SUMMARY OF CHANGES

A Phase 2 Evaluation of VGX-3100, a Synthetic DNA Immunotherapy Targeting Human Papillomavirus 16 and 18 E6 and E7 Proteins, for Anal High-Grade Squamous Intraepithelial Lesions (HSIL) in HIV-Positive Individuals

Version 8.0

NCI Protocol #: AMC-103
Local Protocol #: AMC-103

NCI Version Date: 08APR2022
Protocol Date: 08APR2022

I. Scientific and Substantive Changes

#	Section	Comments
1.	3.2.10	The exclusion criterion for the presence of acute or chronic bleeding or clotting disorders was modified to clarify that over-the-counter aspirin and non-steroidal anti-inflammatory drugs are permitted. The modification also give investigator more leeway on enrolling participants on blood thinners.
2.	7.0	Added guidance text regarding patients rescheduling injection visits as well as requirements for timeframe between injections in an effort to provide consistent guidance to sites in the event of missed visits.

II. Administrative and Editorial Changes

#	Section	Comments
3.	Global	The version number has been updated to version 8.0 and the date has been updated to 08APR2022.
4.	Global	Hyperlinks have been added to references, and missing in-text references to tables and figures have been added globally.
5.	Global	Grammatical and editorial corrections have been applied throughout the document.
6.	Global	Updated email contact information from amcpm@emmes.com to amc-103-emmes.com
7.	AMC-103 Medical Monitor Approval	Removed Inovio medical monitor signature page

#	Section	Comments
8.	Protocol Roster	Shelley Lensing was replaced by Himanshu Joshi as the lead statistician. Second protocol statistician, Mayuri Jain, added to roster.
9.	Participating centers	Laser Surgery Care Center and Weill Cornell Medical College have been added to the list of participating centers. University of Miami has been removed as a participating site.
10.	Abbreviations	The list of abbreviations has been updated, and abbreviations in-text have been revised.
11.	Abbreviations 7.1.3 7.2.2 7.3.2 7.5.2 7.7.2 7.10.2 7.12.2 7.13 Appendix I	Urinalysis was removed from safety laboratory testing as it was found to be low yield in terms of identifying toxicity. Urinalysis results were likely to have low impact on participant safety.
12.	4.2	A timeframe for data upload to Inovio was added along with more specific instructions on required file type, the site URL, and back-up email address.
13.	6.3.2	Added language to clarify that arrays past expiration should not be used in patient treatment.
14.	Appendix IX	Revised wording of "pathology" specimens and slides to "biopsy" specimens and slides. Added clarification in the event of a discrepancy between local and central pathology reviews.

AIDS MALIGNANCY CONSORTIUM

AMC PROTOCOL #103:

A Phase 2 Evaluation of VGX-3100, a Synthetic DNA Immunotherapy Targeting Human Papillomavirus 16 and 18 E6 and E7 Proteins, for Anal High-Grade Squamous Intraepithelial Lesions (HSIL) in HIV-Positive Individuals

A Trial of the AIDS Malignancy Consortium (AMC)

Sponsored by:	National Cancer Institute Office of HIV and AIDS Malignancy (OHAM)
NCT Registration Number:	NCT03603808
Pharmaceutical Support Provided by:	Inovio Pharmaceuticals, Inc. Inovio Protocol Number: 04105
Investigational Agents Provided by Inovio Pharmaceuticals:	VGX-3100 (NSC 797774) CELLECTRA® 5PSP Device
IND#	BB-IND #18450 IND Sponsor: Chia-Ching Jackie Wang, MD
Protocol Chair:	Chia-Ching Jackie Wang, MD
Protocol Co-Chair:	Joel Palefsky, MD

Version 8.0, 08APR2022
NCI Version Date 08APR2022

AMC INVESTIGATOR PROTOCOL SIGNATURE PAGE

I, _____, Principal Investigator at site _____, have read and agree to conduct and follow this protocol, **AMC Protocol #103 – A Phase 2 Evaluation of VGX-3100, a Synthetic DNA Immunotherapy Targeting Human Papillomavirus 16 and 18 E6 and E7 Proteins, for Anal High-Grade Squamous Intraepithelial Lesions (HSIL) in HIV-Positive Individuals (Version 8.0, 08APR2022),** as written according to AMC, NCI, FDA, ICH/GCP, and ISO guidelines. I understand that this protocol must be reviewed by the Institutional Review Board or Independent Ethics Committee overseeing the conduct of the trial and approved or given favorable opinion before implementation. 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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PROTOCOL ROSTER

AMC Protocol #103
A Phase 2 Evaluation of VGX-3100, a Synthetic DNA Immunotherapy Targeting Human Papillomavirus 16 and 18 E6 and E7 Proteins, for Anal High-Grade Squamous Intraepithelial Lesions (HSIL) in HIV-Positive Individuals

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Participation is planned at the above-mentioned centers. Additional sites may participate with the protocol chair and Inovio's approval.

Contact information is subject to change. For current contact information for clinical sites, please see the protocol roster in the password-protected section of the AMC Operations web site at www.AIDSCancer.org.

PROTOCOL SYNOPSIS

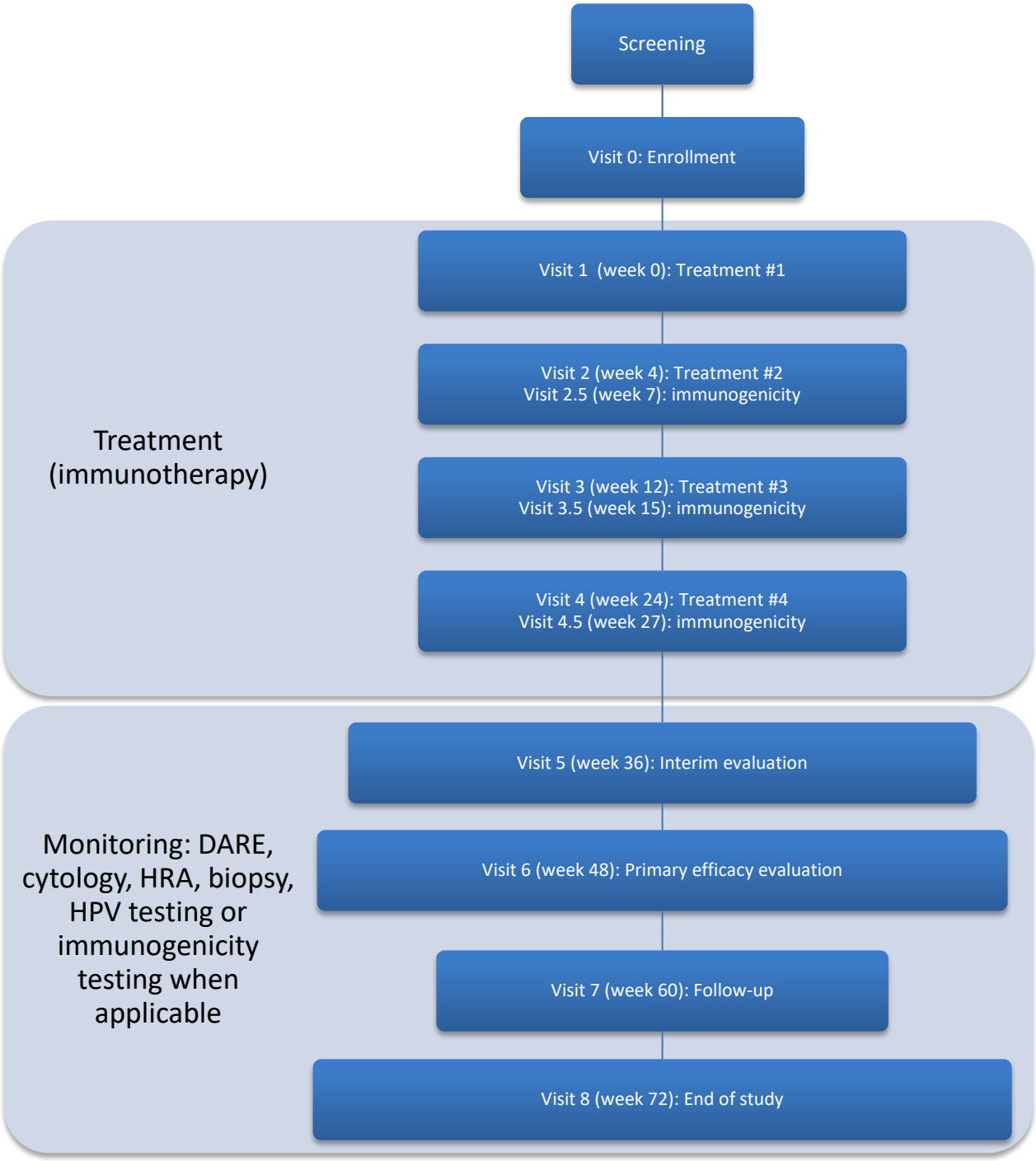
Title:	A Phase 2 Evaluation of VGX-3100, a Synthetic DNA Immunotherapy Targeting Human Papillomavirus 16 and 18 E6 and E7 Proteins, for Anal High-Grade Squamous Intraepithelial Lesions (HSIL) in HIV-Positive Individuals
Phase of Study:	Phase II
Accrual Target:	Minimum 35 participants, maximum 92 participants
Population:	HIV-positive participants with HPV-16 and/or-18 positive anal HSIL (defined as AIN2 with a positive p16 stain, AIN2-3, or AIN3 on histological specimen). Participants will be excluded if they have a history of HPV-associated cancer.
Regimen:	At study week 0, 4, 12, and 24, participants will receive 6 mg VGX-3100 (3 mg plasmid targeting HPV-16 E6 and E7, and 3 mg plasmid targeting HPV-18 E6 and E7) administered intramuscularly in 1 mL, followed by electroporation using the CELLECTRA®-5PSP (Inovio Pharmaceuticals, Inc.) constant current device. All participants will be monitored for safety and response for 48 weeks after the first injection. At that visit, participants with residual HSIL will be offered standard of care treatment or the option to be monitored for delayed response through 72 weeks after the first injection.
Anticipated Trial Duration:	The total study duration is estimated to be 3 years
Primary Objective:	To determine the proportion of participants with HPV-16 and/or HPV-18-positive anal HSIL that achieve either complete or partial response (which is defined as histopathological regression from HSIL to LSIL or normal) at 48 weeks after the first dose of VGX-3100
Secondary Objectives:	1. To determine the safety and tolerability as assessed by Common Terminology Criteria for Adverse Events version 5.0 (CTCAE v5.0) 2. To determine the proportion of participants with HPV-16 and/or HPV-18-positive anal HSIL who achieve complete response (which is defined as histopathological regression from HSIL to normal) at 48 weeks after the first dose of VGX-3100

3. To determine proportion of participants who clear HPV-16 and/or HPV-18 (defined as changing from presence to absence of HPV-16 and/or 18 by anal histological specimen) at 48 weeks after the first dose of VGX-3100
4. To determine proportion of participants who clear HPV-16 and/or HPV-18 (defined as changing from presence to absence of HPV-16 and/or 18 by anal swab) at 48 weeks after the first dose of VGX-3100
5. To compare the proportion of participants with HPV-16 and/or HPV-18-positive anal HSIL who achieve either complete or partial response (which is defined as histopathological regression from HSIL to LSIL or normal) versus those who do not at 72 weeks after the first dose of VGX-3100

Exploratory Objectives:

- To determine the proportion of non-HPV-16 or HPV-18-positive anal HSIL lesions that achieve either complete or partial response (which is defined as histopathological regression from HSIL to LSIL or normal) at 48 weeks after the first dose of VGX-3100
- To determine the T-cell response to VGX-3100 as measured by IFN-γ ELISpot, flow cytometric assessments, and T-cell infiltration into anal mucosal tissue
- To determine the antibody response to VGX-3100 as measured by enzyme-linked immunosorbent assay (ELISA) against HPV16 E7 and HPV18 E7 target antigens
- To determine the association of the addition of a fourth dose of VGX-3100 with T-cell and antibody responses
- To determine the association of VGX-3100 immune response with CD4+ lymphocyte count over time
- To determine the association of VGX-3100 immune response with HIV-1 RNA over time
- To determine if CD4 + lymphocyte count affects the overall or complete response rate at 48 weeks after the first dose of VGX-3100
- To assess the effect of tissue PD-L1 expression and T-cell infiltration on clinical benefit

PROTOCOL SCHEMA



LIST OF ABBREVIATIONS

ACSR	AIDS and Cancer Specimen Resource
ACD	Acid citrate dextrose
AE	Adverse event
AESI	Adverse events of special interest
AIDS	Acquired immunodeficiency syndrome
AIN	Anal intraepithelial neoplasia
AMC	AIDS Malignancy Consortium
AML	Acute myelocytic leukemia
ANC	Absolute neutrophil count
ART	Antiretroviral therapy
CBC	Complete blood count
CDC	Centers for Disease Control and Prevention
CDUS	Clinical Data Update System
CIN	Cervical intraepithelial neoplasia
CMP	Comprehensive metabolic panel
CR	Complete response
CRF	Case report form
CTEP	Cancer Therapy Evaluation Program
CTEP-AERS	Cancer Therapy Evaluation Program Adverse Event Reporting System
CV	curriculum vitae
DARE	Digital Anorectal Exam
DARF	Drug accountability record form
DHHS	Department of Health and Human Services
DNA	Deoxyribonucleic acid
DSMB	Data and Safety Monitoring Board
ECOG	Eastern Cooperative Oncology Group
ELISA	Enzyme-linked Immunosorbent Assay
EMLA	Eutectic Mixture of Local Anesthetics
EP	Electroporation
FDA	Food and Drug Administration
FTP	File transfer protocol
GUI	Graphical user interface
HIV	Human immunodeficiency virus
HPV	Human papillomavirus

HRA	High resolution anoscopy
HSIL	High-grade squamous intraepithelial lesions
IEC	Institutional ethics committee
IFN-γ	Interferon-gamma
IM	Intramuscular
IBC	Institutional biosafety committee
IRB	Institutional review board
IRC	Infrared coagulation
IND	Investigational new drug
IP	Investigational product
LEEP	Loop electroexcision procedure
LSIL	Low-grade squamous intraepithelial lesions
KPS	Karnofsky Performance Score
MDS	Myelodysplastic syndrome
MSM	Men who have sex with men
NCI	National Cancer Institute
NSAIDs	Non-steroidal anti-inflammatory drugs
ODMC	Operations and data management center
PCR	Polymerase chain reaction
PD-L1	Programmed death-ligand 1
PDC	Participant diary card
PR	Partial response
REB	Research ethics board
RNA	Ribonucleic acid
SAE	Serious adverse event
SCC	Squamous cell carcinoma
SIL	Squamous intraepithelial lesion
SOP	Standard Operating Procedure
TNF	Tumor necrosis factor
UADE	Unanticipated Adverse Device Effect
VIN	Vulvar intraepithelial lesions

1.0 OBJECTIVES

1.1 Primary Objective

To determine the proportion of participants with HPV-16 and/or HPV-18-positive anal HSIL that achieve either complete or partial response (which is defined as histopathological regression from HSIL to LSIL or normal) at 48 weeks after the first dose of VGX-3100

1.2 Secondary Objectives

1.2.1 To determine the safety and tolerability as assessed by Common Terminology Criteria for Adverse Events version 5.0 (CTCAE v5.0)

1.2.2 To determine the proportion of participants with HPV-16 and/or HPV-18-positive anal HSIL that achieve complete response (which is defined as histopathological regression from HSIL to normal) at 48 weeks after the first dose of VGX-3100

1.2.3 To determine the proportion of participants who clear HPV-16 and/or HPV-18 (defined as changing from presence to absence of HPV-16 or 18 by anal histological specimen) at 48 weeks after the first dose of VGX-3100

1.2.4 To determine proportion of participants who clear HPV-16 and/or HPV-18 (defined as changing from presence to absence of HPV-16 and/or 18 by anal swab) at 48 weeks after the first dose of VGX-3100

1.2.5 To compare the proportion of participants with HPV-16 and/or HPV-18-positive anal HSIL who achieve either complete or partial response (which is defined as histopathological regression from HSIL to LSIL or normal) versus those who do not at 72 weeks after the first dose of VGX-3100

1.3 Exploratory Objectives

1.3.1 To determine the proportion of non-HPV-16 or HPV-18-positive anal HSIL lesions that achieve either complete or partial response (which is defined as histopathological regression from HSIL to LSIL or normal) at 48 weeks after the first dose of VGX-3100

1.3.2 To determine the T-cell response to VGX-3100 as measured by IFN-γ ELISpot, flow cytometric assessments, and T-cell infiltration into anal mucosal tissue

1.3.3 To determine the antibody response to VGX-3100 as measured by enzyme-linked immunosorbent assay (ELISA) against HPV16 E7 and HPV18 E7 target antigens

1.3.4 To determine the association of the addition of a fourth dose of VGX-3100 with T-cell and antibody responses

1.3.5 To determine the association of VGX-3100 immune response with CD4+ lymphocyte count over time

1.3.6 To determine the association of VGX-3100 immune response with HIV-1 RNA over time

1.3.7 To determine if CD4 + lymphocyte count affects the overall or complete response rate at 48 weeks after the first dose of VGX-3100

1.3.8 To assess the effect of tissue PD-L1 expression and T-cell infiltration on clinical benefit

2.0 BACKGROUND

2.1 HPV and Anal HSIL

2.1.1 Anal cancer, precancerous lesions, and the relationship to human papillomavirus

Squamous cell carcinoma of the anus (SCCA) is an uncommon cancer in the general population, but its incidence is considerably increased among men who have sex with men (MSM) [1]. The risk of SCCA among HIV-positive MSM has continued to increase since the introduction of highly active antiretroviral therapy (HAART) [2]. Currently, rates of SCCA in HIV-positive MSM are higher than cervical cancer reported anywhere in the world [3]. This trend is likely to continue with the aging of the HIV-positive population. Persistent high-risk human papillomavirus (HPV) infection is the causal link to the vast majority of SCCA among HIV-positive individuals [4]. Current data support the 2-tiered system of classifying HPV-associated lesions as anal low-grade squamous intraepithelial lesions (LSIL) and anal high-grade squamous intraepithelial lesion (HSIL). Anal LSIL includes condyloma and anal intraepithelial neoplasia 1 (AIN1) and are not considered to be precancerous. In contrast, anal HSIL includes p16-positive AIN 2/3, which are considered to be true precursors to cancer [5].

Screening for and treating anal HSIL prior to progression to SCCA is likely to be the most effective approach for individuals who have already been exposed to HPV and who have developed HPV-associated precancerous lesions. Compared with removal of cervical lesions using loop electroexcision procedure, complete eradication of HSIL can be comparatively difficult because a high proportion of anal lesions are large and multifocal, especially in HIV-positive individuals [6]. Recurrences of anal HSIL are common post-treatment and vary widely from 22-79% in HIV-positive individuals, depending on treatment and length of follow-up [7-10]. There are limited therapeutic options for the treatment of anal HSIL. Infrared coagulation (IRC) is an effective ablative 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, 11, 12]. Ablation is also inappropriate for extensive circumferential disease. Targeted therapeutic approach against HPV is clearly needed.

2.1.2 Targeting HPV-16/18 as a therapeutic approach

Prophylactic vaccines provide protection against HPV-16 and HPV-18, the genotypes that cause 70% of cervical cancer and 90% of anal cancer. However, the uptake of prophylactic HPV vaccines has been disappointing, leaving many women and men at risk [13]. Fortunately, many HPV types respond to therapeutic agents based upon HPV-16 E7. A phase 2 study, conducted by the New York Phase 2 Consortium, immunized 31 healthy women with biopsy-proven cervical HSIL with three, monthly subcutaneous vaccinations with 500 mcg of SGN-00101 [Heat Shock Fusion Protein-Based Immunotherapy (HspE7)] [14]. 32% (10/31) had a complete pathologic response; 39% (12/31) had a partial response and 29% (9/31) had stable disease. The overall response rate was 71% (22/31, 95% CI=55-87%). No patient progressed. 55% (17/31) were HPV-16 positive prior to vaccination and one patient had HPV-16

subsequently detected. These results suggest cervical cancer patients (not just those who are HPV-16 positive) would benefit from the vaccine targeted at HPV-16 E7.

Available evidence also supports the idea that HPV epitopes are sufficient similar to allow efficacy in other HPV types. Research completed by Nventa, formerly Stressgen, reports extremely high efficacy using the HPV-16 E7 antigen even in non-HPV-16 positive patients; which demonstrates the antigen has immunotherapeutic activity across HPV types [15]. Davidson et al. reported cross reactivity to the E7 antigen and responses in patients with other HPV types in a trial of a vaccinia based HPV-16 E7 vector tested in the clinical treatment of vulvar HSIL [16]. Luxton et al. reported that 22% of responding women treated with HPV-16 E7 peptide epitopes for the treatment of cervical dysplasia or neoplasia were negative for HPV-16 DNA [17]. Selvy et al. reported that B cell epitopes of HPV-16 E7 are cross-reactive with HPV-18 [18]. Krchnak et al found while investigating HPV-16 E7 epitopes that of the nine overlapping peptides that were made to map the immunogenic domains, some epitopes were type-specific and others were cross-reactive with other HPV types [19]. Additional support for the cross reactivity of HPV-16 E7 with other HPV types has been demonstrated in human HLA-A2 transgenic mice [20]. It further appears that certain HPV-16 E7 epitopes are conserved across genera, as Nilges et al. demonstrated that "cross reactivity represents the inherent nature of the T-cell repertoire" with cross reactivity between HPV-16 E7 and coronavirus protein OC43 NS2 [21].

2.1.3 Electroporation

VGX-3100 is delivered using the CELLECTRA® *in vivo* electroporation (EP) device. EP is a physical method of tissue transfection whereby the generation of short, controlled electrical pulses creates a localized electric field at the injection site of the DNA plasmid which increases cell membrane permeability and improves the transfection of DNA and subsequent immunogenicity [22, 23]. EP is believed to increase the uptake and processing of plasmid DNA, thereby enabling increased expression and enhanced immunogenicity of the treatment by 10 to 100-fold [24, 25]. This technology has been used for more than three decades by molecular biologists for cell transfection and has demonstrated versatility in use, as shown by its functionality in combination with a range of molecules, tissue types, disease indications, and across species [26].

The CELLECTRA device developed by Inovio is used for investigational purposes to administer plasmid-based DNA, in both prophylactic and therapeutic settings. Following successful proof-of-concept studies in animals, Inovio has optimized both pulse pattern and voltage to increase transfection efficiency, and has demonstrated that the device helps to elicit favorable immunogenicity in several species (e.g., mice, pigs, and rhesus macaques) [27, 28].

2.2 Study Agents

2.2.1 Investigational Agent: VGX-3100

Investigational product (IP) is defined as a pharmaceutical form of an active ingredient being tested or used as a reference in the study, whether blinded or unblinded. The active formulations to be used in this study are described in Table 2-A. VGX-3100 will be provided by Inovio Pharmaceuticals, Inc., or its designee.

Table 2-A: Formulation of VGX-3100

Product	Formulation	Dose
VGX-3100	6 mg (1:1 mix of SynCon™ HPV-16 E6/E7 and HPV-18 E6/E7 plasmids) in 150 mM sodium chloride and 15 mM sodium citrate	1 mL

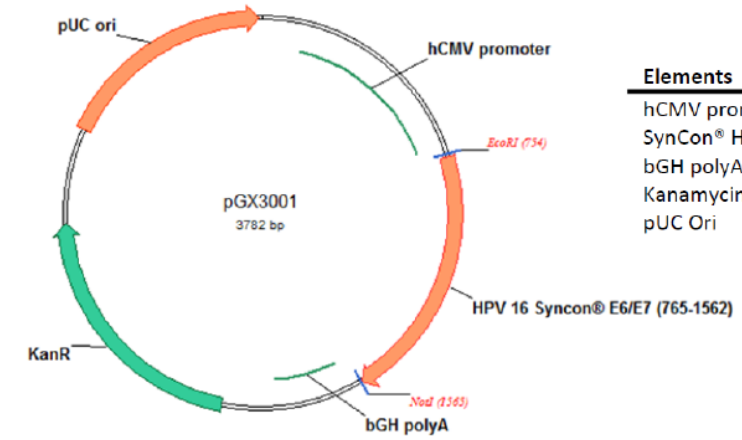
The VGX-3100 drug product contains DNA plasmids for expression of HPV-16 E6/E7 (pGX3001) and HPV-18 E6/E7 (pGX3002) antigens that have been designed and constructed using proprietary synthetic consensus DNA (SynCon®) technology. The drug product will be presented in clear glass cartridges and will be injected intramuscularly (IM).

The backbone of pGX3001 and pGX3002 is the expression vector pGX0001 under the control of the cytomegalovirus (CMV) immediate-early promoter. pGX3001 is a DNA plasmid encoding HPV-16 consensus E6 and E7 antigens, driven by the hCMV promoter with the bovine growth hormone 3'end and bGH polyA signal. pGX3002 is a DNA plasmid encoding HPV-18 consensus E6 and E7 antigens, driven by the hCMV promoter with the bovine growth hormone 3'end and bGH polyA signal (see Figure 2-A).

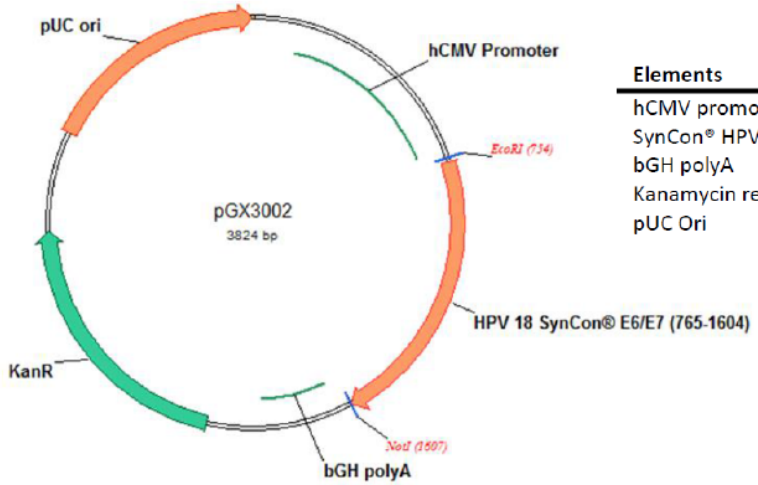
Fermentation and purification of the bulk plasmids is performed in batch mode. E. coli containing a specific plasmid are grown from working seed, fermented to high density, and harvested. The bacteria are then lysed to release their contents, including the plasmid, into solution. The lysate is subjected to three significant purification steps: solid/liquid separation, anion exchange chromatography, and hydrophobic interaction chromatography. The purified plasmid is concentrated and desalted by ultrafiltration/diafiltration using water for injection, filtered through a 0.2-µm sterilizing filter into bulk containers, and placed into frozen storage.

Drug product manufacturing consists of thawing and pooling the appropriate number and type of drug substance containers to create separate SynCon HPV-16 E6/E7 and HPV-18 E6/E7 plasmid pools. The individual plasmid pools are combined in a 1:1 ratio (w/w) and the concentration is adjusted with excipient solution. This final solution is then sterilized via filtration through redundant 0.2-µm filters and filled into glass cartridges.

Figure 2-A: DNA plasmids for expression of HPV-16 E6/E7 and HPV-18 E6/E7



Elements	Base Pairs
hCMV promoter	137–724
SynCon® HPV16 E6 & E7 coding sequence	765–1562
bGH polyA	1613–1837
Kanamycin resistance gene	2010–2804
pUC Ori	3103–3776



Elements	Base Pairs
hCMV promoter	137–724
SynCon® HPV18 E6 & E7 coding sequence	765–1604
bGH polyA	1655–1879
Kanamycin resistance gene	2052–2846
pUC Ori	3145–3818

Non-clinical development of VGX-3100

VGX-3100 focuses on the HPV-16 and HPV-18 proteins E6 and E7 for therapeutic application. They represent tumor-specific antigens in HPV-associated carcinomas and pre-malignant HPV-transformed cells. The integration of E6 and E7 genes into the host cell genome may lead to constitutive over-expression of E6 and E7 proteins, mediating the transformation of the cells to a malignant phenotype [29]. These genes are also required for the maintenance of the transformed phenotype. As a consequence, E6 and E7 are ideal immunotherapeutic targets to induce cellular immune responses against HPV-transformed cells [30].

Clinical development of VGX-3100

The Phase 1 program for VGX-3100 (water for injection [WFI] + low molecular weight poly-L-glutamate [LGS] formulation) and CELLECTRA™ was comprised of two clinical studies, HPV-001 and HPV-002 which enrolled adult women post-surgical or ablative treatment of grade CIN2/3. HPV-001 was an open-label dose-escalation study to evaluate the safety, tolerability, and immunogenicity of three

doses of VGX-3100 followed by EP with CELLECTRA at Day 0, Week 4 and Week 12. HPV-002 was designed to evaluate the safety and tolerability of a fourth 6 mg booster dose of VGX-3100 administered by CELLECTRA at least 6 months after the third dose of VGX-3100 from HPV-001. The Phase 1 clinical trials demonstrated that VGX-3100 followed by EP was generally safe, well-tolerated, and capable of eliciting robust humoral and cell mediated antigen-specific immune responses [31].

VGX-3100 (WFI+LGS formulation) + EP was evaluated in HPV-003, entitled "Phase 2 placebo-controlled study of VGX-3100 (HPV-16 E6/E7, HPV-18 E6/E7 DNA vaccine) delivered IM followed by electroporation (EP) with CELLECTRA-5P for the treatment of biopsy-proven CIN2/3 or CIN3 with documented HPV-16 or 18." This trial was designed to evaluate the histopathologic response to VGX-3100, administered in three 6 mg doses + EP in adult females with biopsy-proven HPV-16 or HPV-18 associated CIN2/3 or CIN3. The primary endpoint was clearance of a CIN2 or CIN3 lesion to CIN1 or less, 6 months following the third and final treatment. The safety of VGX-3100 was assessed over 88 weeks. Participants in this trial were women aged 18-55 years at trial entry and met inclusion/exclusion criteria as defined in the protocol. 169 women were randomized to achieve 148 evaluable subjects. The trial allocation was a 3:1 double-blind randomization (VGX-3100: placebo) followed by EP. Subjects were randomized to receive either 6 mg VGX-3100 or placebo (sterile water for injection) followed by EP three times during the study (at Day 0, Week 4, and 12). All subjects were to undergo repeat cervical biopsy or LEEP of the cervix at Week 36 to assess efficacy. In the modified intention-to-treat analysis 55 (48.2%) of 114 VGX-3100 recipients and 12 (30.0%) of 40 placebo recipients had histopathological regression (percentage point difference 18.2 [95% CI 1.3-34.4]; p=0.034). Treatment with VGX-3100 was well-tolerated. The most common adverse events were administration site reactions although only injection site erythema had a statistically higher incidence in the VGX-3100 group than in the placebo group (24/42, 57.1%) within the 28-day period after a dose (percentage point difference 21.3, 95% CI 5.3–37.8; p=0.007). Other common adverse events included fatigue, headache, myalgia, malaise, nausea, and arthralgia. No abnormalities were observed in creatine phosphokinase concentrations or electrocardiograms s in the first 40 patients enrolled. There was no difference in tolerability between VGX-3100 and placebo treatment groups [32].

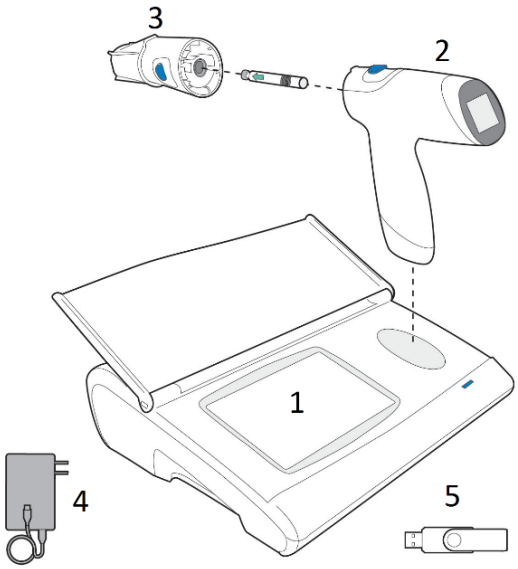
Toxicology

It is not known whether VGX-3100 is excreted in human milk. Because of the unknown potential for serious adverse drug reactions in nursing infants, investigational product should not be administered to nursing mothers.

2.3 Investigational Device

The investigational product will be delivered using the CELLECTRA 5PSP device. The device consists of five (5) main components (see Figure 2-B).

Figure 2-B: CELLECTRA® 5PSP base station with handset



1) 5PSP Base Station which serves as a charging dock for the Handset and can accept limited data inputs as well as store records.

2) 5PSP Handset, a reusable handset which is battery powered and delivers the electroporation pulse pattern. The Handset accepts the disposable array.

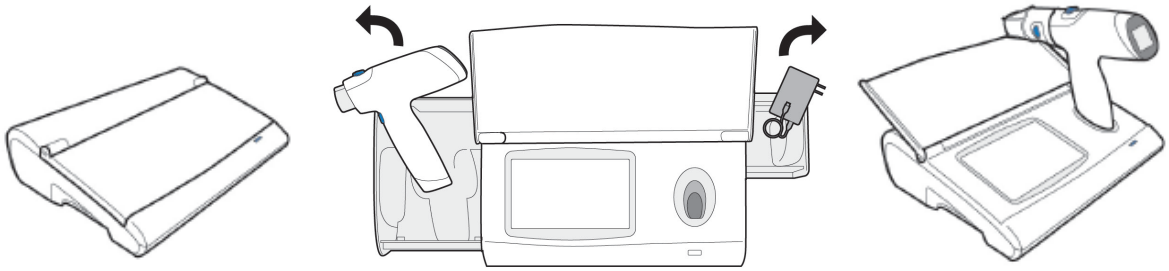
3) 5PSP Sterile Single Use Array which consists of five (5) needle-electrodes molded into a plastic housing that also accepts the (shipped separately) Drug Cartridge. The array accepts a standard, commercially available glass cartridge.

4) USB International Power Supply

5) Flash Drive

The base station communicates instructions, alerts, and error messages to the user through a touch screen graphical user interface (GUI). It also accepts data inputs from the user, e.g., patient identification code, height, and weight; it communicates with the handset, provides energy access for the system through connection with standard wall electrical power supply sources (100-220V, ~0.5Amp, 50-60Hz), and serves as a docking and recharging station for the handset. The base station is illustrated below in Figure 2-C.

Figure 2-C: CELLECTRA® 5PSP base station



Handset

The handset facilitates delivery of the needles for injection and electroporation into the muscle tissue and executes the treatment sequence (drug injection, impedance check, and electroporation pulses). It has a display screen and speakers for user feedback and an embedded processor for running the system software that controls and measures all of the elements of the handset. The handset is powered by a custom, rechargeable battery pack that has its own safety circuit and is compliant to IEC 62133 and UL 2054. The handset is designed with three independent fail-safes to minimize the risk of electrical shock, short, or fire. The handset is illustrated below in Figure 2-D.

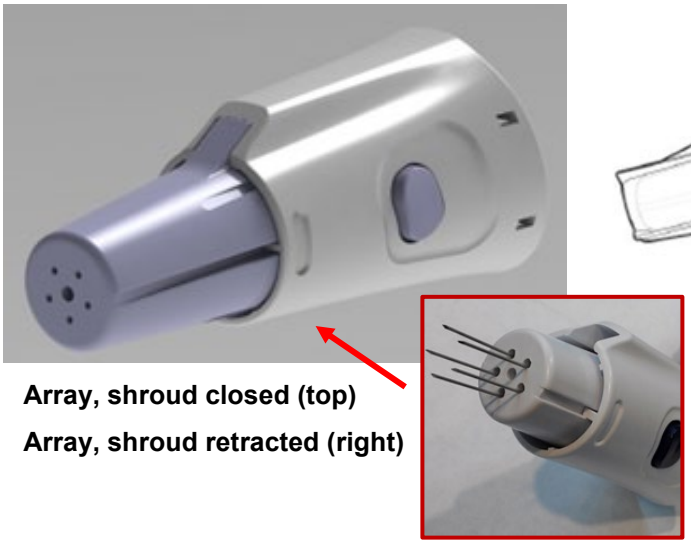
Figure 2-D: CELLECTRA® 5PSP handset

Handset without array attached

Handset with array attached

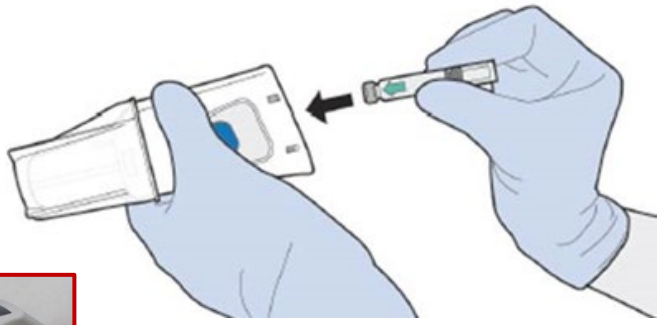
The array is a single-use, sterile, disposable component constructed of commonly used medical plastics and metals, with five fixed, stainless-steel electrodes (needles) and one intramuscular injection needle. The needles are covered by a plastic shroud that retracts as the needles are inserted into the patient, and then returns to the starting position as the needles are removed and locks out to prevent accidental needle sticks. The opposite end of the array accepts the drug cartridge, which is inserted cap-end-first until the bottom of the cartridge is flush with the opening of the array (indicated by an audible click). When properly inserted, the septum is punctured, and the cartridge cannot be removed. The array is illustrated below in Figure 2-E. The array features no software.

Figure 2-E: CELLECTRA® 5PSP array



Array, shroud closed (top)

Array, shroud retracted (right)



Insertion of cartridge into array

2.4 Study Design and Rationale

Given the similar biology of anal HSIL compared to cervical HSIL, it is logical to consider using VGX-3100 as nonsurgical treatment of anal HSIL. Currently there are few FDA-approved or cleared treatments for anal HSIL. None of them target HPV-16/18 specifically and the recurrence rates after treatment are high. New non-invasive targeted treatment options for anal HSIL are clearly needed. We envision that synthetic HPV-16/18 DNA vaccines will become a promising anti-cancer immunotherapy, and it is important to test them in HIV-positive individuals given the significant HPV-associated disease burden in that population. For HIV-positive individuals with exposure to HPV and HPV-associated precancerous lesions, this need for treating anal HSIL is likely to be even more urgent. Therefore, we propose a single-arm phase 2 study to show that this type of therapeutic HPV treatment can be used to treat HIV-positive individuals with anal HSIL.

2.4.1 Rationale for addition of fourth dose of immunotherapy

In our study, we are planning to use a four-dose intramuscular regimen of VGX-3100, instead of the previously tested 3-dose regimen. There is no established threshold of anti-HPV immune response correlating with efficacy in any population studied to date. HIV-positive individuals may have lower seroconversion rates against immunotherapy compared to HIV-negative individuals [33-35]. However, in a recent study testing prophylactic quadrivalent HPV vaccine HIV-positive men with CD4 of 200 cells/mL or higher, the proportion of men exhibiting seroconversion was 95% or greater for each of the HPV types included in the vaccine. For those with pre-existing anti-HPV antibodies, the prophylactic vaccine induced a marked increase in antibody concentrations [12].

Data from prior VGX-3100 studies in women have shown that antibody levels against HPV-16 or 18 levels off around week 24 [31, 32]. In a small pilot study using a similar immunotherapy to VGX-3100 for treatment of head and neck cancer, a 4-dose regimen produced increased HPV-specific cellular and humoral immune responses [36]. The increase between the third and the fourth dose of immunotherapy was not statistically significant, but the sample size was small. In order to ensure adequate immune response in our HIV-positive patients as well as test for the possibility of anamnestic response against HPV-16 or 18, we propose to add a fourth dose of immunotherapy at week 24. When VGX-3100 was tested for treatment of CIN2/3, the primary efficacy endpoint was set at 36 weeks after the first dose, or 24 weeks after the third dose. In our study, we will add a fourth dose of immunotherapy to be administered at week 24. We want to allow adequate time for the fourth dose to take effect. Therefore, we decide to move the primary efficacy evaluation to week 48 (24 weeks after the fourth dose).

2.4.2 Rationale for eligibility criteria

VGX-3100 has not been tested in HIV-positive individuals before and CD4 cells may be important contributors to the response. The investigators believe that for the first study, the trial should include participants with CD4 counts above 350. If VGX-3100 does not show evidence of response in this group, it is highly unlikely to work in those with even lower CD4 counts. Should there be any signs of efficacy

in this study, the investigators plan to include participants with lower CD4 counts in future studies.

2.4.3 Overview of study procedures

HIV-positive participants will be screened to verify that all eligibility criteria have been met within the protocol stated timeframes. Those eligible will then provide informed consent and HIPAA authorization form (if applicable). Enrollment should occur no more than one week before administration of the first dose of the immunotherapy.

All centers should refer to the study manual of procedures (MOP) for details on specimen collection, processing, and shipment to study laboratories.

We will collect one anal swab for anal cytology and one anal swab for HPV genotyping. The study clinician will perform a digital anorectal exam (DARE), high resolution anoscopy (HRA), and HRA-guided biopsies. Anal biopsy and cytology samples will be read by the local pathologist with these results used to guide enrollment and ongoing decisions during follow-up. The anal swab for HPV genotyping will be shipped by local sites in real time to DDL Laboratory. Institutions will send all their remaining paraffin-embedded blocks to the AMC Biorepository after the initial diagnostic slides are read. The AMC Biorepository will send the blocks to DDL Laboratory for HPV genotyping in the histological specimen. The AMC Biorepository will also send tissue slides to the Central Pathology Laboratory at UCSF for central pathology review.

Serum or urine pregnancy tests will be performed within 24 hours prior to each treatment for women of reproductive potential. Participants will be evaluated by study staff at approximately 30 minutes after each treatment, and they will be instructed to record oral temperatures and any local or systemic adverse events (AEs) on diary cards for 7 days after each dose. Adverse events will be graded and assessed according to CTCAE v5.0 by trial investigators.

Participants will be followed with safety labs at baseline and at weeks 0, 4, 12, 24, 48, and 72. CD4/CD8 count will be performed at weeks 0, 12, 24, 48, and 72. HIV viral load will be performed at baseline and then every 12 weeks. Blood for immunogenicity tests will be performed at screening and on weeks 0, 7, 15, 27, 48, and 72 by Inovio Analytical Sciences. The local sites will ship the peripheral blood specimens by overnight express to arrive at Inovio Analytical Sciences within 24 hours. Residual PMBC and serum will be sent from Inovio Analytical Sciences to the AMC Biorepository for storage and future research, if the participant has consented.

At certain study visits, the clinician will collect an anal swab for cytology and for HPV genotyping (see Appendix I). HPV genotyping on anal swab and biopsy specimens will both be accomplished using the SPF10 assay and/or Cobas® HPV test at DDL Laboratory (using same procedure as screening visit). A cytology swab for genotyping and DARE and HRA will be performed every 12 weeks starting at week 24. At HRA the clinician will be asked to determine whether a lesion at a given visit is a recurrence in an area previously noted, or an incident lesion in a new area. At each visit the clinician will carefully map the location of each lesion and

where they did their biopsies. Sites will also be requested to photograph each lesion at every visit using Second Opinion or other software that allows for easy sharing of images between study sites.

At week 36 (12 weeks after fourth and final dose of VGX-3100), HRA with biopsies (if appropriate) will be performed to ensure that no anal lesions are suspicious for cancer (not for response assessment). Participants whose anal HSIL disease is histologically confirmed to be progressing to invasive cancer at week 36 (and any subsequent assessment) will be taken off the study and referred for appropriate treatment.

At week 48, all participants will receive HRA with biopsies of all visible lesions and anal cytology will be performed for the primary efficacy endpoint. HPV genotyping on anal swab and biopsy specimens will both be accomplished using the SPF10 assay and/or Cobas HPV test at DDL Laboratory (using same procedure as screening visit). All participants with histopathological evidence of residual anal HSIL will be offered standard of care therapeutic interventions per investigator's choice. Participants may choose to continue observation for delayed treatment response of VGX-3100. The AMC Biorepository will also send tissue slides to the Pathology Laboratory at UCSF for central pathology review.

At week 72, all participants will receive HRA with biopsies of all visible lesions and anal cytology will be performed. HPV genotyping on anal swab and biopsy specimens will both be accomplished using the SPF10 assay and/or Cobas HPV test at DDL Laboratory (using same procedure as screening visit). The AMC Biorepository will also send tissue slides to the Pathology Laboratory at UCSF for central pathology review.

2.5 Correlative Studies

2.5.1 Overview of sample collection

Participants who sign the informed consent and agree to be screened at Visit 0 will provide a sample of blood and two anal swabs for anal cytology and HPV testing. The anal cytology will be processed at each local site. The anal HPV swab will be shipped to DDL Laboratory for central testing to determine eligibility.

Blood for immunology tests will be collected at screening and at weeks 0 (pre-immunization), 7, 15, 27, 48, and 72, or at early discontinuation. Peripheral blood mononuclear cells (PBMC) and serum will be cryopreserved for immune analysis. Anal swabs for cytology will be collected at weeks 0, 24, 48, and 72; anal swabs for HPV testing will be collected at weeks 0, 24, 36, 48, 60, and 72.

Paraffin-embedded anal biopsy specimens will be collected at weeks 0, 48, and 72 (biopsies may be collected at weeks 24, 36, or 60 if cancer is suspected), and processed for routine hematoxylin and eosin (H&E) histopathologic assessment. Institutions will send all their remaining blocks to the AMC Biorepository after the initial diagnostic slides are read. Biopsy slides will be sent to the Central Pathology Laboratory at UCSF for central review. Tissue blocks will be returned to clinical sites after correlative studies and central review are completed.

All centers should refer to the study manual of procedures (MOP) for details on specimen collection, processing, and shipment to study laboratories.

2.5.2 CD4 and CD8 T-cell counts

CD4+ and CD8+ T-cell total number and percent total lymphocytes will be performed at the study sites in a CLIA-compliant laboratory. Results will be used for determining eligibility and monitoring for changes in T-cell counts over time, for possible toxicity in the form of falling CD4+ and/or CD8+ T-cells.

2.5.3 HIV-1 RNA viral load

HIV-1 plasma RNA viral load will be performed at the study sites in a CLIA-compliant laboratory. Results will be used for monitoring for changes in viral load over time, for possible toxicity in the form of rising HIV-1 RNA levels.

2.5.4 HPV 16/18 genotyping

HPV genotyping in the anal swabs and biopsy specimens will be performed at DDL Diagnostic Laboratory. HPV-16/18 presence in the initial screening anal swab will be used for eligibility. Subsequent HPV genotyping by PCR in both anal swabs and the biopsy specimens will be used as the measures of viral clearance. HPV-16/18 presence in the anal HSIL tissue will be used to determine the response rate of HPV-16/18-positive anal HSIL to VGX-3100 (primary efficacy endpoint).

DNA extraction will be accomplished using the fully automated cobas x 480 instrument (Cobas HPV test). Specimens will be digested under denaturing conditions at elevated temperatures and then lysed in the presence of chaotropic reagent. Released HPV nucleic acids, along with the b-globin DNA serving as process control, will be purified through adsorption to magnetic glass particles, washed, and finally separated from these particles, making them ready for polymerase chain reaction amplification (PCR) and detection. The amplification plates were then manually transferred to the cobas z 480 analyzer for real-time PCR amplification of high-risk HPV (hrHPV) and b-globin DNA. The cobas HPV test uses primers that define a sequence of approximately 200 nucleotides within the polymorphic L1 region of the HPV genome. A pool of HPV primers present in the master mix is designed to amplify HPV DNA from 14 high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) [33]. Fluorescent oligonucleotide probes bind to the polymorphic regions within the sequence defined by these primers. An additional primer pair and probe targeting the human b-globin gene (330-bp amplicon) will be included as an internal control to provide a measure of specimen adequacy as well as to monitor the quality of the extraction and amplification process. Interpretation of the amplification results will be carried out using proprietary software provided with the cobas z 480 analyzer. The cycle threshold cutoffs will be set at 40.5 for HPV 16 and at 40 for HPV 18 as well as the remaining 12 hrHPV genotypes [34]. Positive and negative controls will be included in each run.

To determine the HPV type in anal HSIL tissue, microdissected portions of the anal mucosa tissue will be placed in Eppendorf tubes and will be processed for PCR and methylation studies. HPV typing will be performed using small amplicon primer

sets known to be optimal for studies of DNA from formalin-fixed, paraffin-embedded (FFPE) specimens, including Spf-10 primers [35]. Tissues shown to contain HPV will be analyzed further to determine the type.

2.5.5 T-cell responses to HPV-16 and HPV-18 E6 and E7

PBMCs will be isolated from whole blood samples. Assessment of cellular immune activity may occur via the application of the flow cytometry or interferon-γ enzyme-linked immunosorbent spot (IFN-γ ELISpot) assay as well as flow cytometry.

T-cell responses to HPV-16 and HPV-18 E6 and E7 may be determined by flow cytometric assessments at weeks 0, 7, 15, 27, 48, and 72, to be performed at the Inovio Analytical Sciences. T-cell responses to HPV-16 and HPV-18 E6 and E7 may additionally be determined by interferon-γ-ELISpot at weeks 0, 7, 15, 27, 48, and 72. The current cellular protocols use two sets of peptides, each containing 15–amino acid residues overlapping by eight amino acids representing the entire consensus E6 and E7 protein sequences of HPV-16 or HPV-18, which are pooled at a concentration of 2 mg/ml per peptide into two pools, spanning the length of the E6 and E7 antigens, respectively [22, 23]. The average number of SFU counted in R10 wells is subtracted from the average in individual HPV peptide wells and then adjusted to 1 x 106 PBMCs for each HPV peptide pool.

Additional assessment of cellular immune activity may occur via the application Flow Cytometry for the purposes of performing a lytic granule loading assay. The Lytic Granule Loading assay may examine the following external cellular markers: CD3, CD4, CD8 (T-cell identification), CD137, CD38 and CD69 (T-cell activation markers) as well as PD-1 (exhaustion/activation marker). The Lytic Granule Loading assay may additionally analyze the following intracellular markers: Granzyme A, Granzyme B, Granulysin, and Perforin (proteins involved in lytic degranulation and cytotoxic potential). Markers examined in this assay may change as new relevant data become available.

2.5.6 Antibody responses to HPV-16 and HPV-18 E7

Binding antibodies may be measured by enzyme-linked immunosorbent assay (ELISA) against immunotherapy antigens at weeks 0, 7, 15, 27, 48, and 72. These assays will be performed at the Inovio Analytical Sciences. Endpoint titers of antibodies are determined by standard methods. Briefly, 96-well enzyme immunoassay plates are coated with HPV-16 or HPV-18 E7 proteins. The optical density created by sera binding to immunotherapy antigens post-treatment is compared to optical density of sera binding at Day 0. Positivity is considered if the average optical density (OD) of a sample is greater than 0.15 absorbance units and greater than the average OD before dose 1 (pre-immunization) plus 2.5 times the standard deviation of OD before dose 1 at the same dilution.

2.5.7 Immune infiltration into tissue

Available tissue sections from the diagnostic biopsy and week 48 and 72 specimens may be stained by immunohistochemistry or immunofluorescence for immune markers after histological diagnoses are finalized, to determine cellular immune

response to VGX-3100. Assessment of markers may include, but are not limited to, CD8, CD4, FoxP3, Perforin, CD137, and CD103 as sample allows. Markers listed here may change as new relevant information becomes available.

2.5.8 Assessment of PD-L1 expression

PD-L1 is a critical ligand for PD-1 and its expression has been identified as a potential important correlative biomarker for clinical response to immunotherapy. Therefore, PD-L1 expression will be quantitated by IHC in baseline formalin-fixed paraffin-embedded tumor specimens as an important part of the correlative studies. For this, formalin-fixed, paraffin-embedded tissue block(s) from anal tissue will be obtained by the clinical site from the diagnostic biopsy and week 48 or the early treatment discontinuation biopsy performed as part of this protocol. The PD-L1 testing will be performed by the AMC HPV Virology Core Laboratory.

3.0 PARTICIPANT SELECTION

A rostered AMC investigator (CTEP-registered physician investigator or CTEP-registered non-physician investigator (NPIVR) who is certified in HRA) must document that each protocol participant meets all stated eligibility criteria. The site's Delegation of Tasks Log should indicate that the Investigator has delegated this to the named individual (NPIVR). Participating sites must have documentation that each eligibility requirement is satisfied prior to participant enrollment. In compliance with CTEP policy, no exceptions to eligibility criteria will be granted under 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 treatment enrollment 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.

AMC Participant ID Number: 103 - ___ ___ ___ - ___ ___ ___ ___ ___

Participant's Initials (F, M (optional), L): ___ ___ ___

Note: A separate Inovio Participant ID number will be assigned at Screening (Segment A enrollment).

NOTE: All questions regarding eligibility should be directed to the study chairs and the AMC ODMC (amc-103@emmes.com).

3.1 Eligibility Criteria

_____3.1.1 Biopsy-proven intra-anal or peri-anal HSIL (PAIN2/AIN2 with a positive p16 stain, PAIN2-3, AIN2-3, or PAIN3/AIN3) within 90 days before study enrollment.

_____3.1.2 At least one focus of HSIL must be large enough to be monitored for response, i.e., not completely removed after the screening biopsy.

_____3.1.3 Must be positive for HPV-16 or -18 on genotyping performed on screening anal swab within 90 days before study enrollment.

_____3.1.4 HIV-positive. Documentation of HIV-1 infection by means of any one of the following:

- Documentation of HIV diagnosis in the medical record by a licensed health care provider;

- Any licensed HIV screening antibody and/or HIV antibody/antigen combination assay confirmed by a second licensed HIV assay such as a HIV-1 Western blot confirmation or HIV rapid multispot antibody differentiation assay.

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

_____3.1.5 Must be documented to be on an effective ART regimen, generally a 3-drug regimen based on Department of Health and Human Services (DHHS) treatment

guidelines 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. Each component agent of a multi-class combination ART regimen will be counted separately.

_____3.1.6 Age ≥ 18 years. Because there is no dosing or adverse event data are currently available on the use of VGX-3100 in participants <18 years of age and HIV-positive, children are excluded from this study.

_____3.1.7 ECOG performance status ≤ 1 (Karnofsky ≥ 70%, see Appendix II).

_____3.1.8 Participants must meet the following laboratory parameters within 90 days before enrollment:

- Leukocytes: ≥3,000/mm^3
- Absolute neutrophil count: ≥1,500/mm^3
- Platelets: ≥100,000/mm^3
- CD4 count ≥ 350 cells/mm^3
- HIV plasma HIV-1 RNA below detected limit obtained by Food and Drug Administration (FDA)-approved assays (limit of detection: 75 copies/mL or less)

_____3.1.9 For females, must have cervical cytology and visual examination of the vulva, vagina, and cervix within 12 months prior to enrollment with confirmation of no evidence of carcinoma. For women who underwent hysterectomy with removal of the cervix, cytology from the vagina within 12 months is required.

_____3.1.10 For women of child-bearing-bearing potential (WOCBP[1]), they must have a negative serum or urine pregnancy test within 72 hours of receiving the first dose of VGX-3100 and be at least 3 months post-partum. The effects of VGX-3100 on the developing human fetus are unknown. It is not known whether VGX-3100 can cross the placenta or cause harm to the fetus when administered to pregnant women or whether it affects reproductive capacity. For this reason, WOCBP and men must agree to use adequate contraception (oral contraceptive pills, intrauterine device, Nexplanon, Depo-Provera, or permanent sterilization, etc., or another acceptable method as determined by the investigator) prior to study entry, for the duration of study participation, and four months after completion of VGX-3100 administration. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.

Men who could father a child must agree to use at least one form of birth control during or continued abstinence from heterosexual intercourse prior to the study, for

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

the duration of study participation, and four months after completion of VGX-3100 administration.

_____3.1.11 Ability to understand and the willingness to sign a written informed consent document.

3.2 Exclusion Criteria

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

_____3.2.1 Treatment or removal of HSIL less than 3 months prior to enrollment.

_____3.2.2 Participants who received prophylactic HPV vaccines (e.g. Gardasil® and Cervarix®) or any other investigational agents within the 4 weeks before enrollment, other than investigational antiretroviral agents for HIV and investigational agents for Hepatitis C. If participants wish to receive prophylactic HPV vaccines, they must complete the series at least four weeks prior to enrollment.

_____3.2.3 Participants should be excluded if they have a condition requiring systemic treatment with either corticosteroids (>10 mg daily prednisone equivalents) or other immunosuppressive medications within 2 weeks of study drug administration. These drugs may interfere with the activity of VGX-3100. Inhaled steroids and physiologic adrenal replacement doses are permitted in the absence of active autoimmune disease. Participants are permitted to use ocular, intra-articular, intranasal, and inhalational corticosteroids (with minimal systemic absorption). A brief course of corticosteroids for prophylaxis (*e.g.*, contrast dye allergy) or for treatment of non-autoimmune conditions (*e.g.*, delayed-type hypersensitivity reaction caused by contact allergen) is permitted. Use of anabolic steroids is permitted. Topical steroids are permitted as long as they are not directly applied to the area of the skin where electroporation is planned.

_____3.2.4 History of anal cancer, penile, vulvar, vaginal, or cervical cancer, or signs of any of these malignancies at baseline. Participants with prior carcinoma in situ will not be considered to have prior cancer for eligibility purposes.

_____3.2.5 Current systemic chemotherapy or radiation therapy that potentially causes bone marrow suppression that would preclude safe treatment of HSIL.

_____3.2.6 History of allergic reactions attributed to compounds of similar chemical or biologic composition to VGX-3100.

_____3.2.7 Warts so extensive that they preclude the clinician from determining the extent and location of HSIL.

_____3.2.8 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, cardiac arrhythmia, or psychiatric illness/social situations that, in the opinion of the investigator, would limit compliance with study requirements or could be negatively affected by the electroporation treatment.

_____3.2.9 Presence of unstable or life-threatening cardiac disease (e.g., unstable angina, class 3 or higher congestive heart failure).

_____3.2.10 Presence of acute or chronic bleeding or clotting disorder that would be a contraindication to IM injections (which may include the use blood thinners such as anticoagulants or antiplatelets drugs within two weeks of enrollment).

Exception: Over-the-counter aspirin or non-steroidal anti-inflammatory drugs is allowed.

_____3.2.11 Participants who have not recovered from adverse events due to prior anti-HSIL therapy (i.e., have residual toxicity > Grade 1), per Common Terminology Criteria for Adverse Events (CTCAE) v5.0.

_____3.2.12 Participants who have any metal implants, implanted medical devices, tattoos, keloids or hypertrophic scars, or active lesions/rashes within 2 cm of all intended potential sites of treatment/electroporation.

_____3.2.13 History of seizures, except if participants have been seizure-free for five years or more with the use of one or fewer anti-epileptic agents.

_____3.2.14 Sustained, manually confirmed, sitting systolic blood pressure >150 mm Hg or <90 mm Hg or a diastolic blood pressure >95 mm Hg at Screening or Day 0.

_____3.2.15 Resting heart rate <50 bpm (unless attributable to athletic conditioning) or >100 bpm at screening or Day 0.

_____3.2.16 Participants who have less than two acceptable sites available for IM injection considering the deltoid and anterolateral quadriceps muscles.

_____3.2.17 Participants who have cardioverter-defibrillator or pacemaker (to prevent a life-threatening arrhythmia) that is located ipsilateral to the deltoid injection site (unless deemed acceptable by a cardiologist).

_____3.2.18 Participants who are breastfeeding a child. It is not known whether VGX-3100 is excreted in human milk. Because of the unknown potential for serious adverse drug reactions in nursing infants, investigational product should not be administered to nursing mothers.

_____3.2.19 Any illness or condition that in the opinion of the investigator may affect the safety of the participant or the evaluation of any study endpoint.

Investigator Signature: _____ Date: _____

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

3.3 Number of Participants to be Enrolled

3.3.1 Proposed Sample Size

This study will enroll a minimum of 35 participants (if the trial is found to be futile based on interim analysis) and a maximum of 92 participants.

3.3.2 Accrual Rate

Approximately 4-6 participants per month.

3.3.3 Supplementation of participants

While being HPV-16 or -18-positive based on the baseline anal swab is sufficient for initial eligibility, the adjudication for inclusion in the primary efficacy endpoint analysis will rely on results of the central pathology review. However, if a participant has HPV-16 or -18 by anal cytology but none of the participant's HSIL lesions are found to be HPV-16 and/or HPV-18-positive in testing of the anal biopsy specimen by the central laboratory, the participant will remain on the study to complete treatment and follow-up (up to a maximum of ten such participants), but the study will recruit the required number of participants for the sample size determined by the primary hypothesis.

If a participant has been enrolled but does not receive any doses of VGX-3100 within 28 days after enrollment, he or she will not count toward the required number of participants determined by the primary hypothesis.

Following protocol enrollment, participants who develop the following HIV-related events (defined as HIV-related events that are unrelated to protocol treatment) may be replaced (up to a maximum of ten such participants):

- HIV plasma viral loads > 400 copies/mL that persist for 4 weeks or more without significant ART interruption
- CD4 count ≤ 200 cells/mm^3 that persists for 4 weeks or more
- any new or exacerbation of existing AIDS-defining condition (other than CD4 below 200 cells/mm^3)

Participants who experience HIV-related events will remain on the study to complete treatment and follow-up. They will also be included in the primary analysis.

HIV-related events should be recorded in the site's source documents. The site investigator must notify the protocol chairs of replacement as soon as possible by reporting the incident in the applicable form in Advantage eClinical.

3.4 Participant Enrollment Procedures

Sites must have this protocol approved by their Institutional Review Board (IRB) and the Institutional Biosafety Committee (IBC).

The investigator and sponsor are responsible for ensuring that the clinical study is reviewed and approved according to local and applicable global regulations (e.g., NIH Office of Biotechnology Activities) governing research that involves recombinant or synthetic nucleic acid molecules.

Once IRB and IBC approvals are received the site must be registered for study participation with the AMC Operations and Data Management Center (ODMC) before they may enroll participants.

A signed informed consent form is to be obtained from each participant, and/or from the participant's legally authorized representative, prior to beginning any screening activities. The process for obtaining consent must also be documented in the participant's medical record.

The participating site will ensure a participant meets all eligibility criteria prior to completing the protocol-specific eligibility checklist in Advantage eClinical for enrollment. Participants will be enrolled on-line via Advantage eClinical no more than 1 week prior to the initiation of treatment (enrollment 1 day prior to or on the day of treatment is strongly encouraged). Once the eligibility checklist is submitted a system-generated confirmation email will be sent to the enroller upon successful completion of the participant enrollment. If the on-line system is inaccessible, the site should notify the AMC ODMC (via email at amc-103@emmes.com or via phone at 301-251-1161) for further instructions.

3.4.1 Registration for screening

After an informed consent form has been signed by the participant, the participant must be registered for screening (AMC-103, Segment A) on-line via Advantage eClinical within one calendar day. After successful registration into screening, the participant will receive an eleven-digit participant ID and will then enter the screening process (Screening and Pre-entry visits). Once assigned, participant IDs cannot be reused for a different participant for any reason.

Note: A separate Inovio Participant ID number will be assigned at Screening (Segment A enrollment) for device input during drug/device administration.

3.4.2 Enrollment

After the screening evaluations have been obtained and the participant is determined to be eligible, the participating site will complete the protocol-specific eligibility checklist and enroll the participant into AMC-103 Segment B (on-line via Advantage eClinical). Enrollment should occur no more than 1 week prior to administration of the first dose of the protocol agent (enrollment 1 day prior to or on the day of treatment is strongly encouraged). Once the eligibility checklist is submitted, a system generated confirmation email will be sent to the enroller upon successful completion of the participant enrollment.

Participants must be enrolled into AMC-103 Segment B prior to receiving the first dose of the protocol agent.

4.0 TREATMENT PLAN

A rostered AMC investigator (CTEP-registered physician investigator [IVR] or CTEP-registered non-physician investigator [NPIVR] who is certified in HRA) must prescribe or issue orders for treatment for this protocol. The site's Delegation of Tasks Log should indicate that the principal investigator has delegated this to the named individual (IVR or NPIVR).

4.1 Agent Administration: Injection and Electroporation

Participants will receive a 4-dose series of 1 ml VGX-3100 by IM injection in the deltoid (or anterolateral quadriceps muscle as an alternate option, if deltoid muscle is not possible or appropriate) followed immediately by EP with the CELLECTRA 5PSP. Study treatment must not be given within 2 cm of a tattoo, keloid, or hypertrophic scar. If there is implanted metal, implanted device, within the same limb then use of the deltoid muscle on the same side of the body is excluded. There is a window of +/- 4 days for each dose of the treatments.

Protocol agents will be administered on an outpatient basis. Reported adverse events and potential risks for VGX-3100 are described in Section 5.3. No dose modifications are allowed. No investigational or commercial agents or therapies may be administered with the intent to treat the participant's anal HSIL. The regimen description is presented in Table 4-A.

Table 4-A: Regimen description

Agent	Dose	Route	Schedule
VGX-3100	6 mg (3 mg plasmid targeting HPV-16 E6 and E7, and 3 mg plasmid targeting HPV-18 E6 and E7)	IM/EP	Week 0, 4, 12, 24

4.2 Use of Investigational Device

The instructions for use of the device are located in the CELLECTRA 5PSP User Manual. Users of the CELLECTRA 5PSP device must successfully complete training before administering study treatment using the device. Training will include review of the entire device user manual and hands-on training. After training on the proper use of the CELLECTRA 5PSP device, intended users at each site will be required to demonstrate their competence in its use to Inovio or its designee. An instructional video is available for review by site personnel on an as-needed basis.

Briefly, the Handset and Array should be prepared according to the instructions in the user manual. Remove the array from its packaging while avoiding contact with the end of the array that attaches to the handset and insert the drug cartridge into the array with the arrow on the cartridge pointing away from the handset.

Attach the Array to the Handset and set the needle depth on the handset to the longest needle length judged to allow safe injection into the muscle per the investigator's assessment.

The participant must be in a safe and secure, braced position. The participant's body should

touch the bed or be braced against the bed or have the study staff performing electroporation or assistant in position to brace the participant's arm or leg (as appropriate) as the EP is administered. The user will then insert the CELLECTRA 5PSP needle array into the deltoid (or anterolateral quadriceps muscle as an alternate option, if deltoid muscle is not possible or appropriate) of the participant in accordance with the CELLECTRA 5PSP user's manual instructions. Once triggered by the user, the device will automatically deliver the treatment followed by the EP pulses. The entire treatment period lasts for about 10 seconds.

After each treatment with VGX-3100, data on treatment administration must be downloaded from the EP device and saved to the site's study files. The data file must be sent to Inovio Pharmaceuticals Inc. or its designee approximately 24 to 72 hours after treatment. Data (.BIN file) should be uploaded to DDM (cellectradata.inovio.com) or, if unavailable, emailed to cellectradata@inovio.com. Instructions on how to download the data and contact information will be provided in the User Manual and during device administration training.

The treatment procedure must be performed by qualified personnel. Any individual designated to perform the procedure should be permitted by the relevant local authorities to administer parenteral injections to patients (e.g., MD, DO, RN) in addition to successfully completing device training from sponsor personnel. Individuals whose credentials do not meet the relevant local requirements may perform the treatment procedure under both of the conditions below:

- The procedure must be performed under the direct supervision of the Investigator or an approved sub-investigator who has already been trained by the sponsor's personnel.
- The *curriculum vitae* (CV) and any relevant qualifications of the individual must be reviewed and approved by the sponsor or its designee to perform the procedure.

Any deviation from the above procedures must be reported in Advantage eClinical and reviewed by the sponsor or its designee.

4.3 General Concomitant Medication and Supportive Care Guidelines

4.3.1 Prohibited therapies

Participants in this study may not use prophylactic HPV vaccines, e.g., Gardasil® and Cervarix®. Concomitant anti-HSIL therapies are also prohibited. Due to the mechanism of action of VGX-3100, systemic or topical steroids near the intended injection site or the use of other immunosuppressive or immunomodulatory/ stimulatory agents should be discouraged but not prohibited if clinically appropriate.

Participants should not use alcohol or drugs that would interfere with study requirements during the course of the study and should report ALL medications/drugs take to the investigator and/or other study personnel.

WOCBP and men must use appropriate contraceptive measures during the study and for four months after the end of treatment (See Eligibility Criterion 3.1.11).

Participants should abstain from sexual activity and refrain from use of douching or vaginal/anal lubricants/medication for a period of 24 hours prior to collection of ThinPrep™ or SurePath™ anal swab samples.

4.3.2 Concurrent medications

Participants MUST receive medically appropriate care and treatment for HIV infection, including antiretroviral medications. HIV infection treatment will be managed as per standards of care with appropriate CD4 and viral load monitoring, including consideration for changing ART in case of loss of HIV control.

Participants may receive standard vaccines that are deemed appropriate by their health care providers. We recommend that these vaccines be given two weeks before or after each VGX-3100 administration in order to better ascertain the attribution of potential adverse events. A live-attenuated vaccine must not be given within four weeks before or after each VGX-3100 administration.

All pain medications (both prescription and over-the-counter), anticoagulants, corticosteroids or immunomodulants, anti-anxiety medications, pain medications, any prior receipt of prophylactic HPV vaccinations, and antiretroviral therapy should be recorded in the appropriate electronic case report forms at the time of study entry, and any changes to the participant's medications belonging to the classes mentioned above should be updated as those changes occur. Any additional therapies prescribed for treatment of study product related adverse events and medication taken for anxiety or pain management should be recorded as well.

4.3.3 Management of non-anal HPV-related disease

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

4.3.4 Management of anxiety and pain due to treatment

Participants may be offered topical anesthetic (e.g., eutectic mixture of local anesthetics [EMLA] or equivalent), to prevent significant discomfort from the treatment procedure. If a topical anesthetic is used, an approximately 1.5 cm diameter amount will be applied with occlusion to the site of injection ~30 minutes prior to treatment.

Participants may be offered a mild sedative (e.g., 0.5-1 mg lorazepam), or equivalent, for anxiety related to the treatment procedure. Mild sedatives may be administered approximately 1 hour prior to treatment at day 0, weeks 4, 12, and/or 24. Participants who receive a mild sedative should not be allowed to operate a motor vehicle for 3-4 hours after receiving medication and should have arranged transportation to depart the study site.

Participants may be offered an analgesic (e.g. ibuprofen, ketorolac) after injection/EP.

Participants who are allergic to or have contraindications to EMLA, ibuprofen, ketorolac, or a mild sedative may be offered a suitable alternative.

Medication taken for anxiety or pain management should be added to the concomitant medications form in Advantage eClinical.

4.3.5 Anal biopsies and monitoring for progression to cancer

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

4.4 Criteria for Holding Study Treatment

Participants experiencing grade 3 adverse events that are at least possibly related to study product must 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 if the AE resolves or reduces in severity to grade 1 within 7 days. If the AE does not resolve or reduce in severity to grade 1 within 7 days but resolves prior to when the subsequent dose is due, the case will be reviewed by site investigator, protocol chair along with the sponsor to decide if the study treatment can be resumed and dosing intervals should be maintained. If the AE does not resolve prior to the subsequent dose, the participant will not receive additional doses. If a participant experiences a grade 3 injection site reaction, no further treatment will be administered regardless of how long it takes the grade 3 injection site reaction to resolve.

Participants experiencing grade 1 or 2 adverse events that are at least possibly related to study product can continue the product.

4.5 Criteria for Discontinuing Study Treatment

The investigator must discontinue the study treatment if there is evidence of grade 3 injection site reactions.

The investigator may discontinue study treatment, at his or her discretion, if the participant experiences grade 3 adverse events at least possibly due to study treatment. Alternatively, study treatment may be held as described in Section 4.4.

The Investigator or trial coordinator must notify the Sponsor or Sponsor's designee immediately when a participant has been discontinued/withdrawn due to an adverse event (AE). If a participant discontinues from the trial or is withdrawn from the trial prior to trial completion, all applicable activities scheduled for the final trial visit should be performed

at the time of discontinuation. Any AEs and/or Serious Adverse Events (SAEs) present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 5.0.

Participants who prematurely discontinue treatment should remain in study follow-up according to the schedule of events. If treatment is discontinued prior to week 24, then the participant should undergo HRA with biopsies for the primary endpoint at week 48. Off study treatment for anal HSIL prior to week 48, other than study treatment, should be avoided.

Similarly, participants who prematurely discontinue study treatment administered beginning at week 24 should undergo HRA with biopsies at week 48.

Participants should discontinue 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.

4.6 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 adverse event (see Section 4.5 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.

4.7 Duration of Follow-Up

All participants will be followed for up to 72 weeks after receiving the first dose of VGX-3100, diagnosis of invasive anal cancer, or until death, whichever occurs first. Participants who develop malignancies other than anal cancer or who develop unacceptable adverse events will remain in follow-up for anal cancer outcomes. Participants who cannot attend study visits should be followed for anal cancer outcomes via telephone contact every 12 weeks until week 72. Medical records will be requested from the participant's provider in the event that cancer is diagnosed. Participants removed from study for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

4.8 Criteria for Pausing of Study

- If at any time during the study one-third (1/3) or more of the participants experience an adverse event of special interest, further enrollment and study treatment will be halted

immediately until a thorough investigation has been conducted by the Medical Monitor, Principal Investigator for the trial, and the AMC Data and Safety Monitoring Board (DSMB).

- If any SAE (or potentially life-threatening AE), or death assessed as related to study treatment occurs, further enrollment and study treatment will be halted immediately until a thorough investigation has been conducted by the Medical Monitor, Principal Investigator for the trial, IRB (if applicable) and the AMC DSMB.
- If three or more participants in this study, experience the same Grade 3 or 4 adverse event, assessed as related to study treatment, further enrollment and study treatment will be halted immediately until a thorough investigation has been conducted by the Medical Monitor, Principal Investigator for the trial, and the AMC DSMB.
- In the event of two identical, unexpected, Grade 4 toxicities, assessed as related to study treatment, further enrollment and study treatment will be halted immediately until a thorough investigation has been conducted by the Medical Monitor, Principal Investigator for the trial, IRB (if applicable) and the AMC DSMB.

The sponsor or designee will notify all investigators and IRBs/IECs (if required) regarding the outcome of any investigation stemming from a Study Pause.

5.0 ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

A rostered AMC investigator (CTEP-registered physician investigator or CTEP-registered non-physician investigator (NPIVR) who is certified in HRA) must assess grading and attribution for all adverse events (AE) according to reporting requirements for the study. The site's Delegation of Tasks Log should indicate that the principal investigator has delegated this to the named individual (NPIVR).

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.

5.1 Expected Adverse Events Related to the Procedures

Study procedures include anal cytology swab collection, including samples for HPV testing, High Resolution Anoscopy (HRA), and anal biopsy. Participants will likely experience pressure and urgency to defecate during the cytology/HPV testing sample collection and HRA. Anal bleeding may occur up to one week after the biopsy is taken. The risk of infection is less than 1%.

5.2 Expected Adverse Events Related to Anal HSIL

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 4.5 of the protocol, the investigator must withdraw the participant from receiving treatment and pursue treatment of anal HSIL or invasive cancer outside of the study as clinically indicated. The participant will be followed for anal cancer outcomes via telephone contact every 12 weeks until week 72.

5.3 Adverse Events for VGX-3100

CELLECTRA electroporation device contraindications are as follows:

- Treatment areas where major blood vessels or nerves are present
- Any significant acute or chronic medical illness if deemed by the practitioner that electroporation treatment could negatively impact the illness
- Presence of unstable or life-threatening cardiac disease (e.g., unstable angina, class 3 or higher congestive heart failure)
- Presence of acute or chronic bleeding or clotting disorder that would contraindicate IM injections or use of blood thinners (e.g., anticoagulants or antiplatelet drugs) within 2 weeks

- A cardioverter-defibrillator or pacemaker (to prevent a life-threatening arrhythmia) that is located ipsilateral to the intended deltoid injection site (unless deemed acceptable by a cardiologist)
- Any metal implants or implantable medical device within the electroporation area

Risks of the drug and device combination (VGX-3100 with the CELLECTRA electroporation device) are listed below in Table 5-A. Injection site reactions should be graded per Table 7-B:

Table 5-A: Risks of VGX-3100 + CELLECTRA electroporation device

Frequency [a]	**Event**
Very Common (>10%)	• Mild injection site pain or tenderness • Moderate injection site pain or tenderness • Injection site erythema or redness • Injection site swelling • Injection site pruritus
Common (1-10%)	• Pyrexia
Uncommon (≥0.1% to <1%)	• None • Severe injection site pain or tenderness
Rare (≥0.01% to <0.1%)	• None
Very Rare (<0.01%)	• Allergic Reaction

[a] Defined per CIOMS

No serious related adverse events to VGX-3100 with CELLECTRA electroporation device have been observed. There may be side effects and discomforts that are not yet known.

5.3.1 Theoretical risks

Although not observed, there are theoretical risks of VGX-3100 + CELLECTRA™ which are as follows:

- Sterile abscess at the injection site
- Secondary bacterial site infection
- Electrical injury
- Disruption of function of implanted electronic medical device(s)
- Worsening of unstable cardiac disease (e.g. arrhythmia)
- Effects on fetus and/or pregnancy
- Autoimmune disease
- Renal insufficiency

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

5.4.1 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).

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

5.4.3 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.

5.4.4 Important Medical Event/Medically Significant: Is an important medical event that may not result in death, be life-threatening, or require hospitalization, but based upon appropriate medical judgment, may jeopardize the participant's health and may require medical or surgical intervention to prevent one of the SAE outcomes listed above.

5.4.5 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.

5.4.6 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.

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

5.4.8 CTEP Adverse Event Reporting System (CTEP-AERS): An electronic system for expedited submission of AE reports.

5.4.9 CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

5.4.10 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.

Per NCI's Adverse Event Reporting Guidelines, AEs with an attribution of possible or greater are considered to have a reasonable possibility of a causal relationship to the investigational agent. AEs with an attribution of unrelated or unlikely related are considered to not have a causal relationship to the investigational agent.

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

Per the definition above, a UADE is a type of SAE that requires expedited reporting on the part of the Sponsor. As a reminder, all SAEs regardless of relationship to device, drug, or procedure are to be reported to the sponsor by the trial Investigator within 24 hours. The sponsor will assess each device related SAE to determine if anticipated based on prior identification within the investigational plan.

5.5 Unexpected and Expedited Adverse Event Reporting

An adverse drug reaction (ADR) is any noxious and unintended responses to a medicinal product related to any dose, for which a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility; i.e., there is evidence to suggest a causal relationship between the product and the adverse event. An unexpected ADR is one, the nature or severity of which is not consistent with the applicable product information (investigator's brochure, protocol, and user manual). Reports that add significant information on specificity or severity of a known, already documented SAE constitute unexpected events. For example, an event more specific or more severe than described in the Investigator's Brochure or protocol would be considered "unexpected." Specific examples would be (a) acute renal failure as a labeled ADR with a subsequent new report of interstitial nephritis and (b) hepatitis with a first report of fulminant hepatitis.

The Sponsor will assess each serious ADR report for expectedness, to determine if it is a serious unexpected suspected adverse reaction (SUSAR) which requires prompt reporting to regulatory authorities and participating investigators as an expedited report, according to the applicable regulatory requirements. Additional occurrences of the SUSAR will be required to be reported on an expedited basis until the applicable product information is amended.

In addition to single-case reports of SUSARs, the Sponsor shall notify regulatory authorities and participating investigators of information that might materially influence the benefit-risk assessment of a medicinal product, sufficient to consider changes in product administration or overall conduct of a clinical investigation. Examples of such information include a clinically important increase in the rate of occurrence of a serious

expected adverse event, the identification of a significant hazard to the participant population, or a major safety finding from a study conducted in animals.

5.5.1 Expedited AE reporting for this study must use CTEP-AERS (CTEP Adverse Event Reporting System), accessed via the CTEP home page (https://eapps-ctep.nci.nih.gov/ctepaers). The reporting procedures to be followed are presented in the "NCI Guidelines for Investigators: Adverse Event Reporting Requirements for DCTD (CTEP and CIP) and DCP INDs and IDEs" which can be downloaded from the CTEP home page (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm). These requirements are briefly outlined in Section 5.5.3.

In the rare occurrence when Internet connectivity is lost, a 24-hour notification is to be made to the AMC ODMC by telephone at 301-251-1161. 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.

5.5.2 CTEP-AERS is programmed for automatic electronic distribution of reports to the following individuals: Principal Investigator and Adverse Event Coordinator(s) (if applicable) of the Corresponding Organization or Lead Organization, the local treating physician, and the Reporter and Submitter. CTEP-AERS provides a copy feature for other email recipients, which will include the Sponsor for this protocol.

5.5.3 Expedited reporting guidelines

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

Note: A death on study requires both routine and expedited reporting regardless of causality. 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"** under the system organ class (SOC) of the same name. 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. Table 5-B presents further expedited reporting requirements.

Table 5-B: Phase 1 and Early Phase 2 Studies: Expedited reporting requirements for adverse events that occur on studies under an IND/IDE within 30 Days of the last administration of the investigational agent/intervention [1, 2]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor **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 participant or participant 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 electronic submission within the timeframes detailed in the table below.

Hospitalization	Grade 1 and Grade 2 Timeframes	Grade 3-5 Timeframes
Resulting in Hospitalization ≥ 24 hrs	10 Calendar Days	24-Hour five Calendar Days
Not resulting in Hospitalization ≥ 24 hrs	Not required	

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; five Calendar Days" - The AE must initially be submitted electronically within 24 hours of learning of the AE, followed by a complete expedited report within five calendar days of the initial 24-hour report.
- "10 Calendar Days" - A complete expedited report on the AE must be submitted electronically within ten 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 five calendar days for:
All Grade 3, 4, and Grade 5 AEs
Expedited ten calendar day reports for:
Grade 2 AEs resulting in hospitalization or prolongation of hospitalization
[2] For studies using PET or SPECT IND agents, the AE reporting period is limited to ten 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

5.6 Pregnancy

Although not an adverse event in and of itself, pregnancy as well as its outcome must be documented via **CTEP-AERS**. In addition, the ***Pregnancy Information Form*** included in Advantage eClinical and within the NCI Guidelines for Adverse Event Reporting Requirements must be completed and submitted to CTEP. Any pregnancy occurring in a

participant from the time of consent to 90 days after the last dose of study drug must be reported and then followed for outcome. Newborn infants should be followed until 30 days old. Please see the "NCI Guidelines for Investigators: Adverse Event Reporting Requirements for DCTD (CTEP and CIP) and DCP INDs and IDEs" (at http://ctep.cancer.gov/protocolDevelopment/adverse_effects.htm) for more details on how to report pregnancy and its outcome to CTEP.

5.6.1 Procedures for monitoring and reporting pregnancy

Participants who are pregnant or expect to become pregnant during the course of the study will be excluded from participation in the study. Should a participant become pregnant after enrolling in the study, she will not be given any further study treatments. A Pregnancy Form will be completed by the site personnel and submitted to the sponsor or its designee within 24 hours after learning of the pregnancy. Site personnel will submit the Pregnancy Form to the Sponsor or its designee, as described above.

The investigator will also report this event to the IRB within 24 hours of becoming aware of the pregnancy. Sites must request the participant's permission to query pregnancy outcome and follow each participant to determine the outcome of the pregnancy. Results will be summarized in the clinical study report (CSR).

Participants who become pregnant at any point during the study will continue to be followed for safety assessments without receiving further study treatment. Procedures that are contraindicated during pregnancy, including additional treatments, must not be performed. Investigators should use clinical judgment regarding subsequent study-related blood collection based on the presence or absence of anemia in each participant.

All pregnancies that occur from the time of first study treatment through the follow-up visits must be reported. The investigator will monitor the participant and follow the outcome of the pregnancy. If the end of the pregnancy occurs after the study has been completed, the outcome will be reported directly to the study team and the medical monitor.

5.7 Adverse Events of Special Interest

Adverse events of special interest (AESI) are the adverse events deemed related to VGX-3100 delivered with CELLECTRA 5PSP that require expedited communication from the Site to the Sponsor and meet any of the following criteria:

- Grade 3 or greater persistent administration site erythema, and/or induration recorded ≥ 2 hours immediately after study treatment
- Grade 4 or greater persistent administration site pain, tenderness recorded ≥ 2 hours immediately after study treatment
- Grade 3 or greater fever
- Grade 3 or greater systemic symptoms, including generalized pruritus

Sites will inform the Sponsor as described below within 24 hours to discuss whether further dosing for the particular participant should continue.

5.7.1 Study reporting period of adverse events of special interest

AESI require expedited communication from the Site to the Sponsor. Within 24 hours of the site's awareness of the event, AESI must be reported by the Investigator through data entry in the Adverse Event electronic case report form (eCRF) via Advantage eClinical. In the event that Advantage eClinical is unavailable, the investigator must notify the Sponsor via email or phone.

AESI reporting if Advantage eClinical is unavailable:

- EMAIL: cancer-sae@emmes.com
- PHONE: 301-251-1161

5.8 Routine Adverse Event Reporting

Adverse events of any grade, regardless of causality or expectedness will be recorded on the Adverse Event Form. These requirements are summarized in Table 5-C below.

Table 5-C: 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)*	Adverse Event Form	Adverse Event Form
Related *(attribution of possible, probable, or definite)*	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.

5.8.1 Clinical laboratory abnormalities

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

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

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

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

5.8.2 Study reporting period for adverse events

All solicited and unsolicited adverse events will be collected from the date of informed consent through study discontinuation, recorded in the source documents, and reported in the appropriate AE CRF Form per Section 5.8.

Investigators are not obligated to actively seek AEs or SAEs beyond the follow-up period for participants. However, if the Investigator learns of an AE or SAE that occurs after the completion or termination visit and the event is deemed by the Investigator to be probably or possibly related to the trial treatment, he/she should promptly document and report the event to the Sponsor.

5.9 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.

5.10 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.

6.0 PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational agents administered in this study can be found in Section 5.3.

6.1 Investigational Drug

[**NOTE:** Please refer to the investigator's brochure for more information.]

Product Description

The VGX-3100 drug product contains DNA plasmids for expression of HPV-16 E6/E7 (pGX3001) and HPV-18 E6/E7 (pGX3002) antigens that have been designed and constructed using proprietary synthetic consensus DNA (SynCon) technology.

Route of Administration

Intramuscular

Storage

The drug product must be stored refrigerated at 2-8 °C (36-46 °F) and must be used within 4 hours of removal from the refrigerator.

Packaging and Labeling of Investigational Product

Each cartridge will be labeled with a single-panel label and then individually packaged within a pouch that will contain an additional, double-panel label with tear-off. VGX-3100 labels will include, at minimum, the following information in Figure 6-A.

Figure 6-A: Example labels for investigational product

Cartridges (primary container)	**Pouches** (secondary package)
VGX-3100 Insert cap end IM administration Inovio Pharmaceuticals, Inc.	Study ID/Material ID VGX-3100, Lot number Single-use, 1 mL Storage temperature, expiration date CAUTION: New Drug – Limited by United States Law to Investigational Use Inovio Pharmaceuticals, Inc.

Handling

When handling VGX-3100 for the AMC-103 Study, personal protective equipment (PPE) such as disposable gloves, laboratory coats, and eye protection must be worn.

Biosafety Level 1 (Biosafety level one), the lowest level, applies to work with agents that usually pose a minimal potential threat to laboratory workers and the environment and do not consistently cause disease in healthy adults. Research with these agents is generally performed on standard open laboratory benches without the use of special containment equipment. BSL 1 labs are not usually isolated from the general building and the practices are sufficient for AMC-103 since the IP is not an infectious agent; however, appropriate precautions to protect the IP from contamination should be followed.

Please note: All persons preparing and administering IP must be delegated appropriately on the Delegation of Responsibility Log prior to undertaking any protocol-specific procedures.

6.2 Drug Orders, Transfers, Returns, and Accountability

6.2.1 Drug accountability

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 Drug 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 DARFs document 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 NCI Investigational Drug Accountability Record and if the paper printout is identical to the NCI accountability record. 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.

6.2.2 Drug orders

Study drug will be provided by Inovio Pharmaceuticals or its designee, and will be distributed by the AMC's designee, Sharp Clinical. All essential documents for trial participation must be current as submitted to the sponsor's designee before placing each order for study agents. The site must complete protocol registration with the AMC ODMC and trial sponsor (UCSF), and will submit an Investigational Agent Shipment Authorization Form to Inovio prior to authorizing the first order of drug.

Once complete and the site has initiated participant screening, to order VGX-3100 and the CELLECTRA device, participating sites are to refer to the study drug ordering form and the study device ordering forms located on the AMC Operations Center web site (www.AIDSCancer.org).

The site will request resupply of IP from Emmes based on the quantities remaining at the study site, and it will be the site's responsibility to ensure that the pharmacy maintain adequate supplies for anticipated participant dosing.

6.2.3 Receipt and storage of investigational product

IP will be shipped in a refrigerated condition with a temperature monitoring device. Upon arrival, the pharmacist (or designee) should ensure that the shipment has arrived in good condition. The pharmacist or designee must also check the quantity numbers against the packing slip, and document that the shipment quantity and contents are correct. Receipt of IP is communicated, at the time of receipt, to the sponsor-investigator and Inovio by emailing amc-103@emmes.com and AMC-103Study@inovio.com.

Immediately upon arrival, IPs should be transferred from the shipping container into 2-8°C (36-46°F) storage, in a secure area, according to local regulations. If the temperature monitoring device denotes temperatures outside the pre-specified

range for any product, the Sponsor-investigator and its designee, the AMC ODMC (amc-103@emmes.com) and INO (AMC-103study@inovio.com) should be contacted immediately, and the site must complete the Inovio Temperature Excursion Notification Form (available on the password-protected side of the AMC Operations website).

Refrigerator temperature logs must be maintained at the clinical site and temperatures must be recorded and monitored regularly, either using a continuous electronic temperature monitoring system or daily manual temperature logs. Following storage at the site, the Sponsor-investigator and Inovio must be notified of any deviations from the required storage condition using the Inovio Temperature Excursion Notification Form. Any drug that experiences a temperature excursion may not be administered to a participant unless Inovio verifies that it remains acceptable for use.

6.2.4 Dispensing of investigational product

It is the responsibility of the Investigator to ensure that the IP is only dispensed to study participants. It must be dispensed only from official study sites by authorized personnel according to local regulations.

When a participant is eligible to receive treatment, remove the pouch from the refrigerator and allow to reach room temperature (20-25 °C). The Pharmacist (or designee) must also include the time and date the drug was removed from the refrigerator, as well as the expiration time ("use by time"), dosing visit and visit date in the subject's source notes/worksheet. This worksheet should be maintained in the pharmacy binder, and a copy kept with the subject's source documentation. The product must be used within 4 hours of removal from the refrigerator.

The product cartridge must not be removed from the pouch until immediately prior to administration. The pouch must not be discarded until 1) administration is completed and 2) all pertinent information from the pouch label has been documented in the DARF.

No mixing or dilution of the IP is required. If the IP is damaged or the solution is not clear, do not use, quarantine the IP, notify Inovio IP@Inovio.com, cc: amc-103@emmes.com and AMC-103Study@inovio.com and complete the Drug Complaint Reporting Form, if directed (available on the password-protected side of the AMC Operations web site).

The treatment procedure must be performed by qualified personnel including the Pharmacist (or designee) who have appropriate training and qualifications to administer intramuscular (IM) injections to participants in addition to successfully completing device training from sponsor personnel. Staff must be delegated this responsibility in the site's delegation of tasks log. The device user manual and instructions for use will inform clinical personnel about placement of the IP cartridge into the device, as well as the steps for injection and electroporation.

For complete information, please refer to the current Investigator's Brochure: Investigator Brochure VGX-3100. Contact AMC ODMC staff at amc-103@emmes.com for the current Investigator's Brochure.

6.2.5 Return and destruction of the investigational agent

Upon completion or termination of the study, all unused IP must be destroyed at site per institution policy or returned to Inovio Pharmaceuticals, Inc. or its designee, if site cannot destroy IP.

The used IP cartridge will be discarded along with the disposable array within a sharps container at site.

It is the Investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local, and institutional guidelines and procedures, and provided that appropriate records of disposal are kept. The return of unused IP(s) should be arranged by the responsible Study Monitor.

The unused IP can only be destroyed after being inspected and reconciled by the responsible Inovio Pharmaceuticals, Inc. or a designated AMC Study Monitor.

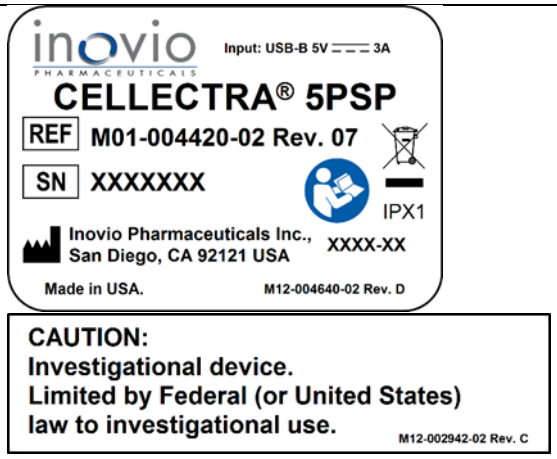
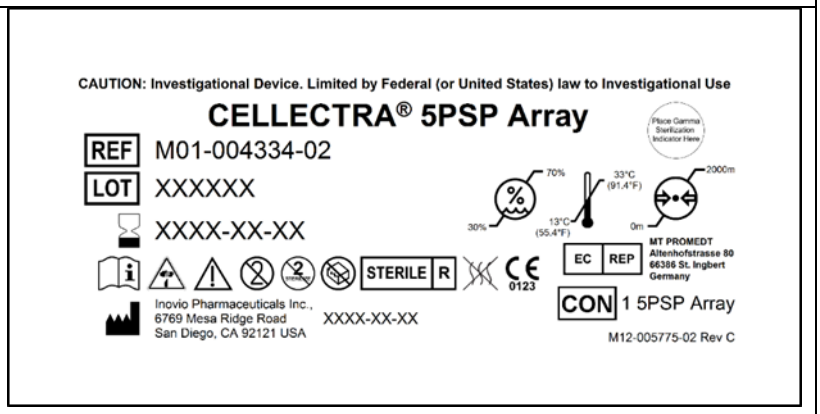
If IP is returned to Inovio Pharmaceuticals, Inc., or its designee, it must be accompanied by the appropriate documentation. Returned supplies should be in the original containers. Empty containers should not be returned to Inovio Pharmaceuticals, Inc. The return of unused IP(s) should be arranged with the responsible Inovio personnel and/or Clinical Monitor.

6.3 Investigational Device

6.3.1 Packaging and labeling of investigational device

See Figure 6-B below for an example of CELLECTRA 5PSP device component labels.

Figure 6-B: Example device labels (base station, handset, array)

Base Station	
Handset	
Array	

6.3.2 Handling of investigational device

The CELLECTRA 5PSP device and its components must be stored in a secure location according to the instructions in the User Manual. Sites are required to track the temperature of the storage conditions for the CELLECTRA 5PSP device and its components. Storage conditions for the device should remain between 13 and 33 degrees Celsius. A temperature log will be provided to sites for monitoring excursions on the password-protected side of the AMC Operations web site. Any temperature excursion affecting the device or arrays must be reported to Inovio Pharmaceuticals, Inc. immediately within one business day of awareness, using the

Inovio form provided for this purpose (available on the password-protected side of the AMC Operations web site). If an array is past the expiration date on its label, it should not be used for patient treatment. Any device or arrays that experience a temperature excursion may not be administered to a participant unless Inovio verifies that it remains acceptable for use.

6.3.3 Investigational device orders and training

Study devices will be provided by Inovio Pharmaceuticals and will be shipped directly to clinical sites. All essential documents for trial participation must be current as submitted to the sponsor's designee before placing each order for study agents. The site must complete protocol registration with the AMC ODMC and trial sponsor (UCSF), and will submit an Investigational Agent Shipment Authorization Form to Inovio prior to authorizing the first shipment of study devices.

Participating centers may request device training by contacting Inovio via email (AMC-103Study@inovio.com) close to the time the site will activate the study and initiate enrollment.

Upon screening the first participant, Inovio will ship two (2) devices with the associate components and arrays directly to the site. The site will request resupply of arrays by emailing the AMC ODMC (amc-103@emmes.com) who will inform Inovio of the request.

6.3.4 Investigational device accountability

The investigative site is responsible for maintaining investigational device and accountability logs. The device must have full traceability from the receipt of the products through the participant use, disposal, or return of the products. The Site must document acknowledgement of receipt and notify Inovio upon receipt of investigational product. This includes the content shipped and condition upon receipt.

For each participant treatment, there must be a record of each product used for that participant, i.e., CELLECTRA 5PSP serial number, array lot number and the study drug lot number. The used sterile disposable array attachment must be discarded after use in accordance with institutional policy regarding disposal of sharp needles/instruments.

6.3.5 Return of investigational device

Upon completion or termination of the study, all investigational devices and unused components must be returned to Inovio Pharmaceuticals, Inc.

All product returned to Inovio Pharmaceuticals, Inc. must be accompanied by the appropriate return documentation. Returned supplies should be in the original containers. The return of all product identified above should be arranged by the responsible Study Monitor.

If product is to be destroyed on site, it is the Investigator's responsibility to ensure that arrangements have been made for the disposal, written authorization has been granted by Inovio Pharmaceuticals, Inc., or its designee, procedures for proper disposal have been established according to applicable regulation and guidelines

and institutional procedures, and appropriate records of the disposal have been documented.

6.4 Reporting of Device Related Complaints or Deficiencies

A product complaint/device deficiency is defined as any written, electronic, or oral communication that alleges deficiencies or inadequacies of the device or components related to the identity, quality, durability, reliability, safety, effectiveness, or performance of the device or components after it is released for distribution within the clinical investigation. All product complaints that meet this definition (with the exception of SAEs requiring 24-hour reporting) must be reported to the Sponsor with 10 days of discovery.

Device deficiencies include malfunctions, use errors, and inadequate labeling. A malfunction is defined as the failure of a device to meet its performance specifications or otherwise perform as intended. The intended performance of a device refers to the intended use for which the device is labeled or marketed.

Any problems experienced during the treatment procedure including potential malfunctions of the device, error messages displayed on the device screen following treatment or errors that occur during the treatment procedure must be reported to the Sponsor or designee immediately for evaluation. The error reporting or complaint form must be completed and emailed to Inovio at clinicalcomplaint@inovio.com.

7.0 CLINICAL AND LABORATORY EVALUATIONS

Schedules shown in the Study Calendar below are provided in Appendix I.

For the injection visits, patients may be rescheduled up to 4 weeks later than the original planned date to allow participants to receive all the study treatment. The injections have to be at least 4 weeks apart. For example, the week 4 dose can be given no later than week 8. Furthermore, the late dose must be administered at least four weeks prior to the next scheduled dose.

Subsequent doses are not impacted by a missed dose. In the above scenario, if the Week 4 dose is not administered until Week 8, the protocol-defined doses for Weeks 12 and 24 remain as scheduled.

If any doses are delayed, the corresponding visit on Week 7, 15, or 27 will need to be moved so it is three weeks after the VGX-3100 injection beforehand. For example, if the Week 4 dose is delayed to Week 8, the original Week 7 visit will be delayed to Week 11.

7.1 Visit 0: Screening/Baseline Evaluations

The screening HRA must be performed by an HRA provider certified by AMC and 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 enrollment registration in Advantage eClinical. Study entry refers to enrollment in segment B for protocol treatment.

Baseline evaluations are to be conducted within 28 days prior to start of protocol therapy, unless otherwise specified below.

7.1.1 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.
- Medication history (as required by Section 4.3.2) 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.
- A listing of all other concurrent medical problems and diagnoses present at baseline.
- 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 the nadir CD4+ cell count report and entered on the CRF. If laboratory results are 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 month/year or year will suffice.

7.1.2 Clinical assessment

- Complete physical examination to include:
  - Performance status (Appendix II), 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 3.1.10.
  - A complete external genital examination in women that includes gross evaluation of the vulva for lesions.
- Digital anorectal exam.
- HRA exam of the anal canal will be performed within 90 days before study entry (Appendix VIII). Lesions must be photographed using Second Opinion or other software that allows for easy sharing of images between sites. If on HRA, 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 enrolled, 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.
- HRA-guided biopsies will be obtained to assure eligibility for the study. At minimum the lesion which appears to have the most advanced disease must be biopsied. When multiple lesions are present, biopsies should be obtained from each lesion, with corresponding HPV typing done. If the participant has had biopsies within 90 days of study entry, new biopsies are not necessary unless there is concern for progression towards invasive cancer. See Appendix VIII for the biopsy protocol.
- The number of involved octants and location of HSIL will be documented. If this is not available from the screening HRA, then this can be documented at study entry prior to treatment.

7.1.3 Laboratory tests must be obtained within 90 days before study entry (unless otherwise noted) and will include the following:

- Anal biopsy: Results of biopsy diagnostic of HSIL. Diagnostic biopsy tissue will be submitted for central pathology review. Biopsy tissue should be sent for central pathology review within 30 days of enrollment. Biopsy tissue will also be submitted for correlative studies and shipped once the participant is enrolled for treatment. Tissue blocks/tissue sandwich samples should be sent to the

AMC Biorepository within 30 days of initial diagnostic reading. Refer to the study MOP for sample submission requirements.

- Anal swab 1 for local cytology result. If anal cytology is not available prior to entry, then this may be obtained at study entry.
- Anal swab 2 for HPV testing. Refer to the study MOP for sample submission requirements.
- Safety laboratory testing: Baseline laboratory testing will include CBC with differential and platelet count, comprehensive metabolic panel (CMP) (includes Na, K, Cl, CO_2, BUN, glucose, calcium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, total protein, albumin), and creatine kinase. Safety laboratory tests may be performed by the local healthcare provider/local laboratory with results sent to the responsible investigator.
- HIV viral load (within 120 days before study entry): Viral load studies will be performed using an assay with a limit of detection of 75 copies/ml or less. A copy of existing laboratory results from a CLIA-certified laboratory may be used.
- T-cells (within 120 days before study entry): CD4/CD8 counts and percentages will be quantified. A copy of existing laboratory results from a CLIA-certified laboratory may be used.
- Pregnancy test: Urine beta-HCG will be performed (and results obtained) within 72 hours prior to enrollment when indicated (women of childbearing potential).
- Electrocardiogram

7.1.4 Optional donation to the AIDS and Cancer Specimen Resource (ACSR). (See Appendix IV for ACSR Informed Consent Form and Appendix III for ACSR Specimen Preparation and Shipping Instructions).

7.1.5 Correlative Studies: At least 34 mL of whole blood and 4 mL of serum must be collected at Screening for peripheral blood immunogenicity assessments (total blood draw: 44 mL) (see Table 7-A). Details of the immunology sample collection and shipment information will be provided in the study Laboratory Manual of Procedures (MOP).

Table 7-A: Correlative study blood samples for peripheral blood immunogenicity assessments

Sample Type	Tubes
Whole Blood (ACD/Yellow Top Tubes)	4 x 8.5 mL tubes
Serum (Plain Red Top Tube)	1 x 10 mL tube

7.2 Visit 1 (Week 0)

7.2.1 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 examination, to include pulse, blood pressure, height, weight, performance status, and any previously identified or new signs or symptoms, including diagnoses that the participant has experienced since the last visit
- Medication review

7.2.2 Laboratory tests

- Pregnancy test: Urine or serum beta-HCG will be performed (and results obtained) when indicated (women of childbearing potential) within 72 hours before IP administration
- Safety laboratory testing: Baseline laboratory testing will include CBC with differential and platelet count, CMP (see Section 7.1.3 for definition), and creatine kinase. Safety labs may be collected up to 30 days before treatment administration. Safety laboratory tests may be performed by the local healthcare provider/local laboratory with results sent to the responsible investigator. Safety lab results must be received before administering treatment.

7.2.3 Assessment of clinical study adverse events

A medical/clinical assessment will be conducted at each visit during which participants will be queried regarding the occurrence of any adverse events, concomitant medications new onset illness or disease, as well as contraceptive compliance. Participants will be reminded to contact study personnel and immediately report any event that happens for the duration of the study. Unsolicited adverse events will be captured from the time of the informed consent to study discontinuation. These events will be recorded on the participant's CRF.

7.2.4 Correlative studies

At least 34 mL of whole blood and 4 mL of serum must be collected again on Day 0 before dosing for immunogenicity assessments (total blood draw: 44 mL). This draw must be collected in addition to the specimens collected during screening. Details of the immunology sample collection and shipment information will be provided in the study Laboratory Manual of Procedures (MOP).

7.2.5 Injection and electroporation #1

7.2.6 Assessment of injection site reactions

The injection site will be assessed by study personnel prior to and approximately 30 minutes after each study treatment and at 2 weeks post study treatment visits. The two week post-treatment study visit may be conducted in person or via phone/video per institutional standards. Participants will also be instructed to

record local and systemic AEs for seven days on a Participant Diary Card (PDC) as shown in Appendix VI.

When evaluating injection site reactions throughout the study, the investigator will use the following grading scale.

Table 7-B: Grading scale for injection site reactions

Local Reaction to Injectable Product (Grade)	Mild (1)	Moderate (2)	Severe (3)	Potentially Life-Threatening (4)
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever >24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room (ER) visit or hospitalization
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization
Erythema/Redness*	2.5-5 cm	5.1-10 cm	>10 cm	Necrosis or exfoliative dermatitis
Induration/Swelling**	2.5-5 cm and does not interfere with activity	5.1-10 cm or interferes with activity	>10 cm or prevents daily activity	Necrosis

September 2007 "FDA Guidance for Industry—Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials"

*In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.

**Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement.

7.3 Visit 2: Week 4 +/- 4 days

7.3.1 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 examination, to include pulse, blood pressure, weight, performance status, and any previously identified or new signs or symptoms, including diagnoses that the participant has experienced since the last visit
- Medication review
- Collect and review PDC

7.3.2 Laboratory tests

- Pregnancy test: Urine or serum beta-HCG will be performed (and results obtained) when indicated (WOCBP) within 72 hours before IP administration
- Safety laboratory testing: CBC with differential and platelet count, CMP (see Section 7.1.3 for definition), and creatine kinase. Safety labs may be collected up to 7 days before treatment administration. Safety laboratory tests may be performed by the local healthcare provider/local laboratory with results sent to the responsible investigator. Safety lab results must be received before administering treatment.

7.3.3 Assessment of clinical study adverse events

7.3.4 Injection and electroporation #2, provide PDC

7.3.5 Assessment of injection site reactions

7.4 Visit 3: Week 7 +/- 7 days

7.4.1 Assessment of injection site reactions and clinical study adverse events

7.4.2 Medication review

7.4.3 Collect and review PDC

7.4.4 Correlative studies: peripheral blood collection for immunogenicity assessments

7.5 Visit 4 (Week 12 +/- 4 days)

7.5.1 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, weight, performance status, and any previously identified or new signs or symptoms, including diagnoses that the participant has experienced since the last visit.
- Medication review

7.5.2 Laboratory tests

- Pregnancy test: Urine or serum beta-HCG will be performed (and results obtained) when indicated (women of childbearing potential, WOCBP) within 72 hours before IP administration.
- Safety laboratory testing: CBC with differential and platelet count, CMP (see Section 7.1.3 for definition), and creatine kinase. Safety labs may be collected up to 7 days before treatment administration. Safety laboratory tests may be performed by the local healthcare provider/local laboratory with results sent to the responsible investigator. Safety lab results must be received before administering treatment.
- CD4/CD8 T-cell counts and HIV-1 RNA viral load. These tests may be collected up to 30 days before the visit and may be performed by the local healthcare provider/local laboratory with results sent to the responsible investigator.

7.5.3 Assessment of clinical study adverse events

7.5.4 Injection and electroporation #3, provide PDC

7.5.5 Assessment of injection site reactions

7.6 Visit 5 (Week 15 +/- 7 days)

7.6.1 Assessment of injection site reactions and clinical study adverse events

7.6.2 Medication review

7.6.3 Collect and review PDC

7.6.4 Correlative studies: Peripheral blood collection for immunogenicity assessments

7.7 Visit 6 (Week 24 +/- 4 days)

7.7.1 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, weight, performance status, and any previously identified or new signs or symptoms, including diagnoses that the participant has experienced since the last visit.
- Medication review
- Digital anorectal examination
- Anal 1 swab for local cytology result
- Anal 2 swab for HPV testing
- High resolution anoscopy. The number of involved octants and location of HSIL will be documented All lesions, all aspects of the anal transformation zone, distal canal and perianus will be photographically documented.

7.7.2 Laboratory tests

- Anal biopsy: If on HRA, the clinician notes suspicious friability, ulceration, mass effect, heaped-up borders, and/or markedly abnormal vascular pattern, these could be indicative of invasive disease. Additional biopsies should be obtained and participant may need to be removed from the study.
- Pregnancy test: Urine or serum beta-HCG will be performed (and results obtained) when indicated (WOCBP) within 72 hours before IP administration.
- Safety laboratory testing: CBC with differential and platelet count, CMP (see Section 7.1.3 for definition), and creatine kinase. Safety labs must be collected up to 7 days before treatment administration. Safety laboratory tests may be performed by the local healthcare provider/local laboratory with results sent to the responsible investigator. Safety lab results must be received before administering treatment.
- CD4/CD8 T-cell counts and HIV-1 RNA viral load. These tests may be collected up to 30 days before the visit and may be performed by the local healthcare provider/local laboratory with results sent to the responsible investigator.

7.7.3 Assessment of clinical study adverse events

7.7.4 Injection and electroporation #4, provide PDC

7.7.5 Assessment of injection site reactions

7.8 Visit 7 (Week 27 +/- 7 days)

7.8.1 Assessment of injection site reactions

7.8.2 Medication review

7.8.3 Collect and review PDC

7.8.4 Correlative studies: peripheral blood for immunogenicity assessments

7.9 Visit 8 (Week 36 +/- 7 days)

7.9.1 Clinical assessment

- Complete physical examination to include:
  - Performance status (Appendix II), 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
  - A complete external genital examination in women that includes gross evaluation of the vulva for lesions
- Medication review
- Digital anorectal exam
- HRA exam of the anal canal: The number of involved octants and location of HSIL will be documented. All lesions, all aspects of the anal transformation zone, distal canal and perianus will be photographically documented.

7.9.2 Laboratory tests

- Anal biopsy: If on HRA, the clinician notes suspicious friability, ulceration, mass effect, heaped-up borders, and/or markedly abnormal vascular pattern, these could be indicative of invasive disease. Additional biopsies should be obtained and the participant may need to be removed from the study.
- Anal swab 2 for HPV testing.
- HIV-1 RNA viral load: This test must be collected up to 30 days before the visit and may be performed by the local healthcare provider/local laboratory with results sent to the responsible investigator.

7.9.3 Assessment of clinical study adverse events

7.10 Visit 9 (Week 48 +/- 7 days)

7.10.1 Clinical assessment

Complete physical examination to include:

- Performance status (Appendix II), 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
- A complete external genital examination in women that includes gross evaluation of the vulva for lesions
- Medication review
- Digital anorectal exam
- HRA exam of the anal canal: The number of involved octants and location of HSIL will be documented. All lesions, all aspects of the anal transformation zone, distal canal and perianus will be photographically documented.

7.10.2 Laboratory tests

- HRA-guided biopsies will be obtained for all visible lesions. This is required for the primary objective. Biopsy tissue will also be submitted for correlative studies. Tissue blocks/tissue sandwich samples should be sent to the AMC Biorepository within 30 days of initial diagnostic reading. Refer to the study MOP for sample submission requirements.
- Anal swab 1 for local cytology result.
- Anal swab 2 for HPV testing.
- Safety laboratory testing: CBC with differential and platelet count, CMP (see Section 7.1.3 for definition), and creatine kinase. Safety labs must be collected up to 7 days before the visit. Safety laboratory tests may be performed by the local healthcare provider/local laboratory with results sent to the responsible investigator. Safety lab results must be received before administering treatment.
- CD4/CD8 T-cell count and HIV-1 RNA viral load. These tests may be collected up to 30 days before the visit and may be performed by the local healthcare provider/local laboratory with results sent to the responsible investigator.
- Correlative Studies: peripheral blood for immunogenicity assessments.

7.10.3 Assessment of clinical study adverse events

7.11 Visit 10 (Week 60 +/- 7 days)

7.11.1 Clinical assessment

- Complete physical examination to include:
  - Performance status (Appendix II), 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
  - A complete external genital examination in women that includes gross evaluation of the vulva for lesions

- Medication review
- Digital anorectal exam
- HRA exam of the anal canal: The number of involved octants and location of HSIL will be documented. All lesions, all aspects of the anal transformation zone, distal canal and perianus will be photographically documented.

7.11.2 Laboratory tests

- Anal biopsy: If on HRA, the clinician notes suspicious friability, ulceration, mass effect, heaped-up borders, and/or markedly abnormal vascular pattern, these could be indicative of invasive disease. Additional biopsies should be obtained and participant may need to be removed from the study.
- Anal swab 2 for HPV testing.
- HIV viral load: This test may be collected up to 30 days before the visit and may be performed by the local healthcare provider/local laboratory with results sent to the responsible investigator.

7.11.3 Assessment of clinical study adverse events

7.12 Visit 11 (Week 72 +/- 7 days)

7.12.1 Clinical assessment

- Complete physical examination to include:
  - Performance status (Appendix II), 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
  - A complete external genital examination in women that includes gross evaluation of the vulva for lesions
- Medication review
- Digital anorectal exam
- HRA exam of the anal canal: The number of involved octants and location of HSIL will be documented. All lesions, all aspects of the anal transformation zone, distal canal and perianus will be photographically documented.

7.12.2 Laboratory tests

- HRA-guided biopsies of all visible lesions are required. Biopsy tissue will also be submitted for correlative studies. Tissue blocks/tissue sandwich samples should be sent to the AMC Biorepository within 30 days of initial diagnostic reading. Refer to the study MOP for sample submission requirements. If any HSIL is diagnosed at this visit, the participant should be referred for treatment off study at the discretion of the treating provider.

- Anal swab 1 for local cytology result.
- Anal swab 2 for HPV testing.
- Safety laboratory testing: CBC with differential and platelet count, CMP (see Section 7.1.3 for definition), and creatine kinase. Safety labs may be collected up to 7 days before the visit. Safety laboratory tests may be performed by the local healthcare provider/local laboratory with results sent to the responsible investigator. Safety lab results must be received before administering treatment.
- CD4/CD8 T-cell count and HIV-1 RNA viral load. These tests may be collected up to 30 days before the visit and may be performed by the local healthcare provider/local laboratory with results sent to the responsible investigator.
- Correlative studies: peripheral blood for immunogenicity assessments

7.12.3 Assessment of clinical study adverse events

7.13 Early Treatment Discontinuation Evaluations

- Clinical assessment: The participant's medical and medication 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 7.2.1).
- Safety laboratory testing: CBC with differential and platelet count, CMP (see Section 7.1.3 for definition), and creatine kinase. Safety labs may be collected up to 7 days before the visit. Safety laboratory tests may be performed by the local healthcare provider/local laboratory with results sent to the responsible investigator.
- Correlative Studies: peripheral blood for immunogenicity assessments
- Anal swab 1 for local cytology result
- Anal swab 2 for HPV testing
- Digital anorectal examination
- HRA exam of the anal canal. The number of involved octants, location, and percent circumference of HSIL will be documented per Section 7.1.2. All lesions, all aspects of the anal transformation zone, distal canal and perianus will be photographically documented.
- All participants should have biopsies obtained as per Appendix VIII.
- If any HSIL is diagnosed at this visit, the participant should be referred for treatment off study at the discretion of the treating provider
- Collect the PDC, if applicable
- Assessment of Clinical Study Adverse Events
- Assessment of Injection Site Reactions, if applicable

8.0 MEASUREMENT OF EFFECT

A rostered AMC investigator (CTEP-registered physician investigator or CTEP-registered non-physician investigator (NPIVR) who is certified in HRA) must assess response at the designated time points. The site's Delegation of Tasks Log should indicate that the principal investigator has delegated this to the named individual (NPIVR).

8.1 Clinical Response

8.1.1 Complete Response (CR): The absence of HPV-16 or -18 associated HSIL or LSIL histology for all biopsies.

8.1.2 Partial Response (PR): The regression of HPV-16 or -18 associated HSIL histology to LSIL or less.

8.1.3 No Response: The presence of HPV-16 or -18 associated HSIL histology with no decrease in the respective lesion size.

8.1.4 Progression of Disease (PD): The presence of new HPV-16 or -18 HSIL lesions or presence of invasive anal cancer

9.0 STATISTICAL CONSIDERATIONS

9.1 Study Design/Endpoints

Primary Objective

Overall response rate (defined as histopathological regression of HPV-16 or 18-positive HSIL to LSIL [AIN1], i.e., partial response, or normal, i.e., complete response) at 48 weeks after the first dose

Hypothesis

Treatment of HPV-16 or 18-positive anal HSIL in HIV-positive individuals by VGX-3100 will lead to the increase of overall response rate at 48 weeks by testing: Null hypothesis: 30% response rate vs. Alternative hypothesis: 45% response rate.

Primary Endpoint Analysis Plan

The one-sample z-test of proportion for the above hypothesis will be used to analyze the overall (complete or partial) response rate at 48 weeks after the first dose. The observed proportion and its corresponding 95% Wald confidence interval will also be used for estimation.

The analysis set for the primary endpoint includes all eligible participants with an HPV-16 or 18-positive HSIL at entry (including those who are replaced for HIV-related events). The participant may have additional HSIL lesion(s) that are non-HPV-16 or 18-positive. Determination of CR and PR for HPV-16 or 18-positive lesions and non-HPV-16 or 18-positive lesions are performed independently.

9.2 Sample Size/Accrual Rate

A sample size of 72 participants with HPV-16 or 18 DNA-positive anal swabs and at least one biopsy-proven anal HSIL will be sufficient to test the null hypothesis: 30% response rate vs. the alternative hypothesis: 45% response rate at the one-sided 0.10 significance level with power of 0.90. The one-sample test of proportion will be used to analyze the overall (complete or partial) response rate at 48 weeks after the first dose.

Our sample size is 72 participants with HPV-16 or 18 DNA-positive anal swabs and at least one biopsy-proven anal HSIL. The target sample size will be 92 participants in order to obtain 72 evaluable participants; an additional 20 participants will be enrolled to adjust for a 10% unevaluable rate, to replace up to ten participants with positive HPV-16/18 in anal swab but negative in their biopsy, and to replace participants with HIV-related events (up to a maximum of ten such participants, see Section 3.3). There are 15 AMC sites that have capability to perform HRA; eight sites that have demonstrated the capacity to enroll multiple participants per month on AMC trials for anal HSIL will be selected. Therefore, we believe accruing on average five participants per month should be feasible.

9.3 Analysis of Secondary Endpoints

- Safety as assessed by Common Terminology Criteria for Adverse Events version 5.0 (CTCAE v.5.0).
- Complete response rate (defined as histopathological regression to normal) at 48 weeks after the first dose.

- Viral clearance rate of HPV-16 and HPV-18 (defined as changing from presence to absence of HPV-16/18 in anal HSIL by anal histological specimen) at 48 weeks after the first dose.
- Viral clearance rate of HPV-16 and HPV-18 (defined as changing from presence to absence of HPV-16/18 in anal HSIL by anal swab) at 48 weeks after the first dose.
- Overall response rate (defined as histopathological regression from HSIL to LSIL or normal) at 72 weeks after the first dose of VGX-3100.

The observed proportion and its corresponding 95% Wald confidence interval will be used to estimate:

a) the proportion of participants with HPV-16 or HPV-18 positive anal HSIL who were complete responders at 48 weeks after the first dose,

b) the proportion of participants with viral clearance of HPV-16 and HPV-18 (defined as changing from presence to absence of HPV-16/18 in anal HSIL by anal histological specimen) at 48 weeks after the first dose,

c) the proportion of participants with viral clearance of HPV-16 and HPV-18 (defined as changing from presence to absence of HPV-16/18 in anal HSIL by anal swab) at 48 weeks after the first dose, and

d) the proportion of participants with HPV-16 or HPV-18 positive anal HSIL who were responders at 72 weeks after the first dose.

The analysis of d) will be stratified by those who do versus do not elect for standard of care prior to Week 72.

The primary analysis will be based upon participants with HPV16/18 positive tissue at entry. Similar secondary analyses will be performed in the population based upon swab positivity at entry (regardless of tissue results).

Potentially 15-20% of patients may have had preventive HPV vaccination prior to enrollment. In addition to the analyses above, results will also be stratified by prior vaccination status to investigate its impact in a sensitivity analysis.

9.4 Analysis of Exploratory Endpoints

The following exploratory endpoints will be analyzed:

- Overall response rate (overall response defined as histopathological regression of non-HPV-16 or HPV-18-positive HSIL to low-grade squamous intraepithelial lesions [LSIL or AIN1] or normal) at 48 weeks after the first dose
- T-cell responses to HPV-16 and HPV-18 E6 and E7 and T-cell infiltration
- Antibody responses to HPV-16 and HPV-18 E7
- The association of the addition of fourth dose of VGX-3100 with T-cell and antibody responses
- The association of VGX-3100 with changes in CD4+ lymphocyte count over time
- The association of VGX-3100 with changes in HIV-1 RNA over time

- The association of CD4 + lymphocyte count with overall or complete response rate at 48 weeks after the first dose of VGX-3100
- The effect of tissue PD-L1 expression and T-cell infiltration on clinical benefit

The observed proportion and its corresponding 95% Wald confidence interval will be used to estimate the proportion of non-HPV-16 or HPV-18-positive anal HSIL that achieve a complete or partial response (which is defined as histopathological regression from HSIL to LSIL or normal) at 48 weeks after the first dose of VGX-3100. Determination of clearance for HPV-16 or 18-positive lesions and non-HPV-16 or 18-positive lesions are performed independently.

T-cell responses will be collected from blood samples. To assess the increase in T-cell responses from blood samples from baseline to 7, 15, 27, 36, 48, 60 and 72 weeks after the first dose, medians and associated exact distribution-free confidence intervals will be used. These increases are left-censored at 0. To assess the change in T-cell infiltration from tissue samples from baseline to weeks 48 and 72 after the first dose, means and associated t-distribution based confidence intervals will be used. To assess antibody responses, geometric mean titers and associated 95% t-distribution based confidence intervals will be used at 27, 48, and 72 weeks after the first dose.

Mean differences and associated t-distribution based 95% confidence intervals will be used to assess the effect of VGX-3100 on CD4+ lymphocyte count over time by comparing the CD4 counts at each time-point to participant baseline values.

Mean differences and associated t-distribution based 95% confidence intervals will be used to assess the effect of VGX-3100 on HIV-1 RNA over time by comparing the viral loads at each time-point to participant baseline values.

Mean differences and associated t-distribution based 95% confidence intervals will be used to compare the CD4+ lymphocyte counts between those with an overall response to those without, and separately, between those with a complete response to those without, at 48 weeks after the first dose.

Mean differences and associated t-distribution based 95% confidence intervals will be used to compare T-cell infiltration and PD-L1 expression between those with an overall response to those without at 48 weeks after the first dose.

9.5 Interim Analysis

One interim analysis of the primary efficacy outcome is planned after 35 participants have complete assessment for the primary endpoint to assess the futility of achieving a significant result if the study continues.

Using the two-stage hybrid design of Herndon [38], accrual does not need to be suspended after the first stage of study while the response is being captured and the data analyzed. This design is well-suited for situations when accrual is slow and the disease under study is rare. Our experience has been that accrual after stopping a study after the first stage often results in much slower accrual.

The design parameters were chosen such that they would be comparable to those of a Simon's Optimum design:

- if X_1 is the number of first stage responses, the decision boundary a_1 for accepting H_0 at the first stage satisfies Binomial $(X_1 \leq r_1|p_A, n_1)=0.0354$. Here, $p_A=0.45$, $n_1=35$, and $r_1=10$.
- if X is the total number of responses after both stages of accrual, the boundary, r_2 for rejecting H_0 after the second stage satisfies Binomial $(X > r_2|p_0, N)=0.0670$. Here, $p_0=0.30$, $N=72$, and $r_2=27$.

As stated by Herndon [38], the error rates associated with the hybrid design can be examined from two points of view: the design point of view (*a-priori* error rates) and after the study has been completed (*post-hoc* error rates). Prior to the start of the study, the goal of a hybrid design is to accrue n_1 participants in the first stage, where n^*, the number of participants in stage 2 whose data are evaluable when the stage 1 analysis indicates suspension, is unknown. The *a-priori* error rates can be calculated based on n_1; these calculations are presented above in the design parameters. Additionally, we can view each one of the additional n* accruals in Table 9-A as their own design with their own corresponding *post-hoc* error rates. The *post-hoc* error rates are presented in Table 9-A. For these reasons, *the a-priori* and *post-hoc* error values need not yield identical calculations.

Table 9-A: Simulation results from 10,000 runs for design parameters for different values of n*

n*	Post-hoc Alpha	Post-hoc Power	Probability of Suspension Under H_0	Probability of Early Termination Under H_0	Probability of Suspension Under H_1	Probability of Early Termination Under H_1	c*
0	0.0707	0.8633	NA	0.5033	NA	0.0365	NA
1	0.0714	0.8669	0.5033	0.4649	0.0365	0.0279	10.5
2	0.0719	0.8693	0.5033	0.4245	0.0365	0.0204	10.9
3	0.0714	0.8678	0.5033	0.4722	0.0365	0.0258	11.4
4	0.0719	0.8699	0.5033	0.4460	0.0365	0.0203	11.8
5	0.0716	0.8684	0.5033	0.4778	0.0365	0.0265	12.3

n* represents the number of participants in stage 2 whose data are evaluable when the stage 1 analysis indicates suspension.

Decision rules: After 35 participants are evaluable, the analysis of stage 1 data is conducted. If there are ten or fewer clinical responses, accrual is temporarily suspended, and data from all evaluable participants, including the n* participants in the 2nd stage, are analyzed. If there are greater than c* responses, then accrual continues until 72 evaluable participants; otherwise, the study terminates early. After 72 participants, if there are more than 27 clinical responses (i.e., 28 or more), the agent is considered worthy of further study.

9.6 Reporting and Exclusions

9.6.1 Evaluation of toxicity – All participants will be evaluable for toxicity from the time of their first treatment with VGX-3100.

9.6.2 Evaluation of response – All participants included in the study will be assessed for response to treatment, even if there are major protocol treatment deviations or if

they are ineligible. Each participant will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease (persistent HSIL/no response), (4) progressive disease (progression to anal cancer), 5) early death from malignant disease (anal cancer), 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). The analysis population for the primary endpoint may be found in Section 9.1.

10.0 ROLE OF DATA MANAGEMENT

10.1 CRF Instructions

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

10.2 Data Quality

It is the responsibility of the AMC ODMC to assure the quality of data for the study (See Appendix V, AMC Data and Safety Monitoring Plan). This role extends from protocol development to generation of the final study database.

10.3 Data Monitoring

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 electronically to CTEP on a quarterly basis by FTP burst of data. 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.

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

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.

All participants must sign the informed consent prior to any trial related procedures being performed. The informed consent documentation must be in accordance with applicable regulations and GCP. Qualified trial personnel will meet with prospective trial participants, explain the trial, and provide them with an informed consent form (ICF) that describes the screening tests, eligibility criteria for entering the trial, trial treatments and follow-up procedures, in a language understandable to the participant. Explanation of the trial includes, but is not limited to, trial objectives, potential benefits and risks, discomforts/inconveniences, and the participant's rights and responsibilities. The participant or participant's legally acceptable representative is then requested to sign and date the ICF. A copy of the signed informed consent documentation must be provided to the participant or participant's legally acceptable representative. The qualified trial personnel will document the process of obtaining informed consent within the source record. Signed ICFs are maintained in the participant's source records and must be accessible for verification at any time.

11.1.1 Office of Biotechnology Activities (OBA)

The investigator and Sponsor are responsible for ensuring that the clinical study is reviewed and approved according to local and applicable global regulations (e.g., NIH Office of Biotechnology Activities) governing research that involves recombinant or synthetic nucleic acid molecules.

11.2 Investigator and Research Associate Registration with CTEP

Food and Drug Administration (FDA) regulations require IND sponsors to select qualified investigators. NCI policy requires all persons participating in any NCI-sponsored clinical trial to register and renew their registration annually. To register, all individuals must obtain a CTEP Identity and Access Management (IAM) account (https://ctepcore.nci.nih.gov/iam). In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) (*i.e.*, clinical site staff requiring write access to Oncology Patient Enrollment Network (OPEN) or Rave or acting as a primary site contact) must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR) (https://ctepcore.nci.nih.gov/rcr). Documentation requirements per registration type are outlined in Table 11-A.

Table 11-A: CTEP registration levels and requirements

Documentation Required	IVR	NPIVR	AP	A
FDA Form 1572	✔	✔		
Financial Disclosure Form	✔	✔	✔	
NCI Biosketch (education, training, employment, license, and certification)	✔	✔	✔	
HSP/GCP training	✔	✔	✔	
Agent Shipment Form (if applicable)	✔			
CV (optional)	✔	✔	✔	

An active CTEP-IAM user account and appropriate RCR registration is required to access all CTEP and Cancer Trials Support Unit (CTSU) websites and applications. In addition, IVRs and NPIVRs must list all clinical practice sites and IRBs covering their practice sites on the FDA Form 1572 in RCR to allow the following:

- Added to a site roster
- Assigned the treating, credit, consenting, or drug shipment (IVR only) tasks in OPEN
- Act as the site-protocol PI on the IRB approval
- Assigned the Clinical Investigator (CI) role on the Delegation of Tasks Log (DTL); the AMC DTL template will be used for this study; see Section 11.7).

Additional information can be found on the CTEP website at https://ctep.cancer.gov/investigatorResources/default.htm. For questions, please contact the RCR ***Help Desk*** by email at RCRHelpDesk@nih.gov.

11.3 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 of the treating institution. A copy of the written approval of the IRB/IEC and the OBA (if applicable) must be sent to the ODMC.

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 participants reflects the percentage representation of these populations in their geographic region and, for the AMC, the United States as a whole. As such, it is expected that the representation of participants on this trial will reflect the constitution of the respective populations. Accrual targets are presented in Table 11-B.

Table 11-B: Accrual targets

DOMESTIC PLANNED ENROLLMENT REPORT					
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	9	14	4	9	36
White	9	19	4	13	45
More Than One Race	2	2	2	2	8
Total	20	38	10	24	92

11.5 Data and Safety Monitoring Board Review

The AMC Data and Safety Monitoring Board (DSMB) will meet annually to review safety data and tissue regression and viral clearance results. The AMC DSMB will be charged with advising the Sponsor or Sponsor's designee if there appears to be a safety issue. The following stopping rules will be applied:

- If at any time during a study one-third (1/3) or more of the participants experience an AESI, further enrollment and study treatment will be halted immediately until a thorough investigation has been conducted by the Medical Monitor, Principal Investigator for the trial, and the AMC DSMB.
- If any SAE (or potentially life-threatening AE), or death assessed as related to study treatment occurs, further enrollment and study treatment will be halted immediately

until a thorough investigation has been conducted by the Medical Monitor, Principal Investigator for the trial, IRB (if applicable) and the AMC DSMB.

- If three or more participants in this study, experience the same Grade 3 or 4 adverse event, assessed as related to study treatment, further enrollment and study treatment will be halted immediately until a thorough investigation has been conducted by the Medical Monitor, Principal Investigator for the trial, and the AMC DSMB.
- In the event of two identical, unexpected, Grade 4 toxicities, assessed as related to study treatment, further enrollment and study treatment will be halted immediately until a thorough investigation has been conducted by the Medical Monitor, Principal Investigator for the trial, IRB (if applicable) and the AMC DSMB.

The sponsor or designee will notify all investigators and IRBs (if required) regarding the outcome of any investigation stemming from a study pause.

The safety data from this study will also be reviewed along with all data from VGX-3100 studies by Inovio using Inovio's program-level safety review process.

If at any time the study enrollment is halted, CTEP and FDA must be notified immediately and the study will not be permitted to reopen until CTEP and FDA provide permission to do so.

11.6 Trial Discontinuation

Inovio and the AMC reserve the right to discontinue the trial at this site or at multiple sites for safety or administrative reasons at any time. In particular, a site that does not recruit at a reasonable rate may be discontinued. Additionally, the trial may be discontinued at any time by an IRB, the FDA or other government agencies as part of their duties to ensure that research participants are protected.

Should the trial be terminated and/or the site closed for any reason, all investigational drugs and devices must be returned to Inovio or its representative. The Principal Investigator should ensure their site file documents are complete prior to archiving and provide copies of any requested documents to the sponsor.

11.7 Protocol Registration and Delegation of Tasks Log

Each site must complete a protocol-specific registration packet, including an AMC DTL using the provided AMC template or local equivalent. The Clinical Investigator (CI) is required to review and sign the DTL prior to the site receiving an approved site registration status and enrolling participants to the study. The AMC DTL template is provided in the protocol registration packet for this protocol, located on the AMC Operations web site at www.AIDSCancer.org. Any individual at the enrolling site on a participating roster may initiate the site DTL. Instructions on completing the DTL are embedded in the AMC DTL template.

The AMC DTL must be updated contemporaneously as personnel are added or removed and/or study roles and delegated tasks change. Changes must be approved by the CI, and documented by his/her initials and date, before they are implemented.

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APPENDIX I: SCHEDULE OF EVALUATIONS

The schedule of evaluations below applies to all consented participants. Baseline evaluations are to be conducted within 28 days prior to study entry, unless otherwise noted. Study entry refers to enrollment in Segment B for protocol therapy.

Evaluation	V0 Screen	V1/ Wk 0	V2/ Wk 4	V3/ Wk 7	V4/ Wk 12	V5/ Wk 15	V6/ Wk 24	V7/ Wk 27	V8/ Wk 36	V9/ Wk 48	V10/ Wk 60	V11/ Wk 72	Early d/c
Medication review	X	X	X	X	X	X	X	X	X	X	X	X	X
History and physical exam[1] Complete(C) or Targeted(T)	X[C]	X[T]	X[T]		X[T]		X[T]		X[C]	X[C]	X[C]	X[C]	X[T]
Electrocardiogram	X												
Adverse event (toxicity) assessment[2]	X	X	X	X	X	X	X	X	X	X	X	X	X
VGX-3100 w/EP, give PDC		X	X		X		X						
Assessment of injection site reactions[10]		X	X	X	X	X	X	X					X
Collect and review PDC			X	X		X		X					X
Safety labs[3]	X[4]	X	X		X		X			X		X	X
CD4/CD8 T-cell count	X[5]				X[9]		X[9]			X[9]		X[9]	
HIV-1 RNA viral load	X[5]				X[9]		X[9]		X[9]	X[9]	X[9]	X[9]	
Pregnancy test (WOCBP)	X	X[6]	X[6]		X[6]		X[6]						
Blood for immunology tests	X	X[8]		X		X		X		X		X	X
Anal swab 1 for cytology	X[7]						X			X		X	X
Anal swab 2 for HPV PCR	X[7]						X		X	X	X	X	X
HRA with DARE	X[7]						X		X	X	X	X	X
Anal biopsy for local diagnosis, HPV genotype(H)	X[7,H]						if cancer suspected		if cancer suspected	X[H]	if cancer suspected	X[H]	X
Cervical cytology and visual exam (WOCBP)	X												
ACSR donation (optional)	X												

1. Refer to applicable visit requirements in Section 7.0 for required medical history and definitions of complete and targeted physical exams.

2. AE assessment at baseline will include documentation of all pre-existing medical conditions in the study source. After treatment, clinical adverse assessment and injection site reactions following IP administration will be evaluated per Section 7.2.3 and 7.2.6.
3. Safety labs include CBC with differential and platelet count, comprehensive metabolic panel (includes Na, K, Cl, CO_2, BUN, glucose, calcium, creatinine, total bilirubin, AST, ALT, alkaline phosphatase, total protein, albumin), and creatine kinase. Safety labs may be collected up to 7 days before treatment administration. Baseline safety labs are permitted to be collected up to 30 days before treatment administration.
4. Up to 90 days before study entry.
5. Up to 120 days before study entry.
6. Up to 72 hours before IP administration.
7. Up to 90 days before study entry.
8. At least 34 mL of whole blood and 4 mL of serum must be collected at Screening (Visit 0) and again at Day 0 (Visit 1). A total of 68 mL of whole blood and 20 mL of serum must be collected prior to dosing on Day 0.
9. These tests may be collected up to 30 days before the visit.
10. The injection site will be assessed by study personnel prior to and approximately 30 minutes after each study treatment and at 2 weeks post study treatment visits. Refer to table 7-B for grading of injection site reactions. Assessment of injection site reactions should be assessed at early d/c, if applicable per Section 7.13.

APPENDIX II: PERFORMANCE STATUS SCALES

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

APPENDIX III: AIDS AND CANCER SPECIMEN RESOURCE (ACSR) SPECIMEN PREPARATION AND 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** in the ACSR.

B. SPECIMEN PREPARATION, PACKAGING, AND SHIPMENT

Blood Specimens

Draw two 8.5 cc (ml) yellow top [acid citrate dextrose (ACD)] tubes from study participant. With a black, water resistant, sharpie pen, label each specimen with the following information:

- AMC Protocol #103
- AMC Participant ID#
- Date and time of collection
- Specimen type, i.e., WB=Whole Blood, P=Plasma, S=Serum, or Tissue
- Specimen purpose: Donation

Specimen Shipment

- Seal the tops of the two 8.5 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 FedEx airbill on blank side of the shipper making sure that it is marked "FedEx 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:

Sylvia Silver, DA
AMC Biorepository
George Washington University Medical Center
2300 I Street, NW
Room 118
Washington, DC 20037
Tel: (202) 994-2945
Fax: (202) 994-5056
Email: ssilver@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

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 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.**

Preparation of Tissue Samples

Refer to the ACSR's SOP on Solid Tissue Collection (T002). It is preferable that tissue is processed as soon as possible. Either cryopreservation method for tissue described below can be used depending upon the availability of the freezing method (dry ice or LN_2).

Optimal Cutting Temperature (OCT) Compound for freezing of tissue using LN_2 (if available):

Fill a wide mouth Dewar halfway with LN_2. Then fill a metal beaker halfway with isopentane, and lower it into the wide mouth Dewar until it touches the bottom. Stir the isopentane using a –1500 C thermometer until the isopentane is quenched to –1500 C. Then fill a 1.0 mL or 1.5 mL cryotube half way with OCT compound and place the tissue on top of OCT in the cryotube and cover the tissue with a layer of OCT. Repeat until all tissue is placed in cryotubes and covered with OCT. Submerge the cryotubes into the isopentane bath for 10 seconds. Store the frozen tissues at –180°C (LN2) until ready to ship.

Optimal Cutting Temperature (OCT) Compound for freezing of tissue using dry ice (-80):
Put two drops of OCT into 1.0 mL or 1.5 mL cryotube(s). Place tissue into the cryotube on top of the OCT. Cover the tissue with OCT. Place the cryotube on top of the dry ice for rapid freezing. Store the frozen tissues at -80°C until ready to ship.

Tissue specimens for donation may be batched for shipping after storage in either -80ºC freezer or -180ºC (LN_2 Tank).

*****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.**

***NOTE**: Specimens can only be accepted Monday through **Friday**. Therefore, specimens can only be shipped **Sunday-Thursday** for delivery the next day. Shipping frozen tissue requires the use of packaging acceptable for dry ice and Class 9 label with weight of dry ice written on package.

TISSUE specimens should be shipped by overnight express to:

Sylvia Silver, DA
AMC Biorepository
George Washington University Medical Center
2300 I Street, NW
Room 118
Washington, DC 20037
Tel: (202) 994-2945
Fax: (202) 994-5056

C. RECORD OF SPECIMENS

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

APPENDIX IV: BIOSPECIMEN COLLECTION AND DONATION TO THE AIDS AND CANCER SPECIMEN RESOURCE (ACSR)

Primary Objective

The objective of this substudy to all AIDS Malignancy Consortium (AMC) protocols is to define the mechanisms to obtain biospecimens and associated clinical information from potential AMC trial participants for AIDS and Cancer Specimen Resource (ACSR) donation.

Background

Basic research into the epidemiology, virology, immunology, and pathogenesis of HIV/AIDS-associated malignancies depends on the availability of biological specimens (biospecimens) collected and processed in compliance with Biobanking Best Practices, which includes biospecimen annotation (accompanying data) to assist in understanding disease and to develop better treatment. The National Cancer Institute's (NCI) Office of the Director, Office of HIV and AIDS Malignancy (OHAM) established the ACSR in 1994 (http://acsr.ucsf.edu/). The ACSR's mission is to acquire, store, and equitably distribute biospecimens and their associated clinical data from persons who have HIV/AIDS-related medical conditions, including HIV/AIDS-related cancers. The ACSR serves to increase access to biospecimens and associated clinical outcome data that will be used by the research community at-large. These biospecimens are used for research purposes only and are greatly needed by scientists and researchers who are studying HIV/AIDS-associated malignancies and other HIV-associated diseases.

OHAM supports both the AMC and ACSR. The ACSR is comprised of four Regional Biospecimen Repositories (RBR) located throughout the United States with a central operations center located at the University California, San Francisco (UCSF). The AMC conducts multi-center clinical trials of innovative therapies of AIDS-related malignancies and the ACSR facilitates access to biospecimens and associated clinical data for HIV-malignancy investigators. The AMC collaborates with the ACSR to collect voluntary biospecimen donations from potential AMC participants for the biorepository to expand the breadth of the ACSR inventory.

AMC Collaboration with the ACSR

In accordance with NCI's directive for the AMC to support the ACSR by collecting voluntary biospecimen donations, all domestic AMC sites are strongly encouraged to participate in this substudy for each AMC protocol. Each AMC member site is expected to offer each potential AMC trial participant the opportunity to participate in this biospecimen donation protocol for the ACSR. Donations may only be collected and submitted to the ACSR if the participant provides informed consent to participate in this optional donation.

Participant Selection

All substudy participants must meet the eligibility criteria below to donate samples to the ACSR. Participation in the primary AMC study is not mandatory to participate in this substudy for the baseline blood and residual tissue donation. Initial contact will be made by participating AMC clinicians and/or research coordinators, who will obtain informed consent and collect biospecimen(s) from donors at the time of inclusion evaluation for the primary AMC protocol. AMC participating sites must have documentation that each eligibility requirement is satisfied prior to participant enrollment. In compliance with CTEP policy, no exceptions to eligibility criteria will be granted in any circumstance.

Eligibility Criteria

- Participants must be at least 18 years of age.
- HIV-1 status must be known. The participant's HIV status may be documented per local standards for medical care.
- Systemic protocol therapy should not yet have been initiated.
- Ability to understand and willingness to sign a written informed consent document.

Number of Donors to be Enrolled

This substudy has no formal accrual target. This substudy will accept participants for the duration of each member site's AMC participation in this protocol and for the duration of the AMC and ACSR grants.

Donor Enrollment Procedures

Participants will be enrolled into this substudy in AdvantageEDC as part of the enrollment process for the primary AMC protocol.

Substudy Procedures

Potential participants will be offered consent to donate 1) a blood specimen directly to the ACSR at baseline, prior to initiating the protocol treatment/intervention on the primary AMC protocol, and 2) donation of all residual specimens, including residual biopsy specimens, remaining after all studies for the primary AMC protocol are complete. Participants will be offered consent to opt in to the use of their biospecimens for any future research projects involving genetic testing. Consented participants for the direct ACSR blood and tissue donation will be asked to provide a blood sample before initiating the protocol treatment/study intervention on the primary AMC protocol. Residual specimens from participants who agree to donate all residual specimens from the primary AMC protocol will be retained after all protocol studies are complete and transferred from the AMC Biorepository to the ACSR per the AMC Biorepository Standards of Procedure. A portion of any diagnostic biopsies performed by the clinical site for the primary AMC protocol may be submitted to the ACSR with the participant's consent to donate residual study specimens. Procedures for submission of blood and residual tissue samples collected at the clinical site for ACSR donation are outlined in the appendix entitled "AIDS and Cancer Specimen Resource (ACSR) Specimen Preparation and Shipping Instructions." Basic clinical and demographic information (birth year, gender, HIV status, and clinical diagnosis of malignancy) will be supplied by the AMC collecting site and entered in the eCRFs for this protocol in AdvantageEDC at the time of enrollment in this substudy and/or the parent AMC protocol. Data from consented participants will be provided by the AMC Operations and Data Management Center (AMC ODMC) via secure electronic data transfer.

Use of Specimens by the ACSR

Biospecimens and accompanying clinical data are made available to researchers for use in prospectively defined studies through application to the ACSR's Central Operations and Data Coordinating Center. Research applications are reviewed by an independent review panel facilitated by the National Cancer Institute (NCI). ACSR biospecimens can only be used for research studies approved through this mechanism. Strict confidentiality protection guidelines are in place for all participant biospecimens and clinical data in the ACSR. Specifically, all

biospecimens and clinical data collected by AMC sites will be fully de-identified prior to ACSR submission, and all AMC identifying codes will be removed from ACSR specimens prior to distribution to external investigators for approved research studies. As such, the results of any studies performed using biospecimens donated to the ACSR will not be identifiable for a given participant and will not be returned to the submitting sites.

Genetic Testing

The ACSR's independent research panel may approve research projects for biospecimens to explore how genetic factors may be related to cancers and other diseases associated with HIV disease. In the future, the results of this testing might help doctors identify who may or may not benefit from treatment as well as host genetic factors that increase susceptibility to HIV-related diseases, particularly malignancies. Biospecimens may be used to learn more about how cancers and other diseases associated with HIV disease develop and/or may result in new tests or discoveries. Biospecimens will only be used for genetic testing with the participant's consent for this use of his or her specimens.

APPENDIX V: AMC DATA AND SAFETY MONITORING PLAN

(Version 9.0 • October 6, 2020)

Introduction

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

Monitoring the Progress of Trials and the Safety of Participants

Routine and expedited AE reporting

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

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

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

Expedited reporting to the Institutional Review Board (IRB)

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

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

Procedures for monitoring trial progress and pharmacovigilance

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

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

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

Data and Safety Monitoring Board Review (DSMB) review

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

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

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

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

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

Cohort trial reviews not subject to DSMB review

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

Plans for Assuring Compliance with Requirements Regarding AE Reporting

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

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

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

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

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

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

Plans for Assuring Data Accuracy and Protocol Compliance

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

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

APPENDIX VI: PARTICIPANT DIARY CARD

Participant Diary

NCI Protocol Number AMC-103

Inovio Protocol Number HPV-202

Participant #: _____

Injection Date: _____

Note to Participant:

For questions or problems, please contact your Site Coordinator.

Name: _____

Telephone: (____) _____

Email (optional): _____

General Instructions

Temperature

Take your temperature orally around the same time each evening, using the thermometer provided and record it in the space provided. Also record the time at which you took your temperature. If you recently drank very hot or cold liquids, wait 15 minutes before taking your temperature.

Injection Site Symptoms

Pain and itching

Some people experience pain in the area where they were injected. Some people may also experience itching in the area where they were injected.

- By pain we mean that the place where you were injected hurts even when it isn't touched.

If you experience pain or itching at the injection site, use the following categories to describe how severe these symptoms were. If you don't experience one or more of these symptoms, mark the **NONE** box.

- **Mild** I only had a little discomfort. I could still use my arm like always.
- **Moderate** I noticed the discomfort and didn't use my arm as much as usual.
- **Severe** I really noticed the discomfort. It kept me from doing something I wanted or had to do.

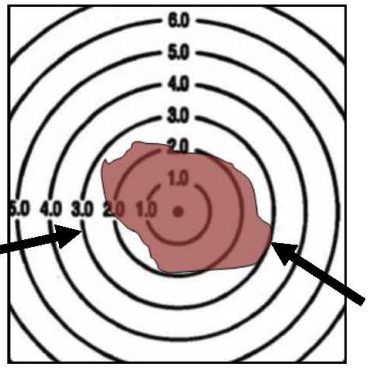
Redness, swelling, and bruising

Some people experience redness, swelling, or bruising in the area where they were injected. If you experience redness, swelling, or bruising, measure the area using the measuring tool you were provided, and record the measurement in the space provided.

To measure the area of redness, swelling, or bruising, do the following:

- Place the measuring tool over the area where you were injected, with the dot over the center of the area.
- Select the circle where the longest part of the area touches the line.
- If the area is in between two circles, select the larger circle.

For example, the area shown to the right measures 3 centimeters (cm) because it is touching the 3cm line.



General and Other Symptoms or Medications

If you have additional symptoms that are not listed, or if you sought medical care for any reason from a health care provider (e.g. doctor's office, emergency room), or if you took any medications, these should be listed in the spaces provided. Please designate any symptoms as Mild, Moderate, or Severe.

- **Mild** I only had minor discomfort. I went about my usual activities.
- **Moderate** I noticed the symptom. It bothered me enough that I didn't do as much as I usually do.
- **Severe** I really noticed the symptom. It kept me from doing something I wanted or had to do.

Day 0: Evening of Injection **Participant #:_____** **Date: ____/____/____**

Sometime during the evening on the day you were injected, fill out the information below. The items on this page refer to the time from your injection to 11:59 p.m. on the day of injection (Day 0). If any of the information changes after you fill out this page but before 11:59 p.m. tonight, make any necessary changes below.

Temperature

Evening Temp.:_____ ^{0}C or ^{0}F (circle one)	Time Taken: _____AM or PM (circle one)

General Symptoms

If you experience any of these symptoms, mark the box that describes your worst symptom until 11:59 p.m. tonight (Day 0). See **General Instructions** on the second page of this diary for more information.

Symptom	None	Mild	Moderate	Severe
Unusually tired/feeling unwell	☐	☐	☐	☐
Muscle aches	☐	☐	☐	☐
Headache	☐	☐	☐	☐
Nausea	☐	☐	☐	☐
Joint pain	☐	☐	☐	☐

Injection Site Symptoms

If you experience an injection site symptom, mark the box that describes your worst symptom until 11:59 p.m. tonight (Day 0). See **General Instructions** on the second page of this diary for more information.

Symptom	None	Mild	Moderate	Severe
Pain	☐	☐	☐	☐
Itching	☐	☐	☐	☐

Redness, Swelling, or Bruising	None	Provide Maximum Measurement
Redness	☐	_____cm at the longest part
Swelling	☐	_____cm at the longest part
Bruising	☐	_____cm at the longest part

Other Symptoms

If you experience symptoms other than the ones above, write them in the space below according to the **General Instructions** on the second page of this diary.

Did you experience any other symptoms? ☐ Yes ☐ No

Symptom or Medical Event	Mild	Moderate	Severe
	☐	☐	☐
	☐	☐	☐
	☐	☐	☐
	☐	☐	☐

Did you take any medications? ☐ Yes ☐ No

If yes, please list out the name(s) and dosage(s) below:

_____

_____

_____

Participant Signature _____ Date _____

Day 1: Day After Injection **Participant #:_____** **Date: ____/____/____**

The items on this page refer to the time between midnight of last night and 11:59 p.m. today (Day 1). If any of the information changes after you fill out this page but before 11:59 p.m. tonight, make any necessary changes below.

Temperature

Evening Temp.:_____ ^{0}C or ^{0}F (circle one)	Time Taken: _____AM or PM (circle one)

General Symptoms

If you experience any of these symptoms, mark the box that describes your worst symptom between midnight and 11:59 p.m. tonight (Day 1). See **General Instructions** on the second page of this diary for more information.

Symptom	None	Mild	Moderate	Severe
Unusually tired/feeling unwell	☐	☐	☐	☐
Muscle aches	☐	☐	☐	☐
Headache	☐	☐	☐	☐
Nausea	☐	☐	☐	☐
Joint pain	☐	☐	☐	☐

Injection Site Symptoms

If you experience an injection site symptom, mark the box that describes your worst symptom between midnight and 11:59 p.m. tonight (Day 1). See **General Instructions** on the second page of this diary for more information.

Symptom	None	Mild	Moderate	Severe
Pain	☐	☐	☐	☐
Itching	☐	☐	☐	☐

Redness, Swelling, or Bruising	None	Provide Maximum Measurement
Redness	☐	_____cm at the longest part
Swelling	☐	_____cm at the longest part
Bruising	☐	_____cm at the longest part

Other Symptoms

If you experience symptoms other than the ones above, write them in the space below according to the **General Instructions** on the second page of this diary.

Did you experience any other symptoms? ☐ Yes ☐ No

Symptom or Medical Event	Mild	Moderate	Severe
	☐	☐	☐
	☐	☐	☐
	☐	☐	☐
	☐	☐	☐

Did you take any medications? ☐ Yes ☐ No

If yes, please list out the name(s) and dosage(s) below:

Participant Signature _____ Date _____

Day 2: 2 Days After Injection **Participant #:_____** **Date: ____/____/____**

The items on this page refer to the time between midnight of last night and 11:59 p.m. today (Day 2). If any of the information changes after you fill out this page but before 11:59 p.m. tonight, make any necessary changes below.

Temperature

Evening Temp.:_____ ^{0}C or ^{0}F (circle one)	Time Taken: _____AM or PM (circle one)

General Symptoms

If you experience any of these symptoms, mark the box that describes your worst symptom between midnight and 11:59 p.m. tonight (Day 2). See **General Instructions** on the second page of this diary for more information.

Symptom	None	Mild	Moderate	Severe
Unusually tired/feeling unwell	☐	☐	☐	☐
Muscle aches	☐	☐	☐	☐
Headache	☐	☐	☐	☐
Nausea	☐	☐	☐	☐
Joint pain	☐	☐	☐	☐

Injection Site Symptoms

If you experience an injection site symptom, mark the box that describes your worst symptom between midnight and 11:59 p.m. tonight (Day 2). See **General Instructions** on the second page of this diary for more information.

Symptom	None	Mild	Moderate	Severe
Pain	☐	☐	☐	☐
Itching	☐	☐	☐	☐

Redness, Swelling, or Bruising	None	Provide Maximum Measurement
Redness	☐	_____cm at the longest part
Swelling	☐	_____cm at the longest part
Bruising	☐	_____cm at the longest part

Other Symptoms

If you experience symptoms other than the ones above, write them in the space below according to the **General Instructions** on the second page of this diary.

Did you experience any other symptoms? ☐ Yes ☐ No

Symptom or Medical Event	Mild	Moderate	Severe
	☐	☐	☐
	☐	☐	☐
	☐	☐	☐
	☐	☐	☐

Did you take any medications? ☐ Yes ☐ No

If yes, please list out the name(s) and dosage(s) below:

Participant Signature _____ Date _____

Day 3: 3 Days After Injection **Participant #:_____** **Date: ____/____/____**

The items on this page refer to the time between midnight of last night and 11:59 p.m. today (Day 3). If any of the information changes after you fill out this page but before 11:59 p.m. tonight, make any necessary changes below.

Temperature

Evening Temp.:_____ ^{0}C or ^{0}F (circle one)	Time Taken: _____AM or PM (circle one)

General Symptoms

If you experience any of these symptoms, mark the box that describes your worst symptom between midnight and 11:59 p.m. tonight (Day 3). See **General Instructions** on the second page of this diary for more information.

Symptom	None	Mild	Moderate	Severe
Unusually tired/feeling unwell	☐	☐	☐	☐
Muscle aches	☐	☐	☐	☐
Headache	☐	☐	☐	☐
Nausea	☐	☐	☐	☐
Joint pain	☐	☐	☐	☐

Injection Site Symptoms

If you experience an injection site symptom, mark the box that describes your worst symptom between midnight and 11:59 p.m. tonight (Day 3). See **General Instructions** on the second page of this diary for more information.

Symptom	None	Mild	Moderate	Severe
Pain	☐	☐	☐	☐
Itching	☐	☐	☐	☐

Redness, Swelling, or Bruising	None	Provide Maximum Measurement
Redness	☐	_____cm at the longest part
Swelling	☐	_____cm at the longest part
Bruising	☐	_____cm at the longest part

Other Symptoms

If you experience symptoms other than the ones above, write them in the space below according to the **General Instructions** on the second page of this diary.

Did you experience any other symptoms? ☐ Yes ☐ No

Symptom or Medical Event	Mild	Moderate	Severe
	☐	☐	☐
	☐	☐	☐
	☐	☐	☐
	☐	☐	☐

Did you take any medications? ☐ Yes ☐ No

If yes, please list out the name(s) and dosage(s) below:

Participant Signature _____ Date _____

Day 4: 4 Days After Injection **Participant #:_____** **Date: ____/____/____**

The items on this page refer to the time between midnight of last night and 11:59 p.m. today (Day 4). If any of the information changes after you fill out this page but before 11:59 p.m. tonight, make any necessary changes below.

Temperature

Evening Temp.:_____ ^{0}C or ^{0}F (circle one)	Time Taken: _____AM or PM (circle one)

General Symptoms

If you experience any of these symptoms, mark the box that describes your worst symptom between midnight and 11:59 p.m. tonight (Day 4). See **General Instructions** on the second page of this diary for more information.

Symptom	None	Mild	Moderate	Severe
Unusually tired/feeling unwell	☐	☐	☐	☐
Muscle aches	☐	☐	☐	☐
Headache	☐	☐	☐	☐
Nausea	☐	☐	☐	☐
Joint pain	☐	☐	☐	☐

Injection Site Symptoms

If you experience an injection site symptom, mark the box that describes your worst symptom between midnight and 11:59 p.m. tonight (Day 4). See **General Instructions** on the second page of this diary for more information.

Symptom	None	Mild	Moderate	Severe
Pain	☐	☐	☐	☐
Itching	☐	☐	☐	☐

Redness, Swelling, or Bruising	None	Provide Maximum Measurement
Redness	☐	_____cm at the longest part
Swelling	☐	_____cm at the longest part
Bruising	☐	_____cm at the longest part

Other Symptoms

If you experience symptoms other than the ones above, write them in the space below according to the **General Instructions** on the second page of this diary.

Did you experience any other symptoms? ☐ Yes ☐ No

Symptom or Medical Event	Mild	Moderate	Severe
	☐	☐	☐
	☐	☐	☐
	☐	☐	☐
	☐	☐	☐

Did you take any medications? ☐ Yes ☐ No

If yes, please list out the name(s) and dosage(s) below:

Participant Signature _____ Date _____

Day 5: 5 Days After Injection **Participant #:_____** **Date: ____/____/____**

The items on this page refer to the time between midnight of last night and 11:59 p.m. today (Day 5). If any of the information changes after you fill out this page but before 11:59 p.m. tonight, make any necessary changes below.

Temperature

Evening Temp.:_____ ^{0}C or ^{0}F (circle one)	Time Taken: _____AM or PM (circle one)

General Symptoms

If you experience any of these symptoms, mark the box that describes your worst symptom between midnight and 11:59 p.m. tonight (Day 5). See **General Instructions** on the second page of this diary for more information.

Symptom	None	Mild	Moderate	Severe
Unusually tired/feeling unwell	☐	☐	☐	☐
Muscle aches	☐	☐	☐	☐
Headache	☐	☐	☐	☐
Nausea	☐	☐	☐	☐
Joint pain	☐	☐	☐	☐

Injection Site Symptoms

If you experience an injection site symptom, mark the box that describes your worst symptom between midnight and 11:59 p.m. tonight (Day 5). See **General Instructions** on the second page of this diary for more information.

Symptom	None	Mild	Moderate	Severe
Pain	☐	☐	☐	☐
Itching	☐	☐	☐	☐

Redness, Swelling, or Bruising	None	Provide Maximum Measurement
Redness	☐	_____cm at the longest part
Swelling	☐	_____cm at the longest part
Bruising	☐	_____cm at the longest part

Other Symptoms

If you experience symptoms other than the ones above, write them in the space below according to the **General Instructions** on the second page of this diary.

Did you experience any other symptoms? ☐ Yes ☐ No

Symptom or Medical Event	Mild	Moderate	Severe
	☐	☐	☐
	☐	☐	☐
	☐	☐	☐
	☐	☐	☐

Did you take any medications? ☐ Yes ☐ No

If yes, please list out the name(s) and dosage(s) below:

Participant Signature _____ Date _____

Day 6: 6 Days After Injection **Participant #:_____** **Date: ____/____/____**

The items on this page refer to the time between midnight of last night and 11:59 p.m. today (Day 6). If any of the symptoms you reported on previous pages have not gone away ("resolved"), you will need to let the Site Coordinator or Doctor know.

Temperature

Evening Temp.:_____ ^{0}C or ^{0}F (circle one)	Time Taken: _____AM or PM (circle one)

General Symptoms

If you experience any of these symptoms, mark the box that describes your worst symptom between midnight and 11:59 p.m. tonight (Day 6). See **General Instructions** on the second page of this diary for more information.

Symptom	None	Mild	Moderate	Severe
Unusually tired/feeling unwell	☐	☐	☐	☐
Muscle aches	☐	☐	☐	☐
Headache	☐	☐	☐	☐
Nausea	☐	☐	☐	☐
Joint pain	☐	☐	☐	☐

Injection Site Symptoms

If you experience an injection site symptom, mark the box that describes your worst symptom between midnight and 11:59 p.m. tonight (Day 6). See **General Instructions** on the second page of this diary for more information.

Symptom	None	Mild	Moderate	Severe
Pain	☐	☐	☐	☐
Itching	☐	☐	☐	☐

Redness, Swelling, or Bruising	None	Provide Maximum Measurement
Redness	☐	_____cm at the longest part
Swelling	☐	_____cm at the longest part
Bruising	☐	_____cm at the longest part

Other Symptoms

If you experience symptoms other than the ones above, write them in the space below according to the **General Instructions** on the second page of this diary.

Did you experience any other symptoms? ☐ Yes ☐ No

Symptom or Medical Event	Mild	Moderate	Severe
	☐	☐	☐
	☐	☐	☐
	☐	☐	☐
	☐	☐	☐

Did you take any medications? ☐ Yes ☐ No

If yes, please list out the name(s) and dosage(s) below:

Participant Signature _____ Date _____

APPENDIX VII: ANAL CYTOLOGY AND HPV SAMPLING

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

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

Procedure for obtaining anal swab specimens:

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

A second swab will be collected after the first swab for local cytology processing, following the same procedures as noted above. The second swab will be shipped to the AMC Biorepository at George Washington University, and will be sent in batches for HPV DNA PCR analysis at DDL Diagnostic Laboratory.

Guidance regarding inconclusive cytology results:

If a cytology specimen is interpreted as insufficient or inconclusive, the specimen collection should be repeated for any visit at which a biopsy is required.

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

Procedure for Performing HRA

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

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

Procedures for Biopsy Review

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

Although p16 IHC staining is not required on all biopsies diagnosed as HSIL by the local laboratories for AMC 103, if it is performed, the immunostained slides are also requested to be sent for Central Pathology review. p16 IHC is useful to adjudicate differential diagnoses on H&E particularly for the following indications:

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

A p16 stain must be obtained if the only area of HSIL among all biopsies taken is AIN 2. At any visit, if other lesions show AIN2-3 or more severe histology, p16 staining is highly recommended for AIN2 biopsies to adjudicate the diagnosis.

The local pathology lab relied upon for this trial must be willing to send slides and tissue blocks as requested by this study. Stained H&E slides from all biopsies will be sent from participants who are biopsied as part of the screening process and randomized at the baseline visit. Slides will be submitted in real time for any cancer diagnoses, as well as all biopsies collected prior to progression to cancer, and any cases that are suspicious for progression to cancer during local pathology review. Tissue blocks will be required for correlative studies on this protocol. Blocks will not be exhausted and will be returned to the local pathology lab.

The baseline pathology review will serve to identify the discrepancy in HSIL vs. no HSIL readings, and HSIL vs. LSIL readings between the local pathologist's finding and the central pathology result. Slides will be reviewed from all subsequent biopsies. Slides will be batched for shipment for central pathology review as outlined in the study Manual of Procedures. The pathology slides will be reviewed later at UCSF, and results of the central pathology review will not be available in real time.

Guidance regarding repeat biopsy and inconclusive biopsy results:

After the screening visit, if all biopsies do not show HSIL and the local cytology result shows high-grade disease, the investigator should bring the participant back for repeat HRA. If the biopsies at screening do not show HSIL and the local cytology result is inconclusive, repeat HRA with biopsy should only be performed if the investigator's clinical impression of the participant's disease is strongly suggestive of HSIL. If the repeat biopsies do not show HSIL, the participant should discontinue screening.

The investigator may request that a participant return for repeat biopsy at any time there is suspicion for cancer.

APPENDIX IX: CENTRAL PATHOLOGY REVIEW

All biopsy specimens will be reviewed by the designated pathologist on site. The HSIL biopsy slides will be reviewed later at UCSF. The central pathology review at UCSF will not be done in real time. In case of discrepancy between the local pathology and central pathology, the pathologist responsible for central review will identify additional pathologist(s) to confirm the final interpretation as she/he deems necessary or appropriate.

For central pathology review, at least one H&E stained slide from each biopsy and any additional stained slide(s) (e.g., p16 immunostain), plus a copy of the pathology report (all PHI redacted) should be sent to the following address:

Sylvia Silver, DA
AMC Biorepository
George Washington University Medical Center
2300 I Street, NW
Room 118
Washington, DC 20037
Tel: (202) 994-2945
Fax: (202) 994-5056

Slide Labeling

Refer to the study MOP for slide labeling, packaging, and preparation requirements.

Record of Specimens

This study will track specimens via GlobalTrace, a component of the AMC Advantage eClinical system. The GlobalTrace 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 via the AMC Biorepository following central pathology review.