SUMMARY OF CHANGES

A Randomized, Phase III Study of Intra-anal Imiquimod 2.5% vs. Topical 5-fluorouracil 5% vs. Observation for the Treatment of High-grade Anal Squamous Intraepithelial Lesions in HIV-positive Men and Women

Version 8.0

NCI Protocol #: AMC-088
Local Protocol #: AMC-088

NCI Version Date: 02AUG2023
Protocol Date: 02AUG2023

I. Scientific and Substantive Changes:

#	Section	Comments
1.	Synopsis 1.2 10.1 10.3 10.6.2	The target accrual was updated from 118 participants to 88 participants due to feasibility concerns with the rate of accrual. Sample size estimation was updated to include the following information: • Due to the slow accrual rate, the sample size was reduced, and the revised sample size was decided by the expected accrual close date (01 July 2023). Thirty-five participants per arm (or 70 in total for observation and 5FU arms) would be the revised sample size. Table 10-A was updated to reflect the updated sample size, H_0 (Futility) Bound Z (P-value), H_1 (Efficacy) Bound Z (P-value) and Overall β (1-power) Spent

II. Administrative and Editorial Changes:

#	Section	Comments
2.	Global	The protocol was updated from version 7.0 to version 8.0 and the date was updated from 28JAN2022 to 02AUG2023.
3.	Global	The AMC Operations & Data Management Center email address was updated to amc-088@emmes.com in roster and throughout the document.
4.	Global	Minor grammatical corrections were applied throughout the document.
5.	Global	Hyperlinks were updated throughout the document.
6.	Protocol Title page Protocol	The Protocol Co-chair was updated to replace Naomi Jay with Cristina Brickman.

#	Section	Comments
	Roster 10.6	
7.	Protocol Roster	Second protocol statistician, Mayuri Jain, added to roster.
8.	Protocol Roster APPENDIX VI APPENDIX VII	The positions of AMC Biorepository Director and AMC Laboratory Resources Committee Chair were combined and updated to AMC Laboratory Director. Sylvia Silver (AMC Biorepository Director) was replaced with Jeff Bethony (AMC Laboratory Director).
9.	List of Abbreviations	The List of Abbreviations table was updated.
10.	10.6	The AMC statistician was updated to replace Lee with Kwon.
11.	References	The reference list was updated to match the references in the protocol.

AIDS MALIGNANCY CONSORTIUM

AMC PROTOCOL #088
A Randomized, Phase III Study of Intra-anal Imiquimod 2.5% vs. Topical 5-fluorouracil 5% vs. Observation for the Treatment of High-grade Anal Squamous Intraepithelial Lesions in HIV-positive Men and Women

A Multi-Center Trial of the AIDS Malignancy Consortium (AMC)

Sponsored by:	National Cancer Institute Office of HIV and AIDS Malignancies
NCT Registration Number:	NCT02059499
IND Status	Exempt
Commercially Available Agents:	5-fluorouracil 5% (NSC #19893) Imiquimod 2.5% (NSC #741062)
Protocol Chair:	Timothy Wilkin, MD MPH
Protocol Co-Chair:	Cristina Brickman, MD MSCE

Version 8.0, 02AUG2023
NCI Version Date: 02AUG2023

AMC PROTOCOL SIGNATURE PAGE

I, _____, Principal Investigator at site ____, agree to conduct and follow this protocol: **AMC Protocol #088: A Randomized, Phase III Study of Intra-anal imiquimod 2.5% vs. Topical 5-fluorouracil 5% vs. Observation for the Treatment of High-grade Anal Squamous Intraepithelial Lesions in HIV-positive Men and Women, (Version 8.0, 02AUG2023),** as written according to AMC, NCI, and FDA guidelines. I understand that no deviations from the protocol eligibility criteria or waivers for protocol deviations will be permitted.

_____ _____

Signature Date (DDMMMYYYY)

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SITES PARTICIPATING IN THE STUDY

This protocol will be open to all interested AMC sites approved by the HPV Working Group. The approval will be based on the certification to perform high resolution anoscopy (HRA).

PROTOCOL ROSTER

AMC Protocol #088

A Randomized, Phase III Study of Intra-anal Imiquimod 2.5% vs. Topical 5-fluorouracil 5% vs. Observation for the Treatment of High-grade Anal Squamous Intraepithelial Lesions in HIV-positive Men and Women

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PROTOCOL SYNOPSIS

Title:	A Randomized, Phase III Study of Intra-anal Imiquimod 2.5% vs. Topical 5-fluorouracil 5% vs. Observation for the Treatment of High-grade Anal Squamous Intraepithelial Lesions (HSIL) in HIV-positive Men and Women
Phase of Study:	Phase III
Study Design:	Prospective, randomized, three-arm, open-label study to evaluate the complete response rate of intra-anal high-grade squamous intraepithelial lesions (HSIL) treated with imiquimod 2.5% or topical 5-fluorouracil 5% (5FU) as compared to spontaneous regression in human immunodeficiency virus (HIV)-infected participants. As of protocol version 5.0, the imiquimod 2.5% arm was dropped because of product unavailability. At randomization, participants will be randomized to intra-anal topical 5FU or observation. Participants will be followed with anal cytology and anoscopy without biopsy through 16 weeks of treatment or observation. At Week 20 (four weeks after completing randomized treatment), high-resolution anoscopy (HRA) with biopsies and cytology will be performed for the primary endpoint. Participants originally assigned to the observation arm having anal HSIL at Week 20 will be offered 5FU for 16 weeks (Weeks 24-40). Participants originally assigned 5FU with anal HSIL at Week 20 will be referred for treatment off study according to the local standard of care. All participants will be seen at Week 44 for HRA with biopsies of visible lesions.
Accrual Target:	88 participants
Population:	HIV-infected participants with biopsy-proven high-grade anal intraepithelial lesions (AIN; AIN2 with positive p16 stain, AIN2-3, or AIN3).
Regimen:	• Arm A: Imiquimod 2.5% cream applied to the intra-anus daily for 16 weeks. Perianal disease will also be treated if present. Closed as of protocol version 5.0. • Arm B: Topical 5FU cream to the intra-anus twice daily for five days followed by nine days off. This cycle is repeated eight times over 16 weeks. Perianal disease will also be treated if present. • Arm C: No treatment is given for 16 weeks.
Duration of Participation:	44 weeks

Primary Objective:	Complete response is defined as no anal biopsies with HSIL and cytology without HSIL at Week 20.
Secondary Objectives:	• To assess the efficacy of intra-anal topical 5FU for treatment of anal HSIL compared to observation only. • To describe the efficacy of intra-anal imiquimod 2.5% for treatment of anal HSIL compared to observation only. • To assess the safety and tolerability of intra-anal imiquimod 2.5% and topical 5FU. • To compare the efficacy of intra-anal imiquimod 2.5% and topical 5FU. • To assess for partial response of intra-anal imiquimod 2.5% or topical 5FU as compared to observation only. • To evaluate the effect of intra-anal imiquimod 2.5% and topical 5FU on human papillomavirus (HPV) persistence. • To evaluate anal HSIL outcomes at Week 44. • To evaluate the effect of behavioral patterns including tobacco smoking and sexual activity on treatment efficacy, tolerability and HPV.

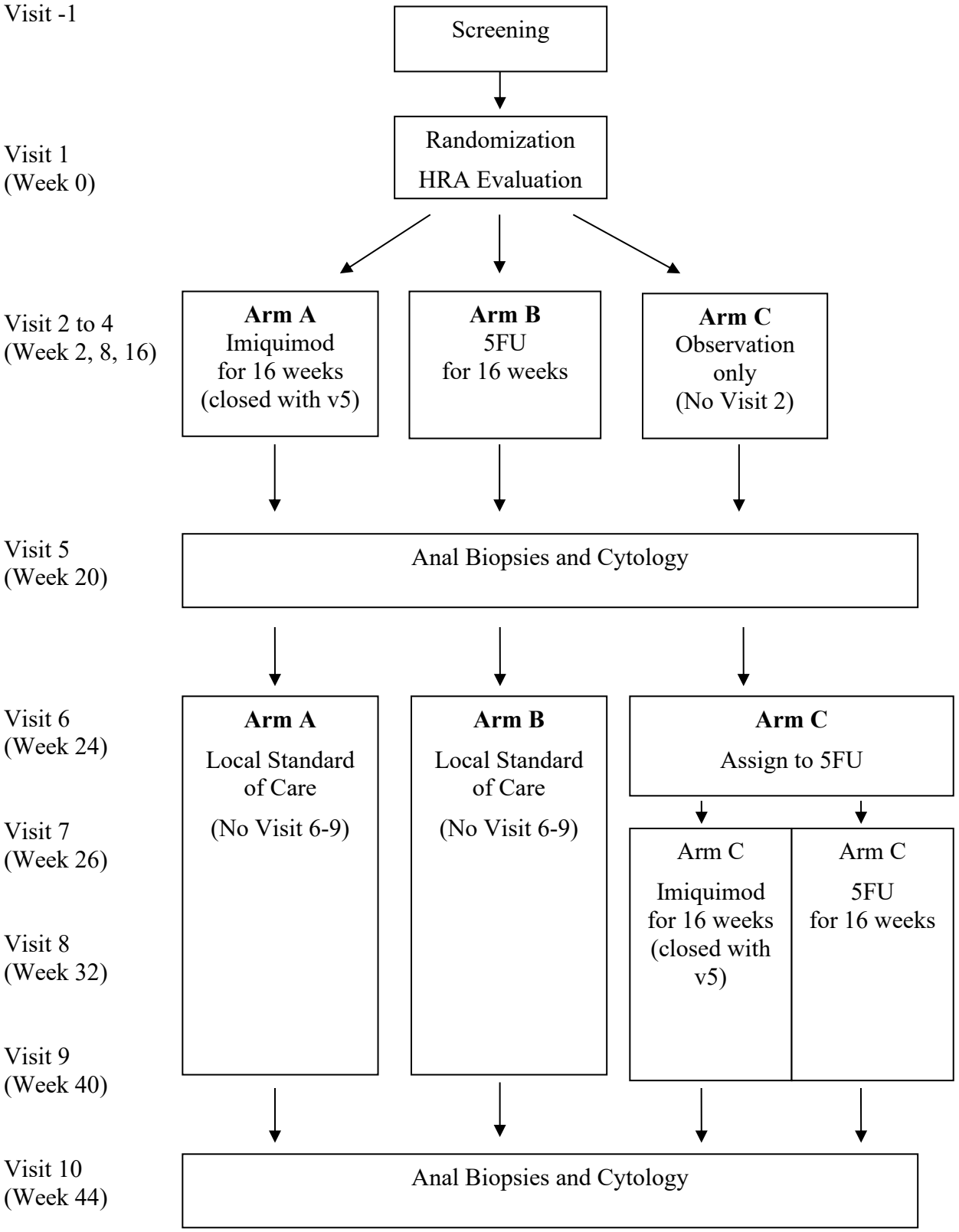
LIST OF ABBREVIATIONS

5FU	5-fluorouracil 5%
ACD	Acid citrate dextrose
ACSR	AIDS and Cancer Specimen Resource
AE	Adverse event
AIDS	Acquired immunodeficiency syndrome
AIN	Anal intraepithelial neoplasia
AMC	AIDS Malignancy Consortium
AML	Acute myelocytic leukemia
ANC	Absolute neutrophil count
ANCHOR	Anal Cancer HSIL Outcomes Research
ART	Antiretroviral therapy
ASCCP	American Society for Colposcopy and Cervical Pathology
Beta-HCG	Beta-human chorionic gonadotropin
BID	Twice daily (*bis in die*)
CBC	Complete blood count
CDC	Centers for Disease Control and Prevention
CDUS	Clinical Data Update System
CIN	Cervical intraepithelial neoplasia
CIP	Cancer Imaging Program
CR	Complete response
CRF	Case report form
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
CTEP-AERS	Cancer Therapy Evaluation Program Adverse Event Reporting System
DARF	Drug accountability record form
DHHS	Department of Health and Human Services
DMU	Data mapping utility
DNA	Deoxyribonucleic acid
DSMB	Data and Safety Monitoring Board
ECOG	Eastern Cooperative Oncology Group

EGW	External genital warts
FDA	Food and Drug Administration
FTP	File transfer protocol
GEE	Generalized estimating equations
H&E	Hematoxylin and eosin
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
HRA	High resolution anoscopy
HSIL	High-grade squamous intraepithelial lesions
IDB	Investigational Drug Branch
IEC	Institutional ethics committee
IL	Interleukin
IME	Important medical event
IRB	Institutional review board
IRC	Infrared coagulation
IND	Investigational new drug
ITT	Intent-to-treat
LEEP	Loop electroexcision procedure
LSIL	Low grade squamous intraepithelial lesions
KPS	Karnofsky Performance Score
LTFU	Lost to follow-up
MDS	Myelodysplastic syndrome
MOP	Manual of Procedures
MSM	Men who have sex with men
NCI	National Cancer Institute
NSAID	Non-steroidal anti-inflammatory drug
ODMC	Operations and data management center
OHAM	Office of HIV and AIDS Malignancy
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
PET	Position emission tomography

PHI	Protected health information
PIN	Penile intraepithelial lesions
PR	Partial response
PrEP	Pre-exposure prophylaxis
REB	Research ethics board
RNA	Ribonucleic acid
SAE	Serious adverse event
SCC	Squamous cell carcinoma
SIL	Squamous intraepithelial lesion
SOP	Standard Operating Procedure
SPECT	Single-photon emission computerized tomography
SWG	Scientific Working Group
TNF	Tumor necrosis factor
TORO	Transfer of Regulatory Obligations
UCSF	University of California San Francisco
VIN	Vulvar intraepithelial lesions
5FU	5% 5-fluorouracil cream

STUDY SCHEMA



1.0 INTRODUCTION

There are limited therapeutic options for treatment of anal high-grade squamous intraepithelial lesions (HSIL), particularly intra-anal HSIL. Topical therapies including 5-fluorouracil 5% (5FU; Efudex) and imiquimod 5% (Aldara) are frequently reported as case studies but there are few controlled studies and these have widely varying results. Results vary in part due to: 1) differences in treatment regimens; 2) differences in treatment site, e.g., perianal versus intra-anal, or both; 3) differences in inclusion criteria with some studies reporting on treatment for both low-grade squamous intraepithelial lesions (LSIL) and HSIL as a single group and others reporting results for HSIL separately; and, 4) differences in treatment populations, e.g., immunocompetent vs. immunocompromised, or both. Study methods also differ with regards to the amount of disease treated, use of high resolution anoscopy (HRA) and histology for determination of efficacy. There are no data available on daily application of imiquimod 2.5% (Zyclara) for treatment of anal HSIL. This multisite, randomized study seeks to determine the efficacy of treatment using 5-fluorouracil 5% compared to observation in human immunodeficiency virus (HIV)-infected individuals with HRA-guided biopsy-proven intra-anal HSIL. Given the lack of drug availability and resulting arm closure, the assessment of the efficacy of treatment using imiquimod 2.5% compared to observation will be exploratory.

1.1 Rationale

Human papilloma virus (HPV)-associated cancers of the anus in HIV-infected individuals are increased at rates that are estimated to be up to 128 times greater than the general population, particularly in men who have sex with men (MSM) (1). The anal cancer precursor lesion, anal HSIL, also disproportionately affects HIV-infected individuals and is found in up to 50% of HIV-infected men and 25% of HIV-infected women (2-4). Anal HSIL can be difficult to treat particularly in HIV-infected individuals who frequently present with multifocal and extensive disease. Recurrences are common post-treatment and vary widely, depending on treatment and length of follow-up, from 22%-79% in HIV-infected individuals (5-8).

There are limited therapeutic options for the treatment of anal HSIL. Infrared coagulation (IRC), an in-office ablative therapy, is an effective treatment but often requires multiple treatments for multifocal lesions and has potential post-procedure complications including pain, bleeding, or abscess formation. While IRC can eliminate a given lesion 64-81% of the time, up to 70% of patients may develop anal HSIL at other untreated sites (7, 9-11). Ablation may be inappropriate for extensive circumferential disease, which is a common presentation, and is contra-indicated in patients on anticoagulant therapies or with thrombocytopenia. New non-invasive treatment options for anal HSIL are needed.

A self-applied topical compound may be better tolerated for treating large areas of disease compared with ablative therapies; a partial response where the disease burden is reduced by a topical treatment may then allow for a less involved ablative therapy. There are no approved topical therapies for anal HSIL. Based on similarities between the pathophysiology of other genital squamous cell intraepithelial lesions to anal HSIL, both imiquimod 2.5% and 5FU have been used for treatment of perianal and intra-anal HSIL, but their usage is considered off-label and they have only been studied sporadically in clinical trials. Imiquimod 5% cream was first approved by the U.S. Food and Drug

Administration in 1997 for topical treatment of external genital and perianal warts. In 2004 it received approval for treatment of clinically superficial basal cell carcinoma and actinic keratosis. Imiquimod 2.5% was approved in 2009. Topical 5-fluorouracil in concentrations of 2% to 5% has been used for more than 50 years in the treatment of vulvar and vaginal high-grade intraepithelial neoplasia and skin pre-cancers. Studies are needed to evaluate any systemic toxicity arising from intra-anal use.

The Anal Cancer HSIL Outcomes Research (ANCHOR) trial is a large, randomized clinical trial that enrolled adults living with HIV with anal HSIL detected on histology. Participants were randomized to treatment of anal HSIL or active monitoring without treatment. The trial followed participants at least every six months. 4,459 adults were randomized. Results indicated that treatment of anal HSIL reduced the risk of anal cancer by 57% (95% CI 6% - 80%). The rate of cancer in the active monitoring group was about 402/100,000 person years (about two people out of 1000 were predicted to develop cancer over the 6-month observation period). The rate of cancer in the treatment group was about 173/100,000 person-years (about one person out of 1000 was predicted to develop cancer over the 6-month period of randomized therapy).

1.1.1 Background: topical 5-fluorouracil 5%

Topical treatment with 5-fluorouracil was first reported in 1962 for treatment of skin cancers following a report that systemic 5-fluorouracil induced regression of keratosis (12). It is a pyrimidine analog made by fluorination of uracil on position 5 of the pyrimidine ring. Its principal mechanism of action is to inhibit deoxyribonucleic acid (DNA) synthesis by blocking the conversion of uracil deoxyribonucleotide to thymine deoxyribonucleotide. It creates a thymine deficiency resulting in a DNA deficiency, thus leading to disordered function resulting in the inability of cells to replicate. A secondary effect of 5-fluorouracil is direct interference with ribonucleic acid (RNA) synthesis and function, inhibiting the incorporation of uracil into RNA by competing with uracil-active enzymes. Its most marked effect is on rapidly proliferating tissues. It is commercially available as 1, 2, and 5 percent fluorouracil topical cream or gel. The most commonly used concentration is 5%. It causes erythema and edema followed by erosion, ulceration, and necrosis when applied topically to a neoplasm. It has been shown to preferentially treat neoplasms with only an inflammatory response on adjacent healthy skin.

Topical 5-fluorouracil has been used since the 1970s to treat female lower genital track disease associated with HPV-related infections and has been well documented (13-15). There are limited data and very few randomized placebo-controlled trials evaluating 5-fluorouracil for genital warts; however, a Cochrane review suggests that in spite of the paucity and heterogeneity of the literature there is evidence for a therapeutic effect against genital warts (16) Topical 5FU has been used for treatment of vulvar intraepithelial neoplasia (41% improvement), vaginal intraepithelial neoplasia (85% improvement), and cervical intraepithelial neoplasia (66% improvement). A randomized study evaluated 5-fluorouracil 5% as prophylactic therapy to prevent recurrence of high-grade cervical intraepithelial neoplasia (CIN) following loop electroexcision procedure (LEEP) in HIV-infected women (14). Recurrence of CIN in the 5FU group was 28% compared to 47% in

the observation group.

There are limited reports of 5FU specifically for treatment of anal lesions. In one study evaluating treatment of patients with perianal HSIL, 7 of 8 patients treated with topical 5FU cream applied twice weekly for 16 weeks had no evidence of Bowen's disease on follow-up biopsies at 12 months post-treatment (17). An open-label study has been reported in 46 patients; multifocal lesions were seen in 76% and 74% had anal HSIL. All were HIV-positive. In this study, 1 g of 5-fluorouracil 5% was inserted using an applicator twice weekly at night for 16 weeks. Complete responses were seen in 12 of 34 patients with anal HSIL and partial responses with a decrease in HSIL to LSIL were seen in an additional eight subjects. Moderate to severe side effects were reported in 48% (18). Results were recently reported in a study of 11 patients using a slightly different schedule in which approximately 0.25 g of 5FU was placed intra-anally at bedtime for a median treatment time of 20 weeks. One patient discontinued therapy due to side effects and 73% of patients experienced some amount of irritation. Six patients had improvement in their disease including one with extensive condyloma who had no lesions on follow-up HRA (19). Jay et al. (20) described the results of a trial from the University of California San Francisco (UCSF) Anal Neoplasia Clinic using intra-anal 5FU twice daily for five days, followed by a nine-day rest period. The cycle was repeated four times and patients re-evaluated. Twenty patients with extensive anal HSIL involving more than 75% of the circumference were evaluable. Complete histologic regression was seen in three, no response in one, and 16 had a significant decrease in volume of disease to 25-50% that allowed in-office ablation using infrared coagulation. Subjects experienced pain, ulceration, bleeding, and exacerbation of herpes simplex. However, only two of the entire group of 27 discontinued treatment because of side effects, and four were considered non-compliant and not evaluable because they completed less than two cycles (20).

While these results are difficult to compare due to differences in treatment protocols, all the studies suggest that anal HSIL may resolve or improve with topical 5FU therapy.

1.1.2 Background: imiquimod 2.5%

Imiquimod has been used for treatment of external genital warts since 1997. It is an imidazoquinoline amine, which is a topical synthetic compound that exhibits antiviral activity by up-regulating the immune response. The compound initially binds to cell surface receptors resulting in secretion of a variety of inflammatory mediators including interferon alpha, tumor necrosis factor (TNF)-alpha, interleukin (IL)-12, IL-1, 6, 8, and 10. These cytokines then activate the cell-mediated immune response and in turn the release of interferon-gamma, responsible for activating virus-infected cell killing cytotoxic T-cells. Imiquimod has no direct antiviral activity or direct effect on T-cells but is a strong inducer of Th-1 type cytokines resulting in recruitment of dense CD4+ infiltrates, which is an important mechanism for HPV clearance and one that is reduced in HIV-infected populations.

Off-label use of imiquimod is common in both skin and mucosal disease (21, 22)

with extensive case reports noted in the medical literature. Treatment with imiquimod has been reported for treatment of infectious diseases including molluscum contagiosum, genital herpes, and leishmaniasis (23-25) Finally, imiquimod has been used for treatment of anogenital intraepithelial neoplasias including vulvar intraepithelial neoplasias (VIN) (26, 27), penile intraepithelial neoplasia (PIN) (28, 29), and anal intraepithelial neoplasia (AIN) (30).

There are relatively few randomized studies of imiquimod for perianal or anal disease. A randomized, controlled trial of 100 patients assigned to imiquimod 5% cream or placebo for 16 weeks showed minimal total clearance of warts in the intent-to-treat analysis (11% drug vs. 6% placebo.) However, 47% of patients in the imiquimod group reported greater than 50% reduction in total wart area vs. 20% in the placebo arm (p=.026). Response rates to imiquimod for genital warts are better in HIV-seronegative patients compared to HIV-infected patients, with response rates of 62% and 31%, respectively (31).

The role of imiquimod in clearing anal HPV infection in patients with anogenital condyloma was investigated in a single-arm study involving 239 HIV-infected and -uninfected men and women. At baseline, 95% had low risk HPV, 60% had high risk HPV, and 56% had both. Condylomata were treated with imiquimod 5% cream for external warts and suppositories for intra-anal disease, three times a week for 26 weeks. At 12 weeks, 30% of subjects with high-risk HPV had cleared their infection as did 66% of subjects with low-risk HPV (32).

There are limited reports using imiquimod for treatment of anal HSIL in cohort studies and case reports that demonstrate efficacy (33). In one study, 14 of 19 (74%) HIV-positive MSM had complete regression of SIL after treatment with imiquimod 5%, and five developed recurrent HSIL. A decrease in HPV types and HPV viral load was also seen following treatment (32). The results from a randomized, double-blind, placebo-controlled study of imiquimod 5% for anal HSIL were reported in 53 patients. In the treatment arm, four resolved and eight downgraded to LSIL versus only one of 25 in the placebo arm that had a spontaneous regression. Patients were treated with open-label imiquimod 5% after completing the study and when grouped together 29 of 47 (61%) had sustained regression of HSIL after treatment with imiquimod 5%. Interestingly, patients were instructed to use only half a packet applied no more than 2 cm into the canal, they were treated for four months and the dosage reduced if significant symptoms developed (34).

Zyclara is 2.5% (or 3.75%) imiquimod cream. It has a Food and Drug Administration (FDA) indication for treatment of actinic keratosis in adults and for treatment of external genital warts (EGW) (Zyclara package insert; http://pi.medicis.us/zyclara.pdf). In two double-blind, randomized, placebo-controlled clinical studies, 601 subjects with EGW were treated with 2.5% imiquimod cream or a matching placebo cream. The baseline wart count ranged from two to 48 warts. Most subjects had two or more treated anatomic areas at baseline. Up to one packet of study cream was applied once daily. Subjects continued applying the study cream for up to eight weeks, stopping if they achieved complete clearance of all (baseline and new) warts in all anatomic areas. The complete clearance rates in the two studies were 27% and 29% compared to 10%

and 9% for placebo creams. Local site reactions to Zyclara were common in these studies: 32% (126/400) of subjects who used Zyclara cream and 2% (4/202) of subjects who used vehicle cream discontinued treatment temporarily (required rest periods) due to adverse local skin reactions, and 1% (3/400) of subjects who used Zyclara cream discontinued treatment permanently due to local skin/application site reactions. These studies did not evaluate intra-anal use.

In summary, few of these studies were randomized and/or placebo-controlled and they are difficult to compare due to different treatment regimens, and differences in whether treatment targeted intra-anal or perianal disease, or whether efficacy was reported for LSIL, HSIL, or a combination. Similar to topical 5FU the studies suggest that anal HSIL may regress or improve with imiquimod 2.5% treatment.

1.2 Study Rationale

Richel et al. (35) reported on 148 HIV-infected MSM with squamous intraepithelial lesions (SIL; 57% with HSIL) randomized to 4-month treatments with 2% 5-fluorouracil, 5% imiquimod, or monthly electrocautery. The response rate was 17%, 24%, and 39%, respectively in the intention to treat group with severe side effects reported as 27%, 43%, and 18%, respectively. Recurrence rates were 58%, 71%, and 68% at 72 weeks post-treatment. In the per protocol group with HSIL, the complete response rate was 21%, 21%, and 50% respectively. This is the first report comparing fluorouracil to imiquimod in a randomized study. While these data indicate that monthly ablation may be superior to either topical cream in the population studied, it may not be appropriate or be the best treatment for those with extensive anal HSIL. In addition, the treatment regimens are differently than what is commonly used for treatment in North America. Richel et al. studied one dose of 2% 5-fluorouracil applied twice weekly, which is lower than what we are proposing in this trial. Further, inserting either agent with suppositories may inadvertently bypass the anal mucosa thereby reducing efficacy. Richel et al. may have confirmed this in that they reported better efficacy of treatment to perianal lesions compared to intra-anal lesions. Our study proposes application of the agents directly to the anal mucosa.

We are proposing an arm with no treatment for up to 20 weeks. This group is necessary to clearly show the activity of these compounds. There are few data on the regression of anal HSIL in the absence of treatment. Fox et al. observed regression in one out of 25 participants randomized to placebo. The risk of progression for this duration of observation is very low, especially with interim anoscopy during the treatment period.

The imiquimod arm was closed as of protocol version 5.0 due to product unavailability.

Rationale in the context of ANCHOR results: The absolute risk of cancer over the course of randomized therapy (24 weeks) was very low in both arms. There are ongoing randomized controlled trials of HPV therapeutic vaccines that randomize women with cervical HSIL to no treatment for a similar short period. We believe the risk is small and is lessened by interim anoscopic monitoring of participants with the option to remove the participant from the study for fear of imminent progression. Participants who appear at risk for progression to cancer are not enrolled into AMC-088. As of 19NOV2021, no cases of invasive cancer have developed in AMC-088.

Version 8.0 reduces the sample size from 118 to 88. The primary analysis is not changing.

The effect of this sample size change on statistical power is discussed in Section 10.0. The motivation for the change is because of the slow accrual despite multiple strategies to increase enrollment. Enrollment closed as of 01JUL2023.

1.2.1 HPV genotyping

Anal swabs will be collected for polymerase chain reaction (PCR) and reverse line blot analysis to determine the HPV genotype at the AMC HPV Genotype Core Laboratory per AMC Standard Operating Procedure (SOP).

2.0 OBJECTIVES

2.1 Primary Objective

Complete response is defined as no anal biopsies with HSIL and cytology without HSIL at Week 20.

- To assess the efficacy of intra-anal topical 5FU for treatment of anal HSIL compared to observation only.

2.2 Secondary Objectives

- To describe the efficacy of intra-anal imiquimod 2.5% for treatment of anal HSIL compared to observation only. Note: this objective is no longer powered to establish efficacy.
- To assess the safety and tolerability of intra-anal imiquimod 2.5% and topical 5FU.
- To compare the efficacy of intra-anal imiquimod 2.5% and topical 5FU.
- To assess for partial response of intra-anal imiquimod 2.5% or topical 5FU as compared to observation only.
- To evaluate the effect of intra-anal imiquimod 2.5% and topical 5-FU on HPV persistence.
- To evaluate anal HSIL outcomes at Week 44.
- To evaluate the effect of behavioral patterns including tobacco smoking and sexual activity on treatment efficacy, tolerability, and HPV.

3.0 PARTICIPANT SELECTION

A rostered AMC investigator (CTEP-registered physician investigator) or a CTEP-registered associate advanced practice clinician (i.e., nurse practitioner or physician assistant) who is certified in HRA by the AMC must document that each protocol participant meets all stated eligibility criteria. Participating sites must have documentation that each eligibility requirement is satisfied prior to subject enrollment. If delegating eligibility review to an HRA-certified, CTEP-registered advanced practice clinician, the site Principal Investigator must document this assigned responsibility in the institution's delegation log. In compliance with CTEP policy, no exceptions to eligibility criteria will be granted in any circumstance.

NOTE: Institutions may use this section of the protocol as an eligibility checklist for source documentation if it has been reviewed, signed, and dated before registration/randomization by the study investigator. If used as source documentation, this checklist must be printed, the investigator must check each item to document their assessment that the participant meets each eligibility criterion, and the completed checklist must be maintained in the participant's chart.

Participant ID Number: 088 - ___ ___ ___ - ___ ___ ___

Patient's Initials (F M (optional) L): ___ ___ ___

NOTE: All questions regarding eligibility should be directed to the study chair.

3.1 Inclusion Criteria

Each participant must comply with the following criteria:

____ 3.1.1 HIV-positive, male or female, ≥ 21 years of age. 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 antiretroviral therapy (ART) (at least two different medications, except pre-exposure prophylaxis [PrEP] regimens [e.g., Truvada]) 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);
- 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 investigational new drug (IND) studies.

____ 3.1.2 Biopsy-proven HSIL (AIN2 with a positive p16 stain, AIN2-3, or AIN3) of the anal canal at either the squamocolumnar junction or distal anus, documented within 60 days prior to enrollment, but not less than seven days prior to randomization.

____ 3.1.3 HSIL comprising two or more lesions, or anal HSIL in at least two octants, or anal HSIL that has recurred or is persistent after prior ablative treatment.

Note: HSIL should be in the anal canal at either the squamocolumnar junction or distal anus on HRA at screening or randomization. The extent of HSIL should be based on available biopsy results and visual appearance.

____ 3.1.4 Anal HSIL lesions are visible at randomization and no lesions are suspicious for invasive cancer.

____ 3.1.5 Ability to understand and willing to provide informed consent.

____ 3.1.6 Participants must, in the opinion of the investigator, be capable of complying with the requirements of this protocol including self-administration of study treatment.

____ 3.1.7 Karnofsky performance status (KPS) of $\geq$ 70% or ECOG performance status $\leq$ 1 (Appendix I).

____ 3.1.8 CD4 count $\geq$ 200 within 120 days prior to enrollment or plasma HIV-1 RNA <200 copies/mL within 120 days prior to randomization.

____ 3.1.9 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 randomization 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 randomization.

____ 3.1.10 Absolute neutrophil count (ANC) >750 cells/mm^3; hemoglobin $\geq$ 9.0 g/dL; platelet count $\geq$75,000/mm^3 within 90 days prior to randomization.

3.2 Exclusion Criteria

Participants meeting the following criteria will be excluded from the study:

____ 3.2.1 History of anal cancer

[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.

____ 3.2.2 Prior intra-anal use of topical 5FU or imiquimod 2.5%, 3.75% or 5% for treatment of HSIL at any point, use of intra-anal or topical 5FU or imiquimod 2.5%, 3.75% or 5% for treatment of condyloma within 6 months prior to randomization or perianal imiquimod 2.5%, 3.75% or 5% or topical 5FU within six months prior to randomization.

____ 3.2.3 Extensive concurrent perianal or lower vulvar HSIL or condyloma requiring a different treatment modality than the study treatment, or treatment that cannot be deferred in observation arm, per examining provider.

____ 3.2.4 Condyloma occupying more than 50% of the circumference of the anal canal or that obscures a satisfactory exam.

____ 3.2.5 Ongoing use of anticoagulant therapy other than aspirin or non-steroidal anti-inflammatory drugs (NSAIDs) that places the participant at increased bleeding risk in the opinion of the site investigator.

____ 3.2.6 Acute treatment for an infection (excluding fungal infection of the skin and sexually transmitted infections) or other serious medical illness within 14 days prior to randomization.

____ 3.2.7 Malignancy requiring systemic therapy. Note: Kaposi sarcoma limited to the skin is not exclusionary unless requiring systemic chemotherapy.

____ 3.2.8 Concurrent systemic corticosteroids, cytokines, and immunomodulatory therapy (e.g., interferons).

____ 3.2.9 Participants who received investigational agents, other than investigational antiretroviral agents for HIV, within the four weeks before randomization, unless approved by the study chair.

____ 3.2.10 Treatment for anal or perianal HSIL, LSIL, or condyloma within four months of randomization. Please note that IRC or electrocautery of a biopsy site to stop bleeding does not constitute treatment.

____ 3.2.11 Female participants who are pregnant or breastfeeding: Women of childbearing potential must have a negative urine or serum pregnancy test within 72 hours prior to randomization. All women of childbearing potential must be willing to comply with an acceptable birth control regimen to prevent pregnancy while receiving treatment and for three months after treatment is discontinued as determined by the investigator. Post-menopausal women must be amenorrheic for at least 12 months to be considered of non-childbearing potential. (Note: A woman of childbearing potential is one who is biologically capable of becoming pregnant. This includes women who are using contraceptives or whose sexual partners are either sterile or using contraceptives.)

Physician Signature: _____ Date: _____

(Optional unless this section is used as an eligibility checklist)

3.3 Recruitment Procedures

This study will be open to all AMC sites certified by the HPV Working Group in HRA. This includes appropriate staff trained and certified by the HPV Working Group in HRA, and the availability of supplies and equipment, including colposcope, biopsy instruments, and the capability for acquiring and maintaining photo documentation of HRA images of dysplasia.

Participants will be selected from established HSIL-positive clinic patients or referrals from physicians, such as primary care physicians, HIV specialists, dermatologists, or colorectal surgeons. Participants may originate from different health care settings. The Investigator or designated individual will review the potential participants' laboratory results, and if the criteria are met, they will be scheduled for the screening visit. Participants who sign the informed consent form and meet all eligibility and screening criteria will be invited to participate in this protocol. If participants do not meet eligibility criteria, they will be informed and offered appropriate alternative treatment and/or follow-up screening.

Note: Participants who were or are in the ANCHOR trial are allowed to enroll into AMC-088.

3.4 Enrollment Procedures

This study will be available for enrollment at all AMC sites that have HRA-certified providers. Sites must have this protocol approved by their Institutional Review Boards (IRB) and be registered with the AMC Operations and Data Management Center (AMC ODMC) before they may enroll participants.

3.4.1 Screening registration

After it is determined that the participant is potentially eligible for the study and the participant has signed the informed consent form, the participant must be enrolled in Segment A (screening segment) of the protocol on-line via the AMC AdvantageEDC^{SM} Internet Data Entry System. After successful registration, the participant will receive a nine-digit participant ID and will then enter the screening process. Participants can immediately proceed to Segment B (randomization) if all necessary data are available.

3.4.2 Randomization

If the participant meets all of the eligibility criteria for the study during the screening evaluation, the participating site will complete the protocol-specific eligibility checklist and will enroll the participant into Segment B of the protocol (online via the AMC Internet Data Entry System, AdvantageEDC). Enrollment should occur no more than one week prior to the initiation of treatment. (Enrollment one day prior to or on the day of treatment is strongly encouraged). Once the eligibility checklist is submitted, a system generated confirmation email with randomization to treatment or observation will be sent to the site staff, AMC ODMC, and Protocol Chair upon successful registration on Segment B. If the online system is inaccessible, the site should notify the AMC ODMC via email at amc-088@emmes.com or via phone at 301-251-1161 for further instructions.

3.4.3 Cross-over

For participants who were assigned to the observation arm upon enrollment into Segment B of the protocol, who have biopsy-proven HSIL present at Week 20, and who agree to receive 5FU at Week 24, the participating site will enroll the participant into Segment C of the protocol (online via AdvantageEDC). Enrollment should occur no more than one week prior to the initiation of treatment. (Enrollment one day prior to or on the day of treatment is strongly encouraged). Once the eligibility checklist is submitted, a system generated confirmation email with the randomization assignment will be sent to the site staff, AMC ODMC, and Protocol Chair upon successful registration on Segment C. If the online system is inaccessible, the site should notify the AMC ODMC via email at amc-088@emmes.com or via phone at 301-251-1161 for further instructions.

4.0 DATA COLLECTION AND MONITORING

4.1 Records to Be Kept

Case report forms (CRFs) will be provided for each participant via AdvantageEDC upon enrollment. Participants must not be identified by name on any study documents. Data will be recorded on the CRFs using the unique participant identification number assigned at registration. Sample CRFs will be available on the AMC ODMC web site (www.AIDSCancer.org).

4.2 Role of Data Management

Instructions concerning the recording of study data on CRFs will be provided by the AMC ODMC. The AMC AdvantageEDC User's Guide can be found on the AMC ODMC web site (www.AIDSCancer.org). Each site is responsible for entering data and submitting forms according to the target submission dates set forth by AdvantageEDC.

It is the responsibility of the AMC ODMC to assure the quality of electronic data reported for each AMC study. This role extends from protocol development to generation of the final study databases.

4.3 Clinical Site Monitoring and Record Availability

This study will be monitored by the AMC Data and Safety Monitoring Board, an independent body to the AMC, as outlined in its Charter and as required by the National Cancer Institute (NCI) Data and Safety and Monitoring Guidelines for randomized phase III investigations. The study will adhere to the AMC Data Safety Monitoring Plan (Appendix VIII).

This study will be monitored in compliance with AMC policies and by the Clinical Data Update System (CDUS) Version 3.0. Cumulative protocol- and participant-specific CDUS data will be submitted via FTP burst 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 AMC ODMC is responsible for compiling and submitting CDUS data to CTEP for all participants and for providing the data to the Principal Investigator for review.

5.0 TREATMENT PLAN

5.1 Summary of Treatment Plan

Entry to Week 16

Arm A (closed as of protocol version 5.0):

Participants randomized to Arm A will begin applying imiquimod 2.5%. The participant will apply one pump actuation of cream intra-anally using a syringe or gloved finger inside the anus as described in Appendix IX. If the participant is tolerating after two weeks (adverse event [AE] of Grade 1 or less), the dose should be increased to one pump actuation to the right side of the anus and one pump actuation to the left side of the anus. An additional pump actuation can be used to cover any areas of perianal or lower vulvar HSIL as needed if instructed by the investigator. The cream should be applied at night before bed or longest period of rest once a day. This should be continued for 16 weeks. Study treatment should be stopped after 16 weeks even if continuous treatment was not completed in the case of treatment interruptions due to lapses in participant adherence or adverse events.

Imiquimod 2.5% (enough for at least 112 pump actuations; approximately 28 actuations per 7.5 gm bottle) should be dispensed at entry and Week 8. Additional containers can be dispensed if needed to treat perianal or lower vulvar SIL. When continuing treatment at Week 8, new containers should be dispensed.

Containers should be weighed before dispensing drug to the participant. Empty or partially used containers should be brought back at each visit to be weighed as a measurement of adherence. Approximately 3.3 gm of cream should be used by Week 2 and 23.1 gm of cream should be used by Week 8. If using additional cream for perianal or lower vulvar HSIL then 6.6 gm should be used by Week 2, and 36.3 gm of cream should be used by Week 8.

Arm B:

Participants randomized to Arm B will begin applying 1 mL of topical 5FU cream. One gram of topical 5FU 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. 5FU contains 5 grams of 5-fluororacil per 100 grams. The daily dose is therefore 50 mg of 5-fluorouracil delivered in divided doses of 25 mg twice daily (BID), and the total weekly dose is 250 mg delivered in 5 grams of cream.

Participants can apply the cream with a syringe or finger. If using a syringe, the participant will apply 0.5 mL (equivalent to 0.5 gm) of cream using a syringe inserted into the anal opening. If applying with a finger, the participant will apply 0.25 mL (equivalent to 0.25 gm) of cream using a finger to the right side of the anus and a second 0.25 mL (equivalent to 0.25 gm) to the left side of the anus as described in Appendix X. The cream should be applied in a thin film twice daily for 5 days followed by 9 days of no treatment. Any areas of perianal or lower vulvar HSIL should be covered with an additional 0.25 mL as needed if instructed by the investigator. This cycle should be repeated to complete eight cycles or 16 weeks. Study treatment should be stopped after 16 weeks even if eight cycles were not completed (in the case of treatment interruptions due to lapses in participant adherence or adverse events).

40 grams of 5FU should be dispensed at Week 0 and Week 8. The partially used 5FU tube should be replaced with a new 40 gm tube at Week 8.

Tubes should be weighed before dispensing drug to the participant. Empty or partially used tubes should be brought back at each visit to be weighed as a measurement of the total amount of cream used during treatment. Approximately 5 gm of cream should be used by Week 2 and 20 gm of cream should be used by Week 8. If using additional cream for perianal or lower vulvar HSIL then 7.5 gm should be used by Week 2, and 30 gm of cream should be used by Week 8.

Arm C:

No treatment.

If receiving topical treatment after Week 20, participants should follow the instructions for Arm A or B as appropriate.

5.2 Concurrent Medications

All pain medications, anticoagulants, HPV vaccines (if ever received), and ART should be recorded in the appropriate electronic case report forms at the time of study entry, and any changes to the participant's medications should be updated as those changes occur. Any additional therapies prescribed for treatment of study product related adverse events should be recorded as well.

5.3 Criteria for Dose Modification

Participants experiencing Grade 3 AEs that are at least possibly related to the study product should have the product held 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 Table 5-A 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 AEs that are at least possibly related to study product can either continue the product at the standard dose (preferred) or dose reduce one level. If the participant tolerates the reduced dose after two weeks, then the dose can be escalated again at the discretion of the site investigator.

Participants experiencing Grade 1 AEs that are at least possibly related to study product 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: Dose modifications

	Topical 5-fluorouracil 5%	Imiquimod 2.5% (closed as of version 5.0)
Standard dose	Twice daily for 5 days followed by 9 days off (5 gm of cream per 2 week cycle)	2 pump actuations once a day at night
Reduced dose	Twice daily for 3 days followed by 11 days off (3 gm of cream per 2 week cycle)	1 pump actuation once a day at night
Minimal dose	Once daily for 3 days followed by 11 days off (1.5 gm of cream per 2 week cycle)	1 pump actuation three times a week at night

5.3.1 Dose delays

Participants may experience dose delays for several reasons including adverse and logistical issues. Sites should strive to complete 16 weeks of study treatment. The end of dosing and the Week 16 visit may be delayed up to three weeks to allow participants to complete 16 weeks of study treatment. The last dose of study treatment should be no later than 19 weeks after randomization. For participants whose last dose is delayed, the Week 20 visit should be delayed to ensure at least two weeks after the last dose of study treatment. For participants who are randomized to topical treatment at Visit 6/Week 24, the visit schedule should be based on the date of cross-over randomization (e.g., Visit 7 should occur two weeks following the Visit 6 date, Visit 8 should occur eight weeks following the Visit 6 date, etc.). Please contact the protocol chairs for advice on specific participants as needed.

5.4 Criteria for Discontinuing Study Treatment

The investigator should discontinue the study treatment if there is evidence of Grade 4 AEs at least possibly due to study treatment.

The investigator may discontinue study treatment, at his or her discretion, if the participant experiences Grade 3 AEs at least possibly due to study treatment. Alternatively, study treatment may be held and dose reduced as described in Section 5.3.

Participants who prematurely discontinue treatment should remain in study follow-up according to the schedule of events. If treatment is discontinued prior to Week 16, then the participant should undergo HRA with biopsies for the primary endpoint at Week 20. Off-study treatment for anal HSIL prior to Week 20, other than randomized treatment, should be avoided.

Similarly, participants who prematurely discontinue study treatment administered beginning at Week 24 should undergo HRA with biopsies at Week 44.

Participants should discontinue assigned study treatment (or observation without treatment) if progression of disease is suspected. For example, the pathologist suspects possible superficial invasion or there is worsening of histology, as evidenced by an

endophytic growth pattern and pushing borders, or if the clinician notes suspicious friability, ulceration, mass effect, heaped-up borders, and/or markedly abnormal vascular pattern on HRA. These participants should remain in study follow-up and receive treatment as determined by the site investigator.

5.5 Criteria for Study Discontinuation

Participants may withdraw from the study at any time, for any reason. Removal of the participant from the study by the physician should occur for any of the following reasons:

- Evidence of invasive carcinoma in any anal cytology or biopsy specimens.
- Worsening of Karnofsky performance status to ≤ 60 (ECOG ≥ 2)

If a participant is terminated because of progression to squamous cell carcinoma (SCC), the adverse finding will be reported to the protocol chair and the NCI as a serious AE (see Section 7.0 for further instructions). Such participants should receive appropriate treatment outside the study. The results of any anal biopsies obtained to evaluate disease progression should be recorded in the CRF.

If a participant fails to attend three study visits in a row, the participant may be withdrawn for failure to attend study appointments.

6.0 STUDY EVALUATIONS

Schedule of Evaluations is provided in Appendix II.

6.1 Screening (Visit -1)

The screening HRA may occur prior to obtaining informed consent provided that the HRA was performed as part of routine clinical care and according to the AMC guidelines for HRA.

All screening procedures must occur prior to randomization in AdvantageEDC.

6.1.1 Complete medical history

To include

- Duration of HIV and AIDS diagnoses, history of opportunistic illnesses, and date of initial diagnosis of anal HSIL. If at all possible, the investigator should obtain a copy of the pathology report from the first diagnosis of anal HSIL.
- CDC HIV risk categories and history of AIDS defining conditions.
- Current medications (as required by Section 5.2) and history of drug allergies.
- All antiretroviral medications taken within the past 30 days will be documented. Current pain medication usage will be quantified.
- Concurrent anal problems including condyloma, hemorrhoids, fissure, skin tag, fistula, sexually transmitted infections, bleeding, pruritus, and pain or irritation.
- Prior treatments of anal HSIL and condyloma.
- T-cell nadir: The participant's prior nadir CD4+ cell count (absolute value and date) must be documented, when possible, with a copy of the nadir CD4+ cell count report and entered on the CRF. If this documentation is not available, then participant recollection of nadir CD4 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) and year will suffice.

6.1.2 Clinical assessment

Complete physical examination to include:

- Performance status (Appendix I), blood pressure, temperature, pulse, weight, and height.
- A complete genital examination in men that includes evaluation of penis and scrotum for lesions, discharge, tenderness, testicular asymmetry, and masses.
- Female participants must have received a routine gynecologic examination including a cervical pap test (if having a cervix) as required in Section 3.1.9.
- A complete external genital examination in women that includes gross evaluation of the vulva for lesions.

- HRA exam of the anal canal will be performed within 60 days but not less than seven days prior to randomization (Appendix IV).
- HRA guided biopsies will be obtained to assure eligibility for the study and to be enrolled and randomized. If the participant has had biopsies within 60 days but not less than seven days of enrollment, new biopsies are not necessary unless there is concern for progression towards invasive cancer. See Appendix V for biopsy protocol.
- The number of involved octants, location, and percent circumference of HSIL will be documented. If this is not available at the screening HRA, then this can be documented at entry prior to randomization.

6.1.3 Laboratory tests must be obtained within 90 days prior to enrollment (unless otherwise noted) and will include the following:

- Anal biopsy: Results of biopsy diagnostic of HSIL (within 60 days to one week prior to enrollment).
- Anal cytology (within 60 days prior to enrollment). If anal cytology is not available prior to entry, then this may be obtained at entry prior to HRA.
- Anal swab for HPV testing. If not obtained at the time of screening HRA, then may be obtained at entry prior to HRA.
- Safety laboratory testing: Baseline laboratory testing will include complete blood count (CBC) with differential and platelet count.
- HIV viral load (within 120 days): Viral load studies will be performed using an assay with a limit of detection of 75 copies/mL or less.
- T cells (within 120 days): CD4/CD8 counts and percentages will be quantified.
- Pregnancy test: Urine or serum beta-human chorionic gonadotropin (beta-HCG) will be performed (and results obtained) within 72 hours prior to enrollment when indicated (women of childbearing potential).
- AIDS and Cancer Specimen Resource (ACSR) donation: If the participant has consented, a whole blood specimen shall be collected for donation to the ACSR. Specimen handling instructions and informed consent are in Appendices VI and VII.

6.2 Randomization Visit/HRA Evaluation (Visit 1)

The baseline visit for study entry and randomization must occur within 60 days after the screening visit and seven to 60 days after the HRA with biopsies used for eligibility (Visit 1).

6.2.1 History and targeted physical exam

- Clinical assessment: The participant's medical history will be updated for any changes. A review of systems for any new or changed local symptoms, such as hematochezia, dyschezia, erythema, swelling, or pruritus. A targeted physical exam should be performed as indicated.

- A targeted physical examination is to include pulse, blood pressure, and 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 AEs.
- Pregnancy test: Urine or serum beta-HCG will be performed (and results obtained) when indicated (women of childbearing potential).
- HRA exam of the anal canal will be performed. The number of involved octants, location, and percent circumference of HSIL will be documented per Section 9.1. If there are any lesions suspicious for invasive anal cancer, then the participant should not be randomized. Entry criteria in Section 3.1.4 should be confirmed prior to randomization (e.g., anal HSIL is visible at study entry and no lesions are suspicious for invasive cancer).
- If during an HRA exam, the clinician notes suspicious friability, ulceration, mass effect, heaped-up borders, and/or markedly abnormal vascular pattern, these could be indicative of invasive disease. This participant should not be randomized, and additional biopsies should be obtained.
- All lesions, all aspects of the anal transformation zone, distal canal and perianus will be photographically documented prior to treatment.
- The study clinician should explain how the study treatment is applied and observe the participant apply the first dose of medication or a proxy white cream to assess for adequate application technique. Sites should use an anesthetic cream (e.g., 4% lidocaine cream) that is used during HRA for the proxy cream. The treatment diary will be distributed to the participant. Topical treatment must be initiated within two weeks after randomization.
- AMC-088 Smoking Status and Recent Sexual History Questionnaire: A review of smoking and recent sexual behavior.
- Study agent must be weighed prior to distribution to the participant to allow for accurate measurement of adherence.

6.2.2 Eligibility review and randomization

Pursuant to NCI policy, no exceptions from the eligibility criteria shall be made. If all criteria are met, eligible participants will enrolled for randomization to treatment or observation (Segment B) through AdvantageEDC and commence the study as specified in the protocol according to the arm of the study indicated on the participant registration screen from randomization. Participants who do not meet all of the eligibility criteria will be considered screen failures and the Screen Failure Form must be completed in AdvantageEDC.

6.3 Visits 2 (Week 2) 14 days after initiating treatment +/- 3 days

6.3.1 For study participants randomized to imiquimod 2.5% or 5-fluorouracil 5% arm only:

- Clinical assessment: The participant's medical history will be updated for any changes. A review of systems for any new or changed local symptoms, such as

hematochezia, dyschezia, erythema, swelling, or pruritus. A targeted physical exam should be performed as indicated (see Section 6.2.1).

- Pregnancy test: Urine or serum beta-HCG will be performed (and results obtained) when indicated (women of childbearing potential).
- Anoscopy or HRA may be performed at the discretion of the treating provider to investigate symptoms that the participant may be experiencing or to evaluate for inflammation and ulceration in the anal canal to assure that the positioning of the study treatment is correct.
- Collect the treatment diary and evaluate treatment adherence. Partially used containers should be weighed to measure the amount of cream used, then returned to participant. Additional instruction on proper administration of study treatment should be given as needed.

6.4 Visit 3 (Week 8) +/- 14 days

6.4.1 The following evaluations will be performed, and laboratory samples will be collected for all participants:

- Clinical assessment: The participant's medical history will be updated for any changes. A review of systems for any new or changed local symptoms, such as hematochezia, dyschezia, erythema, swelling, or pruritus. A targeted physical exam should be performed as indicated (see Section 6.2.1).
- Pregnancy test: Urine or serum beta-HCG will be performed (and results obtained) when indicated (women of childbearing potential).
- Anal swab for HPV testing.
- For study participants randomized to the 5-fluorouracil 5% arm only: HRA will be performed provided that the participant is not experiencing significant anal discomfort from the study treatment. In such a case, anoscopy should be performed without soaking the anal canal with acetic acid. Acetic acid and/or Lugol's solution should only be used sparingly to avoid further participant discomfort. The number of involved octants, location, and percent circumference of HSIL will be documented. Anal biopsies should be performed only if the clinician notes suspicious friability, ulceration, mass effect, heaped-up borders, and/or markedly abnormal vascular pattern. This could be indicative of progression of the lesion toward invasive disease.
- HRA may be performed in the observation arm if disease progression is suspected.
- If performing HRA, all lesions, all aspects of the anal transformation zone, distal canal and perianus will be photographically documented.
- Collect study diary and evaluate treatment adherence. Additional instruction on proper administration of study treatment should be given as needed. Partially used containers should be weighed to measure the amount of cream used. Participants will receive new replacement containers as needed for their

appropriate study arm. Study agent must be weighed prior to distribution to the participant to allow for accurate measurement of adherence.

6.5 Visit 4 (Week 16) +/- 14 days

- Clinical assessment: The participant's medical history will be updated for any changes. A review of systems for any new or changed local symptoms, such as hematochezia, dyschezia, erythema, swelling, or pruritus. A targeted physical exam should be performed as indicated (see Section 6.2.1).
- Pregnancy test: Urine or serum beta-HCG will be performed (and results obtained) when indicated (women of childbearing potential).
- Anal swab for HPV testing.
- Anoscopy or HRA may be performed at the discretion of the treating provider to investigate symptoms that the participant may be experiencing, to evaluate for inflammation and ulceration in the anal canal to assure that the positioning of the study treatment is correct, or if disease progression is suspected. If performing HRA or anoscopy and the participant is experiencing significant anal discomfort from the study treatment, HRA or anoscopy should be performed without soaking the anal canal with acetic acid. Acetic acid and/or Lugol's solution should only be used sparingly to avoid further participant discomfort. If performing anoscopy or HRA, the number of involved octants, location, and percent circumference of HSIL will be documented. Anal biopsies should be performed only if the clinician notes suspicious friability, ulceration, mass effect, heaped-up borders, and/or markedly abnormal vascular pattern. This could be indicative of progression of the lesion toward invasive disease.
- If HRA is performed, then all lesions, all aspects of the anal transformation zone, distal canal and perianus will be photographically documented.
- Collect the treatment diary and evaluate adherence. All containers of study treatment should be collected. Partially used containers should be weighed to measure the amount of cream used.

6.6 Visit 5 (Week 20) +/- 21 days

Note this visit must occur at least two weeks after the last dose of study drug.

6.6.1 The following evaluations will be performed, and laboratory samples will be collected for all participants.

- Clinical assessment: The participant's medical history will be updated for any changes. A review of systems for any new or changed local symptoms, such as hematochezia, dyschezia, erythema, swelling, or pruritus. A targeted physical exam should be performed as indicated (see Section 6.2.1).
- Pregnancy test: Urine or serum beta-HCG will be performed (and results obtained) when indicated (women of childbearing potential).
- Anal cytology (standard of care). If the result is not evaluable, then the site should repeat the cytology to obtain an interpretable result.
- Anal swab for HPV testing.

- HRA exam of the anal canal will be performed per Section 9.1. The number of involved octants, location, and percent circumference of HSIL will be documented.
- All participants should have biopsies obtained as per Appendix V. Please note that pathologists should remain blinded to the treatment assignment.
- If anal HSIL is present, then participants originally randomized to observation have the option of receiving 5-fluorouracil 5%. Cross-over should occur as outlined in Section 3.4.3. If these participants decline cross-over, then they should be referred off study to be treated according to the local standard of care as described below.
- Participants originally randomized to 5-fluorouracil 5% arms, with HSIL at Week 20 should be referred off study to be treated according to the local standard of care. Participants can be treated with ablative methods such as office-based infrared coagulation, hyfrecation, electrocautery, laser, or 80-85% trichloroacetic acid if preferred. Topical treatments such as 5-fluorouracil 5%, imiquimod, Veregen 15%, or cidofovir should not be used.
- Condyloma can be treated at the discretion of the local provider with ablative methods such as office-based infrared coagulation, hyfrecation, electrocautery, laser, or 80-85% trichloroacetic acid if preferred. Topical treatments such as 5-fluorouracil 5%, imiquimod, Veregen 15%, or cidofovir should not be used.
- All lesions, all aspects of the anal transformation zone, distal canal and perianus will be photographically documented.
- AMC-088 Smoking Status and Recent Sexual History Questionnaire: A review of smoking and recent sexual behavior.

6.7 Visit 6 (Week 24) +/- 14 days

This visit is only necessary for participants who started on the observation arm, still have HSIL at Week 20, and agree to be randomized to treatment in the crossover portion of the study. They will be randomized to a study-provided topical therapy (intra-anal topical 5-fluorouracil 5%) for 16 weeks of treatment. Participants who started on the observation arm and do not have HSIL at Week 20 will be treated with local standard of care and only return for Visit 10 (same as Arm A and B). Participants who started on the observation arm who do not agree to the second randomization will be treated with local standard of care and only return for Visit 10 (same as Arm A and B).

6.7.1 History and physical exam

- Clinical Assessment: The participant's medical history will be updated for any changes. A review of systems for any new or changed local symptoms, such as hematochezia, dyschezia, erythema, swelling, or pruritus. A targeted physical exam should be performed as indicated (see Section 6.2.1).
- Pregnancy test: Urine or serum beta-HCG will be performed (and results obtained) when indicated (women of childbearing potential).
- Participants will be assigned to 5-fluorouracil 5%.

- The study clinician should explain how the study treatment is applied and observe the participant apply the first dose of medication or a proxy white cream to assess for adequate application technique. Sites should use an anesthetic cream (e.g., 4% lidocaine cream) that is used during HRA for the proxy cream. The treatment diary will be distributed to the participant.
- All lesions, all aspects of the anal transformation zone, distal canal and perianus will be photographically documented prior to treatment.
- AIDS and Cancer Specimen Resource (ACSR) donation: If the participant has consented, a whole blood specimen shall be collected for donation to the ACSR. Specimen handling instructions and informed consent are in Appendices VI and VII.
- Study agent must be weighed prior to distribution to the participant to allow for accurate measurement of adherence.
- Treatment diary distributed to participant.

6.8 Visit 7 (Weeks 26): 14 days after initiating treatment +/- 3 days

This visit is only required for those participants starting a topical treatment at Week 24.

- Clinical assessment: The participant's medical history will be updated for any changes. A review of systems for any new or changed local symptoms, such as hematochezia, dyschezia, erythema, swelling, or pruritus. A targeted physical exam should be performed as indicated (see Section 6.2.1).
- Pregnancy test: Urine or serum beta-HCG will be performed (and results obtained) when indicated (women of childbearing potential).
- Anoscopy or HRA may be performed at the discretion of the treating provider to investigate symptoms that the participant may be experiencing or to evaluate for inflammation and ulceration in the anal canal to assure that the positioning of the study treatment is correct.
- Collect the treatment diary and evaluate treatment adherence. Partially used containers should be weighed to measure the amount of cream used, then returned to participant. Additional instruction on proper administration of study treatment should be given as needed.

6.9 Visit 8 (Week 32) +/- 14 days

This visit is only required for those participants starting a topical treatment at Week 24.

The following evaluations will be performed, and laboratory samples will be collected for all participants:

- Clinical assessment: The participant's medical history will be updated for any changes. A review of systems for any new or changed local symptoms, such as hematochezia, dyschezia, erythema, swelling, or pruritus. A targeted physical exam should be performed as indicated (see Section 6.2.1).

- Pregnancy test: Urine or serum beta-HCG will be performed (and results obtained) when indicated (women of childbearing potential).
- Anal swab for HPV testing.
- HRA will be performed provided that the participant is not experiencing significant anal discomfort from the study treatment. In such a case, anoscopy should be performed without soaking the anal canal with acetic acid. Acetic acid and/or Lugol's solution should only be used sparingly to avoid further participant discomfort. The number of involved octants, location, and percent circumference of HSIL will be documented. Anal biopsies should be performed only if the clinician notes suspicious friability, ulceration, mass effect, heaped-up borders, and/or markedly abnormal vascular pattern. This could be indicative of progression of the lesion toward invasive disease.
- If performing HRA, all lesions, all aspects of the anal transformation zone, distal canal and perianus will be photographically documented.
- Participants receiving imiquimod 2.5% or 5-fluorouracil 5% should bring back empty or partially used study drug containers, which are to be weighed by staff.
- Collect study diary and evaluate treatment adherence. Additional instruction on proper administration of study treatment should be given as needed. Partially used containers should be weighed to measure the amount of cream used. New containers will be dispensed according to treatment arm. Study agent must be weighed prior to distribution to the participant to allow for accurate measurement of adherence.

6.10 Visit 9 (Week 40) +/- 14 days

This visit is only required for those participants starting a topical treatment at Week 24.

- Clinical assessment: The participant's medical history will be updated for any changes. A review of systems for any new or changed local symptoms, such as hematochezia, dyschezia, erythema, swelling, or pruritus. A targeted physical exam should be performed as indicated (see Section 6.2.1).
- Pregnancy test: Urine or serum beta-HCG will be performed (and results obtained) when indicated (women of childbearing potential).
- Anal swab for HPV testing.
- Anoscopy or HRA may be performed at the discretion of the treating provider to investigate symptoms that the participant may be experiencing, to evaluate for inflammation and ulceration in the anal canal to assure that the positioning of the study treatment is correct, or if disease progression is suspected. If performing HRA or anoscopy and the participant is experiencing significant anal discomfort from the study treatment, HRA or anoscopy should be performed without soaking the anal canal with acetic acid. Acetic acid and/or Lugol's solution should only be used sparingly to avoid further participant discomfort. If performing anoscopy or HRA, the number of involved octants, location, and percent circumference of HSIL will be documented. Anal biopsies should be performed only if the clinician notes suspicious friability, ulceration,

mass effect, heaped-up borders, and/or markedly abnormal vascular pattern. This could be indicative of progression of the lesion toward invasive disease.

- If performing HRA, all lesions, all aspects of the anal transformation zone, distal canal and perianus will be photographically documented.
- Participants receiving 5-fluorouracil 5% must bring back all study drug containers, which are to be weighed by staff.
- Collect the treatment diary and evaluate treatment adherence. All containers of study treatment should be collected. Partially used containers should be weighed to measure the amount of cream used.

6.11 Visit 10 (Week 44) / Study Discontinuation +/- 21 days

This visit is required for **all participants**. Please note that this visit must occur at least two weeks after the last dose of study drug.

6.11.1 The following evaluations will be performed, and laboratory samples will be collected for all participants.

- Clinical assessment: The participant's medical history will be updated for any changes. A review of systems for any new or changed local symptoms, such as hematochezia, dyschezia, erythema, swelling, or pruritus. A targeted physical exam should be performed as indicated (see Section 6.2.1).
- Pregnancy test: Urine or serum beta-HCG will be performed (and results obtained) when indicated (women of childbearing potential).
- Anal swab for HPV testing.
- Anal cytology (standard of care).
- HRA exam of the anal canal. The number of involved octants, location, and percent circumference of HSIL will be documented per Section 9.1. This only applies to participants who began on Arm C (observation) and were randomized at Visit 6 (Week 24).
- All participants should have biopsies obtained as per Appendix V. Please note that pathologists should remain blinded to the treatment assignment.
- Any HSIL diagnosed at this visit should be treated off study at the discretion of the treating provider.
- Ablative treatments should not be performed within two weeks of the Week 44 visit if at all possible.
- All lesions, all aspects of the anal transformation zone, distal canal and perianus will be photographically documented.
- AMC-088 Smoking Status and Recent Sexual History Questionnaire: A review of smoking and recent sexual behavior.

6.12 Early Treatment Discontinuation Visit

- Clinical assessment: The participant's medical history will be updated for any changes. A review of systems for any new or changed local symptoms, such as hematochezia, dyschezia, erythema, swelling, or pruritus. A targeted physical exam should be performed as indicated (see Section 6.2.1).
- Anal swab for HPV testing.
- Anal cytology (standard of care) and anal swab for HPV testing.
- HRA exam of the anal canal. The number of involved octants, location, and percent circumference of HSIL will be documented per Section 9.1.
- All participants should have biopsies obtained as per Appendix V. Please note that pathologists should remain blinded to the treatment assignment.
- Any HSIL diagnosed at this visit should be treated off study at the discretion of the treating provider.
- All lesions, all aspects of the anal transformation zone, distal canal and perianus will be photographically documented.
- Collect the treatment diary and evaluate adherence. All containers of study treatment should be collected. Partially used containers should be weighed to measure the amount of cream used.
- AMC-088 Smoking Status and Recent Sexual History Questionnaire: A review of smoking and recent sexual behavior.

7.0 ADVERSE EVENT REPORTING

CTEP Version 5.0 of the NCI Common Terminology Criteria for Adverse Events (CTCAE) will be utilized for AE reporting. CTCAE Version 5.0 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 CTCAE Version 5.0.

This study will be monitored by the Clinical Data Update System (CDUS). Cumulative CDUS data will be submitted quarterly to CTEP by electronic means. Reports are due January 31, April 30, July 31, and October 31.

7.1 Adverse Events and Potential Risks

7.1.1 Expected adverse events related to the procedure

Pap smear collection, High Resolution Anoscopy (HRA) and anal biopsy.

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

The risk of infection is less than 1%.

7.1.2 Expected adverse events related to 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, 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.

7.1.3 Expected adverse events related to topical 5-fluorouracil 5%

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, 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 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.

7.1.4 Expected adverse events related to the study disease

Anal HSIL may progress to anal carcinoma, whether observed or treated with study provided treatments. If suspected progression to SCC occurs, as outlined in Section 5.5 of the protocol, the investigator may withdraw the participant from the randomized strategy and pursue treatment of anal HSIL or invasive cancer outside of the study as clinically indicated. If progression to SCC occurs, then the

participant must be withdrawn from the protocol and appropriate therapy instituted. Such participants should remain in study follow-up.

7.2 Classification of AEs by Severity and Relationship to Study Drug Administration

7.2.1 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).

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

7.2.3 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.

7.2.4 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.

7.2.5 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.

7.2.6 Unexpected Adverse Event: Any AE that is not listed in available sources including the package insert, the Investigator's Brochure, or the protocol.

7.2.7 Cancer Therapy Evaluation Program Adverse Event Reporting System (CTEP-AERS): An electronic system for expedited submission of AE reports.

7.2.8 Attribution: The determination of whether an AE is related to a medical treatment or procedure. Attribution categories:

Definite – The AE is clearly related to the investigational agent.

Probable – The AE is likely related to the investigational agent.

Possible – The AE may be related to the investigational agent.

Unlikely – The AE is doubtfully related to the investigational agent.

Unrelated – The AE is clearly NOT related to the investigational agent.

7.3 Expedited Adverse Event Reporting

7.3.1 Expedited AE reporting for this study must use CTEP-AERS, accessed via the CTEP home page (http://ctep.cancer.gov). The reporting procedures to be followed are presented 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 in the table below (Section 7.3.3).

A 24-hour notification is to be made to the AMC 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 CTEP-AERS by the original submitter at the site.

7.3.2 CTEP-AERS is programmed for automatic electronic distribution of reports to the following individuals: Study Coordinator of the Lead Organization, Principal Investigator, and the local treating physician. CTEP-AERS provides a copy feature for other email recipients.

7.3.3 Expedited reporting guidelines

Use the NCI protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

Note: A death on study requires both routine and expedited reporting regardless of causality, unless as noted below. Attribution to treatment or other cause must be provided.

Death due to progressive disease should be reported as **Grade 5 "General disorders and administration site conditions – Disease Progression"**. Evidence that the death was a manifestation of underlying disease (*e.g.*, radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

FOR USE IN CIP STUDIES INVOLVING COMMERCIAL (NON-IND/IDE) AGENTS ONLY

CIP Commercial Agent Studies: Expedited Reporting Requirements for Adverse Events that Occur in a CIP Non-IND/IDE trial within 30 Days of the Last Administration of a Commercial Imaging Agent [1, 2]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)

NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)

An adverse event is considered serious if it results in **ANY** of the following outcomes:

- Death
- A life-threatening adverse event
- An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.

Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria **MUST** be immediately reported to the NCI via CTEP-AERS within the timeframes detailed in the table below.

<table>
<tr><th>Hospitalization</th><th>Grade 1 Timeframes</th><th>Grade 2 Timeframes</th><th>Grade 3 Timeframes</th><th>Grade 4 & 5 Timeframes</th></tr>
<tr><td>Resulting in Hospitalization ≥ 24 hrs</td><td colspan="3">10 Calendar Days</td><td rowspan="2">24-Hour 5 Calendar Days</td></tr>
<tr><td>Not resulting in Hospitalization ≥ 24 hrs</td><td colspan="2">Not required</td><td>10 Calendar Days</td></tr>
</table>

NOTE: Protocol specific exceptions to expedited reporting of serious adverse events are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR

Expedited AE reporting timelines are defined as:

- "24-Hour; 5 Calendar Days" - The AE must initially be reported via CTEP-AERS within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
- "10 Calendar Days" - A complete expedited report on the AE must be submitted within 10 calendar days of learning of the AE.

[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:

Expedited 24-hour notification followed by complete report within 5 calendar days for:

- All Grade 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

- Grade 2 adverse events resulting in hospitalization or prolongation of hospitalization
- Grade 3 adverse events

[2] For studies using PET or SPECT agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

Effective Date: May 5, 2011

7.4 Routine AE Reporting

All Grade 3 or greater AEs, whether or not ascribed to the study drug administration, will be recorded on the Adverse Event Form. Unexpected Grade 1 and 2 AEs that are considered to be related to study drug administration will be captured on the AMC Adverse Event Form. Expected Grade 1 and 2 AEs that are related to study drug administration will be captured on the Expected Toxicity form, and do not require reporting in the Adverse Event form. Grade 1 and 2 adverse events that are not considered related to study drug administration will not require reporting for this protocol. These requirements are summarized in the chart below.

Table 7-A: Routine AE reporting requirements by grade and relationship to study agents

Relationship to Study Agents	Grade 1 or 2	Grades 3, 4, or 5
Unrelated *(attribution of unrelated or unlikely)*	Not Required	Adverse Event Form
Related *(attribution of possible, probable, or definite)*	*Expected*: Expected Toxicity Form *Unexpected*: Adverse Event Form	Adverse Event Form

Participants withdrawn from the study due to AEs will be followed by the Investigator until the outcome is determined and, when appropriate, additional written reports and documentation will be provided.

7.4.1 Clinical laboratory abnormalities

Clinical laboratory results that are outside of the normal ranges will be considered AEs if deemed clinically significant by the Investigator.

An abnormal lab value should be deemed clinically significant if either of the following conditions is met:

- The abnormality suggests a disease and/or organ toxicity that is new or has worsened from baseline.
- The abnormality is of a degree that requires additional active management, e.g., change of dose, discontinuation of the drug, close observation, more frequent follow-up assessments, or further diagnostic investigation.

7.5 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.

All secondary malignancies that occur following treatment with an agent under this protocol will be reported via CTEP-AERS. Three options are available to describe the event:

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

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

7.6 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.

8.0 PHARMACEUTICAL INFORMATION

8.1 Efudex (5-fluorouracil 5%)

8.1.1 Product description

Efudex cream is a topical preparation, available in a 40 gm tube, containing the fluorinated pyrimidine 5-fluorouracil, an antineoplastic antimetabolite. 5% 5-fluororacil contains 5 grams of 5-fluororacil per 100 grams. Efudex 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 which 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 $C_4H_3FN_2O_2$.

8.1.2 Storage

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

8.1.3 Route of administration

The agent will be administered per rectum. Participants will self-apply the medications with a syringe or their gloved fingers as described in Section 5.1.

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

8.2 Zyclara (imiquimod 2.5%)

Zyclara was discontinued on this protocol as of version 5.0 due to product unavailability.

8.2.1 Product description

Imiquimod belongs to the chemical class of substances known as imidazoquinoline amines. 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.

Imiquimod cream (Zyclara), 2.5% is available in 7.5 and 15 gm pump bottles, each gram of cream contains 25 mg of imiquimod. Pump bottles dispense 5.9 mg of imiquimod in 0.235 gm of cream per full actuation of the pump after priming. The 7.5 gm pump delivers no fewer than 28 full actuations and the 15 gm pump delivers no fewer than 56 full actuations.

8.2.2 Storage

Store at 25ºC (77ºF); excursions permitted to 15º to 30ºC (59º to 86ºF). Avoid freezing. Store ZYCLARA Cream pumps upright.

8.2.3 Route of administration

The agent will be administered per rectum. Participants will self-apply the medications with a syringe or their gloved-fingers as described in Section 5.1.

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

8.3 Study Agent Supply, Distribution and Accountability

Study agents will be supplied by the manufacturers.

8.3.1 Study agent acquisition

Participating sites are to refer to the study drug request form located on the AMC Operations Center web site (www.AIDSCancer.org).

8.3.2 Study agent accountability

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 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.

8.3.3 Study agent transfers

Agent may not be transferred from one subject to another subject, from one center to another center, or from one protocol to another protocol.

8.3.4 Study agent destruction

Agent returned by participants must be weighed prior to destruction and return must be documented in source documents.

Expired agent may be destroyed according to local policies. At the end of the study, unused agent may be destroyed according to local policy with written authorization from the AMC ODMC.

9.0 RESPONSE CRITERIA

CTEP-registered advanced practice clinicians who are non-physician investigators (i.e., NP or PA) may perform toxicity and response assessment as allowed per local licensure.

9.1 Definition of Clinical Response (by Subject)

9.1.1 Complete Response (CR): The absence of HSIL histology for all biopsies and the absence of HSIL cytology.

9.1.2 Partial Response (PR): 1) The regression of HSIL histology but HSIL cytology is present, or 2) Reduction in number of octants with HSIL.

9.1.3 No Response: The presence of HSIL histology with no change in the number of octants with HSIL with or without HSIL cytology.

9.1.4 Spontaneous Regression: The absence of HSIL histology and HSIL cytology following no treatment.

Please note that LSIL is treated the same as no SILs for the purpose of these definitions. For example, a participant with all biopsies showing LSIL and cytology showing LSIL would be considered a CR.

10.0 STATISTICAL CONSIDERATIONS

10.1 Rate of Accrual

This study is expected to accrue eight participants per month for a total of 118 participants (50 per arm for observation and 5FU arms and 18 to the imiquimod arm, which closed because of lack of drug availability). However, the rate of accrual has been approximately one participant per month from 2021-2023. With Version 8.0, the sample size has been reduced to 88 participants (35 per arm for observation and 5FU arms and 18 to the imiquimod arm).

10.2 Randomization

The study will use a stratified, random permuted block randomization scheme with strata being sites. This approach ensures that arms are balanced within sites.

10.3 Sample Size Estimation

The original sample size was based on the assumption that treatment of anal HSIL lesion with topical therapy (topical 5-fluorouracil 5%) will have a minimum CR rate of 40% as compared to a maximum regression rate of 10% in the observation arm. Due to the slow accrual rate, the sample size was reduced, and the revised sample size was decided by the expected accrual close date (01 July 2023). Thirty five participants per arm (or 70 in total for observation and 5FU arms) would be the revised sample size. Eighteen participants were accrued to the imiquimod arm prior to this arm being closed because of lack of drug availability. The comparison of imiquimod to observation is no longer sufficiently powered to test efficacy; comparisons to imiquimod will be considered exploratory.

10.4 Analysis Population

The primary analysis population for efficacy and safety is intent-to-treat (ITT) population, which consists of all participants who were randomized.

10.5 Safety Analysis

AEs will be summarized at the event level and participant level according to severity. Proportions and their exact 95% confidence intervals will be calculated.

10.6 Monitoring

10.6.1 Safety monitoring.

The occurrence of at least two Grade 3 SAEs that are at least possibly related to study conduct or study treatment (including progression to SCC), or a single Grade 4 or 5 AE that is at least possibly related to study conduct will cause the study to halt enrollment. The study will restart only after a review of available safety data and SAE reports by the safety review team: The AMC Medical Monitor (located at the AMC ODMC), the study chairs (Wilkin and Brickman), HPV Working Group chairs (Palefsky and Stier) and the AMC statistician (Kwon). If consensus about restarting the study is not reached among these members, then the decision will be made by the AMC Executive Committee. This process will be repeated after every two serious and related Grade 3 AEs or after each Grade 4 or 5 AE that is at least possibly related to study conduct or treatment. For unanticipated adverse effects that present an unreasonable risk to participants, the study must be terminated

within 5 days of the determination and not later than 15 days after notification of the event. The study may not be resumed without approval from the FDA and reviewing IRBs.

10.6.2 Futility and efficacy halting rules

An interim analysis is planned after 50% of the ITT population has completed assessment for the primary endpoint to assess the futility of achieving a significant result if the study continues and to potentially demonstrate efficacy before all participants are enrolled. For the comparing topical 5-fluorouracil 5% vs observation, group sequential methods will be used to compare efficacy endpoints preserving the overall one-sided significance level of 0.025 for each comparison. To assess superiority of a treatment arm in comparison to observation the Lan-DeMets stopping boundaries will be used. To assess futility, the O'Brien-Fleming futility boundaries will be used. The interim analyses will be reviewed by the AMC Data and Safety Monitoring Board (DSMB) by the 50% point.

Table 10-A: Statistical test boundaries and associated operating characteristics for testing efficacy of 5-fluorouracil 5% vs. observation

Analysis	Total Number (%) of Subjects*	H_0 (Futility) Bound Z (P-value)	H_1 (Efficacy) Bound Z (P-value)	Overall α Spent	Overall β (1-power) Spent
1 (Interim)	35 (50%)	0.258 (0.398)	2.963 (0.002)	0.002	0.020
2 (Final)	70 (100%)	1.955 (0.025)	1.955 (0.025)	0.025	0.100

*Total number of subjects equals the number in 5FU arm plus observation arm.

10.6.3 Lost to follow-up (LTFU) rate

Since the LTFU rate in potential complete responders would affect the primary endpoint (see Section 10.7), the LTFU rate for each arm will be closely monitored by the study team. After 50% of the ITT population has completed assessment for the primary endpoint, a report will be sent to the DSMB documenting the LTFU rate by arm, as well as the response at any visit(s). When assessing this rate, all participants not providing evaluable endpoint data will be included. Consideration will be given to halting the study if the absolute LTFU rates differ by at least 15%, since a significant arm difference in the CR rate could be an artifact of differential LTFU rates.

10.7 Primary Analyses

The primary analysis population for efficacy and safety is an intent-to-treat (ITT) population, which consists of all participants who are randomized to 5-fluorouracil 5% or observation. The proportion of participants achieving complete response in the 5-fluorouracil 5%, and the proportion of participants with spontaneous regression in the observation arm will be calculated as the number achieving complete response or experiencing spontaneous regression through the primary endpoint visit (Week 20) divided by the number in the intent-to-treat population. For the treatment comparison (topical 5-

fluorouracil 5% vs. observation) the proportions will be compared across sites using stratified Mantel-Haenszel-Cochran tests at the one-sided 0.05 alpha level. This means that participants who drop out or do not provide evaluable data contribute to the analysis since they are included in the denominator, i.e., they are essentially treated as failures.

Since the imiquimod arm was stopped early, we will describe comparisons between imiquimod and observation using only the participants randomized to imiquimod and observation prior to closure of the imiquimod arm. As a secondary objective, imiquimod 2.5% and topical 5-fluorouracil 5% will be compared using a stratified Mantel-Haenszel-Cochran test at the two-sided 0.05 alpha level using participants randomized prior to the closure of the imiquimod arm.

The primary endpoint will include intra-anal HSIL present on cytology or histology. Perianal HSIL will be descriptively reported separately, as well as combined with the primary endpoint. In addition to the primary intent-to-treat analysis (which includes all randomized participants), we will perform a sensitivity analysis that treats participants who receive HSIL treatment other than randomized treatment (or observation participants that receive any treatment) prior to Week 20 as failures; participants who are lost to follow-up or inevaluable are also still treated as failures. We will also perform a per-protocol analysis using the subset of participants who were adherent to the protocol.

10.8 Secondary Analyses

To examine the tolerability and safety of the three arms, descriptive statistics for AEs will be computed. AEs will be summarized at the event level and subject level according to severity. AEs will be stratified according to those reported at or before Week 20 and after Week 20 since those in the observation arm have to option of being randomized to imiquimod or 5FU at Week 20. Proportions and their exact binomial 95% confidence intervals will be calculated. We will compute summary statistics for the amount of study drug taken by measuring the mass of study drug dispensed (weight of study drug container at baseline – weight of study drug at the end of treatment).

Similarly, we will assess for PR in two ways. We will compare between arms the number of quadrants with HSIL found on biopsies treating the response as an ordinal variable. The biopsy plan for Week 20 and 48 (see Appendix V) specifies that at least one biopsy be obtained from each quadrant. Biopsies used for eligibility will generally be obtained through routine care and will not necessarily have four quadrant biopsies, so we are not able to look at the within participant change in the number of quadrants with HSIL on biopsy. Therefore, this comparison between arms assumes that the extent of disease at enrollment is balanced between arms, which is reasonable given randomization. We will also compare the proportion of participants achieving CR or PR (as per Section 9) with imiquimod or 5FU as compared to observation only using similar approaches as the primary objective.

We will evaluate the effect of imiquimod and 5FU on persistence of HPV type specific infections. We will report the frequency and proportion of HPV types present at baseline that are no longer detected at Week 20. We will also report the frequency and proportion of new HPV infections detected at Week 20 that were not present at baseline. Proportions and their exact binomial 95% confidence intervals will be calculated.

HSIL outcomes at Week 44 will be analyzed similarly to those at Week 20. Results for the observation arm will be stratified into cross-over treatment groups.

To evaluate the relationship between smoking and sexual behaviors on treatment efficacy, tolerability, and HPV, regression models will be fit for each treatment arm. For treatment efficacy, a logistic regression model will be fit; for endpoints that are available over time, a generalized estimating equations (GEE) model may be fit. A model that incorporates treatment arm will be developed to assess the role of treatment on these endpoints in the presence of behavioral covariates.

10.9 AMC Policy for Monitoring of Phase III Trials

10.9.1 This study will be monitored by the AMC DSMB, as outlined in its Charter and as required by the NCI Data and Safety Monitoring Policy for Phase III investigations. The study will adhere to the AMC Data Safety Monitoring Plan (Appendix VIII).

10.9.2 Central Pathology Review

Slides from all biopsies used for eligibility and slides from all biopsies obtained post-randomization will be submitted for central review by a single pathologist. Any discrepancies of interpretation (normal, low-grade AIN, or high-grade AIN) will be referred for adjudication.

10.10 Publication of Research Findings

Publication of the results of this trial will be governed by AMC policies.

11.0 ETHICAL AND REGULATORY CONSIDERATIONS

11.1 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 Subjects 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 (AMC ODMC) must receive a copy of the letter of approval from the IRB, which specifically approves the protocol and informed consent, before subject enrollment. The IRB must also approve any significant changes to the protocol and documentation of this approval must be sent to the AMC ODMC. 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 AMC ODMC.

Records of all study review and approval documents must be kept on file by the Investigator and are subject 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 subject. The nature, significance, and risks associated with the study must be explained to the subject. 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 subject 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.

11.2 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/IEC/REB of the treating institution. A copy of the written approval of the IRB/IEC/REB must be sent to the ODMC.

11.3 Subject Confidentiality

In order to maintain subject privacy, all data capture records, drug accountability records, subject number. The Investigator will grant monitor(s) and auditor(s) from the AMC or the NCI and regulatory authorities (FDA) access to the subject's original medical records for verification of data gathered on the data capture records and to audit the data collection process. The subject's confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

11.4 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 subjects 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 subjects on this trial will reflect the constitution of the respective populations.

Table 11-A: Accrual targets

Racial Categories	**Ethnic Categories**				**Total**
	Not Hispanic or Latino		**Hispanic or Latino**		
	Female	**Male**	**Female**	**Male**	
American Indian/ Alaska Native	0	1	0	0	**1**
Asian	0	1	0	0	**1**
Native Hawaiian or Other Pacific Islander	0	1	0	0	**1**
Black or African American	3	17	1	4	**25**
White	5	57	3	17	**82**
More Than One Race	0	5	0	3	**8**
Total	**8**	**82**	**4**	**24**	**118**

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APPENDIX I: 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 II: SCHEDULE OF EVALUATIONS

Shaded visits should only be performed for those receiving study-provided topical treatments.

EVALUATIONS	Screening Visit -1	Visit 1 Week 0	Visit 2 Week 2	Visit 3 Week 8	Visit 4 Week 16	Visit 5 Week 20	Visit 6 Week 24	Visit 7 Week 26	Visit 8 Week 32	Visit 9 Week 40	Visit 10 Week 44	Premature d/c
Informed Consent	X											
HIV Documentation	X											
Complete Medical History	X											
Clinical Assessment[7]	X	X	X	X	X	X	X	X	X	X	X	X
Dispense Drug and Treatment Diary		X	X	X[4]			X[9]	X	X[4]			
Collect Diary / Adherence Assessment			X	X	X			X	X	X		X
Safety Bloodwork (CBC w diff, platelets)	X[1]											
CD4/CD8 (Counts + %)	X											
HIV-1 Plasma RNA	X											
Pregnancy Test	X	X	X	X	X	X	X	X	X	X	X	
Anal Biopsies	X			X[5]	X[5]	X			X[5]	X[5]	X	X
Anal Cytology	X					X					X	X
Anal Swab for HPV testing	X[6]			X	X	X			X	X	X	X
HRA	X	X		X[2]		X			X[2]		X	X
Optional anoscopy or HRA[8]			X[2]		X[2]			X[2]		X[2]		
Photo Documentation of Lesions		X		X	X	X	X		X	X	X	X
Smoking and Recent Sexual History Questionnaire		X				X					X	X
ACSR Donation	X[3]						X[3]					
Randomization		X					X[9]					

[1] At subsequent visits, routine laboratory testing and/or referral for testing for sexually transmitted infections should be conducted only if clinically indicated.
[2] See description in Appendix IV for modification to standard HRA procedure and criteria for biopsies at this visit with respect to the use of acetic acid. These HRA procedure modifications are for participants receiving study treatment only.
[3] Whole blood specimens to be collected at Visit -1 and Visit 6 (Week 24) for participants randomized at this visit, with participant consent for the ACSR donation.

[4] Cream will be weighed for adherence assessment and returned to the participant at Visits 3 and 8.
[5] Anal biopsies should only be performed at visits 3, 4, 8, and 9 if clinician notes any markers indicative of progression as listed in Section 6.4.1.
[6] If the anal swab for HPV testing is not collected at the screening visit, it can be collected at entry (Visit 1) prior to HRA.
[7] As defined per Section 6 for each visit.
[8] If participant can tolerate.
[9] Participants originally randomized to observation with HSIL at Week 20 may receive 5FU beginning at Week 24.

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 an anal swab specimen:

A synthetic 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 distal end of 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.

For anal HPV testing: A second swab should be obtained and placed in Digene STM.

Specimen packaging and shipment instructions are provided in the Manual of Procedures (MOP). Specimens will be sent to the AMC Biorepository for storage before batch shipment to the AMC HPV Genotyping Core Laboratory.

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. Biopsy abnormal appearing areas (colposcope-directed biopsies) clinically suspicious for HSIL; areas suspicious for LSIL may also be biopsied if more severe lesions are not seen. Local anesthetic (e.g., 1% lidocaine with or without epinephrine or .5% bupivacaine) may be used at the provider's discretion prior to biopsy.
8. Attain hemostasis prior to removal of the anoscope at the discretion of the provider (with pressure, Monsel's solution, or silver nitrate).
9. An external genital exam should be performed to note the presence of condyloma and other abnormalities.
10. Apply acetic acid for one minute to perianal area and examine carefully with colposcope.
11. Biopsy any external (perianal) areas clinically suspicious for HSIL, using a local anesthetic (e.g., 1% lidocaine with or without epinephrine or 0.5% bupivacaine) prior to biopsy. Perianal condyloma or LSIL can be biopsied at the discretion of the provider.
12. Participants with signs or symptoms consistent with proctitis or sexually transmitted infections other than HPV should be referred for appropriate diagnosis and treatment.

APPENDIX V: BIOPSY PLAN FOR AMC-088

Biopsies used for eligibility may be obtained through routine care. If performing baseline HRA though AMC-088, then every quadrant must be sampled for eligibility purposes. **HSIL lesions that were biopsied for clinical care prior to the visit may be used to document eligibility and will not require additional rebiopsy at screening** if performed within 60 days but not less than 1 week prior to enrollment. Subsequent study visits requiring biopsies will include 4-quadrant biopsies collected by a certified HRA-provider who is registered with CTEP as follows:

- The anal mucosa should be divided into four quadrants: Anterior, Left, Posterior and Right
- Lesions suspicious for HSIL should be identified and biopsied.
- For quadrants without a lesion suspicious for HSIL, any areas suggestive of LSIL, condyloma, squamous metaplasia, or areas of prior HSIL lesions present at baseline should be biopsied. If no such areas are present, then a biopsy of normal appearing mucosa should be biopsied.
- Please note that more than one biopsy can be obtained per quadrant if multiple HSIL lesions exist or if multiple biopsies of an extensive HSIL lesion are deemed necessary by the anoscopist.
- Distal anal and perianal lesions concerning for HSIL should be biopsied.
- For each anal biopsy obtained, the anoscopist should characterize the suspected result of the biopsy as invasive cancer, HSIL, LSIL or condyloma, squamous metaplasia or atypical squamous metaplasia, or normal mucosa.
- Biopsies from different quadrants must go in separate formalin containers, and get separate pathologic interpretations.

APPENDIX VI: ACSR SPECIMEN PREPARATION & SHIPPING INSTRUCTIONS

A. GENERAL

To ship blood specimens, use a diagnostic shipper approved for a volume of at least 30 cc. The use of the SAF-T-PAK STP 210 diagnostic cardboard shipper is recommended. These shippers may be ordered at the SAF-T-PAK website: www.saftpak.com. The following instructions below are for use with the recommended STP-210 shipper. If using another federally approved diagnostic shipper, please follow instructions provided for that specific shipper.

NOTE: Specimens MUST BE SHIPPED **Mondays** through **Thursdays** as an OVERNIGHT PRIORITY shipment. Specimens are **NOT ACCEPTED ON SATURDAYS OR SUNDAYS** in the ACSR.

B. SPECIMEN PREPARATION, PACKAGING, AND SHIPMENT

BLOOD SPECIMENS

Draw two 10 cc (mL) yellow top [acid citrate dextrose (ACD)] tubes from study patient. With a black, water resistant, sharpie pen, label each specimen with the following information:

- AMC Protocol #088
- AMC Participant ID#
- Date and time of collection
- Specimen type, i.e., WB=Whole Blood, P=Plasma, S=Serum, or Tissue
- Specimen purpose: Donation

<u>Specimen Shipment</u>

- Seal the tops of the two 10 cc yellow tops with parafilm.
- Place the two sealed tubes into bubble wrap (provided in STP-210 kit).
- Tape around the bubble wrap so that the roll stays together and the tubes cannot fall out or break.
- Place absorbent material sheet around the bubble wrapped tubes and slip into a biohazard poly-bag and "self-seal."
- Place poly-bag containing tubes into the white TYVEK bag and seal.
- Place the TYVEK bag into the STP-210 diagnostic cardboard shipper. Seal the cardboard shipper with clear packing/shipping tape.
- Affix the FED-EX airbill on blank side of the shipper making sure that it is marked "FED-EX PRIORITY OVERNIGHT."
- Mark "OTHER" in the airbill under "Packaging." Please use the FedEx # available on the AMC member's only website.

- Under airbill section “Special Handling” indicate “YES-SHIPPERS DECLARATION NOT REQUIRED.”
- Place “From/To” information onto areas provided on the shipper.

Blood specimens should be shipped by overnight express at room temperature to:

Jeff Bethony, PhD
George Washington University Medical Center
2300 I Street, NW
Washington, DC 20037
Tel: (202) 994-2663
Fax: (202) 994-5056
Email: jbethony@gwu.edu

- Make certain that shipper is already either pre-labeled with ‘UN#3373’ stamp, or make a paper label with ‘UN#3373” and affix it to the shipper.
- Make certain that the net volume of the specimen being shipped is written in the space provided on the shipper or make a separate label with the volume in mL and affix to the shipper.
- Affix airbill to shipper so that the ‘UN’ and ‘VOLUME’ labels are visible.
- RETAIN THE TOP COPY OF THE AIRWAY BILL FOR YOUR RECORDS.
- Place the box in the FedEx pickup area at your site or call to request a package pickup.

Please Note: The shippers will be mailed back to each AMC site.

INSTRUCTIONS FOR BLOOD SPECIMENS COLLECTED ON FRIDAY OR SATURDAY

Preparation of Plasma and Mononuclear Cells

Refer to the ACSR’s SOP on Separation of Plasma and Mononuclear Cells on the AMC Operations web site for instructions on preparing plasma and peripheral blood mononuclear cell (PBMC) aliquots. It is preferable that separation occurs as soon as possible. If necessary, whole blood in ACD (yellow top tubes) can be held at room temperature for no more than 24 hours.

Freeze the cell suspension in 0.5 mL aliquots in sterile NUNC vials by placing the NUNC tubes in a room temperature, alcohol saturated, control rate freezer container and store in the -80°C freezer overnight. Transfer the cell suspension into the liquid nitrogen temperature freezer for long-term storage the next working day.

*****PLEASE DOUBLE CHECK PACKAGING OF SHIPPER AND DO NOT DEVIATE FROM REQUESTED LABELING. Shipping frozen aliquots requires the use of packaging acceptable for dry ice and Class 9 label with weight of dry ice written on package.**

C. RECORD OF SPECIMENS

This study will track specimens via GlobalTrace[SM], a component of the AMC AdvantageEDC system. The GlobalTrace shipment manifest must accompany all specimen shipments.

APPENDIX VII: PATHOLOGY SPECIMENS

All pathology specimens will be reviewed by the designated pathologist on site. The pathology slides will be reviewed later at UCSF. The central pathology review will not be done in real time. Slides at each site may be sent in two batch shipments each year (every 6 months). For central pathology review, one hematoxylin and eosin (H&E) stained slide from each biopsy and a copy of the pathology report (all protected health information [PHI] redacted) should be sent to the following address:

Jeff Bethony, PhD
George Washington University Medical Center
2300 I Street, NW
Washington, DC 20037
Tel: (202) 994-2663
Fax: (202) 994-5056

Slide Labeling

Refer to MOP for biopsy and slide labeling and preparation requirements.

Shipping Instructions

1. Place the labeled slides into a specimen container in bubble wrap or other adequate cushioning. Use sturdy outer packaging to prevent breakage.
2. Affix the FedEx airbill on blank side of the shipper.
3. Mark "OTHER" in the airbill under "Packaging."
4. Under airbill section "Special Handling" indicate "YES-SHIPPERS DECLARATION NOT REQUIRED."
5. Enter FedEx account (available on the AMC members only website).
6. Place "From/To" information onto areas provided on the shipper. Specimens are accepted MONDAY through THURSDAY only. All specimens should be shipped by **FedEx 2-day service** to Dr. Darragh at the address listed above:
7. Make certain that shipper is visibly labeled "Exempt human specimen."
8. RETAIN THE TOP COPY OF THE AIRWAY BILL FOR YOUR RECORDS.
9. Place the box in the FedEx pickup area at your site or call to request a package pickup.

Record of Specimens

This study will track specimens via GlobalTrace^{SM}, a component of the AMC AdvantageEDC system. The GlobalTrace^{SM} shipment manifest must accompany all specimens.

Distribution of Slides

The AMC Biorepository will distribute slides received to Dr. Teresa Darragh for central pathology review upon receipt.

Teresa M. Darragh, MD
UCSF/Mt. Zion Medical Center
Dept. of Pathology, Box 1785

1600 Divisadero Street, Room B621
San Francisco, CA 94143
Tel: (415) 353-7861
Fax: (415) 353-7447

All slides will be returned to the site by the AMC Biorepository following central pathology review.

APPENDIX VIII: AMC DATA 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 RB, 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 IX: PARTICIPANT APPLICATION INSTRUCTIONS AND DIARY FOR IMIQUIMOD (ZYCLARA)

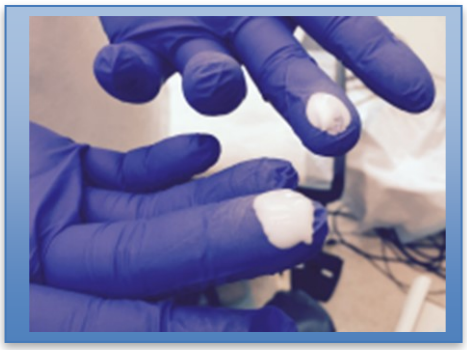
Instructions for Inserting Imiquimod 2.5% Cream

You have been randomized to receive topical Imiquimod cream once a day at night for 16 weeks. Please follow the directions below for the topical cream you have been given.

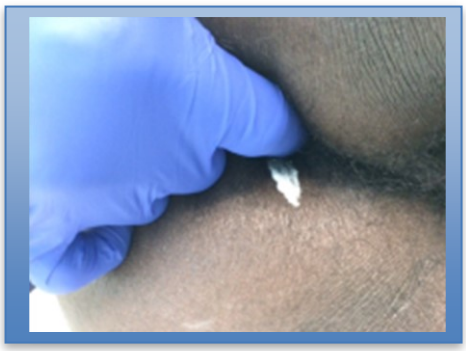
On weeks 1 and 2, you will apply one actuation (pump) daily and it will be divided as shown below; after two weeks this will be increased to two actuations, or one per side.

1. Imiquimod Pump

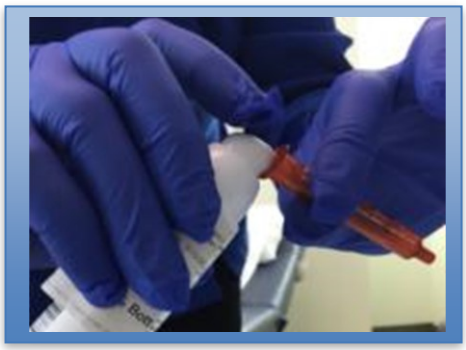
2. Apply one actuation (pump) to either right or left finger.



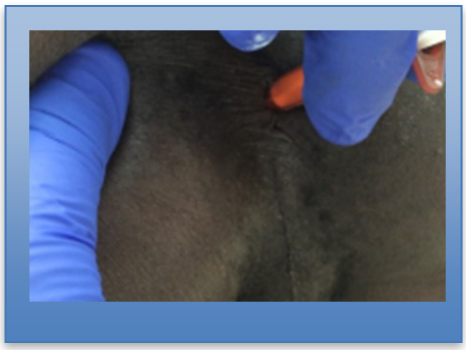
3. Divide this dose in half to one finger on the left and right hand.



4. Apply one finger inside the anus and spread the cream as far as you can reach from side to side. Repeat with opposite hand.



5. Alternatively, remove plunger from syringe and pump the cream into the top.



6. Insert left finger to the first joint and spread the cream evenly as far as you can reach; repeat on right side.

Repeat this once a day; at the two-week follow-up visit the clinician may instruct you to increase this to two actuations a day (one to each side). Inform the study staff of any severe side effects.

If instructed to also treat the outside (perianus) apply an additional actuation around the anal opening.

Record on the diary each application.

Inform the study staff of any severe side effects.

Store the bottle of Imiquimod upright at room temperature.

PROTOCOL AMC-088 IMIQUIMOD PARTICIPANT DIARY

2.5% TOPICAL IMIQUIMOD FOR TREATMENT OF HIGH-GRADE ANAL SQUAMOUS INTRAEPITHELIAL LESIONS IN HIV-INFECTED MEN AND WOMEN

Only the recipient of the study treatment should complete this card. Study personnel may write on this card to document their interview and clarification of information recorded by the participant. However, all primary information should be the subject's; all entries by study personnel should be dated and initialed. Study personnel who annotate this record should sign the last page of this document and print their name clearly.

Study cycle: 2 weeks

Today's date: _____/_____/_____

Subject ID: _____ - _____ - _____

STUDY PARTICIPANTS MUST BRING THIS COMPLETED DIARY CARD AND ALL STUDY DRUG CONTAINERS TO EACH STUDY VISIT DURING TREATMENT ADMINISTRATION.

Complete this card every day for the first 14 days of the study; day number 1 is _____You will complete this card until (_____/_____/_____) and return the card to the study site in person at your next visit.

You may receive a telephone call from the study nurse or coordinator to clarify information you have written. Feel free to write any concerns or questions in the boxes at the bottom of each page.

COORDINATORS: Distribute the applicable section(s) of the imiquimod diary(ies) at each treatment visit. Please ensure all pages of the diary are provided to the participant at each visit when the diary distributed. The diary(ies) **must** be collected at each visit.

Subject ID#: _____ - _____ - _____ Dates: ____ / ____ / _____ to ____ / ____ / _____

Starting on your office visit day, please record the date and time of the application of medication and any reactions that occur at the treatment site. Estimate the severity of the following reactions AT THE APPLICATION SITE using the following definitions:

Mild: An awareness of symptoms, easily tolerated

Moderate: Discomfort is enough to interfere with your usual daily activities.

Severe: Incapacitating and you are unable to participate in activities that you usually do.

Week	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Date (MM/DD/YY)	____/____/____	____/____/____	____/____/____	____/____/____	____/____/____	____/____/____	____/____/____
Applied?	☐ Yes ☐ 1 pump ☐ No ☐ 2 pumps	☐ Yes ☐ 1 pump ☐ No ☐ 2 pumps	☐ Yes ☐ 1 pump ☐ No ☐ 2 pumps	☐ Yes ☐ 1 pump ☐ No ☐ 2 pumps	☐ Yes ☐ 1 pump ☐ No ☐ 2 pumps	☐ Yes ☐ 1 pump ☐ No ☐ 2 pumps	☐ Yes ☐ 1 pump ☐ No ☐ 2 pumps
Pain or Tenderness	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe
Bleeding	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe
Other: _____	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe
Other: _____	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe
Comments							

Starting on your office visit day, please record the date and time of the application of medication and any reactions that occur at the treatment site. Estimate the severity of the following reactions AT THE APPLICATION SITE using the following definitions:

Mild: An awareness of symptoms, easily tolerated

Moderate: Discomfort is enough to interfere with your usual daily activities.

Severe: Incapacitating and you are unable to participate in activities that you usually do.

Week 2	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Date (MM/DD/YY)	____/____/____	____/____/____	____/____/____	____/____/____	____/____/____	____/____/____	____/____/____
Applied?	☐ Yes ☐ 1 pump ☐ No ☐ 2 pumps	☐ Yes ☐ 1 pump ☐ No ☐ 2 pumps	☐ Yes ☐ 1 pump ☐ No ☐ 2 pumps	☐ Yes ☐ 1 pump ☐ No ☐ 2 pumps	☐ Yes ☐ 1 pump ☐ No ☐ 2 pumps	☐ Yes ☐ 1 pump ☐ No ☐ 2 pumps	☐ Yes ☐ 1 pump ☐ No ☐ 2 pumps
Pain or Tenderness	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe
Bleeding	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe
Other: _____	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe
Other: _____	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe
Comments							

Subject ID#: _____ - _____ - _____ Dates: ____ / ____ / _____ to ____ / ____ / _____

Other Complaints or Illnesses	Date (month/day/year)		Severity
	Started	Last Present	
			☐ Mild ☐ Moderate ☐ Severe
			☐ Mild ☐ Moderate ☐ Severe
			☐ Mild ☐ Moderate ☐ Severe
			☐ Mild ☐ Moderate ☐ Severe
			☐ Mild ☐ Moderate ☐ Severe
			☐ Mild ☐ Moderate ☐ Severe
			☐ Mild ☐ Moderate ☐ Severe
			☐ Mild ☐ Moderate ☐ Severe

Comments or Concerns:

Please list any NEW prescription or over the counter medications or supplements that you took beginning on Day 1 and ending on Day 14 during this cycle. You do not have to list your HIV medications or any other drugs that you were taking regularly since the start of the study.

Name of Medication	Date (mm/dd/yy)		Maximum Daily Dose	Reason for Medication
	Started	Last Taken		

If during the 14 days you did not take any medications check the box here: □ None

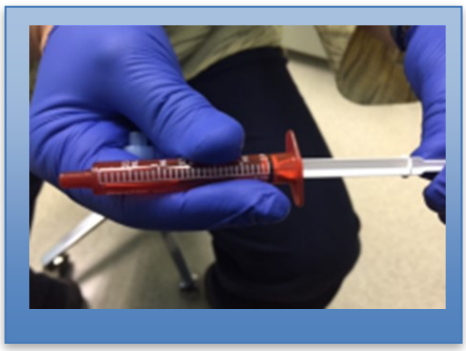
Comments or Concerns:

APPENDIX X: PARTICIPANT APPLICATION INSTRUCTIONS AND DIARY FOR EFUDEX (5-FLUOROURACIL 5%)

Instructions for Inserting 5-FU Cream

You have been randomized to receive topical 5-FU cream for 16 weeks. Please follow the directions below for the topical cream you have been given.

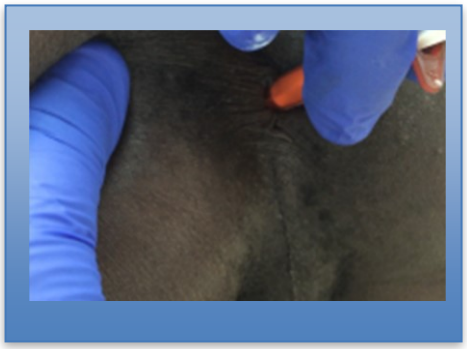
1. Open tube of 5-FU



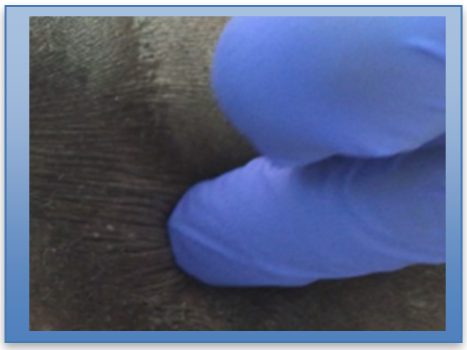
2. Remove plunger from applicator.

3. Insert opening of tube into top of applicator and squeeze a small amount into applicator.

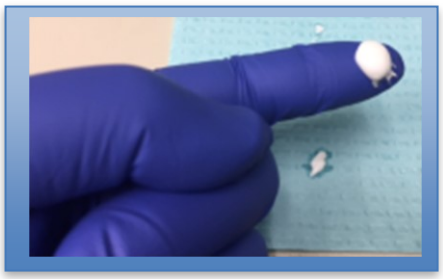
4. Re-insert the plunger until it reaches the .5ml mark on the applicator; any excess cream can be wiped off.



5. Find a comfortable position ex. squatting, lying on side, one leg raised, and insert the applicator into the anus approx. ½", then push the plunger until it stops.



6. Insert left finger to the first joint and spread the cream evenly as far as you can reach; repeat on right side.



7. Alternatively, place ½ the amount (or .25ml) on your left or right finger then follow instruction in #6.

8. Insert any remaining cream on the outside into the anus, then clean off any excess.

Repeat this procedure for 5 days, twice a day. Then stop for 9 days. Repeat this two week cycle for 16 weeks.

Inform the study staff if you have severe side effects.

PROTOCOL AMC-088 5-FU PARTICIPANT DIARY

5% TOPICAL 5-FU FOR TREATMENT OF HIGH-GRADE ANAL SQUAMOUS INTRAEPITHELIAL LESIONS IN HIV-INFECTED MEN AND WOMEN

Only the recipient of the study treatment should complete this card. Study personnel may write on this card to document their interview and clarification of information recorded by the participant. However, all primary information should be the subject's; all entries by study personnel should be dated and initialed. Study personnel who annotate this record should sign the last page of this document and print their name clearly.

Study cycle: 2 weeks

Today's date: _____/_____/_____

Subject ID: _____ - _____ - _____

Doses you should apply: _____

STUDY PARTICIPANTS MUST BRING THIS COMPLETED DIARY CARD AND ALL STUDY DRUG CONTAINERS TO EACH STUDY VISIT DURING TREATMENT ADMINISTRATION.

Complete this card every day for the first 14 days of the study; day number 1 is _____You will complete this card until (_____/_____/_____) and return the card to the study site in person at your next visit.

You may receive a telephone call from the study nurse or coordinator to clarify information you have written. Feel free to write any concerns or questions in the boxes at the bottom of each page.

COORDINATORS: Distribute the applicable section(s) of the imiquimod diaries at each treatment visit. Please ensure all pages of the diary are provided to the participant at each visit when the diary distributed. The diaries **must** be collected at each visit.

Starting on your office visit day, please record the date and time of the application of medication and any reactions that occur at the treatment site. Estimate the severity of the following reactions AT THE APPLICATION SITE using the following definitions:

Mild: An awareness of symptoms, easily tolerated

Moderate: Discomfort is enough to interfere with your usual daily activities.

Severe: Incapacitating and you are unable to participate in activities that you usually do.

Cycle ___ Week 1	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Date (MM/DD/YY)	____/____/____	____/____/____	____/____/____	____/____/____	____/____/____	____/____/____	____/____/____
Applied?	AM PM ☐ Yes ☐ Yes ☐ No ☐ No	AM PM ☐ Yes ☐ Yes ☐ No ☐ No	AM PM ☐ Yes ☐ Yes ☐ No ☐ No	AM PM ☐ Yes ☐ Yes ☐ No ☐ No	AM PM ☐ Yes ☐ Yes ☐ No ☐ No	Day Off	Day Off
Pain or Tenderness	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe
Bleeding	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe
Other: _____	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe
Other:	☐ None	☐ None	☐ None	☐ None	☐ None	☐ None	☐ None

_____	□ Mild □ Moderate □ Severe	□ Mild □ Moderate □ Severe	□ Mild □ Moderate □ Severe	□ Mild □ Moderate □ Severe	□ Mild □ Moderate □ Severe	□ Mild □ Moderate □ Severe	□ Mild □ Moderate □ Severe
Comments							

Starting on your office visit day, please record the date and time of the application of medication and any reactions that occur at the treatment site. Estimate the severity of the following reactions AT THE APPLICATION SITE using the following definitions:

Mild: An awareness of symptoms, easily tolerated

Moderate: Discomfort is enough to interfere with your usual daily activities.

Severe: Incapacitating and you are unable to participate in activities that you usually do.

Cycle ___ Week 2	**Day 8**	**Day 9**	**Day 10**	**Day 11**	**Day 12**	**Day 13**	**Day 14**
Date (MM/DD/YY)	____/____/____	____/____/____	____/____/____	____/____/____	____/____/____	____/____/____	____/____/____
Applied?	Day Off	Day Off	Day Off	Day Off	Day Off	Day Off	Day Off
Pain or Tenderness	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe
Bleeding	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe
Other: _____	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe	☐ None ☐ Mild ☐ Moderate ☐ Severe
Other: _____	☐ None ☐ Mild ☐ Moderate	☐ None ☐ Mild ☐ Moderate	☐ None ☐ Mild ☐ Moderate	☐ None ☐ Mild ☐ Moderate	☐ None ☐ Mild ☐ Moderate	☐ None ☐ Mild ☐ Moderate	☐ None ☐ Mild ☐ Moderate

	☐ Severe	☐ Severe	☐ Severe	☐ Severe	☐ Severe	☐ Severe	☐ Severe
Comments							

Other Complaints or Illnesses	Date (month/day/year)		Severity
	Started	Last Present	
			☐ Mild ☐ Moderate ☐ Severe
			☐ Mild ☐ Moderate ☐ Severe
			☐ Mild ☐ Moderate ☐ Severe
			☐ Mild ☐ Moderate ☐ Severe
			☐ Mild ☐ Moderate ☐ Severe
			☐ Mild ☐ Moderate ☐ Severe
			☐ Mild ☐ Moderate ☐ Severe
			☐ Mild ☐ Moderate ☐ Severe

Comments or Concerns:

Please list any NEW prescription or over the counter medications or supplements that you took beginning on Day 1 and ending on Day 14 during this cycle. You do not have to list your HIV medications or any other drugs that you were taking regularly since the start of the study.

Name of Medication	Date (mm/dd/yy)		Maximum Daily Dose	Reason for Medication
	Started	Last Taken		

If during the 14 days you did not take any medications check the box here: □ None				

Comments or Concerns:

APPENDIX XI: PROTOCOL AMC-088 SMOKING STATUS/ RECENT SEXUAL HISTORY QUESTIONNAIRE

Subject ID number: _____

Date completed: _____ Study Week number: _____

NOTE TO STAFF: Please answer question #1, then give to subject. It is preferable that the questionnaire be self-administered.

1. Was this questionnaire self-administered or interviewer-administered?

   ____ Self-administered

   ____ Interviewer administered

Thank you for taking this questionnaire. This form will ask about cigarette smoking. It will also ask about sex you may have had in the last 6 months.

2. How often do you currently smoke cigarettes? (Choose one)

   ____ Not at all (skip to question 4)

   ____ Some days

   ____ Every day

   ____ I prefer not to answer (skip to question 4)

3. On average over the past 6 months, how many cigarettes have you smoked per day? (1 pack equals 20 cigarettes)

   ____

The next few questions will ask about men or women you may have had sex with in the past 6 months. Let's review some words so that we agree on what we are talking about. By "having sex" we mean oral sex, anal sex, oral-anal sex, or vaginal sex.

ANAL SEX is when a man puts his penis into someone's rectum, anus, or butt.

ORAL SEX is when someone puts his or her mouth or tongue to a woman's vagina or clitoris or on a man's penis.

ORAL/ANAL SEX is when someone puts his or her mouth or tongue to someone's anus (also known as rimming).

VAGINAL SEX is when a man puts his penis in a woman's vagina.

1. Have you ever had sex with a man (even only once)?

   ____ Yes

   ____ No (skip to question 8)

   ____ I prefer not to answer (skip to question 8)

2. Have you ever had sex by having a penis inserted into your anus (even only once)?

____ Yes

____ No

____ I prefer not to answer

3. Have you had sex of any kind with a man in the past 6 months?

____ Yes

____ No (skip to question 8)

____ I prefer not to answer (skip to question 8)

4. How many men have you had sex with in the past 6 months?

____ 1

____ 2 to 5

____ 6 to 10

____ More than 10

5. With how many men have you had receptive anal sex with in the past 6 months (i.e. have sex by putting their penis into your anus)?

____ 0 (skip to question 7)

____ 1

____ 2 to 5

____ 6 to 10

____ More than 10

6. How often did your sex partner use a condom during receptive anal sex?

____ Every time (100%)

____ Mostly

____ Half the time

____ Occasionally

____ Never (0%)

7. In the past 6 months, how many men put their mouth or tongue on your anus?

____0

____1

____2 to 5

____6 to 10

____More than 10

8. In the past 6 months, have you had sex with a woman?

____Yes

____No (skip to the end of the questionnair**e)**

____I prefer not to answer (skip to the end of the questionnaire)

9. In the past 6 months, how many women put their mouth or tongue on your anus?

____ 0

____ 1

____2 to 5

____ 6 to 10

____More than 10

Thank you for taking this questionnaire!