or colder until shipment to the ANCHOR Biorepository. See the study manual of procedures for shipping instructions.

8.1.13 Retention follow-up

Site staff may contact the participant between 6-month visits to identify and attempt to assist with issues related to visit attendance and study retention. Instructions are identified in the protocol Manual of Procedures.

8.2 Screening Evaluations (Visit 0)

Screening evaluations are to be conducted within 12 weeks before randomization (Visit 1), except as noted below. It is recommended that screening occur within 4 weeks after the participant attends a study information session. Screening evaluations for Visit 0 may be performed at Visit 1 (except informed consent, the anal swab for cytology, the screening HRA, and anal biopsies), provided that all Visit 0 evaluations are performed before randomization.

8.2.1 Informed consent and comprehension assessment administration at least one week before, and no more than 6 weeks before randomization. Participants may be verbally re-consented at Visit 1 before randomization if more than 6 weeks have elapsed since signing the informed consent document.

8.2.2 Documentation of HIV status (in compliance with Section 3.1.1), medical history (including nadir CD4 count from participant recall), medication review, and all eligibility criteria

8.2.3 Venipuncture for:

8.2.3.1 Complete blood count with differential and platelets (may occur up to 90 days prior to randomization)

8.2.3.2 Serum (red top tube) for the ANCHOR Biorepository for correlative studies (may occur up to 12 weeks prior to randomization)

8.2.4 Urine pregnancy test for females of childbearing potential (FCBP)

8.2.5 Complete physical examination, height, and weight

8.2.6 Anal swab for cytology (may occur up to 12 weeks prior to randomization (the local cytology result must be known before randomization enrollment) and additional swabs for correlative studies.

8.2.6.1 Leftover material from anal swab cytology suspension (Appendix III). If the anal cytology result is available within 12 weeks prior to randomization but the residual is not available to ship to the Biorepository, the first anal swab may be recollected and submitted directly to the Biorepository for correlative studies.

8.2.6.2 Anal swab for DNA

8.2.6.3 Anal swab for RNA/protein

8.2.7 Digital anorectal examination and examination of inguinal nodes

8.2.8 High resolution anoscopy with biopsies of visible lesions (may occur 1-12 weeks prior to randomization, including before the initial screening visit). Participants who have only a single lesion that is likely to be removed entirely with the initial screening biopsy should be excluded from the trial because the participant cannot be followed for 5 or more years after randomization.

HSIL lesions that were biopsied for clinical care prior to the visit may be used to document eligibility, and will not require repeat HRA and rebiopsy during the screening visit only if all three criteria below are satisfied:

- The biopsies were obtained within 12 weeks before the randomization visit;
- The HSIL lesion(s) are observed by the study clinician during the HRA at Visit 1 preceding randomization; and,
- The pathology report and slides from those prior biopsies are available to submit for central pathology review.

8.2.9 Participants who fail screening due to suspected cancer diagnoses or cancer diagnoses will undergo central pathology review to confirm the diagnosis, if biopsies are available from screening procedures performed by the site. Participants diagnosed with cancer during screening may provide an optional cancer biopsy (placed in RNALater) only if both of the following criteria are satisfied:

- Consent is provided for use of the participant's specimens following screen failure
- The participant is willing to return to the clinic for biopsy before referral to treatment and study discontinuation.

8.2.10 *Substudy completed in February 2020:* ANCHOR Health-Related Symptom Index (A-HRSI; Appendix X) and patient-reported ECOG questionnaire completed via telephone-facilitated interview (by MSKCC staff) or electronic patient-reported outcomes (ePRO) platform (independently by the participant) at any time following screening enrollment until time of randomization (if the participant consents to participate in the optional HRQoL substudy).

8.2.11 *QoL objective*: If the participant consented and is eligible to complete questionnaires for the QoL objective, A-HRSI completion up to 2 weeks before randomization (see Section 8.9).

8.2.12 *SARS-CoV-2 ancillary study:* If the participant consented for this study, an oropharyngeal swab will be collected at the same screening visit as anal swabs 1-3, as outlined in Section 8.1.12.

8.3 Baseline Visit with Randomization Evaluations (Visit 1)

Visit 1 is to be conducted 1-12 weeks following informed consent (re-consent is required if written informed consent occurred more than 6 weeks before randomization, as outlined in Section 8.2.1) and may occur up to 12 weeks after the screening HRA was performed.

8.3.1 Eligibility review and choice of initial HSIL therapy (required for randomization checklist)

8.3.2 ANCHOR Visit 1 Questionnaire (Risk Factor Questionnaire, Appendix V). The questionnaire will be administered to the participant via AdvantageEDC. The questionnaire may be administered up to two weeks before a planned randomization visit. Paper versions of the questionnaire completed by the participant will be submitted to the ANCHOR DMC for data entry.

8.3.3 Review changes in medications and any adverse events from screening procedures

8.3.4 Venipuncture for CD4 level and HIV viral load within 6 weeks before randomization (copies of test results from clinical care will be acceptable). Venipuncture may be performed on the day of randomization, even after enrollment in the system.

8.3.5 Urine pregnancy test for all FCBP (must be performed within 7 days prior to randomization)

8.3.6 Digital anorectal examination and examination of inguinal nodes

8.3.7 High resolution anoscopy for all participants to verify the presence of HSIL lesions to be treated or monitored. Only new HSIL lesions or lesions that are suspicious for cancer should be biopsied at this visit. This HRA at the randomization visit must precede randomization enrollment in AdvantageEDC (Segment B). The participant will discontinue screening if no biopsy-proven HSIL diagnosed from the screening HRA is located during the randomization HRA.

8.3.8 Randomization enrollment in AdvantageEDC

8.3.9 For treatment arm participants being treated with IRC/electrocautery/laser, the initial study treatment should be performed at the randomization visit (within 2 weeks of the randomization visit will be permitted). New lesions that are empirically identified as HSIL and biopsied at the randomization visit should be treated as part of the initial study treatment, or at a subsequent interim visit if staged treatments will be performed.

For those being treated with 5-FU or imiquimod, treatment must be initiated within 2 weeks of randomization. The clinician will demonstrate proper application of treatment to the areas affected by HSIL at the randomization visit. Demonstration using the first dose of study agent is strongly encouraged; an inert cream will be used if treatment initiation will be delayed. If the participant has a biopsy at the randomization visit, the initial treatment will be delayed within this window to permit healing.

For those being treated with TUA, the participant will be referred to surgery and should be performed within 2 months. The costs of TUA will only be covered by the study with the QA committee's approval.

8.3.9.1 If the participant is to be treated with an ablative therapy, one additional biopsy will be collected prior to treatment from the lesion with most severe appearance of disease. This biopsy will be placed in RNAlater for banking only. A maximum of one biopsy for banking may be obtained every 6 months.

8.4 Evaluations between Six Month Visits (Interim Visits)

For treatment arm participants, evaluations are to be conducted per the treatment algorithms in Figure 4-A and Figure 4-B and in the schedule of evaluations in Appendix I for each treatment modality. Monitoring arm participants may be seen between six month visits as clinically indicated for monitoring of potential progression for cancer.

8.4.1 Medication review

8.4.2 Adverse event evaluation

8.4.3 Urine pregnancy test for FCBP in the treatment arm only before treatment procedures, unless otherwise needed at the clinician's discretion

8.4.4 High resolution anoscopy, digital anorectal examination, and examination of inguinal nodes, as clinically indicated

8.4.5 For treatment arm participants, initiation of additional cycles of treatment (as determined by the investigator)

8.4.5.1 If the participant is to be treated with an ablative therapy, one additional biopsy will be collected prior to treatment from the lesion with most severe appearance of disease. This biopsy will be placed in RNAlater for banking only. A maximum of one biopsy for banking will be obtained during each 6 month treatment block, if treatment is administered.

8.4.6 *Completed in February 2020:* If the participant consents to participate in the optional HRQoL substudy for A-HRSI scale responsiveness, the A-HRSI, Patient ECOG PS, and PGIC measures will be completed via telephone-facilitated interview (performed by MSKCC staff) or ePRO platform (independently by the participant) at the following time points:

8.4.6.1 Time 2: At least 14 days (2 weeks) post-randomization, and at most 70 days (10 weeks) post-randomization (Time 2).

8.4.6.2 Time 3: At least 71 days (10 weeks + 1 day) post-ANCHOR randomization, and at most 112 days (16 weeks) post-randomization.

8.4.7 *QoL objective*: If the participant consents and is eligible to complete questionnaires for the QoL objective, A-HRSI completion at 2-7 week (+3 days) and 4 weeks (±1 week) after randomization (see Section 8.9 and Appendix I).

8.5 Evaluations at Visit 2 (6 Months) for All Participants

Evaluations are to be conducted ± 4 weeks.

8.5.1 Medication review

8.5.2 Adverse event evaluation

8.5.3 Serum (one red top tube), and plasma and whole blood fraction (one lavender top tube) for the ANCHOR Biorepository for correlative studies

8.5.4 Urine pregnancy test for FCBP on the treatment arm only before treatment procedures, unless otherwise needed at the clinician's discretion.

8.5.5 Targeted physical exam (per Section 8.1.7)

8.5.6 Anal swab for local cytology and additional swabs for correlative studies

8.5.6.1 Leftover material from anal swab cytology suspension (Appendix III)

8.5.6.2 Anal swab for DNA

8.5.6.3 Anal swab for RNA/protein

8.5.7 Digital anorectal examination and inguinal lymph node examination

8.5.8 High resolution anoscopy

8.5.9 Anal biopsy depending on the assigned arm (Section 4.1 or Section 4.2)

8.5.9.1 HSIL in the treatment arm may be empirically treated at 6-month visits if the lesion is biopsied before treatment at the same visit and if cancer is not suspected on HRA.

8.5.9.2 If the participant is to be treated with an ablative therapy, one additional biopsy will be collected prior to treatment from the lesion with most severe appearance of disease. This biopsy will be placed in RNAlater for banking only. A maximum of one biopsy for banking will be obtained during each 6-month treatment block, if treatment is administered.

8.5.9.3 If all biopsies do not show HSIL or are inconclusive, and the local cytology result shows high grade disease (ASC-H or HSIL), the investigator should bring the participant back for repeat HRA at 3 months (interim visit; window of ±4 weeks). If a biopsy is performed at an even-numbered visit for a monitoring arm participant and the biopsy results are discordant, the participant will not be asked to return for repeat biopsy until the next 6-month visit unless there is suspicion for progression to cancer. If at least one biopsy shows HSIL, repeat biopsies of lesions with inconclusive histology may be deferred to the next 6-month visit so long as there is no suspicion of progression to cancer. If the visit window for the next 6-month visit is within 8 weeks of the last biopsy, repeat HRA and biopsies will occur as part of the next 6-month visit.

8.5.10 *SARS-CoV-2 ancillary study:* If the participant consented for this study and completed swab collection before randomization, an oropharyngeal swab will be collected, as outlined in Section 8.1.12.

8.5.11 COVID-19 telephone follow-up: If the 6-month visit cannot be conducted due to COVID-19 -related restrictions, a telephone follow-up visit may be conducted in lieu of a 6-month visit to obtain follow up on treatment completion (if relevant), safety, medical history, medication history, and study arm compliance. See study source documents for instructions. If a telephone follow-up occurs, a makeup-6 month visit must be conducted as soon as an in-person visit is feasible again. **If a site performs a telehealth visit because the 6-month visit cannot be performed, it will still require reporting as a missed visit and is still considered a protocol deviation.**

8.6 Evaluations at Follow-Up (Visits 3-10+), Every 6 Months until Study Completion

Evaluations are to be conducted ± 4 weeks of the 6-month follow-up visit.

Interim visits may occur as specified in algorithms for each specific treatment, or for clinicians wanting to examine participants more frequently in the active monitoring arm.

Evaluations are to occur in both study arms unless otherwise specified.

8.6.1 Medication review

8.6.2 Recording of HIV viral load and CD4 count laboratory results (annually, only if available from clinical care)

8.6.3 Adverse events evaluation

8.6.4 Serum (red top tube) for the ANCHOR Biorepository for correlative studies

8.6.5 Urine pregnancy test for FCBP on the treatment arm only before treatment procedures, unless otherwise needed at the clinician's discretion.

8.6.6 Targeted physical exam (per Section 8.1.7)

8.6.7 Anal swab for local cytology and additional swabs for correlative studies

8.6.7.1 Leftover material from anal swab cytology suspension (Appendix III)

8.6.7.2 Anal swab for DNA

8.6.7.3 Anal swab for RNA/protein

8.6.8 Digital anorectal examination and inguinal lymph node examination

8.6.9 High resolution anoscopy

8.6.10 Anal biopsy depending on the assigned arm. Participants in the treatment arm will be biopsied every six months if persistent or metachronous HSIL is suspected, or if visible changes can be seen in areas of prior treatment. Areas of prior treatment that appear completely normal (e.g., not acetowhite epithelium (AWE), no vascular changes, and strongly Lugol's positive) do not need to be biopsied to confirm the success of treatment. Participants in the active monitoring arm will have anal biopsies of HSIL only every 12 months, per Section 4.1.

8.6.10.1 HSIL in the treatment arm may be empirically treated at 6-month visits if the lesion is biopsied before treatment at the same visit and if cancer is not suspected on HRA.

8.6.10.2 If all biopsies do not show HSIL or are inconclusive, and the local cytology result shows high grade disease (ASC-H or HSIL), the investigator should bring the participant back for repeat HRA at 3 months (interim visit, window of ±4 weeks). If a biopsy is performed at an even-numbered visit for a monitoring arm participant and the biopsy results are discordant, the participant will not be asked to return for repeat biopsy until the next 6-month visit unless there is suspicion for progression to cancer. If at least one biopsy shows HSIL, repeat biopsies of lesions with inconclusive histology may be deferred to the next 6-month visit so long as there is no suspicion of progression to cancer. If the visit window for the next 6-month visit is within 8 weeks of the last biopsy, repeat HRA and biopsies will occur as part of the next 6-month visit.

8.6.11 Initiate new treatment cycle as needed or schedule surgery for those being treated with TUA

8.6.11.1 If the participant is to be treated with an ablative therapy, one additional biopsy will be collected prior to treatment from the lesion with most severe appearance of disease. This biopsy will be placed in RNAlater for banking only. A maximum of one biopsy for banking will be obtained during each 6-month treatment block, if treatment is administered.

8.6.12 *QoL objective*: If the participant consents and is eligible to complete questionnaires for the QoL objective, A-HRSI completion at 12, 24, 36, 48, and 60 months after randomization (see Section 8.9 and Appendix I).

8.6.13 COVID-19 telephone follow-up: If the 6-month visit cannot be conducted due to COVID-19, a telephone follow-up visit may be conducted in lieu of a 6-month visit to obtain follow up on treatment completion (if relevant), safety, medical history, medication history, and study arm compliance. See the study source documents for instructions. If a telephone follow-up occurs, a makeup-6 month visit must be conducted as soon as an in-person visit is feasible again. **If a site performs a telehealth visit because the 6-month visit cannot be performed, it will still require reporting as a missed visit and is still considered a protocol deviation.**

8.7 Final Evaluations, Off Study

At the completion of the follow-up period for the study, the Off-Study Summary Form should be completed in AdvantageEDC. Evaluations are to occur in both study arms unless otherwise specified.

8.7.1 Medication review

8.7.2 Recording of HIV viral load and CD4 count laboratory results (only if final visit is an annual visit and results are available from clinical care)

8.7.3 Adverse events evaluation

8.7.4 Serum (red top tube) for the ANCHOR Biorepository for correlative studies

8.7.5 Targeted physical exam (per Section 8.1.7)

8.7.6 Anal swab for local cytology and additional swabs for correlative studies

8.7.6.1 Leftover material from anal swab cytology suspension (Appendix III)

8.7.6.2 Anal swab for DNA

8.7.6.3 Anal swab for RNA/protein

8.7.7 Digital anorectal exam and inguinal node examination

8.7.8 High resolution anoscopy

8.7.9 Anal biopsy. Monitoring arm participants will have biopsies of all visible lesions and any prior areas of HSIL that appear to have regressed. Treatment arm participants will have biopsies of all visible lesions and of normal-appearing areas that were previously treated. For participants in both arms, if this totals less than 4

biopsies, normal-appearing tissue in remaining quadrants will be biopsied so that a minimum of 4 areas are sampled.

8.7.10 *QoL objective*: If the participant consents and is eligible to complete questionnaires for the QoL objective, A-HRSI completion at 60 months after randomization (if applicable given the participant's visit schedule at study discontinuation; see Section 8.9 and Appendix I).

8.8 Early Study Discontinuation Evaluations

All participants who notify the site of the intent to discontinue the study early will be asked to consent to be contacted once per year for the remainder of the study for follow-up for anal cancer diagnoses only. Instructions for this contact are outlined in the study MOP. Medical records will be requested from the participant's provider if anal cancer is diagnosed. If more than 3 months have elapsed since the prior 6-month visit, the participant will be asked to complete a final visit before discontinuing participation, as outlined below.

8.8.1 Adverse events evaluation

8.8.2 Recording of HIV viral load and CD4 count laboratory results (only if final visit is an annual visit and results are available from clinical care)

8.8.3 Medication review

8.8.4 Serum (red top tube) for the ANCHOR Biorepository for correlative studies

8.8.5 Targeted physical exam (per Section 8.1.7)

8.8.6 Anal swab for local cytology and additional swabs for correlative studies

8.8.6.1 Leftover material from anal swab cytology suspension (Appendix III)

8.8.6.2 Anal swab for DNA

8.8.6.3 Anal swab for RNA/protein

8.8.7 Digital anorectal exam and inguinal node examination

8.8.8 High resolution anoscopy

8.8.9 Anal biopsy. Monitoring arm participants will have biopsies of all visible lesions and any prior areas of HSIL that appear to have regressed. Treatment arm participants will have biopsies of all visible lesions and of normal-appearing areas that were previously treated. For participants in both arms, if this totals less than 4 biopsies, normal-appearing tissue in remaining quadrants will be biopsied so that a minimum of 4 areas are sampled.

8.8.9.1 Participants diagnosed with cancer may provide an optional cancer biopsy (placed in RNALater) if willing to return to the clinic for biopsy before referral to treatment and study discontinuation.

8.8.10 If the participant is discontinuing treatment due to progression to cancer, stage at diagnosis must be recorded.

8.8.11 *QoL objective*: If the participant consents and is eligible to complete questionnaires for the QoL objective, A-HRSI completion at the applicable time point given the participant's visit schedule at study discontinuation (see Section 8.9 and Appendix

I).

8.9 QoL Objective

If the participant consents to complete A-HRSI questionnaires for the QoL objective, the A-HRSI will be completed via telephone-facilitated interview (performed by clinical site staff) or ePRO platform (independently by the participant) at the following time points. See Appendix I for the window for accepted forms.

Time Point	Target Date	Target Completion Window
T1	Visit 1, before randomization	- 14 to 0 days
T2	2-7 days after randomization	2-10 days
T3	4 weeks after randomization	± 1 week
T4	12 months	± 4 weeks
T5	24 months	± 4 weeks
T6	36 months	± 4 weeks
T7	48 months	± 4 weeks
T8	60 months	± 4 weeks

9.0 BIOMARKER STUDIES

Any use of biospecimens collected as part of this trial requires specific review and approval by NCI CTEP or other mechanisms as will be set up for the NCI Clinical Trials Network (NCTN) or by OHAM, and by the ANCHOR Correlative Science Committee. For a request for use of specimens to be considered, a protocol for use of banked specimens (or an amendment to the protocol if the trial is still active) must be submitted to NCI CTEP; the protocol submitted for review must contain a clear statement of the scientific objectives and hypotheses, a statistical section that provides a brief description of the statistical design and analysis strategy along with sample size/power justification, a description of the assay methodology, and identification of the laboratory/individuals that will perform the assays. For non-standard assays, information about the assay's analytical performance (e.g., sensitivity, specificity, bias, linearity, reproducibility, as applicable) also may be requested by the reviewers.

This correlative science section contains specific aims, hypotheses, and experimental designs to address correlative science questions that could potentially be addressed using specimens collected as part of the ANCHOR study. These include studies of viral factors in HSIL progression to cancer, identification of host factors in HSIL progression to cancer, and viral and host biomarker predictors of risk of progression from HSIL to cancer. The purpose of this section is not to describe the exact experiments that will be done, since the aims, methods, and choice of reagents will likely change in whole or in part by the time sample sets of sufficient size to perform the experiments have been assembled. The information provided in this section is therefore meant only to provide examples of experiments that could be performed, and to illustrate the thinking behind the approach to specimen collection in the ANCHOR protocol.

9.1 Viral Factors in HSIL Progression to Cancer

9.1.1 Study aims

1) Determine the HPV type in cancer and compare to that of overlying HSIL and HSIL biopsies collected concurrently that did not progress to cancer.
2) Determine the strain variant of HPV 16 in participants who progressed to anal cancer and compare to participants with HSIL biopsies who did not progress to cancer.
3) Determine the HPV integration site in overlying anal cancer to that of HSIL overlying the cancer and HSIL biopsies collected concurrently that did not progress to cancer.

For Aims 1 and 2, microdissected portions of the tissue will be placed in Eppendorf tubes and will be processed for PCR and methylation studies. HPV typing will be performed using small amplicon primer sets known to be optimal for studies of DNA from formalin-fixed, paraffin-embedded (FFPE) specimens, including Spf-10 primers (66). Tissues shown to contain HPV will be analyzed further to determine the type. Results from those with HPV16 will be confirmed using E6 sequencing to identify strain variants. Strain variants will be classified as published previously and grouped into non-European and European strain variants (67)(68).

For Aim 3, HPV integration will be assessed using the Amplification of Papillomavirus Oncogene Transcript (APOT) Assay in which HPV-cellular fusion transcripts are sought using a 3`RACE protocol. This allows for PCR amplification for subsequent sequence analysis (69). RNA will be isolated from sections of FFPE biopsies and treated with DNase treatment for 15 min. The APOT assay will be performed as previously described. Total RNA will be reverse-transcribed using an oligo(dT) primer coupled to a linker sequence (59-AAG CAG TGG TAT CAA CGC AGA GTA $CT_{(30)}$VN-3'), referred to as CDS-primer (Clontech, Heidelberg, Germany). HPV-derived fusion transcripts will be amplified using the APOT assay based on a rapid amplification of cDNA ends (RACE) performed in a nested PCR format. HPV E7 primers will be used as forward primers. An adapter primer complementary to the linker sequence in the CDS primer will be used as first reverse primer and the CDS-primer as second nested primer. Cellular fusion transcripts will be excised from the gel and extracted using the Zymoclean Gel DNA Recovery Kit (Analy-tikJena, Jena, Germany). The isolated products will be sequenced and the integration locus will be determined by database alignments using National Centre for Biotechnology Information (NCBI) human megaBlast tool and the University of California, Santa Cruz (UCSC) genome browser.

9.1.2 Specific hypotheses

HPV 16 will be the predominant HPV type in anal cancer even if the participant who progressed to cancer has HSIL with multiple HPV types. Single HPV 16 infection will be found in most anal cancers. HPV 16 has been found to be the predominant HPV type in anal cancers overall (18), but little is known about the distribution of HPV in anal cancers among HIV-infected men and women. Recent data suggest that HPV 16 may play a less prominent role in cervical cancers of HIV-infected women compared with HIV-uninfected women (51)(63). It will be important to know whether this is also true of anal cancers, and whether other HPV types may be playing an important role.

1) Non-European variants will be associated with cancers and will be found in HSILs that progressed to cancer more often than in HSILs that did not progress to cancer after matching for age, race/ethnic background, gender, smoking history, and nadir CD4 level. Non-European variants have been associated with increased risk of developing HSIL compared with European variants (67)(68)(70), but little is known about their role in progression from HSIL to cancer.

2) Different concurrent areas of HSIL represent outgrowth of biologically distinct foci of HPV infection and this will be manifest in varying patterns of HPV integration. HPV integration sites will differ between HSILs that progressed to cancer and concurrent HSILs that did not progress to cancer. In the HSIL that progressed to cancer the integration sites will be same between the invasive cancer and the overlying HSIL, and reflects a site of integration that is pathogenetically important in progression to cancer. Although several studies show that HPV integration sites are not consistent in different cancers, recent studies show some hot spots, including integration at sites that may be pathogenetically important (69). Little is known about HPV integration sites in

either anal HSIL or anal cancer, and it will be important to determine if and how HPV integration is playing a role in anal cancer pathogenesis.

9.1.3 Statistical design

The HPV type(s) in invasive cancer portion of the tissue, overlying HSIL and from biopsies of concurrent HSIL that did not progress will be described in a descriptive analysis. HPV 16 strain variants will be classified as European and non-European. The strain variant of HPV 16 in HSIL from participants who did not progress to cancer will be compared with the HPV 16 from those who progressed to cancer. The results will be controlled for race and ethnic background since non-European strain variants are found more frequently in non-White individuals.

We expect that non-European variants will be found in 75% of HSIL that progressed to anal cancers and 25% of those that did not. If we have 18 cancer cases, 36 well-matched controls will be sufficient to detect a difference in the proportion of non-European variants of 75% in the anal cancer cases vs. 25% in the controls, at the two-sided 0.05 significance level with power of 0.89, assuming a correlation of 0.25 between matched cases and controls. Similar samples sizes will suffice for comparing sets of HSIL biopsies as described above.

Descriptive statistics will be used to describe the integration locus of HPV in the invasive cancers and whether they differ from those of the overlying HSIL. Descriptive statistics will also be used to determine if the loci differ in HSIL that progressed and concurrent HSIL biopsies that did not progress. In each case we will only analyze tissues that contain HPV 16.

9.2 Identification of Host Factors in HSIL Progression to Cancer

9.2.1 Study aims

We will have access to tissues that contain early invasive anal cancers, with the expectation that most will also contain overlying HSIL. This will allow for comparison of molecular characteristics of HSIL with overlying invasive disease. It will also allow for comparisons between HSIL that progressed to cancer and HSIL that did not progress within the same individual, since many participants will have multiple foci of HSIL. In so doing we will elucidate some of the steps involved in progression from HSIL to cancer, potentially opening up new avenues for treatment of HSIL and cancer, and identifying biomarkers that predict progression to cancer. Comparing cancers to overlying HSIL offers different information from comparing HSIL that progressed with HSIL that did not progress. Comparing cancers and overlying HSIL may identify some of the last steps in progression to cancer. Similar comparisons between HSIL that progressed and HSIL that did not progress and between HSIL that progressed and the same lesion at earlier time points will provide insight into some of the penultimate steps in cancer progression.

The goal of performing gene expression analysis is identify genes involved in progression from HSIL to cancer and whose expression may serve as biomarkers of progression to cancer. The goal of performing whole exome sequencing is to identify genetic changes involved in progression from HSIL to cancer and whose expression may serve as biomarkers of progression to cancer.

1) Perform gene expression array analysis comparing expression in anal cancer with HSIL overlying the cancer. Perform gene expression array analysis comparing expression in HSIL biopsies that progressed to cancer with non-progressing HSIL biopsies at other locations. Perform similar analyses comparing expression in HSIL biopsies that progressed to cancer with the same lesion at earlier time points prior to progression.

2) Characterize genetic changes in anal cancers compared with HSIL overlying the cancer. Characterize genetic changes in HSIL biopsies that progressed to cancer compared with non-progressing HSIL biopsies at other locations. Characterize genetic changes HSIL biopsies that progressed to cancer with the same lesion at earlier time points prior to progression.

For Aim 1, RNA will be extracted from sections of microdissected anal cancer and HSIL using the Ambion RecoverAll Kit following the RNA protocol. This kit is designed for FFPE tissue and removes the secondary structure caused by the formalin. RNA will be amplified, fragmented, and labeled using NuGEN Technologies WT- Ovation Kits according to the manufacturer's instructions (NuGEN Technologies, San Carlos, CA). Labeled samples will then hybridized to Affymetrix Human Gene 1.0 ST GeneChips (Affymetrix, Santa Clara, CA). The GeneChips will be washed and stained on Affymetrix 450 Fluidics Stations and scanned on an Affymetrix 3000 Scanner according to standard protocols.

For Aim 2, the projected number of cases of incident invasive anal cancer (n=18) makes most genome-wide association approaches untenable. However, the sample size of non-progressors for the ANCHOR study in the active monitoring arm is considerable (n= approximately 2000). Consequently, the depth of phenotypic characterization and the availability of optimally-matched controls (matched on age, race/ethnic background, gender, smoking history, and nadir CD4 level) can permit the discovery of rare mutations enriched in incident cancer cases compared with matched controls using next-generation sequencing. Accordingly, we will perform whole exome sequencing of all incident cancer cases available for analysis. We will perform several comparisons: 1) compare the genomes of the cancers to the overlying HSIL, 2) the cancers to normal tissue from the same individuals will serve as controls to identify sequencing artifacts, 3) HSIL from the time of progression to cancer to concurrent HSIL at other locations in the same individual that did not progress to cancer, 4) HSIL from the time of progression to the same lesion at different time points prior to progression; and 5) compare HSIL DNA from participants who progressed to cancer with a HSIL from well-matched participants who did not. An excellent review of paired tissue analysis was published by Meyerson et al. and we will use the approaches described in that publication (71).

9.2.2 Specific hypotheses

1) Tumor promoter and suppressor genes that play a role in some of the last steps in progression from HSIL to cancer will differ between cancer and overlying HSIL.

2) Tumor promoter and suppressor genes that play a role in some of the penultimate steps in progression from HSIL to cancer will differ between HSIL that progressed and concurrent HSIL that did not progress.

3) Tumor suppressor genes important in some of the last steps in progression from HSIL to cancer will have mutations in invasive cancers compared with overlying HSIL, with mutations predicted to cause loss of function. Tumor promoter genes may also be mutated.

4) Tumor suppressor genes important in some of the penultimate steps in progression from HSIL to cancer will have mutations in HSIL that progressed, compared with HSIL that did not progress. Tumor suppressor genes important in some of the penultimate steps in progression from HSIL to cancer will have mutations in HSIL that progressed, compared with HSIL at the same location at time points prior to progression. Tumor promoter genes may also be mutated in these tissues.

Many studies have characterized chromosomal changes and genes that are over or under-expressed in cervical HSIL compared with normal tissues, or between cervical cancer and normal tissues or cervical HSIL; a few have looked at anal cancer and HSIL (72)(73)(74)(75)(76)(77)(78). These studies have also shown that cervical swab material can be used to identify markers of increasing grade of abnormality (79). In AMC 046 we performed microarray analysis of perianal HSIL tissues compared with adjacent normal perianal tissues (Chein A and Palefsky J, manuscript in preparation). We found that several members of the immune response gene family and cell cycle genes were up-regulated in the HSIL tissues compared with normal. There are few studies of changes in gene expression in anal cancer, and few at any other anatomic site at which HPV causes cancer that examine changes in gene expression as HSIL progresses to cancer.

Characterization of the genetic risk factors for progression from HSIL to invasive cancer is also of great importance. Tumor samples from individuals with invasive anal cancer are not well represented in the NCI Cancer Genome Atlas project. An understanding of the genomes of incident cancers may provide additional insight into the genomic alterations that provide these invasive tumors a proliferative advantage. In the ANCHOR study, it is most likely that any cancers will be diagnosed in an office-based biopsy on a FFPE tissue, and we will therefore perform genetic studies that can be done on FFPE (80).

9.2.3 Statistical design

Microarrays from all available (up to 18 anticipated) pairs of invasive cancer tissues and overlying HSIL will be compared and analyzed to compare gene expression in cancer to that of overlying HSIL. Likewise array data from pairs of 18 HSIL tissues that progressed to cancer and matched HSIL that did not progress from the same

participant or which were obtained at time points prior to progression. Microarrays from HSIL that progressed will also be compared with those from HSIL from well-matched control participants (matched on for age, race/ethnic background, gender, smoking history, and nadir CD4 level) who did not progress. Microarrays will be normalized for array-specific effects using Affymetrix's "Robust Multi-Array" (RMA) normalization. Normalized array values will be reported on a log2 scale. Average normalized expression is typically around 7.0. For statistical analyses, all array probe sets where no experimental groups have average log2 intensity greater than 3.0 will be removed. It is a standard cutoff below which expression is indistinguishable from background noise.

Linear models will be fitted for each gene using the Bioconductor "limma" package in R (81)(82). Moderated t-statistics, fold-change and the associated p values will be calculated for each gene. To account for the fact that thousands of genes will be tested, false discovery rate (FDR)-adjusted values will be calculated using the Benjamini-Hochberg method (83). FDR values indicate the expected fraction of falsely-declared differentially-expressed (DE) genes among the total set of declared DE genes, i.e. FDR=0.15 would indicate that 15% of the declared DE genes were expected to be false due to experimental noise instead of actual differential expression. The target FDR will be a maximum of 0.05 or less.

To analyze the results, the GO-Elite software tool designed by the J. David Gladstone Institutes will be used (http://genmapp.org/go_elite/). This tool was designed to identify a minimal non-redundant set of Gene Ontology (GO) biological terms or pathways to describe a particular set of genes (https://code.google.com/p/go-elite/wiki/AboutUs#CitingGO-Elite). A z-score and permuted p value will be calculated to assess over-representation of GO terms and pathways in the gene set. The permuted p value represents the chance of randomly selecting the same number of regulated probe sets from all probe sets examined and recalculating z-scores for all terms 2000 times.

We will perform whole exome sequencing of DNA for Aim 2 using DNA from the matched tissue sets as described. Whenever possible, we will perform these studies with approximately 5 controls per 1 case. A ratio of 5:1 was selected to maximize the precision of the estimates of association.

9.3 Viral and Host Biomarker Predictors of Risk of Progression from HSIL to Cancer

9.3.1 Study aims

1) Study different measures of HPV activity as biomarkers of progression from HSIL to invasive cancer (HPV DNA methylation, E6 RNA and E6 protein quantity, serum E6 antibodies, circulating HPV DNA).
2) Study host genetic and epigenetic changes as biomarkers of progression from HSIL to invasive cancer.
3) Study host immune and inflammatory response markers (serum inflammatory cytokines and IL-10, CXCL 12, HLA-G and CCR-2 polymorphisms) as biomarkers of progression from HSIL to invasive cancer.

4) Study cell proliferation proteins as biomarkers of progression from HSIL to invasive cancer (phosphatase and tensin (PTEN), survivin, U3 small nucleolar ribonucleoprotein protein IMP3, epithelial mesenchymal transition proteins).

For Aim 1, HPV DNA methylation studies will be performed on anal swabs as described previously for cervical swabs. Briefly, the extent of CpG methylation will be determined by pyrosequencing (Qiagen, Valencia, CA) according to the manufacturers' recommendations. DNA documented to contain HPV 16 DNA will be treated with bisulfite using the EZ DNA methylation kit (Zymo Research, Orange, CA) according to the suggested protocol. All segments will be amplified by Platinum Taq high fidelity DNA polymerase (Invitrogen, Carlsbad, CA) with a primer containing biotin, captured with streptavidin-coated beads, and annealed to a sequencing primer (available from RDB). Sequence extension will be performed with PyroMark Gold Q96 Reagents (Qiagen, Valencia, CA) that uses DNA polymerase with sequential addition of each dNTP; the released pyrophosphate will be catalyzed by ATP sulfurylase, luciferase, and apyrase sequentially to generate light signals, which are detected by a charge-coupled device (CCD) camera on a PSQ96 system (Qiagen, Valencia, CA). Because the bioluminometric response is linear for the sequential addition of dNTPs, the signal intensity reflects the original methylation ratio producing continuous values between 0 and 1. We will examine methylation in HPV DNA from anal swabs in individuals who progressed to cancer in comparison with swab DNA from matched controls (matched on for age, race/ethnic background, gender, smoking history and nadir CD4 level) who did not progress. Methylation will also be compared in swabs in a given individual as they approach the diagnosis of cancer.

E6 RNA levels will be measured using the commercially available Aptima test. Although this test is currently configured for clinical diagnostic purposes to be reported as "positive or negative," the quantity of E6 RNA can also be measured in relative light units (RLU). We have already shown that the test can successfully be performed on anal swab material (J. Palefsky, personal communication). Participants in the active monitoring arm who develop anal cancer will be compared with matched participants who did not develop cancer. Anal swab specimens at baseline and at least annually up to the time of development of cancer will be analyzed, classified as positive or negative for HPV 16, 18 or 45, and the RLU as measure of RNA quantity will also be analyzed. The quantity of E6 RNA from anal swabs in individuals who progressed to cancer will be compared with matched controls who did not progress. E6 RNA levels will also be compared in swabs in a given individual as they approach the diagnosis of cancer.

Similar analyses will be done for HPV 16, 18, and 45 E6 protein analysis, using the Arbor-Vita E6 protein detection assay. We have already shown that the test can successfully be performed on anal swab material in pilot studies (J. Palefsky, personal communication). Samples are described as positive or negative for E6, and quantitation is performed using a densitometer.

Measurement of serum E6 antibodies will be performed as described previously. Results will be classified as positive or negative and titers will be measured and analyzed. Participants in the active monitoring arm who develop anal cancer will

be compared with matched participants who did not develop cancer. Antibody positivity and titer levels will be compared between the groups as will changes over time in a given individual as they approach the diagnosis of cancer.

Measurement of circulating (serum) HPV 16 DNA will be performed as described previously. Results will be described as positive or negative and changes over time in a given individual will be described as they approach the diagnosis of cancer. Levels will be compared among those who progressed to those who did not.

For Aim 2, approaches similar to those described for analysis of HPV DNA methylation in anal swab specimens will be used to study select cellular genes for methylation. We will study methylation of nine hypermethylated differentially methylated regions (DMRs) shown to be important in cervical HSIL, and zinc finger protein 582. Frequency and level of methylation of these genes in anal swab specimens from individuals who progressed to cancer will be compared with those who did not progress to cancer. Among those who developed cancer we will also study changes in anal swab specimens over time leading up to the diagnosis of anal cancer.

For Aim 3, standard ELISAs will be performed for host inflammatory response proteins (TNF-alpha, interferon), IL-10, and others. Results will be classified as positive or negative and titers will be measured and analyzed. Participants in the active monitoring arm who develop anal cancer will be compared with matched participants who did not develop cancer. Antibody positivity and titer levels will be compared between the groups as will changes over time in a given individual as they approach the diagnosis of cancer.

HLA-G2 and CCR-2 polymorphisms will similarly be studied in progressors and a set of matched non-progressors. DNA from normal tissue as well as lesional tissues will be available for analysis.

For Aim 4, standard immunohistochemical protocols will be used to examine HSIL tissues that progressed to cancer for proteins involved in stimulation or repression of cell proliferation, including PTEN, survivin and U3 small nucleolar ribonucleoprotein protein IMP3. To perform immunohistochemistry, antibodies to each of the proteins are subjected to a standardized test procedure to develop an individual and optimized immunostaining protocol for each antibody (124). Initially one dilution, based on antibody stock concentration of the primary antibody, is tested. The outcome of this test staining is used to guide further dilutions and optimization of the protocol.

TMA slides are deparaffinized and hydrated in xylene and graded ethanol to distilled water prior to immunostaining. A blocking step to quench endogenous peroxidase is performed in 0.3 % H_2O_2 in 95% ethanol for 5 minutes. Heat-induced epitope retrieval (HIER) is performed in a retrieval buffer pH 6, using a pressure boiler as a heat source for 4 minutes at 125°C. After boiling is completed, slides remain in the pressure boiler and are allowed to cool to 90°C. The total processing time for HIER is approximately 45 minutes. If no conclusive result is obtained in the initial test, further tests are done based on the observed staining pattern. Immunohistochemistry (IHC) conditions may be modified, including changing

antibody incubation times, antibody dilutions and diaminobenzidine (DAB) incubation times.

For staining all incubations are done at room temperature. Slides are rinsed in wash buffer, incubated with Ultra V block for 5 minutes, rinsed again in wash buffer, and incubated with primary antibody for 30 minutes. After rinsing twice in wash buffer the slides are incubated with labeled horseradish peroxidase-polymer for 30 minutes. After rinsing twice in wash buffer the slides are developed in DAB solution for 10 minutes, rinsed and counterstained in Mayers hematoxylin for 5 minutes. The slides are rinsed in lithium carbonate water, diluted 1:5 from saturated solution for 1 minute, rinsed in tap water for 5 minutes, dehydrated in graded ethanol, and cover-slipped.

Results will be scored as positive or negative, and graded according to intensity (weak, moderate, strong) and location of staining. Tissue microarrays will be used for this purpose since this will allow for analysis with a small amount of the biopsy specimen. Signal in invasive cancers will be compared with overlying HSIL. Signals in HSIL that progressed will be compared to HSIL that did not progress, as well as arrays of tissues from earlier time points prior to progression. Tissues will also be stained for proteins associated with epithelial-mesenchymal transition (EMT), including e-cadherin, ZO-1, cyclin-D1 and beta-catenin.

9.3.2 Specific hypotheses

Biomarkers of prevalent or incident cervical HSIL will have value as biomarkers of risk of progression from HSIL to cancer:

1) Higher levels of HPV DNA methylation, HPV E6 RNA and HPV E6 protein levels will be associated with progression from HSIL to cancer.
2) Hypermethylation of cellular genes shown to be associated with risk of cervical HSIL (nine selected markers) will be associated with progression from HSIL to cancer.
3) Higher levels of inflammatory response proteins or polymorphisms associated with higher levels of these proteins, and polymorphisms that lead to reduced immune response to HPV will be associated with progression from HSIL to cancer. Detection of any level of HPV DNA in serum will be associated with progression to invasive cancer.
4) Higher levels of proteins associated with increased cellular proliferation and vice versa, will be associated with progression from HSIL to cancer, as will expression of proteins associated with EMT.

Progression from HPV infection to HSIL and then to cancer is a multistep process involving many different pathways. Viral gene expression plays a key role, as do the host immune response, cell cycle pathways, and many others. Given the high proportion of individuals who become infected with HPV but the relatively small proportion who develop cancer, host genetic susceptibility and epigenetic regulation of host gene expression play an important role. Many studies of biomarkers for cervical cancer risk have been performed examining each of these areas (84). Relatively few if any have examined biomarkers for anal HSIL, and

more importantly, few have examined biomarkers of progression from HSIL at any mucosal to site to invasive cancer. In this correlative study, we will examine a group of biomarkers shown to be of interest to predict incident cervical HSIL, and determine whether, using similar or different cutoff levels, they may also be useful to predict progression from anal HSIL to cancer. To do we will use different kinds of samples that will be available through the ANCHOR study. Anal swab material and serum are particularly suitable for screening in the community should these be shown to be of value in the proposed studies. In general markers that involve immunohistochemical analysis of tissue are not as suitable for screening, but proteins shown to be interest can then be tested in swab material in future studies. Biomarkers determined to be of interest in the ANCHOR study can also then be validated for use in the cervix and oropharynx.

We have framed this study according to the overall nature of the pathways involved. Clearly there is some degree of overlap. For example, epigenetic modifications of cellular may alter the level of expression of one or more of the genes being studied in the other specific aims. Likewise, polymorphisms in host genes may alter the function of the proteins or their level of expression.

We are specifically proposing studies of HPV DNA methylation since it has been shown to be important in epigenetic regulation of HPV gene expression, and is a marker of progression to cervical HSIL (85)(86)(87)(88). Serum E6 antibodies have been correlated with HPV-associated oropharyngeal cancer, but their role as markers for prevalent or incident anal cancer is not known (89)(90)(91). HPV only infects epithelial cells, and detection of HPV DNA in serum may reflect circulating, potentially metastasizing HPV-infected tumor cells. Circulating HPV DNA has been detected in patients with oral cancers and some cervical cancers, but not patients without cervical cancer (92)(93). Detection of serum HPV DNA may also reflect tumor burden, and it is therefore possible that serum HPV DNA tests in patients with very early anal cancer will be negative. Nonetheless it is an issue worth exploring since serum testing could be a convenient test to predict prevalent anal cancer, particularly in patients with very widespread anal HSIL in whom it might be difficult to exclude a cancer. E6 RNA has been shown to be nearly as sensitive as HPV DNA tests with increased specificity for cervical HSIL (94)(95). Similar results have been found in ASIL (96). E6 protein-based tests offer additional potential predictive value and are in early stages of development (97). We have pilot tested E6 protein testing in anal samples in our clinic and have shown that the test can work in anal samples (J. Palefsky, personal communication). Like E6 RNA, E6 protein testing data can be quantified as we will explore the value of doing so.

Methylation of cellular genes may be important biomarkers of prevalent cancers or potentially, progression from HSIL to cancer (98). Although there are limited data on host gene methylation in ASIL (99) its role as a diagnostic test for anal disease and its predictive value for progression from HSIL to cancer are not yet known. We have identified several genes of interest in cervical disease that will be of value to study in the setting of anal disease. These include but are not limited to methylation of genes regulating miRNA expression including hsa-miR-203 and -375 (100),

differentially methylated regions in cervical cancer (101), zinc finger protein 582 (102), genes involved in one-carbon metabolism (103) and CADM1 (104).

The host immune and inflammatory responses likely play a critical role in pathogenesis of HPV-related cancers. We will explore host inflammatory markers and relevant gene polymorphisms that have been shown to be important in cervical HSIL or cancer. These include, but are not limited to serum IL-10 levels, and serum IL-6, tumor necrosis factor-alpha, macrophage inflammatory protein-1 alpha, granulocyte macrophage colony-stimulating factor, IL-1 beta, and IL-1 alpha (105). Immunohistochemical staining for CD32B+ lymphocytes, GATA3+ and T-bet+ lymphoid cells (106), CXCL-12 (107), FoxP3+ lymphocytes (106)(108)(109) have all been shown to correlate with cervical HSIL or cancer as have HLA-G (108) and CCR-2 polymorphisms (110).

Finally, markers of cellular proliferation may represent important biomarkers of progression to cancer. As with the other areas discussed above, little is known about their expression in anal HSIL or progression from anal HSIL to cancer. Here again we looked to the cervical literature to identify markers shown to be associated with cervical HSIL or cancer that may be of value for anal disease. These include phosphatase and tensin (PTEN) (111), survivin (111), U3 small nucleolar ribonucleoprotein protein Imp3 (112), p16Ink4a (108)(113)(114), MCM 3 and 5, CDC6, Geminin, cyclins A-D, TOPO2A, CDCA1, and BIRC5 (116), and protein markers of epithelial mesenchymal transition proteins ZO-1, e-cadherin and beta-catenin (114).

9.3.3 Statistical design

DNA methylation will be studied in anal swab specimens at three CpG sites in each of the L1, L2, and E2/E4 genomic regions that were previously shown to be associated with an increased risk of CIN3. Percent methylation will be recorded and the distribution of percent methylation will be compared between those who develop cancer and matched controls who do not. Similarly, the frequency and level of methylation of 9 selected DMR markers in anal swab specimens from individuals who progressed to cancer will be compared with those who did not progress to cancer.

Patients who progress to cancer and matched participants who do not develop cancer will be compared with respect to anal swab E6 RNA and E6 protein, and serum E6 antibodies. Each will be classified as positive or negative, and quantitated with the levels used for data analysis. A sample of all available incident anal cancer case patients will be obtained (sample of 18 estimated). For each case patient, a matching sample of 2 control patient(s) will also be obtained. If a minimum sample of 54 patients is obtained, this will achieve 89% power to detect an odds ratio of 9.00 versus the alternative of equal odds using a Chi-Square test with a 0.05 significance level, assuming a correlation of 0.25 between matched cases and controls.

Changes in the levels of methylation, E6 RNA, protein and antibodies will be compared between the groups as will changes over time in a given individual as they approach the diagnosis of cancer using general estimating equations. The

relationship between E6 RNA, protein, and antibody levels will also be explored in this way. Serum HPV DNA detection results will be classified as positive or negative and their association with the development of cancer will be assessed using a proportional hazards model.

Levels of serum inflammatory response proteins will be measured and analyzed using the study design described above. Polymorphisms in HLA-G 2 and CCR-2 will be described as present or absent.

Immunohistochemical studies will compare the detection of protein expression (positive or negative) between available anal cancer tissues (up to 18 tissues estimated) with invasive anal cancer and overlying HSIL. We will also compare HSILs that progressed to cancer with HSILs that did not and with tissues from pre-progression time points. Similar comparisons will include location of staining (superficial, basal, diffuse) and weak, moderate or strong) among these tissues.

9.4 Medical History and Behavioral Risk Factors for Progression from HSIL to Cancer among Those Who Are Enrolled

9.4.1 Study aim

1) Identify medical history and behavioral risk factors for HSIL progression to cancer

   A brief questionnaire containing questions on medical history and behavioral factors that may be risk factors for progression from HSIL to cancer will be administered to all participants who are being enrolled. The questions were selected from among those used in prior studies of anal LSIL and HSIL and shown to be of interest in univariate or multivariate analysis of prevalent or incident disease. The questionnaire is shown in Appendix V. Data from this questionnaire will be used in a case-control analysis of potential risk factors for progression to cancer among those who were enrolled into the study.

9.4.2 Specific hypotheses

Smoking and chronic irritation induced by sexual activity and chronic hemorrhoids will be risk factors along with anal HPV infection for progression of anal HSIL to anal cancer.

9.4.3 Statistical design

For each risk factor of interest, Fisher's exact test or Pearson's chi-square test will be used to determine if there is an association between the risk factor and development of invasive anal cancer. Factors associated with invasive anal cancer at the 0.10 significance level will be incorporated into a logistic regression model to determine if they are independently associated with invasive anal cancer. Cox regression analyses will also be used to evaluate the association between risk factors and time to diagnosis of invasive anal cancer.

To further elucidate the risk factors associated with invasive anal cancer, a matched control analysis will be done. For each invasive anal cancer case, multiple controls (e.g., 2 or 3) matched by age, gender, and race/ethnicity will be randomly selected. Using a stratified logistic regression model where each stratum is comprised of a

case and its matching controls, risk factors will be assessed to determine whether they are associated with the diagnosis of invasive anal cancer.

10.0 STATISTICAL CONSIDERATIONS

10.1 Study Design/Endpoints

10.1.1 Primary objective

To determine whether treating anal high-grade intraepithelial neoplasia (HSIL) is effective in reducing the incidence of anal cancer in HIV-infected men and women.

10.1.2 Secondary objectives

- To determine the safety of infrared coagulation, electrocautery, imiquimod, laser, and 5- fluorouracil treatments for anal HSIL.
- To assess the responsiveness (sensitivity to change) and clinical significance of the A-HRSI subscales by comparing change scores within groups of participants as defined by participant responses to the PGIC item.

10.1.3 Exploratory objectives

Collect clinical specimens and data to create a bank of well-annotated specimens that will enable correlative science: a) Identification of viral factors in HSIL progression to cancer; b) identification of host factors in HSIL progression to cancer; c) identification of biomarkers of HSIL progression to cancer and d) identify medical history and behavioral risk factors for HSIL progression to cancer.

Ancillary study aims are outlined in Section 2.6.

10.1.4 Quality of Life Objectives

- Primary QOL Objective: To compare arms in terms of changes in physical symptoms and impacts from T2 to T3, adjusting for T1.
- Secondary QOL Objective: To compare arms in terms of changes in psychological symptoms from T3 to T4, adjusting for T1.
- Exploratory QOL Objective: To assess of long-term HR-QoL changes in physical symptoms/impacts and psychological symptoms from T4 through the subsequent T5-T8 follow-ups overall and by arm.

10.2 Sample Size/Accrual Rate

For this study we are assuming an incidence rate of anal cancer of 100/100,000 among all HIV-infected men and women, which by definition includes those with and without prevalent anal SIL. We are assuming that the obligate anal cancer precursor is HSIL, and that all cases of cancer develop from HSIL. If half of the population develops HSIL then the incidence of cancer among those with HSIL would be expected to be 200/100,000. Of note, in their meta-analysis, Machalek et al estimated that 1/377 HIV-infected MSM progress from anal HSIL to anal cancer each year (16). This is equivalent to 265/100,000 per year. All participants must have HSIL to be enrolled in the ANCHOR study and thus we estimate that an incidence of 200/100,000 among study participants in the active monitoring arm is conservative to perform an intent-to-treat (ITT) analysis of our primary objective. The ITT analysis will include all randomized study participants, including those who fail treatment of anal HSIL in the treatment arm, and those who develop new, metachronous lesions, which may or may not have been fully treated at the end of study

follow-up.

Sample size estimates were based on using a log-rank test to compare the treatment and active monitoring arms under the following assumptions: three-year accrual period, five years of follow-up, 5% annual drop-out rate for both arms, and 7% annual drop-in rate for the active monitoring arm (116)(117). The 7% annual drop-in rate is the rate at which participants in the active monitoring arm opt to have treatment for HSIL. The 5% annual dropout rate includes individuals who are lost to follow-up or who die over the course of the study. For both drop-ins and drop-outs, observation time will be censored at the time of drop-in or drop-out. The annual incidence rates of anal cancer were assumed to be constant over time. To detect a difference between an annual incidence of anal cancer of 0.2% (200/100,000) in the active monitoring arm and 0.05% in the treatment arm at the two-sided 0.05 significance level with power of 0.90 will require 2529 study participants per arm for a total of 5058 study participants. Under these assumptions, the expected number of events is 7.0 and 23.7 in the treatment and active monitoring arms, respectively.

The power that we would attain under a range of assumptions is shown in the table that follows. Assuming that we enroll 2529 in each arm (5058 total), power was estimated for three levels of incidence rates for the active monitoring arm, and 3 levels of drop-in rate from the control to the treatment arm under the following assumptions:

- Constant hazard rates (incidence rates) over time for both groups
- Two-sided 0.05 significance level
- Sample size of 5058 evenly divided between treatment and active monitoring arms
- 3-year accrual period and minimum follow-up period of 5 years
- Annual drop-out rate of 5% for both observation and treatment arms
- No drop-ins from treatment to active monitoring arm.
- Drop-in rate of 7% from observation to treatment
- Alternative hypothesis is a 75% reduction in the incidence of anal cancer from the active monitoring arm

Table 10-A: Power associated with varying drop-out rates and drop-in rates from the active monitoring arm with fixed sample size (N=5058), two-sided significance level of 0.05, incidence rate for the active monitoring arm (200/100,000) and the treatment arm (50/100,000)

Drop-out rate	Drop-in rate from the active monitoring arm to the treatment arm		
	7%	10%	15%
5%	90.0	84.9	74.6
10%	85.0	79.4	69.2
15%	79.2	73.5	63.7
20%	72.8	67.2	58.3

Please note that the exact incidence of progression to cancer among HIV-infected individuals is not known. We have estimated 200/100,000 as described above (data shown in bold in the table), but the estimate of Machalek et al yields an estimate of 265/100,000.

We believe that the recruitment goal is a bit larger than it needs to be but should serve to ensure that we will have sufficient power for the primary study objective.

We will monitor the incidence of anal cancer in both arms on a regular basis, and can either discontinue the trial early if the numbers warrant it as determined by the DSMB, or extend follow-up beyond 5 years if cancer is developing at a rate lower than projected.

Over the course of the recruitment period, we estimate that we will need to screen 11065 men to diagnose 4426 (40%) with anal HSIL. We estimate that we will need to screen 6320 women to find 632 (10%) with anal HSIL. The proportion of HIV-infected men and women expected to have HSIL was based on multiple studies of HSIL prevalence demonstrated on biopsy, as determined using HRA. An assumption of 40% prevalence of HSIL among HIV-infected men is conservative based on the literature (118)(119)(120)(121) as is the estimate of 10% prevalence among HIV-infected women (122). Although the study of HSIL in women showed 9% with anal HSIL, that estimate only included women who were referred for HRA because of abnormal cytology. Women in that study with normal cytology were not referred for HRA. Since it is well known that anal cytology underestimates the true grade of disease, and some high-risk individuals with normal anal cytology may have anal HSIL (121)(123), the 9% figure represents a low-end estimate of the prevalence of HSIL among HIV-infected women.

Table 10-B: Sample size = 5058, two-sided significance level = 0.05, 3 year accrual, 5 year follow-up

Power (%)	Events Active Monitoring Arm	Events Treatment Arm	Anal cancer incidence per 100,000		Dropout rate (%)	Drop-in rate from the active monitoring arm to the treatment arm
			Active monitoring Arm	Treatment Arm		
78.8	17.8	5.2	150	37.5	5	7
72.1	15.3	4.4	150	37.5	10	7
72.1	16.7	5.2	150	37.5	5	10
65.6	14.4	4.4	150	37.5	10	10
90.0	23.7	7.0	200	50	5	7
85.0	20.4	5.9	200	50	10	7
84.9	22.2	7.0	200	50	5	10
79.4	19.2	5.9	200	50	10	10
95.6	29.6	8.7	250	62.5	5	7
92.4	25.4	7.4	250	62.5	10	7
92.3	27.8	8.7	250	62.5	5	10
88.3	23.9	7.4	250	62.5	10	10

The sample size of 5058 evenly divided between treatment and active monitoring arms was based on detecting a difference in the incidence of anal cancer of 200/100,000 in the active monitoring arm and 50/100,000 in the treatment arm at the two-sided 0.05 significance level assuming a 5% annual dropout rate across both arms, and a drop-in rate from the active monitoring arm to the treatment arm of 7%. The following table shows the power estimates for changing levels of dropout rate (5%, 10%, 15% and 20%) and changing levels of drop-in rate from the active monitoring to the treatment arm (7%, 10%, 15%). Study participants randomized to the active monitoring arm who drop-in to the treatment will be

followed, if feasible, to determine if they develop anal cancer.

Table 10-C: Power associated with varying drop-out rates and drop-in rates from the active monitoring arm with fixed sample size (N=5058), two-sided significance level of 0.05, incidence rate for the active monitoring arm (200/100,000) and the treatment arm (50/100,000)

Drop-out rate	Drop-in rate from the active monitoring arm to the treatment arm		
	7%	10%	15%
5%	90.0	84.9	74.6
10%	85.0	79.4	69.2
15%	79.2	73.5	63.7
20%	72.8	67.2	58.3

10.3 Stratification Factors

Randomization will be stratified by study site, nadir CD4 count (less than or equal to 200, greater than 200), and lesion size at baseline (greater than 50%, less than or equal to 50% of anal canal/perianal region). Nadir CD4 count will be self-reported; if the participant's lowest CD4 count is unknown (specifically or categorically), the site may rely on a positive history of opportunistic infections as a surrogate to categorize the participant as having a CD4 count less than or equal to 200. In order to document lesion size at baseline, the site must report this stratification factor based on the clinician's evaluation of these results as documented in the source. Three zones are to be evaluated for extent: 1) from the squamocolumnar junction (SCJ)/transformation zone (TZ) to the dentate line; 2) dentate line to the verge; and 3) the verge outward to the perianus. If any one of these zones have HSIL involvement of more than 50% , or if two of these zones have more than 25% HSIL involvement, the participant is considered to have more than 50% extent of HSIL. Lesions do not have to be contiguous to qualify for this definition. The site must report this stratification factor based on the clinician's evaluation of these results as documented in the source.

10.4 Statistical Analysis

10.4.1 Primary analysis

The primary analysis population for this study will be the ITT population which will include all randomized study participants. For each study participant, time to anal cancer will be defined as the time from randomization to diagnosis of anal cancer, and censored at the date of last follow-up. The log-rank test will be used to compare the treatment and control arms with respect to time to detection of anal cancer. For each arm, the hazard rate and its 95% confidence interval will be estimated. The proportional hazards model will be used to assess the association of study site, lesion size, and nadir CD4 level with time to detection of anal cancer.

10.4.2 Analysis of safety of anal HSIL treatments

Adverse events will be summarized at the event level and at the participant level by type of adverse event and severity grade for each of the treatments (infrared coagulation, laser, electrocautery, imiquimod, and 5- fluorouracil treatments), for

the treatment arm as a whole and for the active monitoring arm over the course of study participation. For adverse events that occur in more than 5% of any of the treatments, the Poisson rates will be used to estimate the number of adverse events per unit time and the binomial proportion and its 95% confidence interval will be used to estimate the proportion of participants who reported the event.

10.5 Quality of Life Analyses

10.5.1 A-HRSI scale responsiveness substudy

This substudy will use the PGIC item to document change between the second and third follow-up time points (T2 and T3): "Since the last time you completed a questionnaire, how would you rate your OVERALL QUALITY OF LIFE?", marked on a 7-point scale from "Very much worse" to "Very much better" with a mid-point of "No Change". Each patient will be categorized into one of 3 groups: change for the worse (a response of "Very much worse", "Moderately worse", or "A little worse"), no change ("About the Same"), and change for the better ("A little better", "Moderately better", and "Very much better"). Changes in the A-HRSI scores will also be calculated (T2 scores minus baseline scores). Responsiveness is supported if changes in the A-HRSI scores correspond reliably with the anticipated changes between the three groups. This will be analyzed by a one-way ANOVA on the changes in the A-HRSI scores across the 3 groups ("worse", "no change", and "better").

The PGIC item has been used with recall periods up to 6 weeks. It may or may not work for recall periods exceeding 6 weeks. We will collect the dates of assessments and thus can assess for trends in PGIC item.

A sample size of 90 will support 80% power if the three groups differ by a standardized change of 0.46, in a one-way ANOVA. We estimated the statistical power by running a simulation where the "worsened" group had a mean change in A-HRSI score of -0.46 (simulated from a standard normal of mean=-0.46 and standard deviation=1.0), the "no change" group had a mean change of 0.0, and the "improved" group had a mean change of +0.46. Additional statistical assumptions included a two-sided type-I error rate of 5%, a correlation of 0.35 between the assessment scores, and n=30 in each of the 3 groups. We understand that this change of ±0.46 may be optimistic. If the changes are only ±0.30, then it will support 80% at a type-I error rate of 0.31 (i.e., considerably reduced confidence in rejecting the null). To allow for 10% dropout, 100 participants (50 in each study arm) will be enrolled in the substudy.

Additionally, the standardized response mean (SRM) will be computed as the mean change in subscale scores divided by the standard deviation of change scores within each change category. The purpose of this statistic is to examine the extent to which patients' quality of life change over time. Values greater than 0.8 will be considered large and values between 0.5 and 0.8 will be considered moderate.

The amount of time spent on each ePRO item screen will be descriptively summarized in order to evaluate participant behavior in completing the questionnaire via the ePRO tool, as well as to verify that the ePRO application is

functioning properly.

10.5.2 QoL objective

In the final implementation of quality of life assessment as measured by the A-HRSI will occur at 8 time points: Pre-randomization (T1), within 2-7 days (T2) and at 4 weeks of treatment/randomization (T3), and then yearly out to 5 years (T4-T8).

The primary endpoint for the QoL objective is changes in physical symptoms and impacts from T2 to T3, adjusting for T1. It is anticipated that participants assigned to the treatment arm will experience significant reduction in physical symptoms and impacts from T2 to T3 versus those in the active monitoring arm.

The secondary endpoint for the QoL objective is changes in psychological symptoms from T3 to T4, adjusting for T1. Due to potential uncertainty related to lack of treatment for those in the active monitoring arm, it is anticipated that participants assigned to the active monitoring arm will experience significant increases in psychological symptoms from T3 to T4 versus those in the treatment arm.

The exploratory endpoint is the assessment of long-term HR-QoL changes in physical symptoms/impacts and psychological symptoms from T4 through the subsequent T5-T8 follow-ups.

In the table below, we show the minimum difference that can be detected with 150, 200, and 250 participants per arm in mean A-HRSI physical symptoms and physical impacts subscale change scores (T3 minus T2) using an analysis of covariance adjusting for the covariate baseline subscale to test for differences between arms at a one-sided 0.025 significance level with approximately 90% power. A one-sided alpha of 0.025 was selected for each comparison to maintain an overall one-sided alpha level of 0.05 after a Bonferroni adjustment for two comparisons. The details of the A-HRSI and subscales may be found in Section 2.3.11.

Table 10-D: Smallest differences that can be detected for comparing change for specified time points according to treatment group for various scenarios based on pilot data with 90% power using a one-sided alpha of 0.025

Endpoint	**Alpha (1-sided)**	**SD for change score**	**R^2 with baseline covariate**	**Minimum difference that can be detected with given sample size (per arm):**		
				n=150	**n=200**	**n=250**
Primary (T3 vs. T2):						
Physical Symptoms	0.025	0.78	0.29	0.29	0.25	0.23
Physical Impacts	0.025	1.09	0.22	0.43	0.37	0.33
Secondary (T4 vs. T3):						
Psychological Impacts	0.05	1.34	0.17	0.50	0.43	0.39

SD=standard deviation

Note: Estimates of SD and R^2 were from responsiveness substudy.

Our plan is to target 250 participants per arm to allow for screen failures in consented participants and nonresponse. In the A-HRSI scale responsiveness study 16% of consented participants were screen failures, and 12% of eligible participants did not return at either follow-up.

The above table shows that with 150-200 participants per arm we would be able to detect differences of roughly 0.3 to 0.4 unit change on the two primary endpoints of change in physical symptoms and physical impacts. Although the timing of T2 and T3 are somewhat different compared to the responsiveness substudy, the minimum differences that can be detected are smaller than the changes observed in the responsiveness substudy for those with worse ECOG PS scores compared to those with no change (mean group differences of 0.43 and 0.53 for the two subscales, section 2.3.11).

Data for the QOL primary endpoints (physical symptoms and physical impacts) will each be analyzed using an analysis of covariance (ANCOVA) with the dependent variable being change in subscale (T3 score minus T2 score) and independent variables for arm and baseline subscale score. A similar approach will be used for analyzing change (T4 score minus T3 scores) in psychological impacts. The exploratory endpoint will be analyzed in a mixed model repeated measures ANCOVA with change from baseline calculated at each follow-up visit (T4 through T8) for each subscale and baseline subscale included as a covariate. Arms will be compared across time points by testing the main effect for arm. Additionally, the interaction between time and arm will be examined and trajectories over time, according to arm and specific treatments, will be explored using graphs and post-hoc tests at specific follow-up times using model-based contrasts.

Procedures to address missing data. As stated above, we are targeting 250 participants per arm to allow for screen failures and varying survey response rates over time with the goal of obtaining evaluable data from n=150-200 per arm, which still allows for meaningful effects to be detected with 90% power (Table 10-D). We would not anticipate missing questionnaire to be differential with respect to known and unknown factors between arms due to randomization. However, as a sensitivity analysis, we plan to use regression-based, multiple random imputations for the primary and secondary endpoints assuming questionnaire are missing not a random (MNAR). We will use participant characteristics and baseline A-HRSI measurements in this predictive model. The analysis will then be carried out in multiple data sets, and the results combined using standard methods to produce arm effect estimates with standard errors that incorporate the imputation error. We will also compare participant characteristics for evaluable and unevaluable participants for each primary and secondary endpoint.

10.6 SARS-CoV-2 Ancillary Study Analyses

Aim 1: Determine the prevalence of SARS-CoV-2 detection in anal and oropharyngeal swabs among people living with HIV (PLWH) being screened for the ANCHOR study. The prevalence of SARS-CoV-2 in anal swabs and in oropharyngeal swabs will be estimated using the binomial proportion and its 95% confidence interval. With a sample

size of 400 for each swab type and a hypothesized prevalence rate of 10%, the 95% confidence interval for the SARS-CoV-2 prevalence will be no greater than ±3.0%

Aim 2: Determine the relationship in the ANCHOR screening population between prevalent anal SARS-CoV-2 positivity, anal HPV infection, and anal high-grade squamous intraepithelial lesions (HSIL) Fisher's exact tests will be used to compare those screened participants with and without SARS-CoV-2 detected on the anal swab with respect to the prevalence of anal HPV infection and anal HSIL.

Aim 3: Determine the 6-month incidence of SARS-CoV-2 detection in anal and oropharyngeal swabs among participants with anal HSIL newly enrolled into the ANCHOR study. For each swab type (anal and oropharyngeal), participants will be divided into two groups: those who tested positive for SARS-CoV-2 at baseline and those who did not. For those who tested positive for SARS-CoV-2 on anal (oropharyngeal) swabs, participants will be categorized as persistent if they test positive on the 6-month anal (oropharyngeal) swab. For each swab type, persistence will be estimated as the binomial proportion of those who tested positive at 6-months divided by those who were positive for SARS-CoV-2 at baseline. The point estimate and 95% confidence interval will be estimated for the binomial proportion. For those who tested negative for SARS-CoV-2 on anal (oropharyngeal) swabs, participants will be categorized as incidence cases if they test positive on the 6-month anal (oropharyngeal) swab. For each swab type, the incidence rate will be estimated as the binomial proportion of those who tested positive at 6-months divided by those who were negative for SARS-CoV-2 at baseline. The point estimate and 95% confidence interval will be estimated for the binomial proportion.

Aim 4. Determine the relationship between prevalent or incident SARS-CoV-2 detection and regression of anal HPV infection or HSIL among participants newly enrolled into the ANCHOR study and who are randomized to the monitoring arm.

For monitoring arm participants, the incidence of regression of anal HPV infection will be estimated for prevalent and incident SARS-CoV-2 cases using the binomial proportion and its 95% confidence interval. Similarly, the incidence of regression of HSIL will be estimated for prevalent and incidence SARS-CoV-2 cases using the binomial proportion and its 95% confidence interval. Study time frame: We will enroll 400 PLWH who are being screened for ANCHOR over a 5-month period from the start of the study. This should be straightforward since the study typically screens over 200 PLHW/month. To reduce costs will perform the study only at the top 5-6 screening/enrolling sites in ANCHOR, and collectively these sites screen more than 1100 individuals/year. By completing enrollment within 5 months, we will also complete the 6-month follow-up analysis for Aims 3 and 4 within a year of starting the study. We will analyze the data in the last month of the study.

10.7 Data and Safety Monitoring

10.7.1 Participant safety will be monitored in accordance with the AMC Data and Safety Monitoring Plan (Appendix VI). The study will be monitored by a Data and Safety Monitoring Board. The DSMB will meet at least annually after study initiation to assess enrollment, retention (drop-out and drop-in rates), and safety data, and may meet more frequently if needed. The DSMB will also review the outcome-based interim analyses described in Section 10.6.2.

10.7.2 Interim analysis

Futility and Efficacy Halting Rules

Three interim analyses of the primary efficacy outcome are planned to assess the futility of achieving a significant result if the study continues and to potentially demonstrate efficacy before all participants are enrolled. The Lan and DeMets spending function was used to specify the O'Brien-Fleming boundaries based on a one-sided log-rank test 0.025. At the final test, an overall two-sided alpha level of 0.05 (which corresponds to a one-sided 0.025 alpha level) and 90% power will be maintained. The boundaries and operating characteristics for the planned analyses are found in the table below. The DSMB will be informed of the results of the interim analysis. Consideration will be given to halting the study if the futility or efficacy boundary is crossed during the interim analysis.

Table 10-E: Statistical test boundaries and associated operating characteristics.

Analysis	Information fraction	Reject H_0 (Efficacy) Bound Z	Reject H_1 (Futility) Bound Z	Overall α spent	Overall β (1-power) spent
1 (Interim)	0.50	$Z > 2.963$	$Z < 0.332$	0.002	0.020
2 (Interim)	0.75	$Z > 2.359$	$Z < 1.292$	0.010	0.058
3 (Final)	1.00	$Z > 2.014$	$Z < 2.014$	0.025	0.100

At the time of the interim analyses, the ratio of the hazard rate for the treatment arm to the active monitoring arm will be estimated using its point estimate and 95% confidence interval. The target hazard ratio is 0.25. If the efficacy stopping boundary is crossed, then stopping the study for efficacy would be considered if the upper bound of the hazard ratio is less than or equal to 0.50 suggesting that HSIL treatment reduces the anal cancer incidence rate by at least half.

10.7.3 Drop-out rate and drop-in rate

The sample size estimate for this study was based on a drop-out rate of 5% annually for both arms, and a drop-in rate of 7% to treatment for participants in the active monitoring arm. On a semi-annual basis, the drop-out and drop-in rates will be calculated for each year of follow-up. The drop-out rate will be defined as the number of participants who drop out in a follow-up year divided by the number of person years observed during that follow-up year. The drop-in rate will be defined as the number of participants in the active monitoring arm who are treated for HSIL (for any reason) in a follow-up year divided by the number of person years observed during that follow-up year in the active monitoring arm.

If the drop-out rate exceeds 5 per 100 person-years for two consecutive 6-month periods, the protocol team will be required to implement a corrective action plan to decrease the drop-out rate. If the drop-out rate remains above 5 per 100 person-years for two additional 6-month periods, and would require the accrual duration to be extended more than one year, then the trial would be stopped.

If the drop-in rate exceeds 7 per 100 person-years for two consecutive 6-month

periods, the protocol team will be required to implement a corrective action plan to decrease the drop-in rate. If the drop-in rate remains above 7 per 100 person-years for two additional 6-month periods, and would require the trial duration to be extended for more than one year, then the trial would be stopped.

10.7.4 Stopping rules for accrual

The planned duration of accrual is 36 months. With the sample size of 5058, accrual would be completed in 36 months if the accrual rate is 140.5 enrollees/month. On a semi-annual basis, the cumulative accrual will be compared with the projected accrual for that timepoint to determine the proportion of the projected accrual that has been achieved. After 18 months, the proportion of the projected accrual will be calculated as the number of study participants enrolled divided by the cumulative projected number of participants for that time period. If it is less than or equal to 0.20, then the DSMB will be asked to review accrual and to determine with the study team if measures can be taken to greatly improve the accrual rate. If the proportion of projected accrual that has been achieved is greater than 0.20 and less than 0.50, then enrollment will continue for an additional 6 months. At 30 months, if the proportion of the projected accrual that has been achieved remains below 0.50, then the DSMB will consider whether to stop the study due to accrual.

10.7.5 DSMB safety review rule

The DSMB will conduct a formal review with recommendations when the rate of grade 3 or higher adverse events that are at least possibly related to a study treatment or procedure exceeds 5%, by arm; accounting for follow-up this would correspond to a rate greater than 5 events per 100 person years. If a safety issue is identified, potential actions the DSMB may consider recommending to address a safety risk may include recommendations to remove any procedures posing excess safety risks from the study, discussing added clinical measures with the protocol team to prevent or address the specific risk if feasible, and/or modification of the expected severe adverse event frequency in the protocol and consent form.

11.0 ROLE OF DATA MANAGEMENT

11.1 CRF Instructions

Access to the internet data entry system for this study, AdvantageEDC[SM], and instructions for recording of study data on CRFs will be provided by the ANCHOR DMC at www.anchordmc.com. Participating institutions are responsible for submitting data and/or data forms via AdvantageEDC in accordance with the ANCHOR Data Entry Guide and specific form instructions, within the timelines specified by the AMC's Standards of Procedure for Site Performance Measures.

11.2 Data Quality

It is the responsibility of the ANCHOR DMC to assure the quality of data for the study. See the AMC Data and Safety Monitoring Plan (Appendix VI) for the study plans for adverse event reporting and data review. This role extends from protocol development to generation of the final study database.

11.3 Data Monitoring

This study will be monitored in compliance with AMC and ANCHOR Study policies and by the Clinical Data Update System (CDUS) Version 3.0. Cumulative protocol- and patient-specific CDUS data will be submitted electronically to CTEP on a quarterly basis. Reports are due January 31, April 30, July 31, and October 31. Instructions for submitting data using the CDUS can be found on the CTEP Web site (http://ctep.cancer.gov/reporting/cdus.html).

The ANCHOR DMC is responsible for compiling and submitting CDUS data to CTEP for all participants and for providing the data to the Principal Investigator for review.

12.0 ETHICAL AND REGULATORY CONSIDERATIONS

12.1 IRB Approval and Informed Consent

The principles of Institutional Review Board (IRB) approval and informed consent described in the Food and Drug Administration (FDA) regulations (21 CFR Part 50 and 56) and Department of Health and Human Services (DHHS) regulations for the Protection of Human Participants regulations (45 CFR Part 46) must be followed. IRB approval of the protocol and the informed consent form must be given in writing.

The sponsor's designee (ANCHOR DMC) must receive a copy of the letter of approval from the IRB, which specifically approves the protocol and informed consent, before participant enrollment. The IRB must also approve any significant changes to the protocol and documentation of this approval must be sent to the ANCHOR DMC. The IRB must review the research project at least once every 365 days during the duration of the project. Continuing approval of the project must also be given in writing and provided to the ANCHOR DMC.

Records of all study review and approval documents must be kept on file by the Investigator and are participant to inspection during or after completion of the study. AEs must be reported to the IRB according to local procedures. The IRB should receive notification of completion of the study and final report within 3 months of study completion and termination. The Investigator will maintain an accurate and complete record of all submissions made to the IRB, including a list of all reports and documents submitted.

Written informed consent will be obtained from the participant. The nature, significance, and risks associated with the study must be explained to the participant. The informed consent will describe the purpose of the study, the procedures to be followed, the risks and benefits of participation, all risks of the investigational agent(s) and/or study participation as listed in the model informed consent form, and all other elements of informed consent as required by regulation. A copy of the consent form will be given to the participant to keep.

In addition, any institution(s) conducting research according to the guidelines of this protocol is required to adhere to local and national laws and regulations governing the confidentiality and disclosure of health information.

12.2 Changes to the Protocol

Any change or addition to this protocol requires a written protocol amendment that must be approved by CTEP and the Investigator before implementation. All amendments require approval by the IRB of the treating institution. A copy of the written approval of the IRB must be sent to the DMC.

12.3 Women and Minorities

This study is being conducted by the NCI-sponsored AIDS Malignancy Consortium (AMC). As part of their contractual obligations, each participating site within the AMC and the AMC as a whole is required to assure that the participation of women and minority participants reflects the percentage representation of these populations in their geographic region and, for the AMC, the United States as a whole. As such, it is expected that the

representation of participants on this trial will reflect the constitution of the respective populations.

Table 12-A: Accrual targets

Ethnic Category	Sex/Gender				
	Females		Males		Total
Hispanic or Latino	191	+	1,074	=	1,265
Not Hispanic or Latino	567	+	3,226	=	3,793
Ethnic Category: Total of all participants	758	+	4,300	=	5,058
Racial Category					
American Indian or Alaskan Native	38	+	215	=	253
Asian	38	+	215	=	253
Black or African American	303	+	1,720	=	2,023
Native Hawaiian or other Pacific Islander	38	+	215	=	253
White	341	+	1,935	=	2,276
Racial Category: Total of all participants	758	+	4,300	=	5,058
	(A1 = A2)		(B1 = B2)		(C1 = C2)

Enter actual estimates, whole numbers only (percentages, fractions, or decimals are not acceptable).
The totals provided for each Ethnic/gender or Ethnic/total combination must match those given for each Race/gender or Race/total combination (i.e., A1 must match A2, B1 must match B2, and C1 must match C2).

13.0 REFERENCES

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APPENDIX I: SCHEDULE OF EVALUATIONS

Participants Randomized to Active Monitoring Arm

	Visit 0 Screening Within 4 weeks of Information Visit	Visit 1* Random-ization Within 1-12 weeks of Screening	Visit 2 6 months [1] ±4 weeks	Visit 3 12 months ±4 weeks	Visits 4-10** Every 6 months ±4 weeks	Final study visit (60 months or later) ±4 weeks	Early Study Discont-inuation
Informed consent	X[2]						
Demographics	X						
Medical history	X						
Concurrent meds	X	X	X	X	X	X	X
Physical exam[3]	X		X	X	X	X	X
Inguinal node exam	X	X	X	X	X	X	X
Risk factor questionnaire		X					
Quality of Life questionnaires[4]	T1	T2 T3					
Performance status	X						
CBC w/diff, platelets[5]	X						
HIV documentation	X						
HIV viral load, CD4 level		X[6]		X[6]	X[6]	X[6]	X[6]
Anal cytology[7]	X		X	X	X	X	X
Digital anorectal exam	X	X	X	X	X	X	X
High resolution anoscopy	X	X	X	X	X	X	X
Anal swabs[8]	X		X	X	X	X	X
Anal biopsy[9]	X		X	X	X	X	X
Adverse event evaluation		X	X	X	X	X	X
Urine HCG[10]	X						
Blood for biorepository[11]	X		X	X	X	X	X
Oropharyngeal swab[12]	X		X				

* Screening evaluations for Visit 0 may be performed at Visit 1 (except informed consent, the anal swab for cytology, the screening HRA, and anal biopsies), provided that all Visit 0 evaluations are performed before randomization.

** Participants who are followed longer than 60 months will follow the same procedures for visits every six months.

1 Clinicians may elect to perform HRA and biopsy lesions between any of the 6-month visits if cancer is suspected. Visits that occur between regularly scheduled 6-month visits will be designated with the appropriate visit number and a, b, c, etc.

2 Informed consent will be obtained 1-6 weeks before randomization, and may be given during a preceding information visit, if done.

3 Complete physical exam will be performed at baseline. Targeted physical exam will be performed at all subsequent 6-month visits (see Section 8.1.7). Height and weight will be collected at the screening visit only.

4 For participants who consent to the optional HRQoL substudy (completed in February 2020), the A-HRSI and Patient ECOG PS will be administered via telephone facilitated interview or ePRO platform between time of enrollment and time of randomization (T1). A-HRSI, Patient ECOG PS and PGIC will be administered at least 14 days (2-weeks) post-randomization, and at most 70-days post-randomization (T2). The third HRQoL follow-up will be scheduled to take place at least 71 days (10 weeks + 1 day) post-ANCHOR randomization, and at most 112 days (16 weeks) post-randomization (T3).

5 CBC with differential and platelets may occur up to 90 days prior to randomization.

6 HIV viral load and CD4 count results are required at Visit 1 (results obtained from routine medical care within six weeks prior to randomization will be acceptable). Results will be collected from routine medical care at annual visits through study discontinuation, if available. Venipuncture may be performed on the day of randomization, even after randomization.

7 Collect per Section 8.1.10. Leftover material is to be shipped to the ANCHOR Biorepository.

8 One swab stored for DNA. One swab stored for RNA for correlative science studies. These swabs may be collected at the randomization visit if not collected at the screening visit. All swabs will be collected every 6 months on the study.

9 Screening biopsy must be performed 1-12 weeks prior to Visit 1. Biopsy of HSIL lesions performed at every other 6-month visit (annually). At the final visit, all visible lesions and any prior areas of HSIL that appear to have regressed will be biopsied. If this totals less than 4 biopsies, normal-appearing tissue in remaining quadrants will be biopsied so that a minimum of 4 areas are sampled per Section 4.1. Biopsies may be done at any time if cancer is suspected.
10 HCG for females of childbearing age potential.
11 Blood for correlative science studies (serum [red top tube] at all indicated visits, plus plasma and whole blood fraction [lavender top tube] at Visit 2 only).
12 Collect only if consented for participation in the SARS-CoV-2 ancillary study at the 5 participating centers. Swabs must be collected at the same visit as anal swab collection.

Participants Randomized to Treatment Arm, Treatment with Imiquimod Or 5-Fluorouracil Cream

	Visit 0 Screening Within 4 weeks of Informa-tion visit	Visit 1* Random-ization Within 1-12 weeks of Screening	Visit 1A, 1B 8, 16 weeks, ±2 weeks[1]	Visit 2 6 months[2] ±4 weeks	Visit 3 12 months ±4 weeks	Visits 4-10** Every 6 months ±4 weeks	Final study visit (60 months or later) ±4 weeks	Early Study Discont-inuation
Informed consent	X[3]							
Demographics	X							
Medical history	X							
Concurrent meds	X	X	X	X	X	X	X	
Physical exam[4]	X			X	X	X	X	X
Inguinal node exam	X	X	X	X	X	X	X	X
Risk factor questionnaire		X						
Quality of Life Questionnaires[5]	T1	T2 T3						
Performance status	X							
CBC w/diff, platelets[6]	X							
HIV documentation	X							
HIV viral load, CD4 level		X[7]			X[7]	X[7]	X[7]	X[7]
Anal cytology[8]	X			X	X	X	X	X
Digital anorectal exam	X	X	X	X	X	X	X	X
High resolution anoscopy	X	X	X	X	X	X	X	X
Anal swabs[9]	X			X	X	X	X	X
Anal biopsy[10]	X			X	X	X	X	X
Adverse event evaluation		X	X	X	X	X	X	X
Urine HCG[11]	X	X	X	X	X	X		
Blood for biorepository[12]	X			X	X	X	X	X
Oropharyngeal swab[13]	X			X				

* Screening evaluations for Visit 0 may be performed at Visit 1 (except informed consent, the anal swab for cytology, the screening HRA, and anal biopsies), provided that all Visit 0 evaluations are performed before randomization.

** Participants who are followed longer than 60 months will follow the same procedures for visits every six months, including interim visits for treatment as needed.

1 If treatment is tolerated at Visit 1A, clinician will continue treatment for an additional 8 weeks or stop if no HSIL is seen. Participant will return for an optional visit at 16 weeks for Visit 1B if treatment is continued.

2 If biopsies show HSIL clinician may opt to initiate another treatment modality. The schedule for that 6-month block of treatment will vary depending on the modality that will be used. Visits that occur between regularly scheduled 6-month visits will be designated with the appropriate visit number and an interim visit sequence letter a, b, c, etc.

3 Informed consent will be obtained 1-6 weeks before randomization, and may be given during the preceding information visit, if done.

4 Complete physical exam will be performed at baseline. Targeted physical exam will be performed at all subsequent 6-month visits, (see Section 8.1.7). Height and weight will be collected at the screening visit only.

5 For participants who consent to the optional HRQoL substudy (completed in February 2020), the A-HRSI and Patient ECOG PS will be administered via telephone facilitated interview or ePRO platform between time of enrollment and time of randomization (T1). A-HRSI, Patient ECOG PS and PGIC will be administered at least 14 days (2-weeks) post-randomization, and at most 70-days post-randomization (T2). The third HRQoL follow-up will be scheduled to take place at least 71 days (10 weeks + 1 day) post-ANCHOR randomization, and at most 112 days (16 weeks) post-randomization (T3).

6 CBC with differential and platelets may occur up to 90 days prior to randomization.

7 HIV viral load and CD4 count results are required at Visit 1 (results obtained from routine medical care within six weeks prior to randomization will be acceptable). Results will be collected from routine medical care at annual visits through study discontinuation, if available. Venipuncture may be performed on the day of randomization, even after enrollment into the system.

8 Collect per Section 8.1.10. Leftover material is to be shipped to the ANCHOR Biorepository.
9 One swab stored for DNA. One swab stored for RNA for correlative science studies. These swabs may be collected at the randomization visit if not collected at the screening visit. All swabs will be collected every 6 months on the study.
10 Screening biopsy must be performed between 1-12 weeks prior to Visit 1. Biopsies of HSIL lesions performed at each 6-month visit. Areas that appear normal following treatment do not require rebiopsy. At the final visit, HSIL lesions and previously treated areas that appear normal will be biopsied (minimum of 4 biopsies per Section 4.2). Biopsies may be done at any time if cancer is suspected.
11 HCG for females of childbearing potential, as needed prior to treatment.
12 Blood for correlative science studies (serum [red top tube] at all indicated visits, plus plasma and whole blood fraction [lavender top tube] at Visit 2 only).
13 Collect only if consented for participation in the SARS-CoV-2 ancillary study at the 5 participating centers. Swabs must be collected at the same visit as anal swab collection.

Participants Randomized to Treatment Arm, Treatment with Infrared Coagulation, Hyfrecation/Electrocautery, or Laser

	Visit 0 Screening Within 4 weeks of Informa-tion Visit	Visit 1* Random-ization Within 1-12 weeks of Screening	Visit 1A 6 weeks, ±2 weeks[1]	Visit 2 6 months[2] ±4 weeks	Visit 3 12 months ±4 weeks	Visits 4-10** Every 6 months ±4 weeks	Final study visit (60 months or later) ±4 weeks	Early Study Discont-inuation
Informed consent	X[3]							
Demographics	X							
Medical history	X							
Concurrent meds	X	X	X	X	X	X	X	X
Physical exam[4]	X			X	X	X	X	X
Inguinal node exam	X	X	X	X	X	X	X	X
Risk factor questionnaire		X						
Quality of life questionnaires[5]	T1	T2 T3						
Performance status	X							
CBC w/diff, platelets[6]	X							
HIV documentation	X							
HIV viral load, CD4 level		X[7]			X[7]	X[7]	X[7]	X[7]
Anal cytology[8]	X			X	X	X	X	X
Digital anorectal exam	X	X	X	X	X	X	X	X
High resolution anoscopy	X	X	X	X	X	X	X	X
Anal swabs[9]	X			X	X	X	X	X
Anal biopsy[10]	X			X	X	X	X	X
Biopsy in RNALater[11]		X	Not more than once each 6-month block during study					
Adverse event evaluation		X	X	X	X	X	X	X
Urine HCG[12]	X	X	X	X	X	X		
Blood for biorepository[13]	X			X	X	X	X	X
Oropharyngeal swab[14]	X			X				

* Screening evaluations for Visit 0 may be performed at Visit 1 (except informed consent, the anal swab for cytology, the screening HRA, and anal biopsies), provided that all Visit 0 evaluations are performed before randomization.

** Participants who are followed longer than 60 months will follow the same procedures for visits every six months, including interim visits for treatment as needed.

1 As needed, if the participant is having staged procedures or requires an additional treatment of the lesion being ablated in the judgment of the clinician.

2 If biopsies show HSIL clinician may opt to initiate another treatment modality. The schedule for that 6-month block of treatment will vary depending on the modality that will be used. Visits that occur between regularly scheduled 6-month visits will be designated with the appropriate visit number and an interim visit sequence letter a, b, c, etc.

3 Informed consent will be obtained 1-6 weeks before randomization, and may be given during the preceding information visit, if done.

4 Complete physical exam will be performed at baseline. Targeted physical exam will be performed at all subsequent 6-month visits (see Section 8.1.7). Height and weight will be collected at the screening visit only.

5 For participants who consent to the optional HRQoL substudy (completed in February 2020), the A-HRSI and Patient ECOG PS will be administered via telephone facilitated interview or ePRO platform between time of enrollment and time of randomization (T1). A-HRSI, Patient ECOG PS and PGIC will be administered at least 14 days (2-weeks) post-randomization, and at most 70-days post-randomization (T2). The third HRQoL follow-up will be scheduled to take place at least 71 days (10 weeks + 1 day) post-ANCHOR randomization, and at most 112 days (16 weeks) post-randomization (T3).

6 CBC with differential and platelets may occur up to 90 days prior to randomization.

7 HIV viral load and CD4 count results are required at Visit 1 (results obtained from routine medical care within six weeks prior to randomization will be acceptable). Results will be collected from routine medical care at annual visits through study discontinuation, if available. Venipuncture may be performed on the day of randomization, even after enrollment into the system.

8 Collect per Section 8.1.10. Leftover material is to be shipped to the ANCHOR Biorepository.
9 One swab stored for DNA. One swab stored for RNA for correlative science studies. These swabs may be collected at the randomization visit if not collected at the screening visit. All swabs will be collected every 6 months on the study.
10 Screening biopsy must be performed between 1-12 weeks prior to Visit 1. Biopsies of visible lesions performed at each 6-month visit. Areas that appear normal following treatment do not require rebiopsy. At the final visit, HSIL lesions and previously treated areas that appear normal will be biopsied (minimum of 4 biopsies per Section 4.2). Biopsies may be done at any time if cancer is suspected.
11 One additional biopsy of the lesion with the most severe appearance will be collected prior to ablative treatment and stored in RNALater for banking. This biopsy may be collected beginning at the randomization and not more than once every six months during the study.
12 HCG for females of childbearing potential, as needed prior to treatment.
13 Blood for correlative science studies (serum [red top tube] at all indicated visits, plus plasma and whole blood fraction [lavender top tube] at Visit 2 only).
14 Collect only if consented for participation in the SARS-CoV-2 ancillary study at the 5 participating centers. Swabs must be collected at the same visit as anal swab collection.

Participants Randomized to Treatment Arm, Surgery

	Visit 0 Screening Within 4 weeks of Informa-tion Visit	Visit 1* Random-ization[1] Within 1-12 weeks of Screening	Visit 1A, 1B[2]	Visit 2 6 months[3] ±4 weeks	Visit 3 12 months ±4 weeks	Visits 4-10** Every 6 months ±4 weeks	Final study visit (60 months or later) ±4 weeks	Early Study Discont-inuation
Informed consent	X[4]							
Demographics	X							
Medical history	X							
Concurrent meds	X	X	X	X	X	X	X	X
Physical exam[5]	X			X	X	X	X	X
Inguinal node exam	X	X	X	X	X	X	X	X
Risk factor questionnaire		X						
Quality of life questionnaires[6]	T1	T2 T3						
Performance status	X							
CBC w/diff, platelets[7]	X							
HIV documentation	X							
HIV viral load, CD4 level		X[8]			X[8]	X[8]	X[8]	X[8]
Anal cytology[9]	X			X	X	X	X	X
Digital anorectal exam	X	X	X	X	X	X	X	X
High resolution anoscopy	X		X	X	X	X	X	X
Anal swabs[10]	X			X	X	X	X	X
Anal biopsy[11]	X			X	X	X	X	X
Adverse event evaluation		X	X	X	X	X	X	X
Urine HCG[12]	X	X	X	X	X	X		
Blood for biorepository[13]	X			X	X	X	X	X
Oropharyngeal swab[14]	X			X				

* Screening evaluations for Visit 0 may be performed at Visit 1 (except informed consent, the anal swab for cytology, the screening HRA, and anal biopsies), provided that all Visit 0 evaluations are performed before randomization.

** Participants who are followed longer than 60 months will follow the same procedures for visits every six months, including interim visits for treatment as needed.

1 Surgery will be scheduled and should be performed within 2 months.

2 Additional visits may occur between 6-month visits if TUA is being performed in stages.

3 If biopsies show HSIL clinician may opt to initiate another treatment modality. The schedule for that 6-month block of treatment will vary depending on the modality that will be used. Visits that occur between regularly scheduled 6-month visits will be designated with the appropriate visit number and an interim visit sequence letter a, b, c, etc.

4 Informed consent will be obtained 1-6 weeks before randomization, and may be given during the preceding information visit, if done.

5 Complete physical exam will be performed at baseline. Targeted physical exam will be performed at all subsequent 6-month visits (see Section 8.1.7). Height and weight will be collected at the screening visit only.

6 For participants who consent to the optional HRQoL substudy (completed in February 2020), the A-HRSI and Patient ECOG PS will be administered via telephone facilitated interview or ePRO platform between time of enrollment and time of randomization (T1). A-HRSI, Patient ECOG PS and PGIC will be administered at least 14 days (2-weeks) post-randomization, and at most 70-days post-randomization (T2). The third HRQoL follow-up will be scheduled to take place at least 71 days (10 weeks + 1 day) post-ANCHOR randomization, and at most 112 days (16 weeks) post-randomization (T3).

7 CBC with differential and platelets may occur up to 90 days prior to randomization.

8 HIV viral load and CD4 count results are required at Visit 1 (results obtained from routine medical care within six weeks prior to randomization will be acceptable). Results will be collected from routine medical care at annual visits through study discontinuation, if available. Venipuncture may be performed on the day of randomization, even after enrollment into the system.

9 Collect per Section 8.1.10. Leftover material is to be shipped to the ANCHOR Biorepository.

10 One swab stored for DNA. One swab stored for RNA for correlative science studies. These swabs may be collected at the randomization visit if not collected at the screening visit. All swabs will be collected every 6 months on the study.

11 Screening biopsy must be performed between 1-12 weeks prior to Visit 1. Biopsies of visible lesions performed at each 6-

month visit. Areas that appear normal following treatment do not require rebiopsy. At the final visit, HSIL lesions and previously treated areas that appear normal will be biopsied (minimum of 4 biopsies per Section 4.2). Biopsies may be done at any time if cancer is suspected.

12 HCG for females of childbearing potential, as needed prior to treatment.

13 Blood for correlative science studies (serum [red top tube] at all indicated visits, plus plasma and whole blood fraction [lavender top tube at Visit 2 only).

14 Collect only if consented for participation in the SARS-CoV-2 ancillary study at the 5 participating centers. Swabs must be collected at the same visit as anal swab collection.

Health-Related Quality of Life (QoL) Objective Schedule of Evaluations

Sites are to instruct the participant to complete the questionnaire within the target completion window. Missed time points will not require submission of a protocol deviation form.

Time Point and Target Completion	**T1 Baseline** **Visit 1 Randomization**	**T2 2-7 days post-randomization**	**T3 4 weeks post-randomization**	**T4-T8** **12, 24, 36, 48, and 60 months post randomization**
Target A-HRSI completion window	14 to 0 days before randomization	2 to 7 days after randomization	± 1 weeks	± 4 weeks
Window for accepted forms	14 to 0 days before randomization	2 to 10 days after randomization	21 to 60 days after randomization	-28 / +56 days of target annual visit date

APPENDIX II: PERFORMANCE STATUS SCALES

Karnofsky Performance Scale		ECOG Performance Status Scale	
Percent	**Description**	**Grade**	**Description**
100	Normal, no complaints, no evidence of disease.	0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
90	Able to carry on normal activity; minor signs or symptoms of disease.		
80	Normal activity with effort; some signs or symptoms of disease.	1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
70	Cares for self, unable to carry on normal activity or to do active work.		
60	Requires occasional assistance, but is able to care for most of his/her needs.	2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
50	Requires considerable assistance and frequent medical care.		
40	Disabled, requires special care and assistance.	3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
30	Severely disabled, hospitalization indicated. Death not imminent.		
20	Very sick, hospitalization indicated. Death not imminent.	4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
10	Moribund, fatal processes progressing rapidly.		
0	Dead.	5	Dead.

APPENDIX III: ANAL CYTOLOGY AND HPV SAMPLING

All anal cytology specimens will be examined at the local institution.

The participant should undress so that buttocks are exposed, and either bend over the exam table or lay on their side in left lateral decubitus. The examiner should use one hand to spread the buttocks and expose the anal verge.

Procedure for obtaining anal swab specimens:

For anal cytology and correlative science studies: A non-scored polyester swab moistened in tap water will then be inserted as far as is comfortable into the anus, a minimum of 1-2 inches. If there is difficulty inserting the swab, the participant should also retract their buttocks and the swab reoriented in the canal. With pressure on the swab rotate it firmly in a circular fashion for approximately 20 seconds and slowly remove from the canal. Do not retract the buttocks when the swab is close to the verge to ensure that it is sampled as well. Immediately immerse the swab in a liquid-cytology vial agitating vigorously over 20 to 45 seconds to disperse the cells. The liquid cytology vial will be sent the local cytopathology lab for processing. The first slide will be used for the study. The leftover material from anal swab cytology suspension will be shipped to the ANCHOR Biorepository, processed according to the ANCHOR Biorepository's procedures, and stored for future correlative studies.

For additional correlative science studies: Two additional moistened polyester swabs should be obtained. The second swab will be used for DNA studies, and the third for RNA/protein studies. These two swabs will be processed for storage in the ANCHOR Biorepository according to the instructions in the ANCHOR Study Manual of Procedures (MOP).

All three swabs will be collected in this order at each visit where required. If a local cytology result exists to satisfy protocol requirements at the time of the visit, the first anal swab will be recollected with the second and third swabs, and the first swab sent to the ANCHOR Biorepository without local processing for the cytology result.

Procedures and schedules for shipping monolayer slides, Thinprep solution, and swabs to the ANCHOR Biorepository are outlined in the ANCHOR Study MOP.

Guidance regarding inconclusive cytology results:

Any insufficient or inconclusive cytology at screening must be repeated. Valid results must be available within 12 weeks before randomization.

If a cytology specimen collected after randomization is interpreted as insufficient or inconclusive for any 6-month visit, repeat cytology is required. If an interim visit is planned, the repeat specimen can be collected at that visit, otherwise the participant should be asked to return specifically for sample collection within 3 months. The two additional swabs for correlative science studies will be recollected if the local cytology specimen requires recollection. The residual media from both cytology specimens should be submitted to the ANCHOR Biorepository for correlative studies, denoting which specimen was found to be insufficient or inconclusive.

Guidance regarding discrepant cytology and histology results:

During screening, if the local cytology result is ASC-H or HSIL and no lesions were observed or no biopsy result showed HSIL, the participant will return for a repeat HRA within 3 months.

APPENDIX IV: HIGH RESOLUTION ANOSCOPY (HRA) AND ANAL BIOPSIES

Procedure for performing HRA:

High resolution anoscopy should only be done **after** the specimens for anal cytology and HPV testing are collected. The patient will already be positioned for anal evaluation. A mixture of an anesthetic cream (e.g., 4% lidocaine cream) and water-soluble lubricating jelly should be used as a lubricant. A digital anal rectal exam should then be performed palpating the entire anal canal, distal colon and perianus, noting any masses or areas of induration. The procedure for HRA is as follows:

1. Insert the anoscope, remove obturator, and place a cotton swab wrapped in gauze soaked in 5% acetic acid into anus.
2. Remove the anoscope over the swab and leave swab in place for 1 to 2 minutes.
3. Remove the swab and re-insert the anoscope. Carefully examine the anal canal with a colposcope.
4. Re-apply acetic acid frequently to ensure adequate detection of lesions and verify that all aspects of the Anal Transformation Zone (AnTZ) have been visualized.
5. If acetowhitening is noted, note vascular characteristics, if present.
6. Lugol's solution (iodine) may be used as desired to aid in identifying areas of possible LSIL and HSIL near the squamocolumnar junction.
7. At each visit the clinician will carefully map the location of each lesion and where they did their biopsies, if any. Sites are requested to photograph each lesion at every visit using Second Opinion or other software that allows for easy sharing of images between study sites, for the purposes of training and quality control.
8. Biopsy abnormal appearing areas according to the protocol requirements for the assigned study arm. Local anesthetic (e.g., 1% lidocaine with or without epinephrine or .5% bupivacaine) may be used at the provider's discretion prior to biopsy. Biopsies from different quadrants must go in separate formalin containers and get separate pathologic interpretations.
9. Attain hemostasis with pressure prior to removal of the anoscope or by removing the anoscope. Monsel's solution or silver nitrate should be used **judiciously and only** after all biopsies have been obtained because it can interfere with histologic interpretation. Electrocautery or infrared coagulation should be used judiciously to stop significant bleeding that does not respond to the above measures. It must be documented if used for hemostasis.
10. Apply acetic acid for one minute to perianal area and examine carefully with colposcope.
11. Biopsy any external (perianal) areas per the assigned study arm using a local anesthetic (e.g., 1% lidocaine with or without epinephrine or 0.5% bupivacaine) prior to biopsy.
12. Participants with signs or symptoms consistent with proctitis or sexually transmitted infections other than HPV should be referred for appropriate diagnosis and treatment.

Procedures for Biopsy Review:

All pathology specimens will be reviewed by the designated pathologist on site.

p16 IHC staining is not required on all biopsies diagnosed as HSIL by the local laboratories for the ANCHOR Study. If performed, the immunostained slides are also requested to be sent for Central Pathology review. p16 IHC is useful to adjudicate differential diagnoses on H&E particularly for the following indications:

- To differentiate between the H &E morphologic diagnosis of HSIL (–IN 2 or –IN 3) and a ***mimic of precancer*** (e.g., processes known to be unrelated to neoplastic risk such as immature squamous metaplasia, reparative epithelial changes, tangential cutting). Strong and diffuse block-positive p16 results support a categorization of HSIL. Negative or non–block-positive staining strongly supports a non–HPV-associated pathology.
- To ***clarify a diagnosis of –IN2*** when the lesion is clearly HPV-associated, but on H&E morphologic interpretation the diagnosis of –IN 2 (under the old terminology) is considered. –IN2 is a biologically equivocal lesion falling between the morphologic changes of a productive HPV infection (LSIL) and precancer (HSIL). Strong and diffuse block-positive p16 results support a categorization of HSIL. Negative or non–block-positive staining strongly favors an interpretation of LSIL.

A p16 stain must be obtained if the only area of HSIL among all biopsies taken is AIN 2. At any visit, if other lesions show AIN2-3 or more severe histology, p16 staining is highly recommended for AIN2 biopsies to adjudicate the diagnosis, but is not mandatory. If, after evaluation of screening biopsies, the only remaining focus of HSIL at randomization is AIN2, p16 staining is required for that lesion.

The local pathology lab relied upon for this trial must be willing to send slides and tissue blocks as requested by this study. Stained H&E slides from all biopsies with a result of AIN2 or greater will be sent from participants who are biopsied as part of the screening process and randomized at the baseline visit. If p16 IHC staining is performed, the immunostained slides are also requested to be sent for Central Pathology review. Participants who fail screening due to suspected cancer diagnoses or cancer diagnoses will undergo central pathology review to confirm the diagnosis, if biopsies are available from screening procedures performed by the site. The protocol team may request additional slides undergo central pathology review. Slides will be submitted in real time for any cancer diagnoses, as well as all biopsies collected prior to progression to cancer, and any cases that are suspicious for progression to cancer during local pathology review. Tissue blocks may be requested for correlative studies, for participants who develop cancer, and for matched controls. Blocks will not be exhausted and will be returned to the local pathology lab.

The baseline pathology review will serve to identify the discrepancy in HSIL vs. no HSIL readings, and HSIL vs. LSIL readings between the local pathologist's finding and the central pathology result. If a significant discrepancy is identified at a study site, 100% of the slides will be reviewed from the site from all subsequent biopsies, and additional slides will be required from all other participating sites. Slides will be batched for shipment for central pathology review at least every six months according to the instructions in the ANCHOR Study Manual of Procedures. The pathology slides will be reviewed later at UCSF, and results of the central pathology review will not be available in real time.

Guidance regarding repeat biopsy and inconclusive biopsy results/discordant cytology results:

- After the screening visit, if all biopsies do not show HSIL and the local cytology result shows high grade disease (ASC-H or HSIL), the investigator should bring the participant back for repeat HRA. If the biopsies at screening do not show HSIL and the local cytology result is inconclusive, repeat HRA with biopsy should only be performed if the investigator's clinical impression of the participant's disease is strongly suggestive of HSIL. If the repeat biopsies do not show HSIL, the participant should discontinue screening.
- After randomization, if all biopsies at a 6-month visit do not show HSIL or are inconclusive, and the local cytology result shows high grade disease (ASC-H or HSIL), the investigator should bring the participant back for repeat HRA at 3 months (interim visit, window of ± 4 weeks). If a biopsy is performed at an even-numbered visit for a monitoring arm participant and the biopsy results are discordant, the participant will not be asked to return for repeat biopsy until the next 6-month visit unless there is suspicion for progression to cancer. If at least one biopsy shows HSIL, repeat biopsies of lesions with inconclusive histology may be deferred to the next 6-month visit so long as there is no suspicion of progression to cancer. If the visit window for the next 6-month visit is within 8 weeks of the last biopsy, repeat HRA and biopsies will occur as part of the next 6-month visit.
- The investigator may request that a participant return for repeat biopsy at any time there is suspicion for cancer.

APPENDIX V: RISK FACTOR QUESTIONNAIRE

ANCHOR Visit 1 Questionnaire

Participant ID#: _____ - _____ - _____ Visit#: _____

Date completed: _____/_____/_____

Interviewer's name *(if administered by study staff)*: _____

Background:

Thank you for agreeing to be part of the ANCHOR Study. Before starting the study, we are giving all volunteers this ANCHOR Visit 1 Questionnaire. This questionnaire is a survey that asks questions about your medical history, including medical conditions related to anal HSIL, your sexual history, and whether you smoke or have used drugs. This questionnaire will take about 25 minutes to complete. Please read the information below before you start the questionnaire.

- Your completion of this questionnaire is voluntary. All of your responses will be confidential.
- Some people may find some of the questions embarrassing. It is important that you answer all questions honestly.
- No one will force you to answer any questions if it is too uncomfortable for you. If you do not feel comfortable answering a question, you may leave the question blank and move on to the next one.
- If you feel that you do not have privacy to fill out this questionnaire, talk to a study team member immediately.
- When you are finished completing the questionnaire, place the questionnaire in the envelope provided by the study staff. After you submit the questionnaire, the study team will not be able to see your answers.

1) What was the month and year of your positive HIV test result? (IF MORE THAN 1 POSITIVE TEST, RECORD THE EARLIEST TEST DATE. IF YOU RECALL THE YEAR BUT NOT THE MONTH, ENTER "06" (JUNE) FOR THE MONTH.)

☐☐	☐☐☐☐
Month	Year

2) Have you ever had anal condyloma or warts, also known as venereal warts?

Yes (1)
No (2)

3) Have you ever had anal herpes?

Yes (1)
No (2)

4) Have you ever had anal gonorrhea?

Yes (1)
No (2)

5) Have you ever had syphilis?

Yes (1)
No (2)

6) Have you had chlamydia infection of the anus? Chlamydia infection of the anus is also known as chlamydia proctitis.

Yes (1)
No (2)

7) Have you ever had any of the following conditions?

a. Inflamed hemorrhoids or piles?
Yes (1)
No (2)

b. Diarrhea, 2 or more loose stools per day for at least 2 weeks?
Yes (1)
No (2)

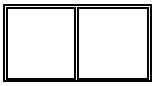
c. A draining or crack-like sore or hole in the anal wall, fissure or fistula?
Yes (1)
No (2)

d. An infection or abscess in your anal area?
Yes (1)
No (2)

e. Swollen or tender lumps around your crotch area?
Yes (1)
No (2)

f. Hardening or narrowing of the anal passage, or stenosis?
Yes (1)
No (2)

8) At what age was your first sexual experience with another person?



Age

9) Have you ever had receptive anal intercourse, that is, anal intercourse in which a man's penis was inserted into your anus?

Yes (1)
No (2) If no, skip to question 12.

10) Using the letter codes below, please tell me the letter that best describes the total number of sexual partners you have had receptive anal intercourse with over your lifetime. _____

Letter Codes for Question 10

1 to 10…….. B
11 to 50……..... C
51 to 100…….. D
101 to 200…… E
201 to 500…… F
501 to 1000…….. G
over 1000……. H

11) Using the letter codes below, please tell me the letter that best describes how often you had receptive anal intercourse during the past 12 months? _____

Letter Codes for Question 11

Never……. A
once/month or less …… B
about once a week ……. C
about 3 or 4 times per week …….. D
about 5 to 10 times per week ……. E
about 11 to 20 times per week …... F
more than 20 times per week ……. G

12) During the past 5 years did you have any objects inserted into your anus, other than a penis, fingers, or a scope by a medical provider?

Yes (1)
No (2)

13) If you replied "yes" to question 12, which letter code below best describes how many times an object was inserted into your anus (other than by a medical provider) during the past 5 years? _____

Letter Codes for Question 13

Less than 10 times total…….. A
10 to 100 times …….. B
101 to 500 times …… C
more than 500 times …….. D

14) Overall, does your sexual partner wear condoms when he inserts his penis into your anus/rectum?

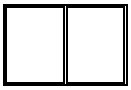
Letter codes for Question 14

Never…….A
Some of the time (<50% of the time)……B
Most of the time (>50% but less than 100% of the time)…….C
All of the time……D
Not applicable……E

15) For women: have you ever had an abnormal cervical Pap smear?

Yes (1)
No (2)

What age?

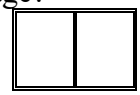


Age

16) For women: have you ever been diagnosed with pre-cancer or high-grade dysplasia of the cervix, vulva, or vagina?

Yes (1)
No (2)

If yes, what age?



Age

17) Have you smoked more than 100 cigarettes in your lifetime?

Yes (1)
No (2) If no, skip to question 23

18) At what age did you first start smoking?

19) At that time, how many cigarettes did you smoke per day?

20) How many years did you smoke this amount of cigarettes?

21) Do you currently smoke?

22) Currently, how many cigarettes/day do you smoke?

23) Have you ever used any of the following substances in the past year? Please indicate how many times you have used the substance using the letter codes below.

Letter Codes for Question 23

Never……..A
Less than 10 times total……..B
10 to 100 times ……..C
101 to 500 times ……D
more than 500 times ……..E

a. Marijuana or hashish _____
b. Cocaine, (blow, snow) or crack cocaine _____
c. Amyl or Butyl nitrite or poppers _____
d. LSD or Acid _____
e. Angel dust, PCP, Ketamine, Special K, or Super K _____
f. Speed, crank, crystal, or amphetamines _____
g. Ecstasy or MDMA _____
h. MDA or Adam _____
i. Ethylchloride _____
j. Molly_____
k. Heroin _____

APPENDIX VI: AMC DATA AND SAFETY MONITORING PLAN

(Version 9.0 • October 6, 2020)

Introduction

The AIDS Malignancy Consortium (AMC) Data and Safety Monitoring Plan (DSMP) outlines the measures employed by the group to monitor the safety of participants and ensure the data validity and integrity for all clinical trials it conducts. This includes methods to: 1) monitor the progress of trials and the safety of participants; 2) comply with regulatory requirements for adverse event (AE) reporting; 3) processes for trial termination or temporary suspension and major modifications; and 4) plans for ensuring data accuracy and protocol compliance. As the AMC conducts protocols of varying research phase, region of conduct (which may include trials conducted in the U.S., international sites, or both), IND sponsor (AMC investigator, CTEP, or industry-sponsored) and clinical data entry system use, this plan addresses broad processes applying to the range of trial designs and requirements. Refer to the individual AMC protocol to identify the applicable study characteristics for the relevant requirements described in this plan.

Monitoring the Progress of Trials and the Safety of Participants

Routine and expedited AE reporting

All AMC protocols that collect safety data adhere to the *National Cancer Institute (NCI), Cancer Therapy Evaluation Program (CTEP) Guidelines: Adverse Event Reporting Requirements* (https://ctep.cancer.gov/protocolDevelopment/adverse_effects.htm), as applicable to the clinical protocol. AEs are to be recorded in the source documents, assessed by a clinical investigator for the AE reporting criteria, and promptly reported in the clinical data entry system as required by each protocol. For AMC trials conducted under a CTEP IND and AMC trials conducted within the U.S., all AEs that meet the NCI's expedited reporting requirements are reported to the NCI via the CTEP Adverse Event Reporting System (CTEP-AERS) web application, either directly or through integration with Medidata Rave where this system is employed for AMC protocols. Use of this system ensures notification to the protocol chair and Investigational Drug Branch (IDB) at CTEP, as required for trials conducted under a CTEP IND, and a uniform expedited reporting and safety review process for AMC domestic trials. The system may also be programmed to include sponsor notification as required for trials with industry support. Alternate process for expedited AE reporting to the AMC protocol chairs and AMC Operations and Data Management Center (ODMC) within the clinical data entry system (AdvantageEDC or Advantage eClinical only) may be defined in the protocol for select trials (international studies and The ANCHOR Study).

All serious adverse events (SAEs) received by the AMC ODMC will be reviewed by the AMC medical monitor at the AMC ODMC for consideration of individual participant safety, safe trial conduct, data reporting quality for AE term selection, and appropriate application of the regulatory criteria for seriousness, expectedness, and relatedness to the investigational therapy. If alternate procedures are followed for SAE review, the process for adequate medical monitoring will be defined in the AMC protocol and the Transfer of Regulatory Obligations (TORO) with the sponsor. AMC medical monitor review includes review of the CTEP-AERS report before CTEP submission for IDB review (if applicable), or review of the SAE report in the data entry system for trials not using CTEP-AERS for expedited reporting. The IND sponsor or its designee will issue the determination as to whether the AE requires IND safety reporting to FDA as a serious and unexpected suspected adverse drug reaction (SUSAR). For protocols not conducted under an IND,

in the event of disagreement between the reporting physician and the AMC medical monitor regarding the relationship of the AE to the investigational agent(s) (i.e., determination of whether the attribution is unrelated or unlikely, or possible, probable, or definite), the AMC medical monitor will provide the final determination of the relationship. IND safety reporting to FDA is performed by CTEP for trials conducted under a CTEP IND; IND safety reporting is performed by the sponsor or sponsor's designee (AMC ODMC or other party defined in the study agreement or TORO) for IND studies sponsored by AMC investigators or industry sponsors.

Expedited reporting to the Institutional Review Board (IRB)

The requirements for IRB review will be identified in the protocol section on ethical and regulatory obligations. All AMC trials initiated before September 1, 2020 and all international sites for all AMC studies are subject to local IRB review; only U.S. sites are subject to the NCI requirement to use a single IRB for protocols initiated on or after September 1, 2020. For trials subject to local IRB review, the site principal investigator is responsible for ensuring that expedited AE reports for its trial participants and any unanticipated problems that affect the local institution only are submitted to the local IRB of the reporting institution, per the local IRB's requirements for such reporting. For studies reviewed by the single IRB, the protocol chair will render a determination as to whether a SAE or other problem constitutes a trial-wide unanticipated problem that requires reporting to that IRB, in accordance with its standards of procedure.

To comply with investigator notification requirements for IND studies under 21 CFR 312.32 and 312.55, IND safety reports from all trials the AMC conducts and reports from external sponsors investigating the same agents are made available to all investigators upon receipt from the sponsor or its designee, either via the password-protected section of the AMC Operations web site (AMC trials subject to local IRB review only) or the CTSU website (U.S. trials subject to single IRB review/CTEP IND agents). The site clinical investigator responsible for the applicable AMC protocol(s) is responsible for reviewing any IND safety reports received and documenting submission to the IRB of record (if required by local policy) within the timeline defined by the Clinical Trials Monitoring Branch (CTMB) audit guidelines.

Procedures for monitoring trial progress and pharmacovigilance

For trials using AdvantageEDC or Advantage eClinical for clinical data entry, the AMC ODMC provides on demand tabular listings of all reported AEs and SAEs on a participant level to the protocol chair and co-chair(s) for review via the password-protected section of the AMC Operations web site, www.AIDScancer.org. For trials using OPEN and Medidata Rave for clinical data collection, data listing will be made available using that system. Summary reports of AEs by frequency and relationship to the investigational agent(s) are provided to all AMC investigators and their staff It is the responsibility of each site to provide trial-specific AE listings to their respective IRB, if required by its policies. For blinded studies, the AE and SAE listings are reviewed and tabulated without treatment assignment.

Accrual summaries for each AMC trial are updated nightly on the password-protected section of the AMC web site. The progress of each AMC trial is reviewed regularly by the protocol chair and also by the appropriate Scientific Working Group (SWG) during scheduled conference calls (monthly SWG calls and as required, protocol-specific monitoring conference calls). Summary accrual, summary AE, and individual SAE reports are provided to SWG leadership and protocol chairs to monitor participant safety during these monthly calls.

The AMC medical monitor reviews listings of all reported AEs on a quarterly basis for assuring compliance with the protocol requirements for AE reporting and the identification of any safety concerns (individual AE or increased frequency/severity of expected AEs) for the agents under investigation. Findings from these reviews are communicated to the protocol chairs and all AMC investigators, and posted to the AMC Operations web site.

Data and Safety Monitoring Board Review (DSMB) review

The AMC has formed an independent Data and Safety Monitoring Board (DSMB) for AMC trials and for the ANCHOR Study. As required by NCI policy, the AMC requires DSMB review for all phase III randomized trials. All other clinical trials that the AMC initiates will be reviewed by the AMC ODMC and AMC Statistical Center during protocol development to issue a recommendation as to whether the study requires DSMB oversight, which will require the approval of the AMC Executive Committee. This determination will be based on the phase of the study, experimental design, risk posed by the investigational approach, extent of data available on the safety of an investigational agent, risk posed by the natural course of the health condition under research, and the categories of vulnerable populations involved. The involvement of a DSMB in reviewing an AMC protocol will be identified in each clinical protocol as approved by CTEP and, as applicable, required by the IRB of record.

Regarding the composition of the AMC DSMB, voting members usually include physicians, statisticians, an ethicist, and a patient advocate. All voting members have no other affiliation to the AMC and are appointed by the AMC Executive Committee with the approval of the OHAM Director. Nonvoting members are the AMC group statistician, the protocol statistician, an AMC ODMC staff member, two representatives (normally a clinician or statistician) from CTEP, and the grant program directors from the NCI Office of HIV and AIDS Malignancy (OHAM).

The DSMB reviews all applicable AMC studies in accordance with the National Cancer Institute's Policy for Data and Safety Monitoring. Confidential reports of all trials under review are prepared by the AMC group statistician with support from the AMC ODMC. A written report containing the current status of each trial monitored, and when appropriate, any toxicity and outcome data, are sent to DSMB members by the AMC ODMC within the timelines specified by the DSMB charter. This report addresses specific toxicity issues and any other concerns about the conduct of the trial, as defined by the protocol plan for DSMB review. The report may contain information for the DSMB to render determinations for participant safety, early trial termination, results reporting, or continuing accrual or follow-up.

The results of each DSMB meeting are summarized in a formal report sent by the DSMB chair to the AMC group chair and AMC ODMC. The DSMB report contains recommendations on whether to close each study reviewed, whether to report the results, and whether to continue accrual or follow-up. A primary recommendation (e.g., continue with no change; recommended or required modification; stop) must be included in the document. The group chair or designee is then responsible for notifying the protocol chair and relevant SWG chair before the recommendations of the DSMB are carried out. In the unlikely event that the protocol chair does not concur with the DSMB, then the OHAM program directors and the NCI division director or designee must be informed of the reason for the disagreement. The protocol chair, relevant SWG chair, group chair, DSMB chair, and NCI division director or designee will be responsible for reaching a mutually acceptable decision about the study. CTEP approval of a protocol amendment will be required prior to any implementation of a change to the study.

Following a DSMB meeting, the DSMB's recommendations are provided to all AMC investigators and staff. It is each site principal investigator's responsibility for conveying this information to its local IRB as relevant for its protocol participation. For trials reviewed by a single IRB, the AMC ODMC will support notification to the IRB as required per its procedures.

Cohort trial reviews not subject to DSMB review

For phase I dose escalation trials, dose escalation (or dose de-escalation) is based on the rules in the protocol and the protocol chair, AMC medical monitor, and protocol statistician determine whether these criteria have been met based on a review of all safety data for the protocol-defined evaluation period. If applicable for phase II trials, stopping the trial for toxicity or efficacy, or suspending enrollment pending observation of responses in a multi-stage phase II trial, is based on meeting criteria stated in the protocol, and the protocol chair, AMC medical monitor, and protocol statistician determine whether these criteria have been met.

Plans for Assuring Compliance with Requirements Regarding AE Reporting

The protocol chair, AMC group chair, and the AMC ODMC share responsibility in assuring that participating investigators comply with applicable regulatory and protocol requirements for AE reporting. The AMC site principal investigator certifies compliance with NCI and FDA requirements for trial conduct by signing the site subaward agreement for the grant and the AMC Adherence Statement for site membership; clinical investigators also certify compliance in completing the protocol signature page for each protocol active at the site, and Form FDA-1572 for CTEP investigator registration, and also for AMC IND studies sponsored by AMC investigators or industry sponsors. Protocol compliance with AE identification, assessment and reporting requirements is assessed by the AMC ODMC using several methods: 1) programmed system checks and messages to instruct the site to complete routine and/or expedited reporting when certain criteria are reported in the clinical data entry system; 2) programmed data reports provided to the protocol chairs that identify reports requiring expedited AE reporting; 3) remote review of data entry or data reports to ensure compliance with protocol and NCI AE reporting requirements; 4) AMC medical monitor review described in the section above; and, 5) routine site audits by reviewing the site's source documentation.

The clinical data entry systems used for AMC studies include the Oncology Patient Enrollment Network, OPEN for enrollment, and Medidata Rave for clinical data entry for enrolled participants; trials activated before September 1, 2020 or that involve only AMC international sites may be reported in AdvantageEDC/Advantage eClinical, a web-based data entry and enrollment system. These data entry systems are programmed to notify the site investigator, protocol chair, AMC medical monitor, and AMC ODMC via email in the event that a site reports an AE that meets expedited reporting criteria to NCI and/or FDA. Additional reporting conditions may be programmed depending on the sponsor reporting requirements of a given protocol (e.g., adverse events of special interest [AESI]). If the site does not follow with an expedited report, the AMC ODMC contacts sites to request compliance with reporting requirements. Additionally, the protocol chair, AMC ODMC, and the AMC medical monitor review reported AEs on a routine basis to identify AEs reported by sites that require expedited reporting. The protocol chair, AMC SWG chairs, AMC group chair, and IND sponsors have general oversight for assuring that routine and expedited adverse reporting requirements are met by the responsible parties.

For studies monitored by CTEP using the Data Mapping Utility (DMU), cumulative protocol- and patient-specific data will be submitted weekly to CTEP electronically via the DMU. For trials

monitored by the NCI's Clinical Data Update System (CDUS), AE information is transmitted electronically to NCI on a quarterly basis. For trials monitored by NCI's Clinical Trials Monitoring Service (CTMS), AE information is transmitted electronically to NCI every two weeks.

Plans for Assuring that any Action Resulting in a Temporary or Permanent Suspension of an NCI-Funded Clinical Trial is Reported to the NCI Grant Program Director Responsible for the Grant

In the event that temporary or permanent suspension of a trial, or major modification to the protocol is under consideration, the protocol chair will convene the AMC ODMC, AMC Statistical Center, and SWG chair by conference call to discuss the options. Suspension actions will also be reviewed by the AMC Executive Committee for program oversight and direct communication of the action with the OHAM program directors. For phase III trials, closure decisions are typically rendered by the AMC DSMB; if the trial in question is under AMC DSMB oversight but rendered by the AMC investigators, the AMC DSMB will be notified of the suspension and the reason. For phase I and II trials, the protocol chair also has the option of asking the DSMB to review the study. The AMC ODMC will inform the CTEP Protocol Information Office (PIO), with copy to OHAM Directors, when studies are temporarily or permanently closed. In the event of major trial modification, CTEP must approve all protocol amendments prior to distributing to the AMC sites.

Plans for Assuring Data Accuracy and Protocol Compliance

All study data for AMC clinical trials are entered directly by AMC clinical site staff into the applicable clinical data entry system for the trial. During data entry, the system performs validation checks on many fields and performs consistency checks between select fields. Range checks are placed on each field to eliminate entry of out-of-range values. Edit check programs are run on the database on a set schedule to identify and resolve inconsistencies between forms or data collected at different points in time. Submitted data entry forms are reviewed for compliance with the protocol and data entry instructions according to the AMC ODMC's standards for data quality processes. AMC ODMC staff routinely interacts with site staff to resolve any data submission problems.

In accordance with NCI guidelines, the AMC ODMC conducts audits at the AMC sites to evaluate compliance with regulatory issues, and to review data for specific cases by checking source documents. These reports are sent to the site principal investigator and to the NCI. In the event that major violations are identified, sites are asked to provide a written corrective and preventative action plan to correct deficiencies. If needed, a repeat site audit is conducted. In the event that a site does not correct deficiencies in a pre-determined time frame, the AMC Executive Committee has the option to implement remedial action(s) for the site. Possible actions include, but are not limited to, suspending enrollment of new patients to AMC trials until deficiencies are corrected; recommending a decrease in funding to the site; and requiring specific training for site investigators or staff members.

APPENDIX VII: PARTICIPANT INSTRUCTIONS FOR APPLYING IMIQUIMOD 5% CREAM

You have been prescribed to receive topical imiquimod 5% cream three times a week at night for 8 weeks. You will receive imiquimod for up to 16 weeks if you are tolerating the treatment. Please follow the directions below for applying the cream you have been given.

The imiquimod dose will be 750 mg of cream per week, administered as one single use sachet (250 mg) applied 3 times per week. You have been given at least 24 sachets with 250 mg of imiquimod for 8 weeks of treatment. If you have perianal HSIL, you will be given extra sachets.

When applying the cream, a latex glove or other protective covering must be used on your hand. Apply the imiquimod cream at night before bed or before your longest period of rest once a day.

You will place half of the contents of the packet onto your right index finger and insert the cream approximately one inch into the anus on the right side, rubbing from side to side as far along the right side as your finger can reach.

Then place the remaining cream on the left index finger, and insert the cream one inch into the anus on the left side, rubbing from side to side as far along the left side as your finger can reach.

You may find it is easier to lie on your side then turn over. Or, you may crouch, or place your right and left leg alternately on the toilet or chair. Use whatever position allows you to reach into the anus.

If perianal HSIL is present, the study clinician will tell you to apply one additional packet of cream using a gloved finger to the perianus. When applying the cream to the perianus, you will place half of the contents of the packet onto your right finger and rub the cream on the right side of your perianus. You will place the remaining cream on the left index finger and rub the cream on the left side of your perianus.

Please speak to the study clinician if you have any side effects that are more than mild. The study clinician will tell you if you should reduce the dose.

If you miss a dose please apply the next dose as scheduled. Do not try to make up for it by adding the contents of more than one sachet at your next dose.

Store the packets of imiquimod at room temperature, away from sunlight.

APPENDIX VIII: PARTICIPANT INSTRUCTIONS FOR APPLYING EFUDEX (5-FLUOROURACIL) 5% CREAM

You have been randomized to receive topical 5-fluorouracil (5-FU) cream for 8 weeks. You will receive 5-FU for up to 16 weeks if you are tolerating the treatment. Please follow the directions below for applying the cream you have been given.

You have been given a 40 gram tube of 5-FU cream. If you have also been given a syringe or applicator, you will join the syringe tip onto the end of the tube. You will squeeze out the cream to the marked line on the applicator (0.5mL of cream). Please continue to do this until you can confidently squeeze the correct volume of cream. If you do not have a syringe or applicator, you may also measure out one inch of cream to apply.

Using gloves or a finger cot, squeeze one half the amount of cream (0.25mL) from the syringe (if using) onto your right index finger.

You will then insert the cream approximately one inch into the anus on the right side, rubbing from side to side as far along the right side as your finger can reach.

Then place the remaining cream (0.25mL) on the left finger, and insert the cream one inch into the anus on the left side, rubbing from side to side as far along the left side as your finger can reach.

You may find it is easier to lie on your side then turn over. Or, you may crouch, or place your right and left leg alternately on the toilet or a chair. Use whatever position allows you to reach into the anus.

Clean any excess cream from the outside of the anus unless you are treating the outside area. The study clinician will let you know if you should treat the outside.

The 5-FU cream will be inserted twice a day (it does not need to be exactly 12 hours apart) for 5 days in a row. You will then stop for 9 days. Each cycle of 5-FU consists of 14 days. You will then repeat the cycle continually for 16 weeks, or 8 cycles.

If you miss a dose please apply the next dose as scheduled. Do not try to make up for it by using extra 5-FU cream at your next dose.

Please speak to the study clinician if you have any side effects that are more than mild. The study clinician will tell you if you should reduce the dose.

APPENDIX IX: REGULATORY STATUS OF INVESTIGATIONAL AGENTS

This appendix serves to outline the regulatory status of the topical drugs and medical devices that will be used in the treatment of anal high grade squamous intraepithelial lesions for the ANCHOR Study: Anal Cancer/HSIL Outcomes Research Study. Questions pertaining to this justification for the regulatory status of these agents may be directed to the ANCHOR Study Chair, Dr. Joel Palefsky, and the ANCHOR Data Management Center via email at anchordmc@emmes.com.

IND Exempt Topical Treatments

Topical imiquimod 5% and topical 5-fluorouracil 5% meet all of conditions of exemption from the requirement for an Investigational New Drug (IND) application at 21 CFR 312.2(b). This investigation will not be used to support a change in the labeling, marketing authorization, advertising, or the indications for use for any of the study agents. ANCHOR Study investigators will use a supply of these drugs that are sourced as legally marketed in the U.S. Participants will not be charged for any of the agents. The protocol will be conducted at participating U.S. AMC member sites in compliance with the requirements for informed consent and institutional review set forth in 21 CFR 50 and 56, respectively. The investigation will not involve an exception from informed consent (21 CFR 50.24).

By review of an IND application for a related AMC trial of topical imiquimod and 5-fluorouracil creams, the Food and Drug Administration (FDA) determined that these agents are exempt from the requirement for an IND when used in the treatment of anal HSIL in persons with HIV infection. (Protocol AMC-088, PI: Dr. Timothy Wilkin, IND 122,224 for Efudex and Zyclara 2.5%). The sponsor-investigator believes that the exemption status of the agents used in protocol AMC-088 apply to the ANCHOR Study as the agents will be used for the same indication for use, patient population, and route. The use of the 5% imiquimod cream in this trial as compared to the 2.5% strength imiquimod cream used in AMC-088 is addressed by a less frequent dosing schedule in the ANCHOR Study and does not significantly increase the risks or decrease the acceptability of the risks associated with the use of imiquimod. The FDA has concurred with this determination of IND exemption for these agents in discussions related to this trial.

IDE Exempt Study Devices

By review of this investigation, FDA has confirmed that the classes of medical devices used in the ANCHOR Study, described in protocol Section 7.3.3, are exempt from the requirement for an Investigational Device Exemption (IDE). A copy of FDA's exemption letter is available upon request.

As a Federally-funded cancer research initiative, the ANCHOR Study protocol chairs and the collaborating consortium, the AMC, have no intention to demonstrate safety and effectiveness of the study devices for clearance or approval in the prevention of cancer, nor will the results of this study be submitted to the FDA to support a change in the labeling or the marketing of the study devices.

Redfield infrared coagulator

FDA regulates the Redfield Infrared Coagulator as an endoscopic electrosurgical unit (21 CFR 876.4300) intended for use in electrosurgical procedures. It is a class II medical device (intermediate risk) that FDA has cleared for use in the treatment of hemorrhoids, tattoo removal, chronic rhinitis, genital condyloma (condyloma acuminata), and general warts. It is

contraindicated in the use of invasive cancer, vulvar or vaginal intraepithelial neoplasia, and in patients with severe photosensitivity reactions.

Hyfrecator and electrosurgical devices

Electrosurgical devices are class II medical devices that are regulated as electrosurgical cutting and coagulation devices intended to remove tissue and control bleeding by use of high-frequency electrical current (21 CFR 878.4400). Specific indications for use vary by device. These devices are cleared for use in general and plastic surgery, in applications including dermatology, gynecology, and proctology, including the removal of hemorrhoids, condyloma, Bowen's disease, basal cell carcinoma, and squamous cell carcinoma.

Laser surgical devices

The generic type of medical devices regulated under 21 CFR 878.4810 includes laser surgical instruments for use in general and plastic surgery and in dermatology, which are regulated as class II medical devices. This classification includes devices intended to cut, destroy, or remove tissue by light energy emitted by carbon dioxide, or argon lasers intended to destroy or coagulate tissue in dermatology by light energy emitted by argon. Laser surgical devices in this classification are indicated for use to cut, destroy, remove, or coagulate tissue, generally soft tissue, for general surgical purposes in medical specialties such as general and plastic surgery, dermatology/aesthetic surgery, podiatry, otolaryngology, gynecology, neurosurgery, orthopedics (soft tissue), dental and oral surgery, and dentistry. Legally marketed carbon-dioxide lasers that are indicated for use in general surgical procedures, including the excision or ablation of tissue, are permitted for use on this trial.

APPENDIX X: ANCHOR HEALTH-RELATED SYMPTOM INDEX

Below is a list of statements that other people diagnosed with anal HSIL (pre-cancer lesion in the anus) and treated or actively monitored have said are important. Please check the box to select your answer based on your experiences in **the past 7 days**.

PHYSICAL SYMPTOMS	**Check if Not Applicable**	**Not at all 0**	**A little bit 1**	**Somewhat 2**	**Quite a bit 3**	**Very much 4**
1. I have anal pain						
2. I have pain other than anal pain						
3. I have pain during bowel movements						
4. I have constipation						
5. I have bleeding from the anus						
6. I have itching in or around the anus						
7. I have discharge (wetness) in my anal area						
8. I have burning sensations in the anal area						
9. I have urgency for bowel movements						
PHYSICAL IMPACTS	**Check if Not Applicable**	**Not at all 0**	**A little bit 1**	**Somewhat 2**	**Quite a bit 3**	**Very much 4**
10. I have problems with my physical ability to move around						
11. I have problems with sitting						
12. I have problems completing daily household chores (e.g., cleaning, cooking, laundry, house maintenance)						
13. I have problems taking care of myself (e.g., bathing, dressing, shaving)						
14. I have problems participating in leisure activities (e.g., watching television, relaxing)						

15. I have problems participating in social activities (e.g., going out to eat, visiting friends)						
16. I have problems with work productivity						
PSYCHOLOGICAL SYMPTOMS	**Check if Not Applicable**	**Not at all 0**	**A little bit 1**	**Somewhat 2**	**Quite a bit 3**	**Very much 4**
17. I have difficulty concentrating						
18. I have a decreased enjoyment of anal sexual activity						
19. I have a decreased enjoyment of forms of sexual activity other than anal sexual activity						
20. I have a decreased desire for anal sexual activity						
21. I have a decreased desire for forms of sexual activity other than anal sexual activity						
22. I am worried about my condition getting worse						
23. I have anxiety						
24. I have depression						
25. I have problems with my intimate relationships						

APPENDIX XI: ANCHOR HEALTH-RELATED SYMPTOM INDEX – SPANISH LANGUAGE VERSION
ÍNDICE DE SÍNTOMAS RELACIONADOS CON LA SALUD ANCHOR

A continuación presentamos una lista de afirmaciones que otras personas diagnosticadas con HSIL (lesión precancerosa en el ano) y que han sido tratadas o monitoreadas activamente han dicho que son importantes. Por favor seleccione la casilla con su respuesta basándose en sus experiencias en **los últimos 7 días**.

SÍNTOMAS FÍSICOS	**Escoja si no aplica**	**Nada 0**	**Un poco 1**	**Algo 2**	**Bastante 3**	**Muchísimo 4**
1. Tengo dolor anal						
2. Tengo dolor pero no en el área anal						
3. Tengo dolor cuando entro al baño a defecar						
4. Tengo estreñimiento						
5. Tengo sangrado del ano						
6. Tengo picazón en el ano o alrededor del ano						
7. Tengo una secreción (mojado) en el área anal						
8. Tengo sensación de ardor en el área anal						
9. Siento urgencia de entrar al baño a defecar						
IMPACTOS FÍSICOS	**Escoja si no aplica**	**Nada 0**	**Un poco 1**	**Algo 2**	**Bastante 3**	**Muchísimo 4**
10. Tengo problemas con mi capacidad física para moverme						
11. Tengo problemas para sentarme						
12. Tengo problemas manteniendo la casa (por ej., limpiar, cocinar, preparar el café, administrar la casa)						
13. Problemas con mi arreglo personal (por ej., bañándome, vistiéndome, afeitándome)						
14. Tengo problemas participando en actividades de relajo (por ej., mirar televisión, relajarme)						

15. Tengo problemas participando en actividades sociales (por ej., salir a comer, visitar amigos)						
16. Tengo problemas siendo productivo en el trabajo debido a mis síntomas						
SÍNTOMAS PSICOLÓGICOS	**Escoja si no aplica**	**Nada 0**	**Un poco 1**	**Algo 2**	**Bastante 3**	**Muchísimo 4**
17. Tengo dificultad concentrándome						
18. Me ha disminuido el disfrute de la actividad sexual anal						
19. Me ha disminuido el disfrute de cualquier forma de actividad sexual diferente a la actividad sexual anal						
20. Me ha disminuido el deseo por la actividad sexual anal						
21. Me ha disminuido el deseo por formas de actividad sexual diferentes a la actividad sexual anal						
22. Me preocupa que mi enfermedad empeore						
23. Tengo ansiedad						
24. Tengo depresión						
25. Tengo problemas con mis relaciones íntimas						

APPENDIX XII: PATIENT VERSION OF THE EASTERN COOPERATIVE GROUP PERFORMANCE STATUS

Questionnaire only required for A-HRSI Scale Responsiveness Substudy (completed February 2020)

Toxicity/Grade	Original Clinician Language	Patient Language Adaptation
ECOG Performance Status		
0	Fully active, able to carry out all predisease performance without restriction	I am fully active and able to carry out activities the same as before my cancer diagnosis, without any restrictions
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, eg, light house work, office work	I have difficulty with physically strenuous activity but I am able to walk and carry out work that is light or based in one location; such as light house-work or office-work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities; up and about more than 50% of waking hours	I can walk and take care of myself, but I am not able to carry out work activities; I am up and about more than half the hours that I am awake
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours	I am capable only of limited self-care and spend more than half of the hours that I am aware in bed or in a chair
4	Completely disabled. Cannot carry on any selfcare; totally confined to bed or chair	I am completely disabled, cannot carry on any self-care, and am totally confined to a bed or chair

APPENDIX XIII: PARTICIPANT GLOBAL IMPRESSION OF CHANGE (PGIC)

Questionnaire only required for A-HRSI Substudy (completed February 2020)

	Very much worse (-3)	Moderately worse (-2)	A little worse (-1)	No change (0)	A little better (+1)	Moderately better (+2)	Very much better (+3)
Since you last completed a questionnaire, how would you rate your OVERALL QUALITY OF LIFE?							