

A5355

Phase II, Double-Blind, Randomized, Placebo-Controlled Trial to Evaluate the Safety and Immunogenicity of a Modified Vaccinia Ankara (MVA)-based anti-Cytomegalovirus (CMV) Vaccine (Triplex), in Adults with Both Human Immunodeficiency Virus (HIV)-1 and CMV Who Are on Potent Combination ART with Conserved Immune Function

A Multicenter Trial of the AIDS Clinical Trials Group (ACTG)

**Sponsored by:
National Institute of Allergy
and Infectious Diseases**

IND # 027476

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PROTOCOL SIGNATURE PAGE

I will conduct the study in accordance with the provisions of this protocol and all applicable protocol-related documents. I agree to conduct this study in compliance with United States (US) Health and Human Service regulations (45 CFR 46); applicable US Food and Drug Administration regulations; standards of the International Conference on Harmonization Guideline for Good Clinical Practice (E6); Institutional Review Board/Ethics Committee determinations; all applicable in-country, state, and local laws and regulations; and other applicable requirements (e.g., US National Institutes of Health, Division of AIDS) and institutional policies.

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STUDY MANAGEMENT

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Protocol E-mail Group

Sites should contact the User Support Group at the Data Management Center (DMC) as soon as possible to have the relevant personnel at the site added to the [actg.protA5355](mailto:actg.protA5355@fstrf.org) e-mail group. Include the protocol number in the e-mail subject line.

- Send an e-mail message to actg.user.support@fstrf.org

Clinical Management:

For questions concerning entry criteria, toxicity management, concomitant medications, and co-enrollment, contact the Clinical Management committee (CMC).

- Send an e-mail message to actg.cmcA5355@fstrf.org. Include the protocol number, patient identification number (PID), and a brief relevant history.

Laboratory

For questions specifically related to immunologic laboratory tests, contact the Protocol Immunologist.

- Send an e-mail message to actg.coreA5355@fstrf.org (ATTENTION: Michael Freeman).

Data Management

- For nonclinical questions about transfers, inclusion/exclusion criteria, electronic case report forms (eCRFs), randomization/registration, and other data management issues, contact the data managers. Completion guidelines for eCRFs and participant-completed CRFs can be downloaded from the FSTRF website at www.frontierscience.org.
- For transfers, reference the Study Participant Transfer SOP 119, and contact **the data manager/s listed on the Affiliations tab of the study's web page on the ACTG Member website**.
- For other questions, send an e-mail message to actg.coreA5355@fstrf.org (ATTENTION: **data manager/s**).
- Include the protocol number, PID, and a detailed question.

Randomization/Participant Registration

For randomization/participant registration questions or problems and study identification number SID lists:

- Send an e-mail message to rando.support@fstrf.org or call the DMC Randomization Desk at 716-834-0900, extension 7301.

DMC Portal and Medidata Rave Problems

Contact DMC User Support.

- Send an e-mail message to actg.user.support@fstrf.org or call 716-834-0900 x7302.

STUDY MANAGEMENT (Cont'd)

Protocol Document Questions

For questions concerning the protocol document, contact the Clinical Trials Specialist (CTS).

- Send an e-mail message to actg.coreA5355@fstfrf.org (ATTENTION: **Lara Hosey**).

Copies of the Protocol

To request a hard copy of the protocol, send an e-mail message to ACTGNCC@dlhcorp.com. Electronic copies can be downloaded from the ACTG website at <https://www.actgnetwork.org>.

Product Package Inserts and/or Investigator Brochures

To request copies of product package inserts or investigator brochures, contact the DAIDS Regulatory Support Center (RSC) at RIC@tech-res.com or call 301-897-1708.

Protocol Registration

For protocol registration questions, send an e-mail message to Protocol@tech-res.com or call 301-897-1707.

Protocol Activation

For questions related to protocol activation contact the CTS.

- Send an e-mail message to actg.coreA5355@fstfrf.org (ATTENTION: **Lara Hosey**).

Study Product

For questions or problems regarding study product, dose, supplies, records, and returns, contact the protocol pharmacist (Shawn Chiambah).

Study Drug Orders

Call the Clinical Research Products Management Center (CRPMC) at 301-294-0741.

IND (Investigational New Drug) Number or Questions

The IND number will be available on the protocol-specific web page (PSWP) within 30 days after the submission to the Food and Drug Administration (FDA). For any questions related to the IND submission, contact the DAIDS RSC at Regulatory@tech-res.com or call 301-897-1706.

Expedited Adverse Event (EAE) Reporting/Questions

Contact DAIDS through the RSC Safety Office at DAIDSRSCSafetyOffice@tech-res.com or call 1-800-537-9979 or 301-897-1709; or fax 1-800-275-7619 or 301-897-1710.

Telephone Calls

Sites are responsible for documenting telephone calls made to A5355 team members.

- Send an e-mail message to actg.coreA5355@fstfrf.org.

Protocol-Specific Web Page

Additional information about management of the protocol can be found on the protocol-specific web page (PSWP).

GLOSSARY

ACC/AHA	American College of Cardiology/American Heart Association
BLQ	below the limit of quantitation
CMV	cytomegalovirus
COH	City of Hope
COVID-19	coronavirus disease 2019
EIA	enzyme immunoassay
HBsAb	hepatitis B surface antibody
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCT	hematopoietic cell transplant
HCV	hepatitis C virus
IgG	immunoglobulin G antibody
IM	intramuscular
MOPS	Manual of Procedures
MVA	Modified Vaccinia Ankara
NAAT	nucleic acid amplification test
PBS	phosphate-buffered saline
pfu	plaque-forming unit
RPR	rapid plasma regain
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
TNF	tumor necrosis factor
VRC	vaccination report card

SCHEMA

A5355

Phase II, Double-Blind, Randomized, Placebo-Controlled Trial to Evaluate the Safety and Immunogenicity of a Modified Vaccinia Ankara (MVA)-based anti-Cytomegalovirus (CMV) Vaccine (Triplex), in Adults with Both Human Immunodeficiency Virus (HIV)-1 and CMV Who Are on Potent Combination ART with Conserved Immune Function

<u>DESIGN</u>	A5355 is a Phase II, double-blind, randomized, placebo-controlled study to evaluate the safety, and immunogenicity of two injections of MVA Vaccine Encoding Cytomegalovirus (CMV) antigens (Triplex) in adults with both HIV and CMV.
<u>DURATION</u>	Participants will be followed for 92 weeks after the last scheduled vaccination at week 4, for a total study duration of 96 weeks.
<u>SAMPLE SIZE</u>	90 participants (of whom at least 25% are individuals assigned female sex at birth not on testosterone or individuals assigned male sex at birth on feminizing hormones)
<u>POPULATION</u>	Adults between the ages of 18 and 65 years with both HIV and CMV. Participants must be HIV virologically suppressed on antiretroviral therapy (ART) with current CD4+ cell count >250 cells/ μ L and nadir CD4+ cell count \geq 100 cells/ μ L.
<u>REGIMEN</u>	<p>Participants will be randomized in a 2:1 ratio to receive either two injections of CMV-MVA Triplex or placebo administered at Day 0 and week 4.</p> <p>Vaccine Group: 60 participants will receive CMV-MVA Triplex containing 5×10^8 plaque-forming unit (pfu) $\pm 0.5 \times 10^8$ pfu of MVA Vaccine Encoding CMV Antigens by intramuscular (IM) deltoid injections.</p> <p>Placebo Group: 30 participants will receive a volume of placebo (7.5% Lactose in phosphate-buffered saline [PBS]) that matches the volume of the active vaccine injection by IM deltoid injections.</p>

1.0 HYPOTHESIS AND STUDY OBJECTIVES

1.1 Primary Hypotheses

- 1.1.1 Safety Two injections of MVA Vaccine Encoding CMV Antigens (Triplex) administered according to a 4-week, two-injection schedule will be safe over 48 weeks.
- 1.1.2 Cellular immunogenicity Anti-CMV CD8+ T cell responses (measured as pp65-specific CD137+ CD8+ T cells) between Day 0 and study week 12 will increase in participants receiving the active vaccine, compared to placebo.
- 1.1.3 Inflammation Blood plasma levels of soluble receptors for tumor necrosis factor type II (sTNFRII) will decrease over the first 48 weeks in participants receiving the active vaccine, compared to placebo.

1.2 Secondary Hypotheses

- 1.2.1 Inflammation The blood plasma levels of selected soluble inflammatory biomarkers (including but not limited to IL-6, sCD163, IP-10, sTNFRII, D-Dimers) at study weeks 12, 24, 48, and 72 will decrease in participants receiving the active vaccine, compared to placebo. Inflammation might temporarily increase early after vaccine administration.
- 1.2.2 Cellular immunogenicity (IE1 and IE2) Anti-CMV CD8+ T cell responses (measured as IE1 and IE 2 -specific CD137+ CD8+ T cells) between Day 0 and study week 12 will increase in participants receiving the active vaccine, compared to placebo.
- 1.2.3 Prolonged cellular immunogenicity The increase in anti-CMV CD8+ T cell responses (pp65, IE1 and IE2) will be higher in participants receiving the active vaccine, compared to placebo, over the first 48 weeks (area under the curve).
- 1.2.4 CMV DNA shedding Vaccination will reduce the frequency and levels of CMV DNA in peripheral blood mononuclear cells (PBMC), urine, genital secretion, and oral secretion at study week 12, 48, and 72 in participants receiving the active vaccine compared to participants receiving placebo.
- 1.2.5 Prolonged safety Two injections of MVA Vaccine Encoding CMV Antigens (Triplex) administered according to a 4-week, two-injection schedule will be safe over the 96-week study period.
- 1.2.6 Detection of persistence of MVA DNA after immunizations Viral DNA derived from the recombinant MVA vaccine will decay in blood and will be undetectable by study week 12.

1.3 Exploratory Hypotheses

- 1.3.1 Inflammation The blood plasma levels of soluble inflammatory biomarkers other than those listed as secondary hypothesis (including but not limited to IL-18, IL-7, sCD14) at study weeks 12, 24, 48, and 72 will decrease in participants receiving the active vaccine, compared to placebo. Inflammation might temporarily increase early after vaccine administration.
- 1.3.2 T cell dysfunction Participants completing the vaccination series will have less evidence for T cell dysfunction (including but not limited to, less activation, proliferation, and exhaustion of T cells) in their blood at study weeks 12 and 48, and 72 when compared to those receiving placebo.
- 1.3.3 Quality of cellular responses (pending external funding) Broad and maintained anti-CMV T cell receptor (TCR) diversity in response to the immunogen will be observed post-vaccination at 12 and 48 weeks, as opposed to lower quality responses resulting from narrowing of the TCR repertoire due to expansion of limited anti-CMV TCRs present at Day 0.
- 1.3.4 HIV Reservoir (pending external funding) Vaccination will reduce the size and transcriptional activity of the HIV DNA reservoir at study weeks 12, 24, 48, and 72 in participants receiving the active vaccine compared to participants receiving placebo.
- 1.3.5 Microbiome (pending external funding) The rectal microbiome composition will predict the magnitude of the vaccine immune response.
- 1.3.6 Substance use Use of non-prescribed stimulatory drugs (e.g., cocaine, methamphetamine) will be associated with increased immune activation in both arms.

1.4 Primary Objectives

- 1.4.1 Safety To determine whether a 2-injection regimen of MVA Vaccine Encoding CMV antigens is safe (over 48 weeks).
- 1.4.2 Cellular immunogenicity To determine the anti-CMV CD8+ T cell responses (pp65) in participants receiving the active vaccine versus placebo (week 12).
- 1.4.3 Inflammation To determine whether the active vaccine decreases inflammation, as measured by sTNFRII, compared to placebo (week 48).

1.5 Secondary Objectives

- 1.5.1 Inflammation To determine if anti-CMV vaccination reduces plasma levels of selected soluble inflammatory biomarkers (e.g., IL-6, sCD163, IP-10, TNFRII, D-Dimers).

- 1.5.2 Cellular immunogenicity (IE1 and IE2) To determine the anti-CMV CD8+ T cell responses (IE1 and IE2) in participants receiving the active vaccine versus placebo (week 12).
 - 1.5.3 Prolonged cellular immunogenicity To determine the anti-CMV CD4+/CD8+ T cell immune responses (pp65, IE1 and IE2) to vaccine in participants over 48 weeks of follow-up.
 - 1.5.4 CMV DNA shedding To determine if vaccination influences the shedding of CMV DNA in PBMC, oral secretion, genital secretion, and urine.
 - 1.5.5 Prolonged safety To determine whether a 2-injection regimen of MVA Vaccine encoding CMV antigens is safe over 96 weeks of follow-up.
 - 1.5.6 Detection of persistence of MVA DNA after immunizations To determine the persistence of viral DNA derived from MVA vaccine in blood specimens.
- 1.6 Exploratory Objectives
- 1.6.1 Inflammation To determine if anti-CMV vaccination reduces plasma levels of soluble inflammatory biomarkers (other than those listed in [section 1.2.1](#)).
 - 1.6.2 T cell dysfunction To determine if anti-CMV vaccination reduces markers of T cell dysfunction.
 - 1.6.3 Quality of cellular responses (pending external funding) To determine the quality of the anti-CMV T cell response (i.e., the breadth and persistence of the anti-CMV TCR repertoire) induced by vaccination at weeks 12 and 48 as compared to Day 0.
 - 1.6.4 HIV Reservoir (pending external funding) To determine if vaccination influences the size and transcriptional activity of the HIV reservoir.
 - 1.6.5 Microbiome (pending external funding) To determine if the composition of the rectal microbiome predicts the magnitude of the vaccine immune response.
 - 1.6.6 Substance use To determine if stimulatory drugs influence immune activation in both arms.

2.0 INTRODUCTION

2.1 Background

HIV and CMV Co-infection

Both HIV and CMV infections persist lifelong, and almost all individuals with HIV also have CMV; these co-infections are neither independent processes nor benign [1]. Since

the first description of AIDS, co-infections with CMV have been responsible for many AIDS-defining complications, and are among the most common opportunistic infections observed in people with AIDS [2]. The considerable overlap between the HIV epidemic and CMV infection does not appear to be random: the acquisition of these two viral infections goes beyond overlapping risk factors for co-infection [3]. Accumulating data show that these two types of pathogens interact with regard to both epidemiology and pathogenesis by driving viral replication and facilitating transmission.

CMV, Inflammation, and Immunosenescence

Among all viruses, CMV infection has one of the most dynamic and comprehensive interactions with the human immune system. In this complex host-virus relationship, the virus elicits and maintains high frequency of CMV-specific T cells that are engaged in a life-long fight to restrain CMV replication and prevent life-threatening disease [4, 5]. CMV devotes some of its genetic information to evade the host system by preventing this immune response to clear infection or interfere with viral transmission. A significant percentage of both CD4+ and CD8+ memory T cells circulating in blood are targeted towards CMV [5], and this percentage can increase even further in older individuals and people with HIV [6]. As people age with their CMV infection, the continual immune activation and response to CMV replication can drive the T cell repertoire towards a more differentiated T cell phenotype [7, 8], which may promote replicative exhaustion and senescence in some cases [9]. CMV infection may compromise the response to other antigens by shrinking the remaining T cell repertoire in favor of expansion of CMV-specific T cells, although this remains controversial [6]. Senescent T cells frequently bear antigen specificity towards CMV [10-12], and the abundance of senescent T cells has been associated with a variety of negative outcomes, including decreased vaccine responsiveness in the elderly, autoimmunity, frailty, reduction in the T cell receptor repertoire, cardiovascular disease, and poor responsiveness to new infections [13-16]. Recurrent CMV shedding may put further stress on the immune resources in participants with HIV and accelerates rapid progression to AIDS [1]. The link between CMV shedding and systemic inflammation during HIV infection has been examined in a randomized controlled study that showed a reduction in T cell immune activation when people with HIV were treated with the anti-CMV drug valganciclovir [17]. While this study was limited in that only 70% of the participants had HIV RNA suppression with ART and the sample size was small (n=30), it did show a sustained reduction in CMV shedding and immune activation 4 weeks after stopping valganciclovir. This suggests that there may be a circular feedback between CMV shedding and immune activation, and if this is true then the anti-inflammatory benefit of reducing CMV replication with anti-CMV-vaccine could be compounded over time.

CMV and HIV DNA Reservoir

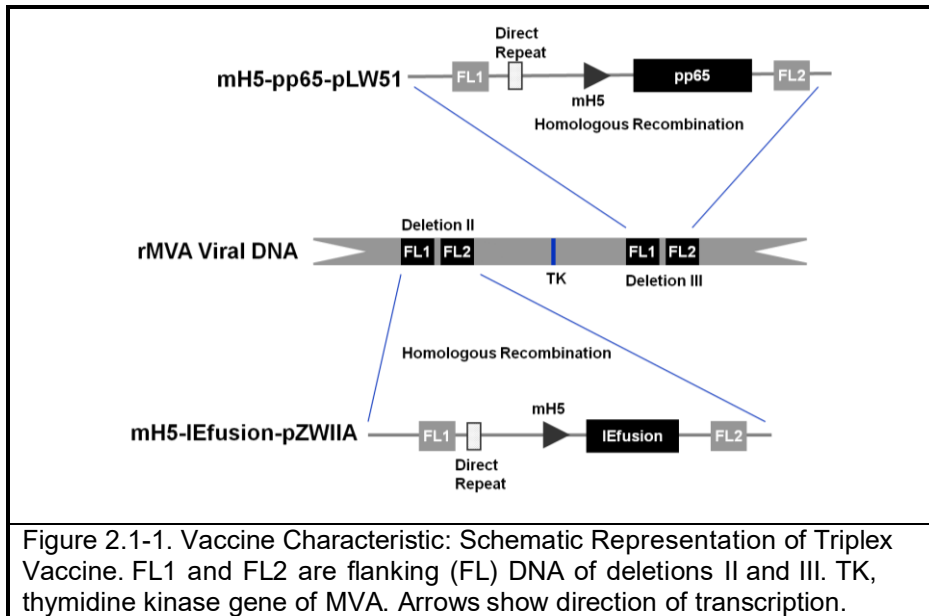
There is convincing evidence that chronic inflammation and immune activation helps to maintain the HIV reservoir during ART [18]. To draw the link between CMV shedding, systemic inflammation and maintenance of the HIV reservoir, recent studies have demonstrated that asymptomatic CMV seminal shedding is associated with increased levels of total HIV DNA in both ART-naïve individuals [19] and in individuals suppressed on long-term ART [20]. Furthermore, in a large longitudinal study of 108 individuals followed since the earliest phase of HIV infection, there was a significant positive

association between longitudinal levels of CD4+ T cell associated HIV DNA in blood and the frequency of detectable CMV DNA in blood cells [21]. Although the observational design of these studies does not allow causality to be inferred, it does support the theory that asymptomatic CMV replication in genital secretions and in blood drives local and systemic immune activation with a subsequent increase in the latent HIV reservoir.

Therefore, it may follow that targeting drivers of chronic inflammation, like CMV, could contribute to HIV curative strategies. As part of this study, we will collect and store aliquots of PBMC and, if warranted, we will request additional external funding to measure the size of the HIV DNA reservoir before and after vaccine administration.

MVA Vaccine Encoding CMV Antigens

The proposed vaccine is a Modified Vaccinia Ankara (MVA)-based vaccine (Triplex), which encodes three full-length CMV antigens (pp65 (UL83), IE1-exon4 (UL123), IE2-exon5 (UL122) [22]. It was constructed using an MVA virus [23] and two plasmid shuttle vectors; mH5-pp65-pLW51 expressing pp65 and mH5-IEfusion-pZWIIA expressing the fusion of IE1-exon4 and IE2-exon5 (Figure 2.1-1) [24]. Parental wild type (wt) MVA virus (MVA 572.FHE-22.02.1974), used to construct the CMV- MVA vector, was obtained by Clinical Trial Agreement from Dr. Bernard Moss, Laboratory of Viral Disease (National Institute of Allergy and Infectious Diseases). The first transgene contains a fusion protein of two CMV antigens, IE1-exon4 and IE2-exon5 that was inserted in the MVA deletion-II locus. The second transgene contains another CMV antigen, pp65 and was inserted in the MVA deletion-III locus. The transcription of each of these transgenes is under the control of separate mH5 promoters [25]. The Good Manufacturing Practice (GMP) grade of CMV-MVA Triplex passed all toxicology and applicable release tests specified by the FDA, and reported in the Certificate of Analysis. Release tests were performed at City of Hope, BioReliance (Gaithersburg, MD), and WuXi-AppTec (Marietta, GA). All toxicology studies were carried out at Southern Research (Huntsville, AL). Triplex was manufactured at the Center for Biomedicine and Genetics (CBG), within the Center for Applied Technology Development at City of Hope (COH). This California Food and Drug Branch licensed manufacturing facility operates under the principles of Current Good Manufacturing Practice regulations for the manufacture of Phase I and II biologics. The product was designated as BB-IND 15792 by the FDA.



Clinical Safety and Tolerability of CMV-MVA Triplex

The attractiveness of an MVA-based vaccine stems from its previous safety record as a smallpox vaccine in the young and elderly, and as a therapeutic vaccine in both cancer [23, 26-29] and immune suppressed HIV-AIDS patients [30-32]. Critical to the clinical development of CMV-MVA Triplex was a trial of wild type non-recombinant MVA conducted in hematopoietic stem cell transplant (HCT) recipients, which showed excellent safety and immunogenicity [33]. CMV-MVA Triplex has already showed very encouraging results in a Phase I trial (NCT01941056) [22]. In brief, the initial Phase I clinical evaluation of the CMV-MVA Triplex vaccine was completed in 24 adults without HIV, with or without immunity to CMV and vaccinia virus (previous DryVax smallpox vaccination). Three escalating dose levels were administered intramuscularly in 8 participants, with an identical booster injection 28 days later and one-year follow-up. Vaccinations at all dose levels were safe with no dose limiting toxicities. No vaccine-related serious adverse events (AEs) were documented. Local and systemic reactogenicity were transient and self-limiting. In a recent Phase II clinical trial in HCT recipients (NCT02506933) the vaccine met the primary endpoint with excellent tolerability and vaccine-associated immunogenicity [34]. Specifically, Triplex displayed potent immunogenicity, as many vaccine recipients with either CMV-positive or -negative donors showed strong reconstitution of both CD4 and CD8 CMV-specific immunity that initiated soon after the first injection and was elevated for at least 100 days post-HCT. Most notably there was a reduction of primary endpoint-defining reactivation through day 100 in the vaccine arm (5 events, 9.8%) compared to the placebo arm (10 events, 19.6%, $p=0.08$).

Clinical Immunogenicity of CMV-MVA Triplex

In a Phase I trial [22], robust, functional, and durable CMV-MVA Triplex driven expansions of CMV-specific T cells were detected by measuring T cell surface levels of

4-1BB (CD137), binding to CMV-specific HLA multimers, and IFN- γ production. Enhanced and durable CMV-specific T cell responses were also detected in CMV-MVA Triplex vaccinated CMV-seronegatives, and in DryVax-vaccinated participants. A long-lived memory effector phenotype is thought by some investigators to be associated with viral control during CMV primary infection. It was convincingly shown that off-target vaccine responses activating memory T cells from the related herpes virus Epstein-Barr Virus (EBV) remained undetectable. In summary, this Phase I study showed that CMV-MVA Triplex is highly immunogenic, and generates expansion of durable CMV-specific T cells in both CMV-seropositive and seronegative participants, and also in those participants who previously received smallpox vaccination. This clinical trial is listed as NCT01941056.

Detection of Persistence of MVA DNA after Immunizations

The persistence of vectored vaccines is always a concern in clinical trials, since the objective is to elicit immunity but have the vaccine decay, so side effects based on indefinite stimulation of immunity or other unwanted side effects are minimized. While the non-recombinant form of MVA has been investigated in over 120,000 people, and recently in HCT recipients, and found to be safe, the recombinant forms are unique, and cannot assume to have the identical safety profile, unless investigated. Previous work in immunocompromised rhesus macaques indicated decay of the viral DNA derived from a recombinant MVA vaccine after a 6-week interval [35]. The COH CBG produced MVAp53 is currently used as an experimental cancer vaccine in a Phase II study (IRB protocol #16448; NCT03113487). When FDA-mandated toxicology evaluations were performed in mice, it was shown that MVAp53 was cleared from the skin injection site within 60 days of administration, and from all other tissues including blood within 2 weeks of administration. Our expectation is similar for this clinical trial [35, 36]. The FDA-mandated toxicology testing for CMV-MVA Triplex vaccine is performed in rabbits and results have been incorporated into the IND (Southern Research Institute; Birmingham, AL; Study # 13928.01.01). Persistence of the CMV-MVA Triplex vaccine given to participants will be monitored during the course of the trial. According to the US Guidance for Industry on Gene Therapy Clinical Trials (FDA Center for Biologics Evaluation and Research), the risk of gene transfer-related delayed AEs is low, since poxviruses do not have a propensity to integrate in the host genome. Long-term follow-up observations are therefore not necessary. The method will be based on an assay we have developed for assessing the presence of MVA sequences during the derivation of the vaccine, taking place in our own Good Laboratory Practice (GLP) facility, and validated by the Quality Assurance Department of the CBG. It is a real-time polymerase chain reaction (PCR) approach, employing separate sets of primers for the MVA backbone and the CMV insert genes utilizing TaqMan reagents. The assay is sensitive to detect <1 copy of MVA per sample, so it can be used to distinguish if any low level residual MVA remains after the time points of blood sampling. A valid plasmid DNA standard will be employed to measure copy number of the experimentally determined levels of MVA and the insert genes from blood specimens that will be taken at intervals specified in the protocol. No additional blood specimens will be needed for this assay.

2.2 Rationale

How a Therapeutic Vaccine Might Decrease Inflammation

We hypothesize that the CMV-MVA Triplex vaccine for CMV will stimulate B-cell and T cell responses, and yet decrease systemic inflammation. We hypothesize that this immune effect will be because the CMV DNA vaccine intervention will generate a CMV-specific immune response that will decrease sub-clinical CMV shedding. Specifically, persistent and intermittent CMV replication is associated with a large bystander activation of non-CMV-specific T cells and monocytes/macrophages [37, 38], likely secondary to the production of several chemokines (IL-1, tumor necrosis factor [TNF]- α , monocyte chemoattractant protein [MCP]-1, macrophage inflammatory protein [MIP]-1 β , RANTES, IL-6 and IL-8) at the site of CMV replication. Further, CMV encodes both viral homologue chemokines (vIL-10, vCXC-1, vCXC-2) and chemokine-like receptors (US33, US78, US27, US28), which also activate immune cells at the sites of CMV replication through a non-antigen-specific mechanism [2, 39-44]. Moreover, a provocative study demonstrated that least at some strains of Rhesus CMV could divert CD8+ T cell responses away from canonical CMV epitopes that likely constitute the most efficient targets for cytotoxicity [45]. As a consequence of these bystander effects, CMV shedding is highly associated with local and systemic increases in inflammation, and we hypothesize that the decrease in CMV shedding secondary to an efficient CMV specific immune response generated by the CMV vaccine will then translate into *less* overall inflammation. Nevertheless, we acknowledge that an alternative hypothesis is that a more robust anti-CMV immune response will increase markers of inflammation/immune activation and this will be also tested as part of this trial (two-sided tests).

Rationale for Upper Age Limit and CD4+ Cell Count Criteria

This study will enroll 90 participants with HIV between the ages of 18 and 65 who started similar ART during chronic infection. Eligible participants will also have CD4+ cell count >250 cells/ μ L at enrollment and a nadir CD4+ cell count \geq 100 cells/ μ L. We decided to exclude older individuals and individuals with lower CD4+ cell counts to enhance the likelihood of having a more robust immune response to the active vaccine since older age and compromised immune system are associated with worse vaccine response. These individuals will need to be evaluated in future work if this current study demonstrates promise.

Rationale for Excluding Participants with High Risk for Cardiovascular Disease

There was no evidence of cardiovascular toxicity for CMV-MVA Triplex in a Phase I trial on healthy volunteers and **a completed study in HCT participants (NCT02506933)** as well as in a prospective surveillance on MVA recipients. Nonetheless, we acknowledge that individuals with HIV/AIDS and on ART might present an increased risk if the vaccine has pro-inflammatory effects and require more caution. Therefore, we are excluding people with high cardiovascular risk from the study.

Rationale for Using CD137 as Primary Immunological Endpoint

CD137 is a member of the tumor necrosis factor (TNF) receptor family. Cellular expression of CD137 (4-1BB) is a specific marker of recent activation, which is uniformly up-regulated 24 hours after antigen stimulation on the surface of all T cells, regardless of

their differentiation stage or profile of cytokine secretion [46]. Analysis of CD137 levels has been shown to be an efficient and sensitive ex vivo technique, to rapidly identify antigen-specific T cells present at low frequencies, and displaying heterogeneous functional profiles [47]. In the context of clinical longitudinal studies, often relying on reduced amounts of patient blood specimens, the assessment of this surrogate marker of T cell activation, which is associated with multiple T cell functions, has become a convenient and informative tool. It has provided an enhanced technique for CMV immune monitoring in a number of different studies [46, 48-52], and importantly has shown to be a reliable method to estimate magnitude and duration of the CMV specific T-cell expansion after Triplex vaccination [22].

Rationale for Selection of Inflammatory Biomarkers

The primary immunologic endpoint for the trial is sTNFRII in blood plasma (measured by ELISA). This endpoint was chosen since sTNFRII is much higher among CMV-seropositive than CMV-seronegative individuals with HIV maintaining ART-mediated viral suppression [6]. Also, sTNFRII strongly predicts all-cause mortality, composite non-AIDS events, and specifically cardiovascular events in this setting [53, 54]. Similarly, the panel of secondary biomarker (IL-6, sCD163, IP-10, TNFRII, D-Dimers) were selected based on published data because they are increased in the setting of CMV co-infection and predictive of non-AIDS events.

Primary inflammation endpoint:

- sTNFRII (elevated in CMV seropositive HIV+ [6], predicts non-AIDS events [53])

Secondary inflammation endpoints:

- D-dimer (elevated in HIV+CMV+ versus HIV+CMV- [6]; predicts non-AIDS events [53])
- sTNFRII (predicts non-AIDS events [53])
- sCD163 (predicts all-cause mortality in HIV [55]; elevated in HIV+CMV+ versus HIV+CMV- [56])
- IP-10 (elevated in HIV+CMV+ versus HIV+CMV- [6]; does not predict non-AIDS events [53])

Exploratory inflammation endpoints:

- IL-6 (not elevated in HIV+CMV+ versus HIV+CMV- [6]; predicts non-AIDS events [53])
- sCD14 (predicts non-AIDS events [53]; trend, but not significantly elevated in HIV+CMV+ versus HIV+CMV- [56])
- suPAR (predicts non-AIDS events [54])

3.0 STUDY DESIGN

A5355 is a phase II, double-blind, randomized, placebo-controlled, multicenter study to evaluate the safety and immunogenicity of two injections of MVA Vaccine Encoding CMV antigens (Triplex) in adults with both HIV and CMV. Participants will be randomized in a 2:1 ratio to receive either two injections of CMV-MVA Triplex or placebo

administered at Day 0 and week 4. Participants will have follow-up visits in person or by phone for 92 weeks after the second injection, for a total of 96 weeks of follow-up. During the study, participants will have blood, urine, genital secretions, and oral secretions collected.

Enrollment will be stratified based on sex and use of gender-affirming hormones with at least 25% of participants being individuals assigned female sex at birth not on testosterone or individuals assigned male sex at birth on feminizing hormones. Special outreach to transgender and gender non-binary persons will be encouraged with exploratory stratified analysis conducted based on both gender and sex assigned at birth.

4.0 SELECTION AND ENROLLMENT OF PARTICIPANTS

4.1 Inclusion Criteria

- 4.1.1 HIV-1 infection, documented by any licensed rapid HIV test, or HIV enzyme or chemiluminescence immunoassay (E/CIA) test kit, at any time prior to study entry, and confirmed by a licensed Western blot, or a second antibody test by a method other than the initial rapid HIV and/or E/CIA, or by HIV-1 antigen, plasma HIV-1 RNA viral load.

NOTE: The term “licensed” refers to a US FDA-approved kit, which is required for all IND studies.

WHO (World Health Organization) and CDC (Centers for Disease Control and Prevention) guidelines mandate that confirmation of the initial test result must use a test that is different from the one used for the initial assessment. A reactive initial rapid test should be confirmed by either another type of rapid assay or an E/CIA that is based on a different antigen preparation and/or different test principle (e.g., indirect versus competitive), or a Western blot or a plasma HIV-1 RNA viral load.

- 4.1.2 **On continuous ART for ≥48 weeks prior to randomization with no ART interruption longer than 7 consecutive days, no anticipated modification during the study or within the 12 weeks prior to randomization, except as noted below.**

NOTE A: The following modifications within 12 weeks prior to randomization are not exclusionary:

- **change in formulation (e.g., from standard formulation to fixed-dose combination)**
- **a within-class single drug substitution (e.g., switch from nevirapine to efavirenz, from atazanavir to darunavir, from tenofovir disoproxil fumarate [TDF] to tenofovir alafenamide [TAF])**

NOTE B: A switch between any other nucleoside reverse transcriptase inhibitor (NRTI) to abacavir is not permitted within 12 weeks prior to randomization.

- 4.1.3 **Documented** HIV-1 RNA level below the limit of detection of clinically certified assays (**with a quantification limit of 75 copies/mL or lower**) for at least 48 weeks prior to study **entry (randomization)** using an FDA-approved assay performed by any US laboratory that has a Clinical Laboratory Improvement Amendments (CLIA) certification or its equivalent. **Additionally**, the participant must have a minimum of two values in the last 48 weeks obtained >30 days apart, with the most recent value obtained within 45 days prior to **randomization**.

NOTE: Single determinations **up to** 500 copies/mL (i.e., “blips”) are allowed as long as the preceding and subsequent determinations are both below the level of quantification. The screening value may serve as the subsequent undetectable value following a blip.

- 4.1.4 CD4+ cell count >250 cells/ μ L, obtained within 45 days prior to **randomization** at any US laboratory that has a CLIA certification or its equivalent.
- 4.1.5 The following laboratory values, obtained within 45 days prior to **randomization** (**except as** otherwise noted) by any US laboratory that has a CLIA certification or its equivalent:
- Hemoglobin ≥ 9.0 g/dL
 - Platelet count $\geq 75,000/\text{mm}^3$
 - Estimated Glomerular Filtration Rate (eGFR) >50 mL/min/1.73m² or creatinine clearance (CrCl) >50 mL/min using the Cockcroft-Gault equation on the FSTRF website.
 - Aspartate aminotransferase (AST) (SGOT), alanine aminotransferase (ALT) (SGPT), and alkaline phosphatase $\leq 3 \times \text{ULN}$
 - Hemoglobin A1c (HgbA1c) <6.5% (within 90 days prior to **randomization**)
- 4.1.6 Positive CMV immunoglobulin G antibody (IgG) serology, using an FDA-approved assay at any US laboratory that has a CLIA certification or its equivalent at any time prior to **randomization**.
- 4.1.7 Participants on statin therapy must be stable on the same dose for at least the 12 weeks **prior to randomization** with no anticipated change in statin or dose during the first 48 weeks of study.
- 4.1.8 For individuals who are able to become pregnant, a negative serum or urine pregnancy test by any US clinic or laboratory that has a CLIA certification or its equivalent or is using a point of care (POC)/CLIA-waived test **at screening**.

NOTE: Individuals who are able to become pregnant are defined as individuals who have reached menarche and who have not been post-menopausal

for at least 12 consecutive months with follicle-stimulating hormone (FSH) ≥ 30 IU/mL or 24 consecutive months if an FSH is not available, i.e., who have had menses within the preceding 24 months, and have not undergone a sterilization procedure (e.g., hysterectomy, bilateral oophorectomy, or salpingectomy).

- 4.1.9 Participants who are able to impregnate or become pregnant (i.e., of reproductive potential) and are participating in sexual activity that could lead to pregnancy must agree to practice contraception/birth control as indicated below or agree to not participate in a conception process (e.g., active attempt to become pregnant or to impregnate, sperm donation, in vitro fertilization) for at least 14 days prior to **randomization** through at least 60 days after the **second** vaccination at week 4.

Individuals who are able to become pregnant are defined above in [section 4.1.8](#). Individuals who are able to impregnate are defined as individuals who do not have documented azoospermia.

Acceptable contraception/birth control for this study includes the use of one or more of the following methods:

- Condoms with a spermicide
- Diaphragm or cervical cap with spermicide
- Intrauterine device (IUD)
- Hormone-based therapy (e.g., contraceptive pills, patches, implants, rings, or injections)

Participants who are not of reproductive potential are eligible without requiring the use of a contraceptive method. Acceptable documentation of lack of reproductive potential includes written documentation or oral communication of one or more of the following:

- Surgically sterile by hysterectomy, bilateral oophorectomy, or bilateral tubal ligation (documented in medical records or by ultrasound)
- Postmenopausal with participant reporting at least a 2-year history of amenorrhea
- Surgically sterile following a successful vasectomy

NOTE: The participant may not be able to provide written proof of a partner's vasectomy, sterilization, or menopausal status, since the participant's partner is not usually enrolled in the same study to provide consent for release of this information. The verbal report from the participant of their partner's status should be written into the source documents.

- 4.1.10 Individuals age ≥ 18 to ≤ 65 years at **randomization**.

- 4.1.11 Ability and willingness of participant to provide informed consent.

4.2 Exclusion Criteria

- 4.2.1 Nadir CD4+ cell count <100 cells/ μ L performed by any US laboratory that has a CLIA certification or its equivalent.

NOTE: If documentation is not available, then participant recall is acceptable.

- 4.2.2 Breastfeeding.

- 4.2.3 History of or active autoimmune disorders, including, but not limited to, inflammatory bowel diseases, scleroderma, severe psoriasis, myocarditis, uveitis, pneumonitis, systemic lupus erythematosus, rheumatoid arthritis, optic neuritis, myasthenia gravis, adrenal insufficiency, untreated hypothyroidism and/or hyperthyroidism, autoimmune thyroiditis, or sarcoidosis.

NOTE: For questions related to the definition of autoimmune disorders, sites should contact the A5355 clinical management committee (CMC) per the [Study Management section](#).

- 4.2.4 Known allergy/sensitivity or any hypersensitivity to components of the vaccine.

- 4.2.5 Use of anticoagulants, bleeding disorder, or condition associated with prolonged bleeding time that would contraindicate IM injection.

NOTE: Use of daily aspirin is not exclusionary.

- 4.2.6 Use of drugs with anti-CMV activity within 14 days prior to **randomization** (including but not limited to ganciclovir, valganciclovir, foscarnet, cidofovir, and letermovir).

NOTE: Acyclovir and valacyclovir may be used.

- 4.2.7 Any episode of symptomatic CMV disease within 12 months prior to **randomization**.

- 4.2.8 Previous receipt at any time of any experimental CMV vaccine.

- 4.2.9 Use of any infusion blood product or immune globulin within 3 months prior to **randomization**.

- 4.2.10 Use of **systemic** immunomodulators (e.g., interleukins, interferons, cyclosporine, and monoclonal antibodies), HIV vaccine, systemic cytotoxic chemotherapy, or investigational therapy within 60 days prior to **randomization**.

NOTE: Participants receiving stable physiologic glucocorticoid doses, defined as prednisone \leq 10 mg/day or the equivalent, will not be excluded. Stable physiologic glucocorticoid doses should not be discontinued for the

duration of the study. In addition, participants receiving inhaled or topical corticosteroids **or immunomodulators** will not be excluded.

4.2.11 Intent to use immunomodulators (e.g., IL-2, IL-12, interferons, or TNF modifiers) during the course of the study.

4.2.12 Pre-existing cardiovascular disease (**e.g., conduction disturbance, repolarization abnormality, significant atrial or ventricular arrhythmia, including frequent ectopy and flutter or fibrillation, evidence of past myocardial infarction**) or diabetes mellitus diagnosed by a medical provider.

NOTE 1: History of or current diagnosis of coronary artery disease, angina pectoris, myocardial infarction, previous coronary artery intervention (stenting, angioplasty), peripheral arterial disease (claudication, peripheral arterial angioplasty, or peripheral arterial bypass procedure), cerebrovascular disease (stroke or transient ischemic attack with documented carotid or aortic atherosclerosis), or abdominal aortic aneurysm are exclusionary for this study.

NOTE 2: Poorly controlled hypertension, defined as $\geq 160/100$ mmHg at two occasions, is exclusionary. A pre-existing history of hypertension alone is not exclusionary.

4.2.13 For those >40 years of age, a 10-year American College of Cardiology/American Heart Association (ACC/AHA) cardiovascular disease (CVD) risk of >15% **calculated using results and data obtained within 45 days** prior to **randomization**. [Links to risk calculators are provided in the protocol MOPS]

4.2.14 Receipt of a vaccine within **28 days** prior to **randomization**.

NOTE: This restriction applies to any non-MVA-based vaccine, including approved and experimental SARS-Cov-2/COVID-19 vaccines.

4.2.15 Receipt of MVA-based vaccines (e.g., for HIV or tuberculosis) within 1 year prior to **randomization**.

4.2.16 Active HIV-associated dementia.

4.2.17 Active hepatitis C (defined as hepatitis C virus (HCV) antibody (Ab) positive and HCV RNA detectable within 24 weeks prior to **randomization**).

4.2.18 Active hepatitis B (defined as hepatitis B surface antibody (HBsAb) negative, hepatitis B surface antigen positive (HBsAg), and/or HBV DNA detectable within 24 weeks prior to **randomization**).

NOTE 1: Participants with HBV DNA suppressed on an antiviral regimen

containing anti-HBV agents are eligible if they have HBV DNA BLQ within the past 24 weeks or at screening.

NOTE 2: Prior documentation of positive HBsAb is acceptable evidence that hepatitis B is not present. If HBsAb is BLQ or documentation is not available, HBsAg and HBcAb should be documented prior to **randomization**. Participants who have positive HBcAb but BLQ HBsAg and HBsAb (isolated HBcAb positive status) must have HBV DNA polymerase chain reaction (PCR) performed and confirmed as BLQ for participant to be eligible.

- 4.2.19 **Documented** active rectal, genital, or pharyngeal chlamydia, gonorrhea, or syphilis (based on screening test results and other clinical information). These **candidates must** be treated. **They may** be rescreened 30 days or more after treatment **ends**. Treatment must be documented.

NOTE: Screening for chlamydia and gonorrhea by nucleic acid amplification test (NAAT) only. In persons with positive syphilis enzyme immunoassay (EIA) or rapid plasma regain (RPR), a treponema-based test must be performed for confirmation, however, only evidence of active infection would exclude the **candidate** from the study.

- 4.2.20 Active drug or alcohol use or dependence that, in the opinion of the site investigator, would interfere with adherence to study requirements.
- 4.2.21 Acute or serious illness requiring systemic treatment and/or hospitalization within 14 days prior to **randomization and that, in the opinion of the site investigator, could increase or decrease systemic inflammation**.

NOTE: See [section 8.3](#) for guidelines related to COVID-19 infection.

- 4.2.22 **Any episode of migraine within 30 days prior to randomization**.

4.3 Study Enrollment Procedures

- 4.3.1 Prior to implementation of this protocol, and any subsequent full version amendments, each site must have the protocol and the protocol consent forms approved, as appropriate, by their institutional review board (IRB)/ethics committee (EC) and any other applicable regulatory entity (RE). Additionally, prior to protocol registration and initiation each site must have the protocol approved by their local Institutional Biosafety Committee (IBC) in accordance with the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines) dated April 2019 and the DAIDS Protocol Registration Manual, dated March 1, 2019. Upon receiving final approval, sites will submit all required protocol registration documents to the DAIDS Protocol Registration Office (DAIDS PRO) at the Regulatory Support Center (RSC). The DAIDS PRO will review the submitted protocol registration packet to ensure that all of the required documents have been received.

Site-specific informed consent forms (ICFs) WILL be reviewed and approved by the DAIDS PRO, and sites will receive an Initial Registration Notification from the DAIDS PRO that indicates successful completion of the protocol registration process. A copy of the Initial Registration Notification should be retained in the site's regulatory files.

Upon receiving final IRB/EC and any other applicable RE approvals for an amendment, sites should implement the amendment immediately. Sites are required to submit an amendment registration packet to the DAIDS PRO at the RSC. The DAIDS PRO will review the submitted protocol registration packet to ensure that all required documents have been received. Site-specific ICF(s) WILL NOT be reviewed or approved by the DAIDS PRO. Sites will receive an Amendment Registration Notification when the DAIDS PRO receives a complete registration packet. A copy of the Amendment Registration Notification should be retained in the site's regulatory files.

For additional information on the protocol registration process and specific documents required for initial and amendment registrations, refer to the current version of the DAIDS Protocol Registration Manual.

Once a candidate for study entry has been identified, details will be carefully discussed with the participant. The participant will be asked to read and sign the approved protocol consent form.

Participants from whom a signed informed consent has been obtained may be screened and enrolled, if they otherwise qualify. An ACTG Screening Checklist must be entered through the DMC Participant Enrollment System.

4.3.2 Protocol Activation

Prior to enrollment, sites must complete the Protocol Activation Checklist found on the ACTG Member website. This checklist must be approved prior to any screening of participants for enrollment.

4.3.3 Randomization/Participant Registration

For participants from whom informed consent has been obtained, but who are deemed ineligible or who do not enroll into the initial protocol step, an ACTG Screening Failure Results form must be completed and keyed into the database. Participants who meet the enrollment criteria will be randomized to the study according to standard ACTG DMC procedures.

4.4 Co-enrollment Guidelines

- Sites are encouraged to co-enroll participants in A5128, “Plan for Obtaining Informed Consent to Use Stored Human Biological Materials (HBM) for Currently Unspecified Analyses.” Co-enrollment in A5128 does not require permission from the A5355 protocol chairs.
- For specific questions and approval for co-enrollment in other studies, sites **should first check the co-enrollment document posted on the study’s PSWP. If the study in question is not listed there, please** contact the protocol team via e-mail as described in the [Study Management section](#).

5.0 STUDY TREATMENT

Study treatment is defined as Modified Vaccinia Ankara (MVA) Vaccine Encoding CMV Antigens (CMV-MVA Triplex), or placebo for CMV-MVA Triplex (phosphate-buffered saline [PBS] containing 7.5% [7.5% lactose/PBS]).

CMV-MVA Triplex and placebo will be provided by the study.

All study participants are required to be on combination ART continuously **while on study**. ART will not be provided by the study.

5.1 Regimens, Administration, and Duration

5.1.1 Regimen

At **randomization**, participants will be randomized 2:1 to CMV-MVA Triplex or placebo. The regimen will be in addition to the participants’ pre-existing combination ART regimen.

Vaccine Group participants will receive two doses of CMV-MVA Triplex on Day 0 and study Week 4. Each dose of CMV-MVA Triplex contains 5×10^8 plaque-forming unit (pfu) $\pm 0.5 \times 10^8$ pfu of MVA Vaccine Encoding CMV Antigens. The volume of vaccine administered will vary depending on the vaccine titer per volume, which can vary by vaccine lot, and is specified in [section 5.2.2](#).

Placebo Group participants will receive two doses of placebo (7.5% lactose/PBS) on Day 0 and study Week 4. The volume of placebo administered will match the dose volume for the CMV-MVA Triplex lot in pharmacy stock.

5.1.2 Administration

The study product will be administered intramuscularly in alternating deltoid muscles on each administration day.

5.1.3 Treatment Duration

Study participants will receive two doses of the study product, on Day 0 and on study week 4.

Participants will be followed for 92 weeks after the last injection, for a total of 96 weeks on study (see [section 6.2.3](#)).

5.2 Study Product Formulation and Preparation

5.2.1 Study Product Formulation

CMV-MVA Triplex is provided as a frozen, sterile, preservative-free solution containing 5.2×10^8 pfu/mL OR 8.0×10^8 pfu/mL. CMV-MVA Triplex is formulated in 7.5% lactose/PBS, and is supplied in 2-mL polypropylene cryogenic vials with a silicone washer seal. CMV-MVA Triplex vial fill volumes vary by vaccine lot; refer to Table 5.2.1-1 for details. CMV-MVA Triplex must be stored frozen at **-70°C to -90°C, with short (i.e., up to 24-hr) excursions to as high as -60°C allowable**.

Table 5.2.1-1: Vial Fill Volume by Lot Number

CMV-MVA Triplex Lot Number	Titer (pfu/mL)	Vial Fill Volume
0786-181-0003-1	5.2×10^8	1.1 mL
0786-181-0004-1	8.0×10^8	1.2 mL

Placebo for CMV-MVA Triplex will be provided as 7.5% lactose/PBS, a sterile, non-pyrogenic solution supplied in 1.2 mL to 2 mL polypropylene cryovials with a fill volume of approximately 1 mL. 7.5% lactose/PBS must be stored at **-70°C to -90°C, with short (i.e., up to 24-hr) excursions to as high as -60°C allowable**.

CMV-MVA Triplex and 7.5% lactose/PBS are described in further detail in the CMV-MVA Triplex Investigator's Brochure.

5.2.2 Study Product Preparation

The investigational pharmacist must be proficient in the preparation of products requiring aseptic technique under a pharmacy biological safety cabinet **or better**. For individual protection measures, such as personal protective equipment, local requirements and regulations are to be followed, and may include safety glasses, mask, gloves, and gowns.

CMV-MVA Triplex and placebo for CMV-MVA Triplex injections will be prepared by the investigational pharmacist. CMV-MVA Triplex injections will be prepared from a vial containing vaccine at

- 5.2×10^8 pfu/mL
- OR
- 8.0×10^8 pfu/mL.

Refer to Table 5.2.2-1 for the CMV-MVA Triplex dose volume by vaccine lot.

Table 5.2-2-1: CMV-MVA Triplex Dose Volume by Vaccine Lot

CMV-MVA Triplex Lot Number	Titer (pfu/mL)	Dose Volume
0786-181-0003-1	5.2×10^8	0.96 mL \pm 0.05 mL
0786-181-0004-1	8.0×10^8	0.625 mL \pm 0.05 mL

The placebo dose volume will match the dose volume for the CMV-MVA Triplex lot in pharmacy stock.

Thawing and preparation of CMV-MVA Triplex:

- Before the scheduled participant injection, remove one vial of CMV-MVA Triplex from the freezer and let thaw at room temperature (approximately 15-30 minutes).
- Record the time at which the vial is completely thawed. This is the start time of the vaccine dose preparation. Once thawed, if there is any delay between thawing and the subsequent steps, place vial in an ice bucket, the refrigerator, or any device that maintains temperature around 4-8°C to keep cold.
- Thoroughly wipe the exterior of the vial with an alcohol swab.
- **Just prior to withdrawing vaccine product, vortex vial for 30 seconds at highest setting.**
- **Immediately spin for 5 seconds in a microfuge at 6000 RPM to maximize the extractable volume.**
 - **Note that use of a microfuge is recommended but is not required if another method (e.g., tapping repeatedly) can be successfully employed to maximize the extractable volume.**

- Unscrew the cap from the vaccine vial and, using **an appropriately sized sterile needle**, withdraw the appropriate volume of solution (depending on vaccine lot titer) into an appropriately sized sterile syringe:
 - If using Lot # 0786-181-0003-1, withdraw 0.96 ± 0.05 mL of solution
 - If using Lot # 0786-181-0004-1, withdraw 0.625 ± 0.05 mL of solution
- Apply an overlay to the syringe and label appropriately to maintain the blind. Place the syringe in a sealable plastic amber bag, and place the plastic bag inside an insulated container with ice packs. Record the time that the syringe is placed inside the insulated container.
- Transport to the clinic for administration with a temperature-monitoring device such as a solid state or liquid thermometer.

Administration of CMV-MVA Triplex must occur within **10** hours after start time of dose preparation (i.e., time the vial is completely thawed).

Thawing and preparation of placebo for CMV-MVA Triplex:

- Before the scheduled participant administration, remove one vial of 7.5% lactose/PBS from the freezer and let thaw at room temperature (approximately 15-30 minutes).
- Record the time at which the vial is completely thawed. This is the start time of the placebo dose preparation.
- Once thawed, if there is any delay between thawing and the subsequent steps, place the 7.5% lactose/PBS vial in an ice bucket, the refrigerator, or any device that maintains temperature around 4-8°C to keep cold. Thoroughly wipe the exterior of the vial with an alcohol swab.
- **Just prior to withdrawing the placebo**, vortex vial for 30 seconds at highest setting.
- **Immediately spin for 5 seconds in a microfuge at 6000 RPM to maximize the extractable volume.**
 - **Note that use of a microfuge is recommended but is not required if another method (e.g., tapping repeatedly) can be successfully employed** to maximize the extractable volume.
- Unscrew the cap from the 7.5% lactose/PBS vial and, using **an appropriately sized sterile needle**, withdraw the appropriate volume of solution into an appropriately sized sterile syringe. Placebo volume withdrawn should match dose volume for CMV-MVA Triplex lot in pharmacy stock.
- Apply an overlay to the syringe and label appropriately to maintain the blind. Place the syringe in a sealable plastic amber bag, and place the plastic bag inside an insulated container with ice packs. Record the time that the syringe is placed inside the insulated container.
- Transport to the clinic for administration with a temperature-monitoring device such as a solid state or liquid thermometer.

Administration of placebo for CMV-MVA Triplex must occur within **10** hours after the start time of dose preparation (i.e., time the vial is completely thawed).

5.3 Pharmacy: Product Supply, Distribution, and Accountability

5.3.1 Study Product Acquisition/Distribution

Triplex is manufactured and provided by City of Hope.

CMV-MVA Triplex and 7.5% lactose/PBS will be made available to study sites through the NIAID Clinical Research Products Management Center (CRPMC). Upon successful completion of protocol registration procedures **and after randomization for each study participant**, these study products may be obtained by the investigational pharmacist by following the instructions in the manual *Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks*.

5.3.2 Study Product Accountability

The investigational pharmacist is required to maintain complete records of all study product received from the NIAID CRPMC and subsequently dispensed. All study product must be stored in the pharmacy.

All unused study product remaining at sites after the study is completed or terminated must be returned to the NIAID CRPMC (unless otherwise directed by the sponsor). Study products may also be returned to the CRPMC for other reasons, as requested by the sponsor. Investigational pharmacists will follow the relevant instructions for return of unused study products provided in the manual *Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks*.

5.4 Concomitant Medications

Whenever a concomitant medication or study agent is initiated or a dose changed, investigators must review the concomitant medication's and study agent's most recent package insert, Investigator's Brochure, or updated information from DAIDS to obtain the most current information on drug interactions, contraindications, and precautions. Additional drug information may be found on the ACTG Precautionary and Prohibited Medications Database located at http://tprc.pharm.buffalo.edu/home/di_search/.

5.4.1 Required Medications

None.

5.4.2 Prohibited Medications

- Anticoagulants
 - NOTE: Use of aspirin is allowed.
- Antivirals: ganciclovir, valganciclovir, foscarnet, cidofovir, letermovir
- Live attenuated virus vaccines and other non-study vaccines, including approved or experimental COVID-19 vaccines are prohibited for at least 28

days after the final study treatment administration.

- Systemic cancer chemotherapy, or immunomodulators
 - Growth factors, immune globulin, interleukins, interferons, anti-TNF therapies, and monoclonal antibodies
 - Immune modulators include, but not limited to:
 - interleukin-2 (IL-2, Proleukin)
 - interferons (Betaseron, Actimmune, Roferon A)
 - pentoxifylline (Trental)
 - thalidomide (Thalomid) and related compounds
- Systemic corticosteroids

NOTE 1: Topical corticosteroids are allowed

NOTE 2: Steroids are permitted if the doses are within ≤ 10 mg/day of prednisone or equivalent not within 7 days of administration of the study treatment.
- Statins

NOTE: Use is allowable for participants on stable statin therapy at entry as long as there are no changes to statin use or dose.
- Any other investigational agents that are not FDA-approved except with **prior** approval of the protocol team.

5.4.3 Precautionary Medications

None.

6.0 CLINICAL AND LABORATORY EVALUATIONS

6.1 Schedule of Evaluations (SOE)

Evaluation	Screening	Randomization/Entry	Day 0 (within 14 days after randomization)	2-3 Days Post- Day 0	Week 4 (-5, +14 days) (see section 6.3.16 for deferment criteria)	2-3 Days Post-Week 4	Post-Day 0 Visit Weeks (Visit windows in weeks)						Confirm Virologic Failure	Premature Study Disc.
							8 (±1)	12 (-1/+2)	24 (±2)	48 (±2)	72 (±2)	96 (±2)		
Documentation of HIV	X													
Medical History	X	X												
Medication History	X	X												
Complete Physical Exam	X													
Targeted Physical Exam			X		X		X	X	X	X	X		X	X
Concomitant Medications	X	X	X		X		X	X	X	X	X			X
Nadir CD4+ T Cell Count	X													
Pregnancy Testing	X (see section 4.1.8)		X		X		If indicated							X
FSH (see sections 4.1.8 and 6.3.5)	X													
Hematology	X		X		X			X		X				

Evaluation	Screening	Randomization/Entry	Day 0 (within 14 days after randomization)	2-3 Days Post- Day 0	Week 4 (-5, +14 days) (see section 6.3.16 for deferment criteria)	2-3 Days Post-Week 4	Post-Day 0 Visit Weeks (Visit windows in weeks)						Confirm Virologic Failure	Premature Study Disc.
							8 (±1)	12 (-1/+2)	24 (±2)	48 (±2)	72 (±2)	96 (±2)		
Blood Chemistry Tests (see section 6.3.5 for possible lipid panel at Screening.)	X		X		X			X		X				
HbA1c	X													
Liver Function Tests	X		X		X			X		X				
Coagulation Panel	X													
eGFR or Calculated CrCl	X		X							X				
Hepatitis B (see section 6.3.5)	X													
Hepatitis C (see section 6.3.5)	X													
CMV Serology	X													
Syphilis Serology	X		X					X		X				X
MVA Levels in Blood			X					X		X	X			X
CD4+/CD8+	X		X		X			X		X	X		X	X
Advanced Flow T-Cell Phenotype			X		X			X		X	X			
Advanced Flow CMV-Specific Immune Response			X					X		X				
Plasma HIV-1 RNA	X		X		X		X	X	X	X	X		X	X

Evaluation	Screening	Randomization/Entry	Day 0 (within 14 days after randomization)	2-3 Days Post- Day 0	Week 4 (-5, +14 days) (see section 6.3.16 for deferment criteria)	2-3 Days Post-Week 4	Post-Day 0 Visit Weeks (Visit windows in weeks)						Confirm Virologic Failure	Premature Study Disc.
							8 (±1)	12 (-1/+2)	24 (±2)	48 (±2)	72 (±2)	96 (±2)		
Stored Plasma for Inflammatory Biomarkers + Future Studies			X		X		X	X	X	X	X			X
Stored PBMC for T cell Response and Flow Cytometry + Future Sorting and Reservoir Studies			X		X			X		X	X			X
Urine for Gonorrhea/Chlamydia	X		X					X		X				X
Rectal Swab			X					X		X				
Urine for CMV DNA			X		X			X		X	X			X
Buccal Rinse for CMV DNA			X		X			X		X	X			X
Genital Secretion			X		X			X		X	X			X
ART Adherence Assessment		X	X		X		X	X	X	X	X		X	X
Scheduling Day 0 Visit (see 6.3.16)		X												
Vaccine/Placebo Injection and Post-injection Observation			X		X									
Vaccination Report Card (VRC) Distribution			X		X									
Vaccination Report Card (VRC) Collection and Review					X		X							X
Substance Use Questionnaire			X		X		X	X	X	X	X			X

Evaluation	Screening	Randomization/Entry	Day 0 (within 14 days after randomization)	2-3 Days Post- Day 0	Week 4 (-5, +14 days) (see section 6.3.16 for deferment criteria)	2-3 Days Post-Week 4	Post-Day 0 Visit Weeks (Visit windows in weeks)						Confirm Virologic Failure	Premature Study Disc.
							8 (±1)	12 (-1/+2)	24 (±2)	48 (±2)	72 (±2)	96 (±2)		
Telephone Contact	Recommended			X		X						X		
Remote Data Collection (see section 6.2.3)		possible					If approved		If approved					

6.2 Timing of Evaluations

6.2.1 Screening Evaluations

Screening evaluations must occur prior to the participant's starting any study medications, treatments, or interventions. See [section 8.3](#) for COVID-19 considerations.

Screening

Screening evaluations to determine eligibility must be completed within 45 days prior to **Randomization** unless otherwise specified. See [section 6.3.19](#) for **guidelines regarding telephone contact prior to Randomization and Day 0**.

In addition to data being collected on participants who enroll into the study, demographic, clinical, and laboratory data on screening failures will be captured in a Screening Failure Results form and entered into the ACTG database.

6.2.2 Randomization (Entry) Evaluations

Randomization

Where permitted and to reduce the number of clinic visits and to ensure sufficient time to order and receive study product on site, randomization may be conducted remotely up to 14 days prior to Day 0.

In all cases, site staff must review eligibility criteria with the participant at the time of randomization to confirm eligibility.

6.2.3 On-study Evaluations

Day 0

It is strongly encouraged that participants receive the first injection of study vaccine/placebo within **5** days after randomization, although vaccine/placebo administration within **14** days after randomization is **permitted**.

Post-Day 0

All **on-study** evaluations will occur per the SOE, **relative to Day 0, the visit at which the first study product vaccination is administered.**

Participants who report fever, or pain or tenderness causing more than minimal limitation of use of limb during the post-injection follow-up telephone **call** will be scheduled to return to the clinic for an interim visit as soon as possible, but prior to the next scheduled visit. Participants who report a rash must be scheduled to return to the clinic for an interim visit within **3 days after** rash onset.

The final study visit, which will occur at Week 96 (± 2 weeks), will be conducted via telephone unless any concerning events, as outlined in the Manual of Procedures (MOPS), warrant a visit in the clinic.

Remote Data Collection

Except for week 4 and week 12, guidelines for which are presented below, post-**Day 0** study visits may be conducted remotely (e.g., telephone, telehealth) in the following situations if the site first receives approval to do so from the A5355 CMC (actg.cmca5355@fstrf.org):

- A participant is unable to attend a visit within the given visit window (e.g., because of personal illness, illness among contacts, local conditions, guidelines restricting travel to the clinic, **travel or moving away from site location**).
- The site is temporarily unable to conduct non-essential visits in the clinic; the site must inform the CMC (actg.cmca5355@fstrf.org) when it has to stop non-essential visits.

Regardless of the situation, sites must document which visits were conducted remotely, attempt to obtain as much of the visit-specific required information, based on the SOE, as possible, and record it. The impacted visits and rationale must be reported and documented.

Week 4 and week 12 visits

If a site expects that either the week 4 visit (second vaccine administration) or the week 12 visit (primary immunological endpoint) cannot be conducted within the existing visit window, the site should inform the A5355 CMC (actg.cmca5355@fstrf.org) as soon as possible. Neither of these visits should be conducted as a Remote Data Collection visit.

6.2.4 Visit to Confirm Virologic Failure

Confirmed virologic failure is defined as two consecutive HIV-1 RNA levels ≥ 200 copies/mL by real-time HIV-1 RNA testing. Participants with a plasma HIV-1 RNA ≥ 200 copies/mL at any visit will have a confirmatory viral load obtained as soon as possible but within 14 days after the first sample was drawn, if possible. If this visit coincides with a regularly scheduled visit, the evaluations should be combined. If the consecutive measurement of HIV-1 RNA is also ≥ 200 copies/mL, the participant will be considered to have confirmed virologic failure and the protocol core team must be notified via e-mail actg.cmca5355@fstrf.org within 48 hours.

6.2.5 Discontinuation Evaluations

Evaluations for Randomized Participants Who Do Not Start Study Treatment

All eCRFs must be keyed for the period up to and including the **Randomization visit (Study Entry)**.

Premature Treatment Discontinuation Evaluations

Participants who receive the Day 0 injection but not the Week 4 injection will continue the follow-up schedule as outlined in the SOE including **all on-study**

evaluations **with the exception of those related to the week 4 injection.**

Premature Study Discontinuation Evaluations

Participants who miss the first dose of study vaccine will have the study discontinuation evaluations performed as soon as possible prior to being taken off the study.

6.3 Instructions for Evaluations

Each study site and laboratory involved in this study will comply with the DAIDS policy on Requirements for Laboratories Performing Testing for DAIDS-Supported and/or Sponsored Clinical Trials, which is available at:
<https://www.niaid.nih.gov/sites/default/files/laboratorypolicy1.pdf>.

All clinical and laboratory information required by this protocol is to be present in the source documents. Sites must refer to the Source Document Guidelines on the DAIDS website for information about what must be included in the source document:
<https://www.niaid.nih.gov/sites/default/files/score-source-documentation-requirements.pdf>.

All stated evaluations are to be recorded on the eCRF unless otherwise specified. Refer to [section 7.0](#) for information on the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table) and AE reporting of adverse events requirements.

The protocol team and/or study monitoring entity may determine that additional source data associated with procedures or evaluations performed per protocol should be entered into eCRFs so that the data can be used for analysis or to otherwise assist with interpretation of study findings. In such cases, sites will be officially instructed to enter the additional data into eCRFs from available source documentation.

6.3.1 Documentation of HIV-1

[Section 4.1.1](#) specifies assay requirements for HIV-1 documentation. HIV-1 documentation is not recorded on the eCRF.

6.3.2 Medical History

The medical history must include all signs and symptoms regardless of grade and all diagnoses identified by the ACTG criteria for clinical events and other diagnoses regardless of grade within the past 30 days.

The following diagnoses should be reported regardless of when the diagnosis was made (verbal history accepted):

- AIDS-defining conditions
- Bone fractures

- Coronary heart disease
- Peripheral vascular disease
- Cancer (exclusive of basal/squamous cell skin cancer)
- Diabetes
- Tuberculosis
- Chronic hepatitis C
- Chronic hepatitis B
- Previous CMV diagnosis
- Herpes simplex virus
- Cerebrovascular disease or stroke
- Heart disease
- Diagnoses of sexually transmitted infections within the past year
- Any allergies to any medications and their formulations
- Smoking history
- Any diagnosis of COVID-19 within the past year

6.3.3 Medication History

A medication history must be present, including start and stop dates. The table below lists the medications that must be included in the history. Documentation is preferred, but self-report is acceptable.

Table 6.3.3-1: Medication History

Medication Category	Timeframe
Antiretroviral therapy	Within 1 year
Immune-based therapy	Complete
Blinded study treatment	Complete
HIV-1-related vaccines	Complete
Prescription drugs for treatment of opportunistic infections	Within 30 days
Prescription drugs for prophylaxis of opportunistic infections	Within 30 days
Previous MVA-based vaccine	Within 1 year
Previous anti-CMV vaccines	Complete
Previous shingles or chicken pox vaccines	Complete
All other previous vaccines	Within 3 months
Other prescription drugs	Within 30 days
Alternative therapies	Within 30 days
Dietary supplements	Within 30 days
Sex-hormone medications or sex-hormone analogues or antagonists*	Last 12 months except as noted below

*Includes: hormone-releasing IUDs (e.g., Mirena inserted in the last 5 years); oral, injectable, implanted, or patch contraceptives; vaginal ring, creams, or inserts;

estrogen, progesterone, or testosterone therapy; leuprolide or other synthetic gonadotropin-releasing hormone; tamoxifen, raloxifene, aromatase inhibitors or any other androgen, estrogen, progesterone analogue or antagonist therapy, or spironolactone.

6.3.4 Clinical Assessments

Complete Physical Exam

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

Targeted Physical Exam

A targeted physical examination is to include vital signs **and evaluation of symptoms, including injection site pain, erythema, induration, nausea, fatigue, myalgia, arthralgia, headache, chills, and fever**, and is to be driven by any previously identified or new signs or symptoms including diagnoses that the participant has experienced within 14 days prior to entry/since the last visit.

On-study, see [section 8.2](#) for collection requirements for pregnancy.

At **Randomization**, refer to [section 6.3.2](#) (Medical History) for reporting requirements. **Beginning with Day 0**, refer to [section 7.2](#) for AE reporting requirements.

Concomitant Medications

All unnecessary medications should be avoided during the study period. Any prescription or over-the-counter medications taken and any non-study vaccine administered during the study period should be recorded.

Nadir CD4+ Cell Count

Record the participant's nadir CD4+ cell count (absolute value and date). Document the nadir CD4+ cell count when possible with a copy of the nadir CD4+ cell count report. If this documentation is not available, then participant recall 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., <150 cells/ μ L) is acceptable.

6.3.5 Laboratory Evaluations

If a participant experiences an acute inflammatory condition, receives a non-study vaccine or experiences a severe concurrent illness, wait 7 days to obtain laboratory evaluations listed below. Examples of inflammatory conditions that would justify delaying a blood draw include an infection requiring hospitalization,

a systemic viral illness such as an influenza-like illness, a severe drug hypersensitivity reaction, myocardial infarction, fever on the day of visit (defined as body temperature $>38^{\circ}\text{C}$), and major trauma. Sites are encouraged to contact the A5355 CMC (actg.cmcA5355@fstrf.org) with any questions regarding whether a specific situation would require a delay in blood collection.

Beginning at screening, all laboratory values for study-required testing must be recorded regardless of grade. Record abnormal laboratory findings per [section 7.2](#).

All laboratory toxicities that led to a change in vaccine treatment, regardless of grade, must be recorded. Further evaluation will be required for those events that meet Expedited Adverse Event (EAE) or International Council on Harmonisation (ICH) reporting requirements.

NOTE: Because of the diurnal variation in biomarkers that may be measured as part of the study (e.g., CD4+ and CD8+ cell counts, other biomarkers that may be measured on stored samples), blood draws and specimen collections for individual participants should be performed consistently in either the morning or the afternoon throughout the study, if possible.

Pregnancy Test

For individuals who are able to become pregnant: Serum or urine β -HCG (urine test must have a sensitivity of 15-25 mIU/mL). Record pregnancy and pregnancy outcomes per [section 8.0](#).

FSH

For individuals with a uterus who report being post-menopausal during a period between 12 and 24 consecutive months and have not undergone a sterilization procedure (e.g., hysterectomy, bilateral oophorectomy, or salpingectomy). If individuals with a uterus report being post-menopausal for >24 months, FSH is not necessary to define postmenopausal status.

Hematology

Hemoglobin, hematocrit, red blood cells (RBC), mean corpuscular volume (MCV), white blood cells (WBC), differential WBC, absolute neutrophil count (ANC), platelets.

Blood Chemistry Tests

Electrolytes (sodium, potassium chloride, bicarbonate), glucose, creatinine, blood urea nitrogen (BUN).

At Screening, for candidates who are >40 years old, a lipid panel that includes HDL and total cholesterol performed within 45 days prior to randomization. Fasting is not required.

Hemoglobin A1c
HbA1c.

Liver Function Tests
AST (SGOT), ALT (SGPT), alkaline phosphatase, and total bilirubin.

Coagulation Panel
Thromboplastin Time (APTT), Prothrombin Time/International Normalized Ratio (PT/INR).

eGFR or Calculated CrCl
eGFR is to be calculated according to local clinical practices. Calculated CrCl is estimated by the Cockcroft-Gault equation. This requires the recording of all serum creatinine values regardless of grade.

NOTE: A calculator for estimating the CrCl can be found on the FSTRF website.

Hepatitis B
HBsAg, **HBcAb**, HBsAb.

NOTE: Prior documentation of positive HBsAb is acceptable evidence that hepatitis B is not present. If HBsAb is BLQ or documentation is not available, HBsAg and HBcAb should be documented prior to study entry. Participants who have positive HBcAb but BLQ HBsAg and HBsAb (isolated HBcAb positive status) must have HBV DNA polymerase chain reaction (PCR) performed and confirmed as BLQ for participant to be eligible.

Hepatitis C
HCV antibody.

NOTE: If HCV antibody is positive, HCV RNA should also be performed.

CMV Serology
CMV IgG serology using a FDA-approved assay at any US laboratory that has a CLIA certification or its equivalent.

Syphilis Serology
RPR or EIA will be performed in a local laboratory. If the initial test yields positive results the alternate treponema test must be performed for confirmation.

MVA Levels in **Blood**
MVA by TaqMan will be performed on **blood** collected before injection at Day 0, as well as on **blood** collected at Weeks 12, 48, and 72, and at Premature Study Discontinuation.

6.3.6 Immunologic Studies

CD4+/CD8+

Obtain absolute CD4+/CD8+ cell count and percentages within 45 days prior to **randomization** from a laboratory that possesses a CLIA certification or equivalent.

For **on-study** evaluations, all laboratories must possess a CLIA certification or equivalent.

6.3.7 Virologic Studies

Plasma HIV-1 RNA

Screening HIV-1 RNA must be performed within 45 days prior to **Randomization** by a laboratory that possesses a CLIA certification or equivalent. Eligibility will be determined based on the screening value.

6.3.8 Stored Plasma

Stored blood plasma will be tested by ELISA for immunological biomarkers including, but not limited to, IL-6, sCD163, IP-10, TNFR11, and D-Dimers. Some plasma will be used to measure additional inflammatory biomarkers, including but not limited to IL-18, IL-7, and sCD14, and for other future studies.

6.3.9 Stored PBMC

These cells will be used to measure levels of CMV DNA. Extra stored PBMC will also be used to perform

- (1) HIV reservoir studies, if warranted
- (2) T cell proliferation and exhaustion marker analysis by flow cytometry with antibodies including but not limited to CD3+, CD4+, CD8+, Ki67, PD-1, Lag-3, Tim-3, TIGIT, T-bet, Eomes, and MAF
- (3) An assessment of the quality of the anti-CMV response at Day 0, Week 12, and Week 48 by stimulating PBMCs with overlapping peptides as described above, followed by single-cell sorting of CD8+CD137+ and CD4+CD137+ T cells. Single-cell TCR sequencing to identify paired $\alpha\beta$ TCRs will be performed on these cells.

Advanced Flow T Cell Phenotype

Monitoring of T cell responses per the SOE will allow analysis of the kinetics of CMV-specific responses and their persistence. PBMCs will be analyzed directly ex vivo by flow cytometry with a panel of antibodies and reagents including, but not limited to:

- markers of viability (Live/Dead Fixable Viability Dye)
- cell identity (CD3+, CD4+, CD8+, CD45RO)
- cell trafficking (CCR7, CX3CR1)

- immune exhaustion (PD-1, Tim-3)
- immune senescence (CD57+)
- cardiovascular interactions (PAR-1, PSGL-1)
- costimulation potential (CD27+, CD28+, CD2+).

Advanced Flow CMV-Specific Immune Response

The frequency of specific T cell responses will be determined by flow cytometric analysis of CD137 expression after 24-hour in vitro stimulation of PBMC with pools of 15-mer overlapping peptides spanning the entire length of CMV pp65, IE1, and IE2 proteins. To measure the functional phenotype of the CD137+ T cells, after stimulation the cells will also be stained by flow cytometric analysis with antibodies including, but not limited to:

- CD3+
- CD4+
- CD8+
- CD27+
- CD137+
- CD69+
- CD28+
- CD57+.

6.3.10 Urine for Gonorrhea and Chlamydia

Urine testing for gonorrhea and chlamydia will be performed in real time using a NAAT at a local laboratory that possesses a CLIA certification or equivalent.

6.3.11 Rectal Swab

Rectal swabs will be collected per the SOE, and stored for future microbiome studies. Instructions can be found in the MOPS. **Self-collection is permitted.**

6.3.12 Urine for CMV DNA

Urine will be collected per the SOE and stored for CMV DNA.

6.3.13 Buccal Rinse

Buccal rinse will be collected according to the SOE and stored for CMV DNA. Refer to the MOPS and the LPC for directions on buccal secretions collection and processing, respectively.

6.3.14 Genital Secretions

Genital secretion samples will be collected for CMV DNA **at the time points indicated in** the SOE. Participants must refrain from sexual intercourse and avoid using intravaginal products 48 hours prior to sample collection. **Refer to**

the MOPS for collection procedures and to the LPC for processing and storage instructions.

Self-collected seminal secretion sample collections may be performed off-site or at the clinic.

Two vaginal swabs may be obtained by study personnel or via self-collection.

6.3.15 ART Adherence Assessment

Site personnel will perform an assessment of adherence to ART according to the SOE. **In the event of ART discontinuation or modifications, participants will be referred for appropriate intervention and follow-up.**

The ART Adherence eCRF is posted on the DMC Portal in the Forms Management Utility.

6.3.16 Scheduling Day 0 Visit

Prior to Randomization, site staff will determine whether study candidates (those who have signed the informed consent form at Screening) remain interested in participating in the study. If so, Randomization should be scheduled to take place at least 1 business day before the participant is expected to receive their first dose of study treatment (Day 0). This separation allows time for the site to order and receive study product from the CRPMC.

Where permitted, the Randomization visit may be scheduled as a remote visit.

Regardless of how the Randomization visit is conducted, site staff must ensure that a study candidate is eligible to participate in the study before completing randomization.

6.3.17 Vaccine/Placebo Injection

Vaccine/placebo should be deferred for fever, defined as body temperature >38°C, on the day of vaccination and until the participant is afebrile for 24 hours. Other situations that may require vaccine/placebo deferral with written approval from the study team include, e.g., a participant's inability to appear in person because of illness and a site's inability to administer the product on the expected day.

It is acceptable to defer the injection for either time point to the maximum deferral as outlined in [section 9.0](#). In the event a study injection is deferred, the regular Day 0 or Week 4 evaluations should be postponed to the next visit/when the

study vaccine/placebo is actually administered.

For 30 minutes after each injection, participants will be observed for any allergic reaction (see [section 8.1.1](#) for monitoring any allergic reaction).

6.3.18 Vaccination Report Card (VRC) Distribution, Collection, and Review

All participants will **use** a vaccination report card (VRC) to record daily temperatures (oral or equivalent, **taken at approximately the same time each day**) and injection-site reactions for 5 days following each vaccination and to record any systemic reactions during the 4-week follow-up following each vaccination.

A VRC will be given to each participant at Day 0 **and** will be collected **and reviewed** at the week 4 visit **prior to administration of the week 4 injection**. If the week 4 vaccination **must be** deferred, then the **participant should be reminded to continue entering data on the VRC** until the vaccination is administered.

A second VRC will be given to each participant at the week 4 visit and will be collected **and reviewed** at the week 8 visit.

Participant-reported entries on VRCs will be reviewed remotely during the telephone contact 2-3 days after each injection and in person at all study visits through 4 weeks after the second injection. **Site staff must evaluate any AEs, grading them as described in the current A5355 MOPS.**

Clinic personnel will review the VRC for completeness, accuracy, and clarity. All comments are to be reviewed by the study personnel and, if necessary, discussed with the participant for clarification.

It is essential that the Day 0 VRC be reviewed BEFORE the week 4 vaccination is administered. Sites may consider delaying preparation of the vaccination until it is certain that the participant is eligible for the week 4 vaccination, based on clinical criteria used for the initial vaccination.

All non-study vaccines or medications taken during the study must be documented on the VRC.

The VRC will be a source document containing data that will be transferred to the appropriate eCRFs.

The following data should be transcribed from each VRC to the eCRF:

- Any oral/equivalent temperature $\geq 101.0^{\circ}\text{F}$ ($\geq 38.3^{\circ}\text{C}$)
- All injection site reactions such as pain, redness, and swelling
- All systemic reactions

- All rash reactions
- All medications taken (including medications taken for injection site pain or reaction)
- Any non-study vaccines received

In case of premature study discontinuation, the VRC will be reviewed for completeness, accuracy, and clarity at the time of discontinuation.

Any additional information obtained by contact with the participant should be clearly documented, initialed, and dated at the bottom of the VRC. The VRC is considered a source document and no original information recorded by the participant should be crossed-out or altered in any manner by study personnel.

Participants will be asked to notify the study personnel immediately if any unexpected or severe reaction occurs. The participant will be asked to immediately notify the study investigator if they experiences a rash. The rash should be examined by the site investigator or clinic personnel within **3 days after** rash onset.

The VRC is posted on the DMC Portal in the Forms Management Utility.

6.3.19 Substance Use Questionnaire

Substance use will be assessed using a self-report questionnaire per the SOE ([section 6.1](#)).

The substance use questionnaire is posted on the DMC Portal in the Forms Management Utility. Refer to the A5355 MOPS for the specific information and instructions for completing the substance use questionnaire.

6.3.20 Telephone Contact

Sites are encouraged to contact study candidates by phone at or before randomization, especially when randomization is conducted remotely. The purpose of this contact is to ensure that the participant remains eligible for and interested in the study.

The study staff will contact each participant by telephone **2 to 3 days** after each study vaccine/placebo injection to determine whether any side effects have occurred. Information from the telephone contact will be recorded. If a participant experiences fever, or pain or tenderness causing more than minimal limitation of use of limb, the participant should be scheduled to come into the clinic for evaluation, including a targeted physical exam focused on the area involved, as soon as possible.

The final study visit (week 96) will also be conducted via telephone.

See the MOPS for more telephone contact details.

NOTE: If telephone contact is not possible, then contact via e-mail or text, or in person is permissible.

7.0 ADVERSE EVENTS AND STUDY MONITORING

7.1 Definition of Adverse Events

An AE is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or diagnosis that occurs in a study participant during the conduct of the study REGARDLESS of the attribution (i.e., relationship of event to medical treatment/study product/device or procedure/intervention). This includes any occurrence that is new in onset or aggravated in severity or frequency from the Day 0 condition.

7.2 Adverse Event Collection Requirements for this Protocol

All AEs must be recorded on the eCRFs if any of the following criteria have been met:

- Grade ≥ 3 AEs
- Vaccine-related AEs regardless of grade
- AEs that led to a change in study treatment/intervention regardless of grade
- AEs meeting the Serious Adverse Event (SAE) definition or EAE reporting requirement
- Any Grade ≥ 0 COVID-19 diagnosis (including any positive SARS-CoV-2 test, regardless of symptoms)

NOTE A: Study drug related SAEs and study drug related EAE should also be reported to the CMC within 24 hours after a site's awareness of the event and be entered into the DAIDS Adverse Experience Reporting System (DAERS), an internet-based reporting system, within 3 days.

NOTE B: All Grade ≥ 3 AEs should be recorded on the eCRF and reported to the CMC within 24 hours after a site's awareness of the event. See the A5355 MOPS for guidance in grading participant-reported post-vaccination injection site reactions.

NOTE C: For any Grade ≥ 3 drug-related toxicity that is identified after the first vaccination, sites must contact the CMC (actg.cmcA5355@fstfrf.org) within 24 hours of when they become aware of the event; the second vaccination must NOT be administered.

All AEs that are reported must have their severity graded **based on** the DAIDS AE Grading Table, Corrected Version 2.1, July 2017, which can be found on the DAIDS RSC website at <https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables>.

Serious Adverse Events (SAEs)

An SAE is defined as any untoward medical occurrence that:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is an important medical event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above).

7.3 Expedited Adverse Event (EAE) Reporting to DAIDS

7.3.1 Expedited Reporting of Adverse Events to DAIDS

Requirements, definitions and methods for expedited reporting of AEs are outlined in Version 2.0 of the DAIDS EAE Manual, which is available on the RSC website at <https://rsc.niaid.nih.gov/clinical-research-sites/manual-expedited-reporting-adverse-events-daids>.

The DAERS, an internet-based reporting system, must be used for EAE reporting to DAIDS. In the event of system outages or technical difficulties, EAEs may be submitted using the DAIDS EAE Form. This form is available on the DAIDS RSC website at <https://rsc.niaid.nih.gov/clinical-research-sites/paper-eae-reporting>.

For questions about DAERS, please contact NIAID CRMS Support at CRMSsupport@niaid.nih.gov. Please note that site queries may also be sent from within the DAERS application itself.

For questions about expedited reporting, please contact the DAIDS RSC Safety Office at DAIDSRSCSafetyOffice@tech-res.com.

7.3.2 Reporting Requirements for this Study

- The SAE Reporting Category, as defined in Version 2.0 of the DAIDS EAE Manual, will be used for this study.
- The study agents for which expedited reporting are required are Modified Vaccinia Ankara-based anti CMV Vaccine (Triplex) and placebo for CMV-MVA Triplex.

7.3.3 Grading Severity of Events

The DAIDS AE Grading Table, corrected Version 2.1, July 2017, must be used and is available on the DAIDS RSC website at <https://rsc.niaid.nih.gov/clinical->

[research-sites/daids-adverse-event-grading-tables](#).

7.3.4 Expedited AE Reporting Period

- The expedited AE reporting period for this study per the EAE manual.
- After the protocol-defined EAE reporting period, unless otherwise noted, only suspected, unexpected serious adverse reactions (SUSARs) as defined in Version 2.0 of the DAIDS EAE Manual, will be reported to DAIDS if the study staff become aware of the events on a passive basis (from publicly available information).

7.4 Study Monitoring

The Protocol Team will monitor the conduct and safety of the study via regular summaries of accrual, AEs, and possibly sample/data availability, pooled over the study arms, as well as **baseline** characteristics and premature treatment and study discontinuations (and reasons).

The DAIDS clinical representative will review and assess **study drug related** EAE reports for potential impact on the study participant safety and protocol conduct per DAIDS policies, guidance documents, and SOPs, as applicable.

The study will undergo interim review by an ACTG-appointed Study Monitoring Committee (SMC). The SMC will review accrual, **baseline** characteristics, conduct of the study (including premature study discontinuations and premature study treatment discontinuations), AEs by study treatment arm (**including protocol team assessment of relationship to study treatment**), CD4+ cell counts and HIV-1 RNA levels/suppression over time by study treatment arm, and possibly sample availability. The first SMC review will occur 6 months after the first participant is enrolled and then every 12 months as long as participants remain in follow-up. The SMC may elect to deviate from this timeline. An interim review may also be convened if a concern is identified by the DAIDS clinical representative, the study chairs, or study statistician in consultation with the team. See [section 10.5](#) for statistical and other considerations related to interim monitoring.

Detailed plans for study monitoring will be outlined in a Study Progress Data and Safety Monitoring Plan (SPDSMP) developed by the Statistical and Data Management Center (SDMC) prior to enrollment of the first participant.

8.0 CLINICAL MANAGEMENT ISSUES

8.1 Toxicity

8.1.1 Toxicities to CMV-MVA Triplex

Expected (known) toxicities associated with CMV-MVA Triplex in **individuals** without HIV with the highest grade indicated are injection site reaction (Grades 1-

2), cutaneous reaction (Grade 3), myalgia (Grades 1-2), malaise (Grades 1-2), and headache (Grades 1-2). **Refer to [section 7.2](#) for instructions if a participant has a Grade ≥ 3 drug-related toxicity after the first vaccination.**

A phase II study entitled “Phase II Randomized Placebo-Controlled Multicenter Trial to Protective Function of a CMV-MVA Triplex Vaccine in Recipients of an Allogeneic Hematopoietic Stem Cell Transplant” (NCT02506933) **has been completed and published [34]**. A total of 102 participants were accrued and randomized to receive Triplex vaccine (n=51) or placebo (n=51). The placebo is identical to the one described for this clinical protocol. Triplex was well tolerated in transplant recipients in this study. There was no increased number or severity of overall AEs in the vaccine arm. A total of four Grade 3-4 AEs were reported as possibly attributable to vaccine, while eight Grade 3-4 AEs were reported as possibly attributable to placebo during the 365-day follow-up period. No participant was removed from the clinical trial because of vaccine-related toxicity. There was no sign of increased mortality or disease relapse associated with Triplex vaccination.

The unblinded efficacy results show that the vaccine demonstrated superiority over the placebo in reducing CMV reactivations as defined in the protocol, meeting its primary endpoint based upon the log-rank test. The full data analysis, including primary, secondary, and exploratory endpoints, **is** publically available. FDA has been fully apprised of events related to the vaccine in our ongoing trials and has permitted new clinical trials that are listed in clinicaltrials.gov in high-risk adult transplant recipients (NCT03438344 & NCT03560752), and pediatric transplant recipients (NCT03354728).

Anticipated toxicities that have not yet been seen from the agent but are foreseeable based on other similar agents include bruising at the site of injection, infection, and transient hypotension.

Participants experiencing any of the expected toxicities listed above may receive the second vaccine, with the exceptions noted in [section 9.1](#).

Participants will be observed for 30 minutes after each injection for any allergic reaction. If an allergic reaction develops, an antihistamine will be administered to counter the reaction.

8.1.2 Toxicities to the Placebo

There are no known toxicities associated with administering the placebo. Anticipated toxicities that have not yet been seen from the placebo but are foreseeable based on other similar agents include bruising at the site of injection and transient hypotension.

8.2 Pregnancy and Breastfeeding

Pregnancy, breastfeeding, and pregnancy outcome will be recorded on the eCRFs. Pregnancies that occur on study should be reported prospectively to The Antiretroviral Pregnancy Registry. More information is available at www.apregistry.com. Telephone: 800-258-4263; Fax: 800-800-1052.

Pregnancy Outcomes and Reporting

If a pregnant participant has completed the study or chooses to discontinue from the study before the end of the pregnancy, then site staff should request permission to contact the participant regarding pregnancy outcomes at the end of pregnancy. If the information is obtained, pregnancy outcomes will be submitted on an eCRF at the end of the pregnancy.

Pregnant and breastfeeding participants will discontinue study medication and will be encouraged to **remain on study and** complete remaining study evaluations.

8.3 SARS-CoV-2/COVID-19 Infection and Vaccination

8.3.1 Screening

Infection

Participants with suspected or confirmed SARS-CoV-2 infection should be referred for appropriate testing and/or treatment. Sites should contact the A5355 CMC (actg.cmca5355@fstf.org) regarding completion of screening.

Vaccination

As noted in [section 4.2.14](#), vaccination with an approved or experimental COVID-19 vaccine is permitted but must be completed at least 28 days before **randomization**.

8.3.2 Entry (**Randomization**) through Week 8

Infection

Participants with suspected or confirmed SARS-CoV-2 infection after **randomization** may remain on study but must be referred for appropriate testing and/or treatment. Local and federal guidelines must be followed regarding the participant's ability to return to the site and resume study visits.

Participants diagnosed with COVID-19 must not receive study treatment until **14 days after resolution of all symptoms**. The site must contact the A5355 CMC (actg.cmca5355@fstf.org) **if this restriction interferes with the** week 4 administration of study treatment.

Vaccination

As noted in [section 5.4.2](#), participants may receive an approved or experimental COVID-19 vaccine but not until at least 28 days after the final administration of study treatment. These participants may remain on study.

8.3.3 After Week 8

Infection

Participants with suspected or confirmed SARS-CoV-2 infection after **week 8** may remain on study but must be referred for appropriate testing and/or treatment. Local and federal guidelines must be followed regarding the participant's ability to return to the site.

As noted in [section 6.2.3](#), most visits after week 8 may be conducted remotely, as necessary. The week 12 visit is an exception, however; sites must contact the A5355 CMC (actg.cmca5355@fstrf.org) to obtain approval to re-schedule the week 12 visit.

Vaccination

Participants may receive an approved or experimental COVID-19 vaccine at any time after week 8.

9.0 CRITERIA FOR DISCONTINUATION

Plans to replace participants **who meet any of the** discontinuation criteria **listed below** are outlined in [section 10.4](#).

9.1 Permanent and Premature Treatment Discontinuation

- Week 4 injection deferral >4 weeks.
- Grade 4 AE.
- Requirement for prohibited concomitant medications (see [section 5.4](#)).
- Pregnancy or breastfeeding.
- Request by participant to terminate treatment.
- Clinical reasons believed life-threatening by the physician, even if not addressed in the [toxicity section](#) of the protocol.

9.2 Premature Study Discontinuation

- Failure by the participant to attend two consecutive clinic visits.
 - This criterion is not applicable if the A5355 CMC has approved remote data collection in lieu of multiple study visits. **Note that the week 4 and week 12 visits must be conducted in person.**
- Failure to receive the first dose of study vaccine within **14 days** after randomization.
- Request by the participant to withdraw.
- Request of the primary care provider if they think the study is no longer in the best interest of the participant.
- At the discretion of the IRB/EC, FDA, NIAID, Office for Human Research Protections (OHRP), other government agencies as part of their duties, or investigator.

10.0 STATISTICAL CONSIDERATIONS

10.1 General Design Issues

A5355 is a phase II, double-blind, randomized, placebo-controlled study to evaluate the safety, and immunogenicity of two doses of MVA Vaccine Encoding CMV antigens (Triplex) in adults between the ages of 18 and 65 years with both HIV and CMV and who are virologically suppressed on potent combination ART. Participants will be randomized in a 2:1 ratio to receive either two doses of CMV-MVA Triplex or placebo administered at Day 0 and Week 4. Participants will be followed for 92 weeks after the last scheduled vaccination at Week 4, for a total study duration of 96 weeks. The total sample size will be 90 participants (60 in the CMV-MVA Triplex group and 30 in the placebo group), at least 25% of whom will be individuals assigned female sex at birth not on testosterone or individuals assigned male sex at birth on feminizing hormones.

While safety is one of the co-primary outcomes, the study was powered for the immunogenicity co-primary outcome and thus a limitation of the study design is low power to detect differences in safety between the arms given the sample size and the low probability of safety events expected.

For A5355, the primary completion data (PCD) will be reached prior to the close of study follow-up. Because of the co-primary outcomes, the PCD will be based on the safety outcome which spans the first 48 weeks of follow-up.

10.2 Outcome Measures

Primary and secondary outcome measures listed below will be addressed in the study's primary Statistical Analysis Plan, which will define the content of the Primary Analysis Report. This report will form the basis for the primary study manuscript and results reporting to <https://ClinicalTrials.gov>.

10.2.1 Primary Outcome Measures

10.2.1.1 Safety Occurrence of Grade ≥ 3 AEs over 48 weeks.

10.2.1.2 Cellular immunogenicity Change in pp65-specific CD137+ CD8+ T cells from Day 0 to Week 12.

10.2.1.3 Inflammation Change in sTNFRII from Day 0 to Week 48.

10.2.2 Secondary Outcome Measures

10.2.2.1 Inflammation Change from Day 0 to Weeks 12, 24, 48, and 72 in levels of inflammatory biomarkers (including but not limited to IL-6, sCD163, IP-10, sTNFRII, D-Dimers).

10.2.2.2 Cellular immunogenicity Change in IE1 and IE2-specific CD137+

CD8+ T cells from Day 0 to Week 12.

- 10.2.2.3 Prolonged cellular immunogenicity Change in the percent of pp65-, IE1- and IE2-specific CD137+ CD8+ T cells over 48 weeks.
- 10.2.2.4 CMV DNA shedding CMV DNA in PBMC, urine, and genital secretion, oral secretions at Weeks 12, 48, and 72.
- 10.2.2.5 Detection of persistence of MVA DNA after immunizations: Viral DNA from recombinant MVA vaccine at Week 12.

10.2.3 Other Outcome Measures

- 10.2.3.1 Inflammation Change from Day 0 to Weeks 12, 24, 48, and 72 in levels of inflammatory biomarkers (including but not limited to IL-18, IL-7, sCD14).
- 10.2.3.2 T cell dysfunction Change from Day 0 to Weeks 12, 48, and 72 in levels of T cell immune activation, proliferation, and exhaustion as well as CD4+/CD8+ T cell ratio.
- 10.2.3.3 Prolonged safety Occurrence of Grade ≥ 3 AEs over 96 weeks.
- 10.2.3.4 Quality of cellular responses (pending external funding): Frequency of unique anti-CMV T cell receptors at Day 0 and at Weeks 12 and 48.
- 10.2.3.5 HIV Reservoir (pending external funding) Change from Day 0 to Weeks 12, 24, 48, and 72 in the size and transcriptional activity of the HIV DNA reservoir.
- 10.2.3.6 Microbiome (pending external funding) Change from Day 0 to Weeks 12 and 48 in the composition of the microbiome and possible effect of the microbiome to the magnitude of the immune response.

10.3 Randomization and Stratification

After eligibility is confirmed, participants will be randomized at a 2:1 ratio (CMV-MVA Triplex: placebo) using permuted blocks without institutional balancing with stratification by sex and use of gender-affirming hormones. To ensure that at least 25% of the study population are individuals assigned female sex at birth not on testosterone or individuals assigned male sex at birth on feminizing hormones, accrual will be capped at 67 for participants who do not meet this criterion.

10.4 Sample Size and Accrual

It is anticipated to take up to 3 years to accrue 90 participants who receive study treatment. Participants not initiating study treatment will be replaced to reach the target

of 90 and will not be included in the analysis. Participants who received at least one dose will not be replaced. The sample size for A5355 was determined based on the primary endpoint of cellular immunogenicity.

10.4.1 Cellular Immunogenicity

The first co-primary objective is to determine if CMV-MVA Triplex influences CMV-specific immune responses. We hypothesize that CMV-MVA Triplex will significantly increase pp65-specific CD137⁺ CD8⁺ T cells compared to participants vaccinated with placebo. Published data from a Phase I vaccine trial [22] was used to estimate pp65-specific CD137⁺ CD8⁺ T cell distributions at Days 0 and 42. Using the minimum, median, and maximum values, and variance estimation methods [57], we assumed a standard deviation of **0.70** cells/ μ L at **Day 0** and **2.0** cells/ μ L at Day 42. For change in pp65-specific CD137⁺ CD8⁺ T cells we assumed a correlation between time points of 0.5 and thus a change standard deviation (SD) of 1.7 cells/ μ L. Because these SD estimates came from a small sample, our SD was further inflated [58] by 80% to 3.06 cells/ μ L.

Using a two-sample t-test, the change SD of 3.06 cells/ μ L and a two-sided 5% alpha, 72 participants (48 vaccine, 24 placebo) would provide 80% power to detect a 2.2 cells/ μ L difference between the arms. This effect size corresponds to the observed change over 42 days in the previously cited vaccine trial [22]. [Table 10.4.1-1](#) provides detectable effect size for various power and SD estimates.

Notably, the sample size calculation determination of using 2.2 cells/ μ L was based on the generation of a CMV-specific response in both CMV-immune and non-immune recipients, and thus was skewed toward a lower value than we expect to observe here. Since all our study participants will be CMV seropositive, we do expect a stronger immune response.

To account for 20% of participants not being included in the primary analysis due to missing samples, laboratory error, LTFU, not meeting per protocol definition, etc., the sample size was inflated to 90 participants (60 vaccine, 30 placebo).

Table 10.4.1-1: Effect Size Detectable with 72 Participants (48 CMV-MVA Triplex, 24 placebo) per Protocol Participants for Varying Power and SD

	Power		
	80%	85%	90%
SD=2.50	1.8	1.9	2.1
SD=2.75	2.0	2.1	2.3
SD=3.06	2.2	2.3	2.5
SD=3.25	2.3	2.5	2.7
SD=3.50	2.5	2.7	2.9

While this study is not fully powered to detect treatment effects within cisgender women or differential treatment effects between men and women, those analyses are of interest. It is also important to understand treatment effects among transgender and gender non-binary participants. Similar to above, we will not have sufficient power to detect treatment effects for each group, so we will conduct exploratory analyses between groups (cisgender men, transmen, cisgender women, transwomen, and gender non-binary).

10.4.2 Safety

The second co-primary objective is to assess the safety of CMV vaccination. With a study sample size of 90 participants and a 2:1 randomization to CMV-MVA Triplex: placebo injection ratio, 60 participants will receive vaccine and 30 will receive placebo. The 60 participants receiving CMV-MVA Triplex will provide at least a 90% probability of observing at least one participant with a Grade 3 or higher AE when the true event proportion is 3.8% or more in participants receiving CMV-MVA Triplex (in a previous Phase I study no vaccine-related SAEs were documented. Local and systemic reactogenicity were transient and self-limiting).

With 60 CMV-MVA Triplex and 30 placebo participants and assuming a Grade 3 or higher AE in 2% of the placebo participants, there will be 80% power to detect a difference between the arms if **23%** or more of the CMV-MVA Triplex participants have a Grade 3 or higher AE. If the proportion with an AE in the placebo arm is 5% there will be 80% power to detect a difference of **24%** (5% vs. **29%**; [Figure 10.4.2-1](#)). These calculations are based on a **two-sided 5% alpha Barnard's unconditional exact test**.

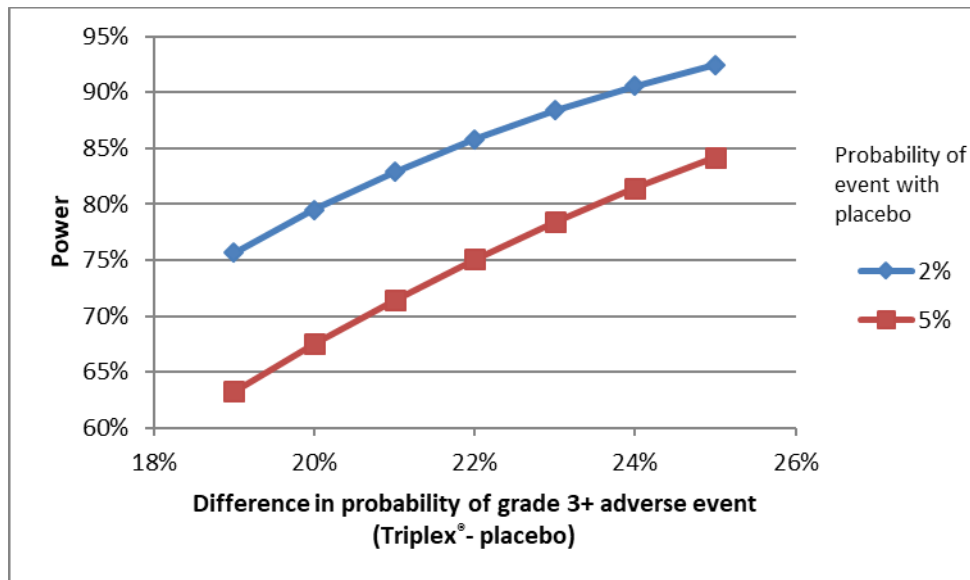


Figure 10.4.2-1: Power to detect a difference (vaccine – placebo) between study arms in the probability of Grade 3 or higher AEs.

10.4.3 Inflammation

The third co-primary objective is to determine if CMV-MVA Triplex influences inflammation. We hypothesize that it will significantly decrease sTNFRII when compared to placebo. Past ACTG trials have consistently shown an sTNFRII change SD between 0.15 and 0.20 \log_{10} pg/mL over treatment and follow-up periods of varying duration. Assuming an SD of 0.20 \log_{10} pg/mL, a Type I error of 5%, and an effective sample size of 72 participants (48 vaccine, 24 placebo), we will have 80% power to detect an effect size as small as 0.14 \log_{10} pg/mL. A reduction of 0.14 \log_{10} pg/mL corresponds to a 27% reduction in the geometric mean fold change compared to placebo. Per NWCS 329, each \log_{10} increase in sTNFRII levels was associated with a 13.6-fold increased odds of a non-AIDS event (using year 1 biomarker assessment), which corresponds to a 44% increased odds of a non-AIDS event for each 0.14 \log_{10} increase in sTNFRII.

These calculations are based on a two-sided two-sample t-test.

10.4.4 Secondary Outcome Measures

Secondary objectives are largely exploratory, without formal sample size calculations. However, for continuous secondary outcome measures an analysis sample size of 48 vaccine and 24 placebo participants will provide 80% power to detect a difference of 0.71 standard deviations (SD) between the arms at a single **on-study** time-point. As an example, for sCD163 change and assuming a SD of 0.21 \log_{10} ng/mL (observed in A5260s) we will have 80% power to detect a difference of 0.15 (0.21*0.71) \log_{10} ng/mL. A reduction of 0.15 \log_{10} ng/mL corresponds to a 29% reduction in geometric mean fold change. These

calculations are based on a two-sided 5% alpha two-sample t-test. The following table summarizes the power to detect various effect sizes as well as the precision of the effect size estimate.

Table 10.4.4-1: Effect Size, Power, and Precision of Cross-sectional Continuous Secondary Outcomes Based on the Outcome SD

Comparison of Two Arms (48 vs. 24 participants in analysis population)		
Effect Size	Power	Width of 95% CI
0.82*SD	90%	± 0.50 *SD
0.71*SD	80%	± 0.50 *SD
0.63*SD	70%	± 0.50 *SD

10.5 Data and Safety Monitoring

At SMC reviews, data will be considered as detailed in [section 7.4](#). Note that biomarker measurements will be run in batches after follow-up is concluded, and therefore these data are not expected to be available at interim reviews.

The protocol team will monitor AEs and virologic failures (**confirmed VL >200 copies/mL**) monthly. **If there are two or more study drug related Grade ≥ 3 AEs from distinct participants, or three or more virologic failures prior to the Week 48 visit, pooled across the treatment arms, an ad-hoc SMC review will be conducted.**

As soon as a trigger for an ad-hoc SMC review is met, study treatment administration (including first and second doses), screening, and accrual to the study will be paused. The SMC will review all accumulated safety data and a decision to either continue, modify, or stop the study will be made in consultation with the DAIDS representative and, if needed, the protocol team.

10.6 Analyses

All statistical tests will be two-sided with a nominal alpha level of 0.05. Because this is a phase II study and the biologic activities of the intervention are of interest, **efficacy analyses will use a per-protocol population defined as** participants who 1) received the Day 0 CMV-MVA Triplex/placebo injection, 2) have not used prohibited medications from Day 0 until the Week 48 visit, and 3) do not have a confirmed virologic failure, defined as the occurrence of two sequential plasma HIV-1 RNA values ≥ 200 copies/mL, unless otherwise noted. **Safety analyses will use a modified intent-to-treat population (mITT) consisting of all participants who have been exposed to CMV-MVA Triplex or placebo.**

10.6.1 Primary Analyses

The first primary efficacy analysis of the study will assess the effect of CMV-MVA

Triplex on pp65-specific CD137+ CD8+ T cells in participants with both HIV and CMV are well-controlled on ART. To address this, changes in pp65-specific CD137+ CD8+ T cells from Day 0 to Week 12 will be compared between the CMV-MVA Triplex arm and the placebo arm by linear regression. For this model each participant will have a single outcome measure of pp65-specific CD137+ CD8+ T cell change from Day 0 to Week 12. The predictor variables will be study arm, sex and use of gender-affirming hormones (the stratification factor).

Three supplemental linear regression analyses will be performed. The first will additionally adjust for Day 0 pp65-specific CD137+ CD8+ T cell values (continuous), while the second will assess differential CMV-MVA Triplex effects by Day 0 pp65-specific CD137+ CD8+ T cell tertile by additionally adjusting for the pp65-specific CD137+ CD8+ T cell tertile main effect and the study arm by pp65-specific CD137+ CD8+ T cell tertile interaction. The third will perform the primary analysis using **the** modified intent-to-treat (mITT) population.

For the primary safety analysis, Grade 3 or greater AEs will be summarized by treatment arm **in the mITT population. The resulting difference in proportions and associated exact 95% CI will be provided.**

For the other primary efficacy analysis, which will assess the effect of CMV-MVA Triplex on sTNFRII change from Day 0 to Week 48, the same analysis approach will be used as with pp65-specific CD137+ CD8+ T cells above, including the three supplemental analyses.

10.6.2 Secondary Analyses

Similar to the initial primary analysis regression model, changes in other continuous outcome measures will be compared between the CMV-MVA Triplex and placebo arms by linear regression. For these models each participant will have a single outcome measure of change from Day 0 to Week 12. The predictor variables will be study arm, sex and gender-affirming hormones.

Additionally, for all continuous secondary outcomes and the primary outcome (pp65-specific CD137+ CD8+ T cells), GEE models with an identity link will use change from Day 0 to all follow-up time points. These models will use appropriate correlation structures and splines if necessary.

For the CMV DNA shedding (yes/no) and Ki67 expression (<7%/≥7%) outcomes, GEE models with a logit link will examine Week 12, 48 and 72 data. Again, these models will use appropriate correlation structures and splines if necessary. The secondary safety analysis of Grade 3 or greater AEs over 96 weeks will be summarized by treatment arm.

10.7 Unblinding

10.7.1 Planned Unblinding

Participants will be unblinded at completion of the study. Please refer to ACTG SOP-123 Unblinding Participants for details.

10.7.2 Sudden/Unplanned Unblinding

The decision to unblind one or more arms of an ongoing study is made by the team in conjunction with the relevant Scientific Committee and the Executive Committee. This can occur based on a recommendation from an SMC or the results of another trial (also see the DAIDS SOP “Termination of a Trial or a Single Treatment Arm”).

If the decision is made to unblind, participants should be unblinded as soon as possible. Unblinding is conducted through the DMC, which sends treatment assignments to the sites soon after the unblinding decision. Every effort should be made by the sites to contact participants who have completed follow-up in order to explain the study results.

When a treatment comparison is unblinded based on an interim analysis, the results of that interim analysis must be reported in publications. Data from visits that occurred before the interim review but that were not in the database at the data cutoff date have little potential for bias and may be reported with a comment. Data from visits that occurred after unblinding are potentially biased and must not be used if the intent is to claim that all the data are from a blinded study. In unblinding due to both “interim analysis” and the “other trial results” situations, if analyses are reported on clinical data or samples taken after the unblinding date, the conditions under which these data were gathered must be made clear in any publication.

11.0 PHARMACOLOGY PLAN

Not applicable.

12.0 DATA COLLECTION AND MONITORING

12.1 Records to Be Kept

Electronic case report form (eCRF) screens will be made available to sites for data entry. Participants must not be identified by name on any data submitted to the DMC. Participants will be identified by the patient identification number (PID) and study identification number (SID) provided by the ACTG DMC upon randomization.

12.2 Role of Data Management

12.2.1 Instructions concerning entering study data on eCRFs will be provided by the ACTG DMC. Each CRS is responsible for keying the data in a timely fashion.

12.2.2 It is the responsibility of the ACTG DMC to assure the quality of computerized data for each ACTG study. This role extends from protocol development to generation of the final study databases.

12.3 Clinical Site Monitoring and Record Availability

Monitoring visits may be conducted on site or remotely. Remote visits may include remote source document verification using methods specified for this purpose by NIAID. Remote monitoring visits may be performed in place of or in addition to onsite visits to ensure the safety of study participants and data integrity. The site **must** make available study documents for site monitors to review utilizing a secure platform that is HIPAA (i.e., the Health Insurance Portability and Accountability Act of 1996) and 21 CFR (Code of Federal Regulations) Part 11 compliant [59]. **The DMC will configure Medidata Remote Source Review (RSR) and make it available to all sites. Sites are encouraged to use the DMC provided Medidata RSR platform but other potential platform options include Veeva SiteVault, site-controlled SharePoint or cloud-based portal, direct access to Electronic Medical Record (EMR). Other secure platforms that are compliant with 21 CFR Part 11 may be used, as allowed by the DAIDS Office of Clinical Site Oversight (OCSO).**

13.0 PARTICIPANTS

13.1 Institutional Review Board (IRB) Review and Informed Consent

This protocol and the [informed consent document](#) and any subsequent modifications will be reviewed and approved by the IRB responsible for oversight of the study. A signed consent form will be obtained from the participant. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the participant and this fact will be documented in the participant's record.

13.2 Participant Confidentiality

All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified by coded number only to maintain participant confidentiality. All records will be kept locked. All computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by the ACTG, IRB/EC, FDA, NIAID, OHRP, other local, US, and international regulatory entities as part of their duties, or the industry supporter or designee.

13.3 Study Discontinuation

The study may be discontinued at any time by the ACTG, IRB/EC, FDA, NIAID, OHRP, or other government agencies as part of their duties to ensure that research participants are protected.

14.0 PUBLICATION OF RESEARCH FINDINGS

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

15.0 BIOHAZARD CONTAINMENT

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, blood products, genital secretions, and blood-contaminated oral secretions, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the CDC and the National Institutes of Health.

All dangerous goods and materials, including diagnostic specimens and infectious substances, must be transported using packaging mandated by CFR 42 Part 72. Please refer to instructions detailed in the International Air Transport Association (IATA) Dangerous Goods Regulations.

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INFORMED CONSENT FORM

Sponsor / Study Title: National Institute of Allergy and Infectious Diseases / "Phase II, Double-Blind, Randomized, Placebo-Controlled Trial to Evaluate the Safety and Immunogenicity of a Modified Vaccinia Ankara (MVA)-based anti-Cytomegalovirus (CMV) Vaccine (Triplex), in Adults with Both Human Immunodeficiency Virus (HIV)-1 and CMV Who Are on Potent Combination ART with Conserved Immune Function"

Protocol Number: A5355

Principal Investigator:
(Study Doctor) «PiFullName»

Telephone: «lcfPhoneNumber»

Address: «PiLocations»

SUMMARY

PURPOSE

This is a research study and your participation in this study is voluntary. The purpose of this study is to see if an investigational vaccine for cytomegalovirus (CMV), called Triplex, is safe when given to people with both Human Immunodeficiency Virus (HIV) and CMV. Triplex is not approved by the United States Food and Drug Administration (FDA) for the treatment of CMV.

NUMBER OF PARTICIPANTS

There will be 90 participants. Sixty (60) people will receive the active study vaccine and 30 people will receive a placebo injection. A placebo looks like the CMV study vaccine, but it does not have any active drug in it.

STUDY TREATMENT

In this study, you will be randomized to one of the study treatment arms. You will receive either Triplex study vaccine or placebo by injection into the muscle of your shoulder 2 times; once **at the beginning of** the study and again about 4 weeks later. You will have double the chance of receiving Triplex versus the placebo. You must continue to take your anti-HIV drugs throughout the study.

LENGTH OF STUDY

The study will last about 96 weeks (about 2 years).

REQUIRED ACTIVITIES

Blood and urine collections

- At all clinic visits, some blood will be collected from a vein in your arm.

- At most clinic visits, you will be asked to provide a urine sample.

Special procedures

- **You will receive study vaccine or placebo as an injection in the muscle of your upper arm.**
- **2-3 days** after each study injection study staff will contact you to check how you are doing.
- For 4 weeks following each study injection you will complete a daily study diary (which is also known as a study vaccination report card); you will need to take and record your body temperature for the first 5 days each time.
- At several of the clinic visits, saliva, rectal swabs, and/or genital fluid (semen or vaginal swab) will be collected. **You will be able to perform some of these collections yourself.**

RISKS

The following are possible:

- Triplex study vaccine side effects (common)
 - Pain, swelling, redness and itching at the injection site
 - Muscular aches
 - Chills
 - Headache
 - Tiredness
- Triplex study vaccine side effects (less common)
 - Cough
 - Nausea
 - **Vomiting**
- Risks associated with injections and blood draws
 - Pain and bruising at the site of injection or blood draw
 - Infection
 - Hypotension (lowering of blood pressure)
- Other blood draw risks
 - Anemia (low red blood cell count)
 - Feeling lightheaded or fainting
- Mild discomfort during rectal or vaginal swab collection.

BENEFITS

No direct health benefits should be expected from participating in this study.

OTHER CHOICES

Instead of being in this study, you have the option of continuing with your current treatment or starting a new treatment under the care of your regular doctor or other health care provider.

Regardless of whether you receive any study- or non-study-treatment or vaccines for CMV, you should continue your current treatment for HIV (or discuss possible alternative treatments for HIV with your treating physician).

INTRODUCTION

You are being asked to take part in this research study because you have both HIV and CMV and because you have been taking anti-HIV drugs that have controlled the amount of HIV in your blood. Your CMV infection was determined by a previous blood test that shows you were exposed to CMV, as most people have been, and it remains in your body indefinitely although it usually doesn't cause specific CMV infectious complications. This study is sponsored by the National Institutes of Health (NIH). The study doctor in charge of this study at this site is listed on the first page of this consent form. Before you decide if you want to be a part of this study, we want you to know about the study and understand what the study involves.

This is a consent form. It gives you information about this study. The study staff will talk with you about this information. You are free to ask questions about this study at any time. If you agree to take part in this study, you will be asked to sign and date this consent form. You will get a copy to keep. After you join the study, you may decide to stop taking part in the study at any time.

WHY IS THIS STUDY BEING DONE?

The purpose of this study is to see if an investigational vaccine for CMV (called Triplex) is safe when given to people with both HIV and CMV. This study will also collect information on the effectiveness of Triplex to reduce inflammation and immune activation markers compared to a placebo. This will be the first time that this type of information will be collected. You should be aware that the current standard of care for individuals with both HIV and CMV includes effective treatment for HIV but does not include treatment of CMV with either medication or vaccination – unless there is evidence that the CMV is causing or contributing to illness.

WHAT DO I HAVE TO DO IF I AM IN THIS STUDY?

Information Collected at Screening

If you decide to take part in this study, you will have a screening visit to determine if you qualify for the study. There is some information that we collect on everyone who is screened for an AIDS Clinical Trial Group (ACTG) study. As part of your screening visit, some demographic (for example, age, gender, sex, race), clinical (for example, disease condition, diagnosis), and laboratory (for example, Cluster of Differentiation [CD4+] cell count, viral load) information will be collected from you. We also collect information on whether you use (or have used) illicit intravenous (IV) drugs. All of this information is collected and stored in a highly confidential manner.

We will collect this information even if you do not enroll in this study. This information is collected so that ACTG researchers may determine whether there are patterns and/or common reasons why people do not join a study.

Tests you will have at screening are described below (see Appendix I). This visit could take between 60 and 90 minutes.

Randomization, Day 0 and Week 4 Visits

If you qualify for the study, you will be randomized to receive a study injection of either the study vaccine or placebo. **You will receive your randomized treatment on Day 0 and again at the week 4 visit.**

“Randomized” means that the **study treatment that you receive will be determined at random, like rolling dice.** In this study, you will have double the chance of receiving the study vaccine versus the placebo. You and the study staff will not know what study treatment you receive. **It is possible that the staff will conduct the randomization remotely – meaning that you would not need to come to the clinic for this “visit.”**

The visits at which you receive a study injection could take between 60 and 90 minutes. At each of these visits, you will be observed for side effects for 30 minutes after the study injection. Before you leave the clinic on each of these days, you will be given a study diary to complete before the next study visit.

If you have a fever (your temperature is above 100.4°F) at either of these visits, the study injection will be delayed until your fever is gone for at least 24 hours.

The assessments you will have during the study entry and week 4 visit are described below (see Appendix I).

About 2 or 3 days after each study injection, someone from the study site will contact you **(probably by phone) to ask how you are feeling. If you have had any serious side effects, you could be asked to seek care right away or might be asked to come to the clinic for an extra visit.** After each study injection, you will record information about how you are feeling in the study diary you were given at the study injection visit. You will bring your completed diary to the visit 4 weeks after each study injection visit.

Follow-up Visits

You will have follow-up visits in person at weeks 8, 12, 24, 48, and 72. Except for week 12, any of these visits could be conducted remotely (for example by telephone or telehealth). There will be a final by phone call 92 weeks after the second study vaccine injection. The schedule of visits and study procedures are explained below (see Appendix I). These visits could take between 30 and 60 minutes.

Throughout the study, blood or urine samples will be tested for chlamydia, gonorrhea, and syphilis, which are three different sexually transmitted infections/diseases. If the tests show that you have one of these sexually transmitted infections/diseases, the study staff will refer you **for treatment. The study will not pay for this treatment.** Study staff may be required to give public health department the names, contact information and treatment records of participants who have a positive test result for chlamydia, gonorrhea or syphilis.

Receipt of Other Vaccines while on Study

You may not receive other non-study vaccines, including approved or experimental COVID-19 vaccines, until at least 4 weeks after your last dose of study **injection. Participants are encouraged to receive non-study vaccines (for example, flu shots or COVID vaccines) before enrolling in the study.**

Other Considerations

As mentioned above, there may be times when a study visit cannot be conducted in person, including when someone with whom you have close contact is ill. This is explained more fully in Appendix I, under the heading Remote Data Collection.

If you or your study doctor feel you may have COVID-19, arrangements will be made for you to be tested. You will not receive a study **injection** until 14 days after your symptoms of COVID-19 have stopped. **If your symptoms last more than 4 weeks and you are not able to receive your second study injection, you will not be able to continue in the study.**

WILL I RECEIVE THE RESULTS OF ANY TESTS?

You will be told the results of all tests with the exception of those tests to look at the levels of study treatment in your body and that are for future ACTG-approved testing.

CAN I CHOOSE THE TYPES OF RESEARCH THAT MY SAMPLES AND INFORMATION ARE USED FOR?

Some of your blood, urine, genital and oral secretions will be stored and used for study-required metabolic, immunologic, and virologic testing.

Your samples **will not be labeled with** any private information that has been collected about you. This means that no one looking at the labels or at other information will be able to know that the samples or information came from you.

The tests described above are required by this study. If you do not agree to the storage or testing that has been described above, you should not join this study.

When samples are no longer needed for this study, the ACTG may want to use them in other studies and share them with other researchers. These samples are called “extra samples.” The ACTG will only allow your extra samples to be used in other studies if you agree to this. If you have any questions, please ask.

Extra samples are stored in a secure central place called a repository. Your samples will be stored in the ACTG repository located in the United States.

There is no limit on how long your extra samples will be stored.

If researchers want to use your samples or information, their research plan must be approved by the ACTG. Researchers’ institutional review board (IRB) will also review their plan. IRBs protect the rights and well-being of participants in research. ACTG will send samples to researchers only if their plans are approved. Researchers who are not part of the study team may use your samples without your being asked again for your consent.

You will not be paid for your samples. Although a researcher may make a new scientific discovery or product based on the use of your samples, there is no plan to share any money with you.

You may withdraw your consent for research on your extra samples at any time, and the specimens will be discarded.

Please choose the response that matches what you want by putting your initials in the space provided. Please ask the study staff any questions that you have before you indicate your selection.

Research without Human Genetic Testing

If you agree, your extra samples may be stored (with usual protection of your identity) and used for ACTG-approved HIV-related research that does not include human genetic testing.

____ (initials) I understand and I agree to this storage and possible use of my samples

OR

____ (initials) I understand but I do not agree to this storage and possible use of my samples

Research with Human Genetic Testing

The ACTG has a study that collects samples for human genetic testing from ACTG study participants in the United States, called A5128, Plan for Obtaining Informed Consent to Use Stored Human Biological Materials (HBM) for Currently Unspecified Analyses.

Your site might ask you if you would like to participate in A5128. If you would like to participate, you will sign and date a separate consent form. Your extra samples will not be used for human genetic testing **unless you are part of A5128**.

HOW MANY PARTICIPANTS WILL TAKE PART IN THIS STUDY?

About 90 people will take part in this study

HOW LONG WILL I BE IN THIS STUDY?

You will be in this study for about 96 weeks (which is about 2 years).

WHY WOULD THE STUDY DOCTOR TAKE ME OFF THIS STUDY EARLY?

The study doctor may need to take you off the study early without your permission if:

- The study is stopped or cancelled
- You miss the first dose of study **agent**
- You miss two visits in a row
 - Note that this criterion may not be enforced if you are able to **participate in** visits remotely.
- Your doctor thinks the study is no longer in your best interest

If you must stop the study early, the study doctor may ask you to return for some study visits and procedures before you leave the study permanently.

The study doctor may also decide not to give you the second study vaccine/week 4 injection without your permission if:

- You have a serious side effect **after** the first study injection
- You need a treatment that you may not take while on the study

- You are pregnant or breast-feeding
- You are not able to receive the second study injection within 4 weeks of the scheduled visit.
 - Note that this criterion may not be enforced if the study team approves a longer time between study injections in special circumstances (for example, if it is unsafe for you to travel to the clinic or the clinic is not able to conduct non-essential visits)

Even if you do not have the second study injection, the study doctor may ask you to continue to be part of the study.

WHAT ARE THE RISKS OF THE STUDY?

The study vaccine used in this study may have side effects, some of which are listed below. In a research study, all of the risks or side effects may not be known before you start the study. You need to tell your study doctor or a member of the study team immediately if you experience any side effects.

Please note that these lists do not include all the side effects seen with this study vaccine. These lists include the more serious or common side effects with a known or possible relationship. If you have questions concerning the additional side effects, please ask the study staff at your site.

There is a risk of serious and/or life-threatening side effects when non-study medications are taken with the study vaccine. Since the effect of the study vaccine taken with other medications may not be known, it is important for your safety, that you tell the study staff about all prescription and non-prescription drugs, herbal preparations, and nutritional supplements that you are taking or planning to take. Also, you must tell the study staff before enrolling in any other clinical trials while on this study.

The study vaccine has been administered to 75 adults who were not living with HIV; these participants reported minimal discomfort.

Commonly reported side effects of the study vaccine, occurring in about one of every three participants, were:

- Pain, swelling, redness, and itching at the injection site
- Muscular aches
- Chills
- Headache
- Tiredness

Less common side effects of the study vaccine, occurring in less than one in ten participants were:

- Cough
- Nausea
- **Vomiting**

These side effects do not generally last more than a few days and usually do not require treatment but you may be given other non-prescription pain medications (similar to aspirin) to help relieve the symptoms.

As with all vaccines or drugs, you could have an allergic reaction such as a rash or hives. Allergic reactions can be dangerous; therefore, the study staff will observe you for 30 minutes after each study injection. None of the participants who received the study vaccine had an allergic reaction to the study vaccine. If you develop an allergic reaction, you will be given medication (Benadryl-like) to counter the reaction.

Other risks that would not be related to this study vaccine but that could occur due to the study injection process include:

- Developing a bruise at the site of injection
- Infection
- Hypotension (lowering of blood pressure)

These side effects have been seen with other vaccines and could occur when you receive the study vaccine, but they were not observed in the participants vaccinated with **the** study vaccine.

Receiving **the** study vaccine may mean that you will not be eligible to receive other investigational CMV vaccines at a later date. There is no approved CMV vaccine currently available.

Risks Associated With Collection of Genital Fluid (Semen or Vaginal Swabs)

- There are no known physical risks associated with semen collection by masturbation.
- Mild discomfort related to the insertion of the vaginal swab **for collection of vaginal fluid** may occur.

Risks of Rectal Swab

You may have mild discomfort when the swab is performed, particularly if you are already suffering from sores or hemorrhoids. If you are already having pain in the rectal area, be sure to let the study team know.

Risks Associated with Blood Draw

The needle used to draw blood from a vein may cause pain and bruising, and rarely, infection at the site of the blood draw. There is also a risk of anemia (low red blood cell count) or hypotension (low blood pressure). Sometimes, having blood drawn will cause people to feel lightheaded or even to faint.

Risks of Social Harm

Although the study site will make every effort to protect your privacy and confidentiality, it is possible that your involvement in the study as a participant could become known to others, if it is not already, and that social harm may result (because you could become labeled as someone with HIV). For example, you could be treated unfairly or discriminated against by family members, friends, and/or the community.

ARE THERE RISKS RELATED TO PREGNANCY?

It is not known whether this study vaccine might hurt an unborn child. You should not become pregnant or impregnate a partner from 14 days before **randomization** until at least 60 days after the second study injection (until week 12).

While participating in this research study, you should not nurse (breastfeed) a baby. You may be provided counseling about preventing pregnancy. Let the study staff know immediately if you become pregnant. At the screening and **randomization** visits, if you are pregnant or if you are nursing a baby and do not want to stop, you cannot take part in this study.

If you are an individual who can become pregnant, a urine or blood pregnancy test will be obtained before you receive the study vaccine and throughout your participation in the study. If you can become pregnant and are participating in sexual activity that could lead to pregnancy, you must use a medically effective form of birth control until at least 60 days after the second study injection (until week 12).

Acceptable medically effective forms of birth control are:

- **Abstinence (in other words, not participating in sex that could lead to pregnancy)**
- Double-barrier methods (in other words, condoms, diaphragm, **or** cervical cap used **in combination** with spermicidal gel or foam)
- Intrauterine device (IUD) (in other words Progestin/hormonal or Copper IUD)
- Hormonal contraceptives (in other words, birth control patches, implants, pills, rings, or injections)

If you are an individual who can become pregnant and have not had your menstrual cycle between 12 and 24 consecutive months and have not had a surgical procedure to prevent pregnancy, you will have blood collected to check your level of follicle-stimulating hormone (FSH: a hormone associated with reproduction). This will help in determining whether you are post-menopausal.

If you become pregnant before week 4, you will not receive the **study** injection scheduled for week 4. You will be encouraged to continue on study to complete **most** study evaluations.

The study staff would like to obtain information about the outcome of the pregnancy. If you are taking anti-HIV drugs when you become pregnant, your pregnancy will be reported to an international database that collects information about pregnancies in individuals taking anti-HIV drugs. This report will not use your name or other information that could be used to identify you.

ARE THERE BENEFITS TO TAKING PART IN THIS STUDY?

You should not expect any direct health benefits from participating in this study. Information learned from this study may help others who have HIV and CMV.

WHAT OTHER CHOICES DO I HAVE BESIDES THIS STUDY?

Instead of being in this study you have the choice of:

- Treatment with prescription drugs for CMV available to you

- Treatment with experimental drugs for CMV if you qualify
- No treatment for CMV

Please talk to your study doctor about these and other choices available to you. Your study doctor will explain the risks and benefits of these choices.

WHAT ABOUT CONFIDENTIALITY?

We will do everything we can to protect your privacy. In addition to the efforts of the study staff to help keep your personal information private, we have gotten a Certificate of Confidentiality from the US Federal Government. This certificate means that researchers cannot be forced to tell people who are not connected with this study, such as the court system, about your participation. Also, any publication of this study will not use your name or identify you personally.

Your records may be reviewed by the US Food and Drug Administration (FDA), the ACTG, the US Office for Human Research Protections (OHRP), or other local, US, and international regulatory entities as part of their duties, Advarra Institutional Review Board (IRB) (a committee that protects the rights and safety of participants in research), National Institutes of Health (NIH), study staff, study monitors, the drug company supporting this study, and its designees. A Certificate of Confidentiality does not prevent you from releasing information about yourself and your participation in the study.

Even with the Certificate of Confidentiality, if the study staff learns of possible child abuse and/or neglect or a risk of harm to yourself or others, we will be required to tell the proper authorities.

All information collected about you as part of the study will be sent securely to the ACTG data management center for combining with information from other study participants and for statistical analyses of study results. Your name and other personal identifiers will not be sent. Your research site is responsible for sending your information in accordance with the US and local laws, regulations, and policies.

A description of this clinical trial will be available on <http://www.ClinicalTrials.gov>, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

WHAT ARE THE COSTS TO ME?

The study will pay for research-related tests and assessments. Taking part in this study may lead to added costs to you and your insurance company. In some cases, it is possible that your insurance company will not pay for these costs because you are taking part in a research study.

Anti-HIV drugs and birth control will not be provided by the study.

WILL I RECEIVE ANY PAYMENT?

«Compensation»

You will be paid up to a total of \$xx.xx if you complete this study. You will be paid for the visits you complete according to the following schedule:

- \$xx.xx for Visits xxx.
- \$xx.xx for Visits xxx.
- \$xx.xx for Visits xxx.

If you do not complete the study, for any reason, you will be paid for each study visit you do complete.

You will be paid _____ [*“after each visit,” “annually,” “bi-weekly,” etc.*]

If you have any questions regarding your compensation for participation, please contact the study staff.

[OR]

You will not receive any monetary compensation for your participation in this study.

WHAT HAPPENS IF I AM INJURED?

If you are injured as a result of being in this study, you will be given immediate treatment for your injuries. The cost for this treatment will be charged to you or your insurance company. There is no program for compensation either through this institution or the National Institutes of Health. You will not be giving up any of your legal rights by signing and dating this consent form.

WHAT ARE MY RIGHTS AS A RESEARCH PARTICIPANT?

Taking part in this study is completely voluntary. You may choose not to take part in this study or leave this study at any time. Your decision will not have any impact on your participation in other studies conducted by NIH and will not result in any penalty or loss of benefits to which you are otherwise entitled.

We will tell you about new information from this or other studies that may affect your health, welfare, or willingness to stay in this study. If you want the results of the study, let the study staff know.

WHOM TO CONTACT ABOUT THIS STUDY

During the study, if you experience any medical problems, suffer a research-related injury, or have questions, concerns or complaints about the study, please contact the study doctor at the telephone number listed on the first page of this consent document. If you seek emergency care, or hospitalization is required, alert the treating physician that you are participating in this research study.

An institutional review board (IRB) is an independent committee established to help protect the rights of research participants. If you have any questions about your rights as a research participant, and/or concerns or complaints regarding this research study, contact:

- By mail:
Study Subject Adviser
Advarra IRB
6100 Merriweather Dr., Suite 600
Columbia, MD 21044
- or call toll free: 877-992-4724
- or by email: adviser@advarra.com

Please reference the following number when contacting the Study Subject Adviser:
Pro00053633.

SIGNATURE PAGE

If you have read this consent form, all your questions have been answered, and you agree to take part in this study, please sign and date your name below.

Participant's Name (print)

Participant's Signature and Date

Study Staff Conducting
Consent Discussion (print)

Study Staff's Signature and Date

APPENDIX I: A5355 STUDY VISITS

The study staff can answer any questions you have about individual study visits, how long they will last, or about the tests that will occur. The table below can be used as a quick reference for you, along with the explanations that follow.

I. Study Schedule

[illegible]

Evaluation or Procedure	Screening	Randomization	Day 0	2-3 Days After 1 st Study Injection	Week 4	2-3 Days After 2 nd Study Injection	Week 8	Week 12	Week 24	Week 48	Week 72	Week 96	Confirm Virologic Failure	Early Study Discontinuation
injection and post-injection observation														
Substance use questionnaire			✓		✓		✓	✓	✓	✓	✓			✓
Study diary distribution			✓		✓									
Study diary return and review					✓		✓							✓
Telephone contact	Possible			✓		✓						✓		
Remote data collection		Possible					Possible		Possible					

II. Explanation of **Visit Timing** (column headings)

Screening: After you have read, signed, and dated the consent form, you will have the evaluations listed in this column to make sure that you meet the requirements for joining the study.

Randomization: This is the day that you enter the study (**even if you do not come to the clinic**).

Day 0: **This is the day that you** receive your first study injection of study treatment. Other study visits are counted from this day. In other words, the study visit listed as “Week 4” will be scheduled approximately 4 weeks after your **Day 0** visit.

2-3 Days after 1st and 2nd Study Injections: **Each of these** “visits” will actually take place as a telephone call between you and the study staff.

Week 4: **This visit will occur about 4 weeks after you receive the first vaccine. This visit, and your second vaccine dose, may be delayed for a few reasons, like you have fever or are unable to attend the visit in person.**

Week 8 through Week 96: These visits are known as “follow-up” visits. The week 96 “visit” will be conducted as a telephone call between you and the study staff.

Confirmation of Virologic Failure: **If this visit is needed, it will occur no more than 14 days after the first test that showed an increase in the level of HIV in your blood.**

Early Study Discontinuation: **If you have to leave the study early, this visit will be done before you are taken off the study.**

III. Explanation of Evaluations or Procedures

Below are descriptions of the **study** evaluations and procedures that are listed in the rows in this column. You will be told the results of all tests with the exception of those tests to look at the levels of study treatment in your body and that are for future ACTG-approved testing.

Consent and contact information collected

After you read the consent and have had a chance to ask questions about the study, you will sign and date the consent form if you want to continue and join the study. The study staff will ask how to contact you for the telephone “visits.”

HIV-1 status checked

If there is no record available, an HIV-1 test will be done. If an HIV-1 test has to be done, you may have to sign and date a separate consent form before this is done. You will be told the result of the HIV-1 test as soon as it is available. The study doctor may be required by law to report the result of these tests to the local health authority.

Physical examination

You will have a physical exam and will be asked questions about your health and about any medicines you have taken or are taking now. You will have your weight measured. At the screening visit, your height will be measured and you will also be asked about the lowest point to which your CD4+ count has ever dropped.

Pregnancy test

If you are able to become pregnant, you will be asked to give a small urine or blood sample (about 5 mL or 1 teaspoon) for a pregnancy test. **A blood sample may also be taken to check the level of follicle-stimulating hormone (FSH) in your blood. This hormone helps maintain the menstrual cycles and is associated with the development of eggs in people who can become pregnant.** You may not enter the study if you are pregnant.

Blood collected

Because results of some of the test results can vary depending on the time of day a sample is taken, blood should be collected around the same time of day throughout the study, if possible. We will try to find a consistent, mutually agreed upon time for your visits. Blood will be collected from you for various tests during the study.

Blood tests include:

- *Hematology, blood sugar, chemistry, liver function tests, coagulation panel, creatinine clearance*
These are routine blood tests for safety. You will have blood collected for some or all of these routine tests at screening, **Day 0**, and at weeks 4, 12, and 48.
- *Hepatitis B and C*
These tests show if you have the hepatitis B and hepatitis C viruses (viruses that can hurt your liver). You will have blood collected for these tests at screening.
- *CMV Serology*
This test shows if you have CMV. You will have blood collected for this test at screening unless you can provide documentation of a prior test.
- *Syphilis Serology*
This test shows if you have syphilis. You will have blood collected for this test at screening, **Day 0**, and at weeks 12 and 48, and if you discontinue the study early.
- *Plasma for study vaccine levels*

This test looks for the presence of the study vaccine in your blood. You will have blood collected for this test at **Day 0**, at weeks 12, 48, and 72, and if you discontinue the study early.

- *CD4+ cell count*
This is a test that shows how many infection-fighting cells you have in your blood. You will have blood collected for this test at screening, **Day 0**, at weeks 4, 12, 48, and 72, at the visit to confirm suspected virologic failure, if needed, and if you discontinue the study early.
- *HIV-1 viral load*
This is a test that shows how much HIV is in your blood. You will have blood collected for this test at screening, every clinic visit from **Day 0** through week 72, at the visit to confirm suspected virologic failure, if needed, and if you discontinue the study early.
- *Stored blood*
Tests on your stored blood will include looking for markers of inflammation and other immune cell responses, and for other future studies. You will have blood collected for storage at **Day 0**, at weeks 4, 8, 12, 24, 48, and 72, and if you discontinue the study early. “Inflammation” refers to your body’s normal response to injury or infection. Sometimes, inflammation can last longer than is helpful and this can lead to other problems.

Urine collected

You will be asked to provide a sample of urine to test for gonorrhea and chlamydia at screening, entry, at weeks 12 and 48, and if you discontinue the study early. You will also be asked to provide urine that will be stored for future use at entry, at weeks 4, 12, 48, and 72, and if you discontinue the study early.

Rectal swab performed (self-collection possible)

Because we are interested in the possible effect of the study vaccine on the microbiome in your gut or gastrointestinal tract, we will be obtaining a rectal swab at **several visits**. “Microbiome” refers to a group of living organisms that are too small to see without a microscope and includes the many bacteria that normally live in our gastrointestinal (GI) tracts.

Mouth rinse collected

You will be asked to rinse your mouth with a special fluid. The fluid will then be collected and stored for future studies to look at the presence of CMV DNA. The collections will take place at entry, at weeks 4, 12, 48, and 72, and if you discontinue the study early.

“DNA” is a molecule that transfers genetic characteristics.

Genital fluid collected

You will be asked to provide a sample **of genital fluid** for future use at **several visits**. Please note that you will be asked to refrain from sexual activity for 48 hours prior to this sample collection.

If you are able to provide a semen sample, you will be asked to provide a self-collected sample by masturbation within 2 hours prior to the study visits. Semen collection can be performed elsewhere if your sample can be transported to the processing laboratory immediately at room temperature. **Study staff will provide information about the timing of the collection.**

If you have a vagina, you will be asked to avoid inserting anything into your vagina 48 hours prior to sample collection. During the study visit, you will be asked to provide vaginal swabs; you may choose to self-collect your own vaginal swab (by placing a cotton swab a short distance into the vaginal opening) or have study personnel obtain the sample from you.

Genital secretions will be tested for the presence of CMV DNA.

Anti-HIV drugs adherence questions

You will be asked questions about how well you remember to take your anti-HIV drugs at all clinic visits from entry through week 72, at the visit to confirm suspected virologic failure, if needed, and if you discontinue the study early.

Study Vaccine/placebo injection

You will receive the CMV study vaccine or placebo injected into the muscle of your shoulder **and will remain at the clinic for 30 minutes after each injection for observation.**

Substance use questionnaire

Because use of certain drugs can increase or decrease inflammation, you will complete a brief self-report drug use questionnaire at entry, at weeks 4, 8, 12, 24, 48, and 72, and if you discontinue the study early.

Study diary

You will be given a study diary **on each of the two days that you receive a study injection. During the 4 weeks until the next study visit, you will be expected** to record:

- Your temperature for 5 days after each study injection. You will be given a thermometer by the site study staff and will be given instructions about how to use it.
- Any side effects for 5 days after each study injection.
- Any rash or skin irritation.
- Any medications taken.
- Any non-study vaccines received.

Each day, it should take you about 10-15 minutes to complete the diary. You will bring the diary back to the clinic 4 weeks after each study injection visit or if you leave the study **early**.

Telephone contact

The study staff may contact you between screening and Day 0 to check that you are still eligible for the study and that you are still interested in participating.

The study staff will contact you via phone **2-3 days** after **each study injection visit to check on** how you are feeling.

Your week 96 visit will also be conducted over the phone.

The study staff will ask if there is another way to contact you (for example, e-mail or text or an in-person visit) in case contacting you by phone is not possible.

Remote Data Collection

Sometimes, the study staff may need to conduct a scheduled visit with you remotely (for example, by telephone, or via telehealth). This could happen for any of the reasons listed below:

- You are not able to attend a study visit in person (for example, because you are not feeling well or it is not safe for you to travel to the clinic, which may be because of illness in someone you are in close contact with)
- The site is temporarily unable to conduct non-essential visits in the clinic (for example, because of a problem at the facility or because of a public health emergency)
- At the discretion of the A5355 study team.

Regardless of the reason, the study staff will attempt to contact you and obtain as much of the required information from you as is possible. If the week 4 or week 12 visits cannot take place in person, these visits will be re-scheduled.