STUDY PROTOCOL

A Phase I Pilot Study to Evaluate the Safety and Immunogenicity of the HIV-1 Vaccines MVA.tHIVconsv3 (M3) and MVA.tHIVconsv4 (M4) Given Alone or In Combination in HIV-1-Infected Adults Suppressed on Antiretroviral Therapy - The M&M Study

NCT number NCT03844386
Document Date 02/19/2021

Letter of Amendment #1

IGHID 11810 - A Phase I Pilot Study to Evaluate the Safety and Immunogenicity of the HIV-1 Vaccines MVA.tHIVconsv3 (M3) and MVA.tHIVconsv4 (M4) Given Alone or In Combination in HIV-1Infected Adults Suppressed on Antiretroviral Therapy – The M&M Study

Protocol Version 3.0, dated 06 January 2021

IND Number: IND 18368

DAIDS-ES ID 38563

Date of Letter of Amendment (LOA) #1: February 19, 2021

Summary of Revisions and Rationale

We would like to allow participants from the UNC IGHID 11627 study (UNC IRB 17-0468 Protocol version 1.0 dated 06-Feb-2017 and Protocol version 2.0 dated 15-Aug-2017) who received HIV-1 Antigen Expanded Specific T Cell Therapy (HXTC) to participate in the M&M vaccine study, provided their last dose of HXTCs was greater than or equal to 12 months from M&M screening.

Preliminary analysis and data from participants who received the dose 2 x $10^7/m^2$ indicate lack of immunogenicity and therefore, we feel this therapy has not increased or changed the nature of the existing HIV specific T-cell responses.

Implementation

This Letter of Amendment does not change the informed consent form.

The plan is to incorporate the modifications included in this Letter of Amendment into the next full protocol amendment. Text noted below with a strikethrough represents deletion; text appearing below in **bold** represents an addition.

Protocol Changes

1. Section 5.2 Exclusion Criteria (page 44)

Item #11

Use of any prior HIV vaccine (prophylactic and/or therapeutic) or HIV immunotherapy.

Note: exceptions allowed for antibody therapies per PI review and approval.

Note: exceptions allowed for IGHID 11627 (UNC IRB 17-0468; NCT 03212989) per PI review and approval provided there is greater than 12 months since receipt of study-provided treatment.

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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 U.S. 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.

Protocol Principal Investigator: _____
Print/Type

Signed: _____ Date: _____

Title: _____

IGHID 11810 - A Phase I Pilot Study to Evaluate the Safety and Immunogenicity of the HIV-1 Vaccines MVA.tHIVconsv3 (M3) and MVA.tHIVconsv4 (M4) Given Alone or In Combination in HIV-1-Infected Adults Suppressed on Antiretroviral Therapy – The M&M Study

DAIDS ES-ID Protocol Number: 38563

National Clinical Trial (NCT) Number: NCT03844386

Grant Principal Investigator: Nilu Goonetilleke, PhD

Protocol Principal Investigator: Cynthia Gay, MD, MPH

IND Number: IND 18368

Funded by: National Institutes of Health (NIH)

Division of AIDS (DAIDS)

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06 January 2021

IGHID 11810 - A Phase I Pilot Study to Evaluate the Safety and Immunogenicity of the HIV-1 Vaccines MVA.tHIVconsv3 (M3) and MVA.tHIVconsv4 (M4) Given Alone or In Combination in HIV-1-Infected Adults Suppressed on Antiretroviral Therapy – The M&M Study

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Protocol Principal Investigator: _____

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Signed: _____ Date: _____

Title: _____

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STATEMENT OF COMPLIANCE

The conduct of this clinical trial will be in accordance with International Conference on Harmonization Good Clinical Practice (ICH GCP) and the following:

United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The Institutional Review Board (IRB) will review and approve the protocol, informed consent form(s), recruitment materials, and all participant materials submitted. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

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1 PROTOCOL SUMMARY

1.1 Synopsis

Title:	IGHID 11810 - A Phase I Pilot Study to Evaluate the Safety and Immunogenicity of the HIV-1 Vaccines MVA.tHIVconsv3 (**M3**) and MVA.tHIVconsv4 (**M4**) Given Alone or In Combination in HIV-1-Infected Adults Suppressed on Antiretroviral Therapy – The M&M Study
Study Description:	This is a Phase 1, double blind, randomized, placebo-controlled, parallel design study to evaluate the safety and immunogenicity of MVA.tHIVconsv3 (**M3**) and MVA.tHIVconsv4 (**M4**) vaccines.
Hypotheses:	Intramuscular (IM) vaccination with **M3** or **M4**, given individually or together (**M3+M4**) in adult HIV-infected participants on suppressive combination antiretroviral therapy (ART) will be safe and increase HIV-1-specific T cell responses targeting conserved regions of HIV-1. The simultaneous administration of **M3** with **M4** (**M3+M4**) will result in a greater increase in the breadth of HIV-1-specific T cells targeting conserved regions of HIV-1 than individual administration of **M3** or **M4**.
Objectives:	**Primary Objective**: Evaluate the safety of vaccination with M3 or M4 given individually or in combination (M3+M4) in HIV-1 infected participants on ART with plasma HIV-1 RNA <50 copies/mL. **Secondary Objectives**: Compare the within-participant relative change in magnitude of HIV-1-specific T cell responses following vaccination with either M3 or M4 or M3+M4 combined. Compare the between-arm change in breadth of HIV-1-specific T cell responses following vaccination with either M3 or M4 or M3+M4 combined.

Other Objectives:

Compare the change in function and phenotype of HIV-1-specific T cell responses in participants from pre-vaccination to post-vaccination with either M3 or M4 or M3+M4.

Evaluate the kinetics of immunologic responses in participants pre- and post-vaccination with either M3 or M4 or M3+M4.

Evaluate the kinetics of $CD8^+$ T cell mediated HIV inhibition pre- and post-vaccination with either M3 or M4 or M3+M4.

Explore cellular activation status of total $CD4^+$ and $CD8^+$ T cells pre- and post-vaccination with either M3 or M4 or M3+M4.

Explore the impact of vaccination with either M3 or M4 or M3+M4 on low-level plasma viremia.

Explore the impact of vaccination with either M3 or M4 or M3+M4 on cell-associated HIV RNA in CD4+ T cells.

Explore the impact of vaccination with either M3 or M4 or M3+M4 on total HIV DNA in CD4+ T cells.

Endpoints:

Primary Endpoint: Safety

Occurrence of at least one ≥ Grade 3 Adverse Event (AE) including signs/symptoms, lab toxicities, and/or clinical events, that is possibly, probably or definitely related to study treatment any time from the first day of treatment through 28 days following vaccination.

Secondary Endpoint: Safety

Occurrence of any ≥ Grade 1 AE including signs/symptoms, lab toxicities, and/or clinical events, that is possibly, probably, or definitely related to study treatment any time from the first day of treatment through Day 168 (Week 24) following vaccination.

Safety data will include local and systemic signs and symptoms, laboratory measures of safety/toxicity, and all adverse and serious adverse events. Safety data will be routinely collected throughout the duration of the study.

NOTE: The occurrence of a Grade 3 elevated blood pressure during a study-related leukapheresis that resolves following completion of the procedure will not be included as a primary safety endpoint.

Secondary Endpoints: Immunogenicity

Relative change in magnitude of T cell responses to HIV-1 conserved regions from pre-vaccination (Day 0) to post-vaccination at Day 7 and Day 14.

Change in breadth of T cell responses targeting HIV conserved regions from pre-vaccination (Day 0) to post-vaccination at Day 28.

Study Population: HIV-infected men and women ≥ 18 and ≤ 65 years of age with viral suppression on ART as measured on standard HIV RNA assays. Eligible participants must have a CD4 cell count ≥ 350 cells/mm^3 at screening.

Phase: Phase 1

Description of Facilities Enrolling Participants: Single site - The University of North Carolina, Chapel Hill, North Carolina, USA.

Description of Study Intervention: This is a double blind, randomized, placebo-controlled, parallel design, study in which 24 HIV-infected participants with durable viral suppression will be randomly assigned to receive vaccination with MVA.tHIVconsv3 (M3), MVA.tHIVconsv4 (M4), M3+M4 combined, or placebo.

Participants will be randomized 7:7:7:3 to one of four study arms, and receive study treatment or placebo at Day 0.

Study Schema

Arm	N	Day	Treatment	Dose (pfu)	Route
1	7	0	M3	2×10^8	IM
2	7	0	M4	2×10^8	IM
3	7	0	M3+M4	1×10^8, each vaccine	IM

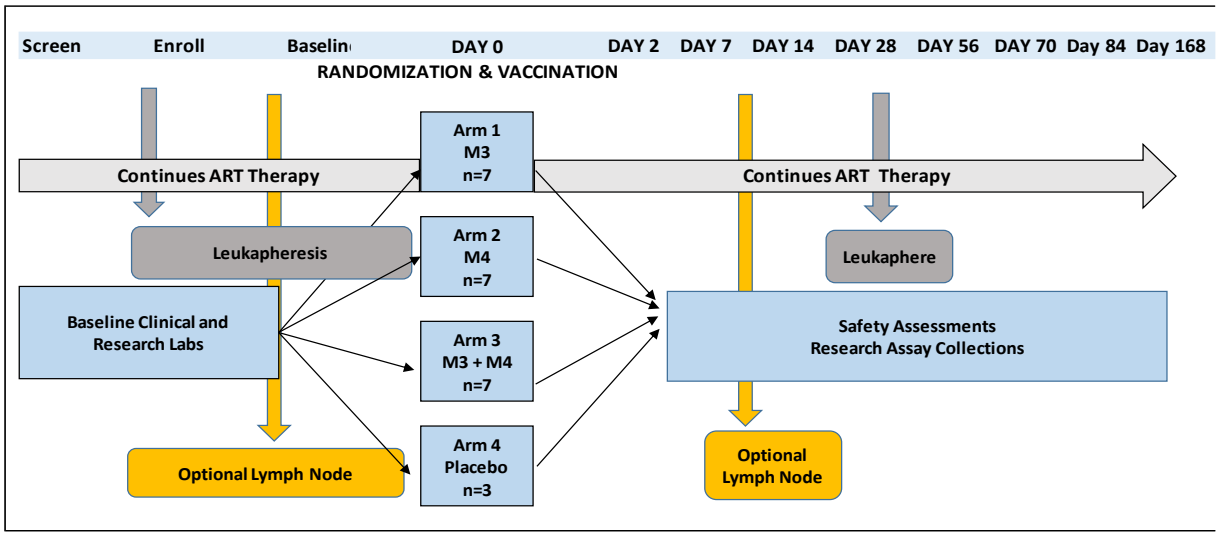
4	3	0	Placebo (saline)	-	IM

M3 = MVA.tHIVconsv3
M4 = MVA.tHIVconsv4
pfu = plaque forming units
IM = intramuscular

Study Duration: The study will take approximately 2.5 years from the time the study opens to enrollment until completion of data analyses.

Participant Duration: Each enrolled participant will complete the study in approximately 33.5 weeks (8.4 months).

1.2 Schema



1.3 Schedule of Events (SOE)

PLEASE NOTE: This study combined Visits 2 and 3, eliminating one study visit from the Schedule of Events prior to Day 0. To avoid issues with data and protocol implementation related to renumbering all study visits, the numbering of the Day 0 through Day 168 Visits will remain unchanged (Visits 4 through 12). We eliminated the Visit 3 from the Schedule of Events.

	Screening	Enrollment and Baseline		Day 0	Day 2	Day 7	Day 14	Day 28	Day 56	Day 70	Day 84	Day 168
Visit #	1	2[1]		4	5	6	7	8	9	10	11	12
Visit Windows in days												
Standard		≤ 60 of Visit 1		≤ 10 of Visit 2			-/+2	- /+7	-/+ 7	- 6/+7	-/ +6	-/+7
Visit anchored to date of Day 0	Day -60	Estimated Day -10				Day 7	Day 12 - Day 16	Day 21 - Day 35	Day 49 - Day 63	Day 64 - Day 77	Day 78 - Day 90	Day 161 - Day 175
Clinical Procedures												
Informed consent	X											
Eligibility and Demographics	X											
Locator Information	X	X		X	X	X	X	X	X	X	X	X
PID assignment	X											
SID assignment		X										
Randomization				X								
Medical and Medication History	X										X	X
Complete Physical Exam	X							X				
Targeted Physical Assessment				X		X	X		X	X		
Vital Signs (VS) [2]	X	X[3]		X		X	X	X	X	X		
Height (in cm)	X											
Calculate Body Mass Index (BMI)	X	X										
Electrocardiogram (ECG)	X											
Review ART and ART adherence	X	X		X	X	X	X	X	X	X	X	X
Targeted Reproductive History	X	X		X	X	X	X	X	X	X		
Update Medical History		X		X	X	X	X	X	X	X	X	X
Update Concomitant medications		X		X	X	X	X	X	X	X	X	X
Assess Signs and Symptoms		X		X	X	X	X	X	X	X	X	X
Assess Adverse Events		X		X	X	X	X	X	X	X	X	X

	Screening	Enrollment and Baseline		Day 0	Day 2	Day 7	Day 14	Day 28	Day 56	Day 70	Day 84	Day 168
Study Treatment or Placebo				X								
Post-vaccine/placebo assessment					X	X	X	X				
Completion of Post-vaccine/placebo Symptom Log by the participant					X	X						
Leukapheresis		X----------X[3]						X[4]				
Lymph Node Procedure [5,6]		X----------X				X ---------- X						
Telephone Assessment					X						X	X
Clinical Laboratory												
CBC with Differential	X	X				X	X	X	X	X		
Screening chemistry [7]	X											
Safety chemistries[8]		X				X	X	X		X		
Pregnancy test [9]	X			X								
Urine Pregnancy – POCT [10]				X		X	X	X	X	X		
Coagulations Test (PT/INR/APTT)	X											
HIV Ag/Ab test[11]	X											
CD4+ T Cell Differential Panel	X					X	X			X		
HIV-1 RNA PCR	X					X				X		
RPR	X											
HBsAg & HCV Ab [12]	X											
Fasting Lipid Panel	X											
Urinalysis	X									X		
Research Laboratory Assays												
HLA typing		X										
Flow cytometry sample[6]		X----------X				X ---------- X						

	Screening	Enrollment and Baseline		Day 0	Day 2	Day 7	Day 14	Day 28	Day 56	Day 70	Day 84	Day 168
Plasma and PBMC for Immunologic and Virologic Studies [13]	X	X[14, 15]		X		X	X	X[15, 16]	X	X		

1. Visit 2 and all associated procedures (inclusive of leukapheresis) will occur within 60 days of Visit 1. If required for scheduling, the leukapheresis visit can occur separately from Visit 2 but within 60 days of Visit 1 and after the Enrollment/Baseline visit. If the leukapheresis is on a separate day, perform the safety labs, vital signs and BMI assessment at the leukapheresis visit and the Day 0 visit within 10 days of the leukapheresis.
2. Pulse (P), blood pressure (BP), respiratory rate (RR), temperature (T) (°C, oral) and weight (kg).
3. Reference Section 8.2.3
4. Reference Section 8.2.3
5. Optional Lymph Node Procedure –consenting participants will complete 2 procedures: the first between Enrollment (Visit 2) and Day 0 and the second between Day 7 and Day 21.
6. Draw blood sample (3 mL EDTA) on day of FNA procedure, unless procedure done on the same day as the leukapheresis or on a visit day where blood sample is already being collected for plasma & PBMCs for Immunology and Virologic Studies.
7. Screening chemistries: sodium, chloride, potassium, CO_2/bicarbonate, glucose, blood urea nitrogen (BUN), creatinine, calcium, phosphate, albumin, magnesium, LDH, total protein, total bilirubin, AST (SGOT), ALT (SGPT) and alkaline phosphatase
8. Safety chemistries: sodium, chloride, potassium, CO_2/bicarbonate, BUN, creatinine, calcium, magnesium, glucose, total bilirubin, AST (SGOT), ALT (SGPT) and alkaline phosphatase
9. Serum pregnancy on all women at screening and on the day of vaccine/placebo only if pregnancy suspected.
10. POCT at Day 0 on all women and at any post vaccine visit where pregnancy suspected in WOCBP only.
11. Optional - HIV testing at screening only if documentation of HIV infection needed.
12. Reactive Hepatitis C Antibody test reflex for Hepatitis C RNA; negative RNA test is acceptable for study participation.
13. Collect research samples in ACD tubes.
14. Collect separate research PBMCs samples at Enrollment (Visit 2).
15. Leukapheresis product
16. Draw 42.5 mL ACD for research assays at Day 28 if leukapheresis scheduled after Day 35.

2 INTRODUCTION

2.1 Study Rationale

2.1.1 HIV Infection and the Need for Alternative Therapies

Multiple strategies to eradicate latent HIV are under investigation. The most advanced include latency reversing agents (LRA) that aim to drive infected cells out of latency. Several groups including ours have now demonstrated that LRAs can increase cell-associated HIV RNA in participants, though no decrease in the overall size of the HIV reservoir has been observed (1,2). These clinical observations are consistent with *in vitro* studies that suggest latency reversal is not itself cytopathic and current LRAs reactivate only a fraction of all infected cells, most of which harbor virus that is not replication competent (1).

Clearance of reactivated cells by $CD8^+$ T cells has been demonstrated in *in vitro* studies (1, 3). These observations are consistent with the extensively documented role of $CD8^+$ T cells in the control of untreated HIV infection, as well as more recent studies suggesting $CD8^+$ T cells contribute to control of HIV reactivation and/or virus rebound (4-6). Immunotherapeutic strategies to eliminate the need for ART by strengthening HIV-specific T-cell immune response have been attempted, although results have been largely disappointing (7-10). Only a small number of HIV vaccine trials have reported an impact of vaccines on the size of the latent reservoir; finding either no sustained impact (11, 12) or, at best, a small, transient decline in the frequency of replication-competent latent infection (13) and below the threshold of what our group identified as necessary to achieve significance (14).

While further iterative testing of both LRAs and immunotherapies are needed, ultimately, it is likely that combination strategies that incorporate both latency reversal and T-cell mediated clearance will be needed to achieve HIV eradication (16). To advance the field of HIV curative therapy, we propose to evaluate the safety and immunogenicity of MVA-vectored vaccines expressing conserved regions of HIV. The proposed clinical trial will administer MVA.tHIVconsv3 (**M3**) and MVA.tHIVconsv4 (**M4**) vaccines either alone or in combination (**M3**+**M4**) in HIV-infected individuals durably suppressed with combination antiretroviral therapy (ART).

2.2 Background

2.2.1 MVA-Vectored Vaccines

Modified vaccinia virus Ankara strain (MVA) is a highly attenuated derivative of vaccinia virus that is unable to replicate efficiently in humans due to a replication defect that occurs late in virion assembly (16). MVA was administered to more than 120,000 people as a

safer alternative to the original smallpox vaccine. MVA in its unmodified form is licensed as a smallpox virus vaccine in Europe and Canada (Imvamune®/Imvanex®, Bavarian Nordic). Recombinant MVAs (rMVAs) have proven excellent vaccines that express high levels of the vaccine transgene product (immunogen) (16, 17). The MVA vector also provides potent adjuvant capacity for the induction and/or boosting of T-cell responses against the passenger immunogen. Comparable to the unmodified form, rMVA vaccines also have extensive and excellent safety profiles in clinical studies, including in HIV-infected (viremic, aviremic) individuals (18-23), infants, (23, 24) and advanced cancer patients (25).

rMVAs have reproducibly increased T-cell responses whether used alone in pre-immune individuals (26) or as a boosting vaccination following priming with recombinant protein, attenuated viruses, DNA and mycobacterial vaccines. rMVAs have been safely delivered by either intramuscular (IM) or intradermal (ID) delivery. Both routes elicit comparable T-cell responses in the blood, though IM delivery may elicit more potent mucosal T cell responses (27). MVA doses of 1-2x10^8 pfu have been both well tolerated and immunogenic (28-30).

2.2.2 HIV Conserved Immunogens

Rationale for HIV conserved-region T-cell vaccines: The first HIV vaccines, including rMVAs, expressed full-length, clade consensus proteins and were tested both in HIV seronegative (31) and HIV positive participants (32). However, T-cell responses elicited by vaccination with full-length HIV immunogens often disproportionately target regions of HIV that are highly variable (32-34) with two implications. Firstly, T cell responses elicited against the more variable or 'high entropy' regions of the HIV proteome are less likely to afford broad coverage of participants' latent reservoir (35). Secondly, $CD8^+$ T cell responses that target variable or 'high entropy' HIV epitopes are more susceptible to rapid virus escape that can occur within weeks of infection (36). These virus escape variants are incorporated into the HIV reservoir (37). By contrast, $CD8^+$ T cells targeting more conserved or low entropy regions of HIV escape significantly more slowly with escape sometimes taking years (38). This suggests that on balance, the 'pre-existing' viral escape mutants in the replication competent viral reservoir exist at higher frequency in variable regions in HIV. Conserved region immunogen designs therefore afford broader population coverage and help limit the negative impact of pre-existing virus escape in the HIV reservoir.

MVA.HIVconsv: HIVconsv is the first HIV conserved-region T cell immunogen designed by Dr Hanke. HIVconsv comprises 14 conserved regions of the HIV-1 proteome (39). Each segment is a consensus sequence from one of the four major HIV-1 clades A, B, C, and D, which alternate to ensure equal clade coverage (39). MVA.HIVconsv has been tested or is in testing in eight trials in both HIV seronegative and seropositive individuals, mostly given IM (1-3 immunizations) at a dose of 2x10^8 pfu (Table 1). In most studies,

MVA.HIVconsv was given as a boosting vaccination following a vaccination with a simian adenovirus (ChAd63.HIVconsv) also expressing, HIVconsv. However, in HIV-CORE 001, MVA.HIVconsv was given three times by IM alone. For all studies, MVA.HIVconsv was manufactured by IDT Biologika, Germany.

Across all studies, safety data indicate MVA.HIVconsv vaccination was well tolerated and safe (Investigator Brochure [IB], MVA.tHIVconsv3 2.0, IB, MVA.tHIVconsv4 version 2.0). Both adverse event (AE) profiles and the frequency of reactions were similar to those observed in HIV seronegative individuals. The few SAEs reported were not attributed to vaccination, AEs associated with MVA boosting were mostly mild or moderate and there were no reported interactions with ART.

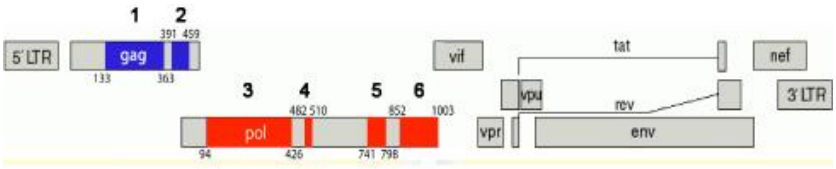
Both MVA.HIVconsv and other rMVA expressing HIV immunogens increased pre-existing HIV-specific T-cell responses in HIV-1 infected, ART-treated study participants (26, 40). In both *ex vivo* ELISpot, and multi-parameter flow cytometry assays, HIV-specific T-cell responses peaked 1-2 weeks after rMVA vaccination (41). MVA.HIVconsv vaccination also increased HIV inhibitory capacity of $CD8^+$ T cells with $CD8^+$ T cells post-vaccination exerting *in vitro* control of replication of four major HIV clades A, B, C, and D (41-43). MVA.HIVconsv delivered as part of combination latency-reversal (romidepsin) and vaccination strategy produced a signal of viremic control during a monitored ART pause in 36% of vaccine recipients (44) (Table 1) .

Table 1 Clinical Testing of MVA.HIVconsv in HIV Seronegative and Seropositive Participants

Study	HIV status	N=	Route	Dose (pfu)	No. doses	Treatment related SAE
HIV-CORE 001 (41)	+	19	IM	$5x10^7$/ $2x10^8$	3	0
HIV-CORE 002 (46)[1]	-	23	IM	$2x10^8$	1	0
BCN01[1,2]	+	24	IM	$2x10^8$	1	0
HIV-CORE 004[1,3]	-	60	IM	$2x10^8$	1	0
HIV-CORE 003[1,4]	-	31	IM	$2x10^8$	1	0
PEACHI 004[1,5]	_	32	IM	$2x10^8$	1	0
BCN01ROMI[1,6]	+	15	IM	$2x10^8$	2	0
RIVER[1,7]	+	30	IM	$2x10^8$	1	0

[1] Heterologous vaccination with simian adenovirus or DNA
[2] ClinicalTrials.gov NCT01712425
[3] ClinicalTrials.gov NCT02099994
[4] ClinicalTrials.gov NCT02425241; combination vaccination with simian adenovirus or DNA and SAP
[5] ClinicalTrials.gov NCT02362217
[6] ClinicalTrials.gov NCT02616874
[7] ClinicalTrials.gov NCT02336074

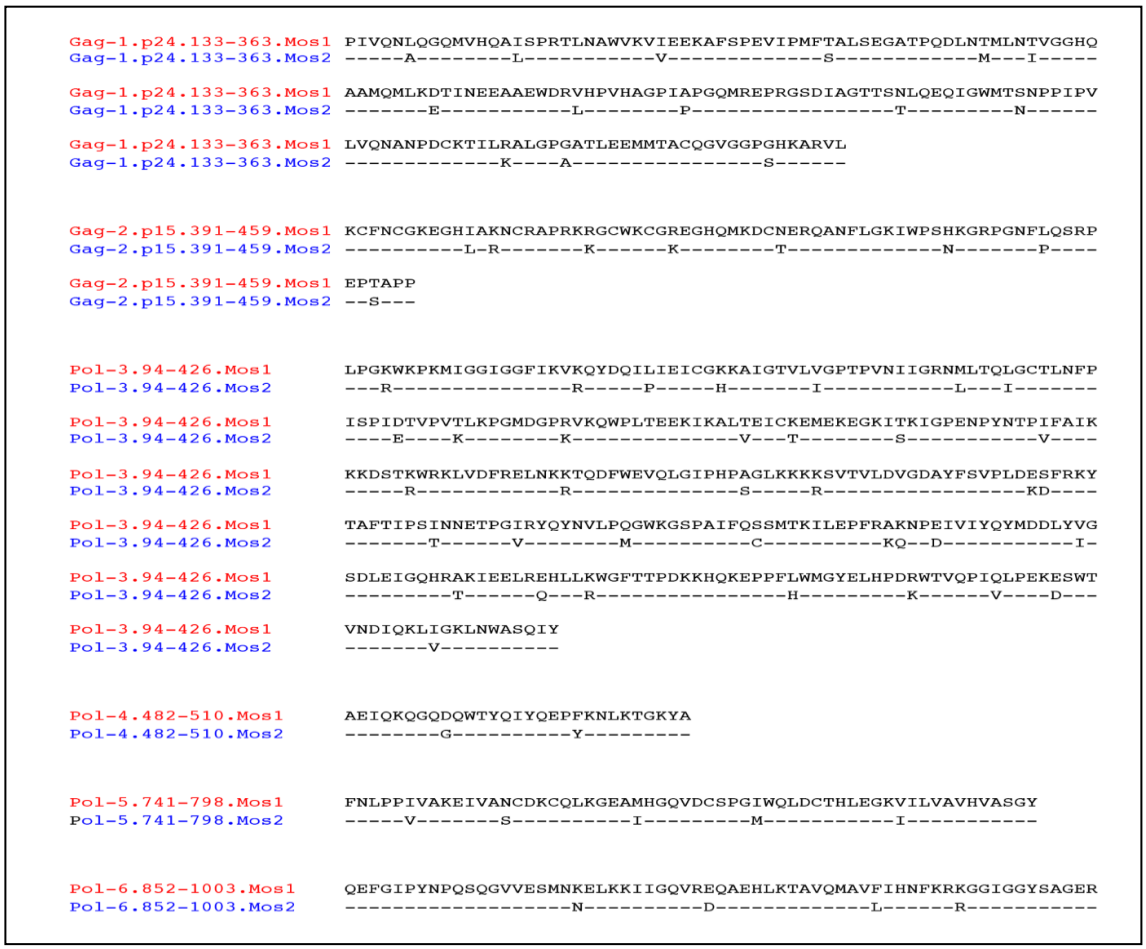
Motivation for design of second generation immunogen: The regions of HIV included in the first generation immunogen HIVconsv were identified on the basis of sequence conservation only. Immunogencity of these regions was not considered. To improve population-level vaccine immunogenicity, second generation HIV immunogens were designed to maximize the inclusion of HIV sequences that spanned regions of HIV that are both conserved and *protective* (i.e. enriched in participants with lower HIV plasma virus loads). The protective T cell epitopes included in the second generation immunogens had been previously identified in studies of treatment-naive patient cohorts on four continents, including North America (46). Figure 1 identifies the six regions of HIV included in the second generation conserved immunogens, collectively called, tHIVconsvX (47).



Six regions (ordered 1-6, highlighted with HXB2 alignment)

Figure 1 Six Regions in the HIV Proteome are Included in tHIVconsvX Vaccine Immunogen

Rationale for mosaic immunogen design: Even in the most conserved regions of the HIV proteome, some sequence variability exists. *In silico* approaches were used to generate two mosaic immunogens, Mosaic 1 and Mosaic 2, which together provide greater coverage of global HIV sequence variability (Figure 2). Both Mosaic-1 and Mosaic-2 are 872 amino acids long. They differ in 84 amino acids or 9.6% of amino acids. Protein alignment using the SerialCloner 2-6-1 software scores the two proteins as 96.44% similar (Figure 2) (48). Mosaic-1 and -2 are complementary sequences and equivalent in their coverage of HIV sequence variability.



(Mosaic-1 red, Mosaic-2 blue) comprised of six conserved regions of HIV-1. Each region is identified by the protein from which it is derived and its position relative to the HIV HXB2 reference strain

Figure 2 Amino acid alignments of the Two Complementary Mosaics

The vaccine immunogen tHIVconsv3 (immunogen of **M3**) is comprised of Mosaic-1 regions; however, the order of the six regions differs from the native proteins (Table 2). tHIVconsv4 (immunogen of **M4**) is comprised of Mosaic-2 regions and again, the order of the six regions differs (Table 2). The aim of scrambling the order of HIV regions is to minimize induction of T-cell responses to the junctional sequences and maximize induction of T cell responses against the common HIV regions. This will be relevant for possible future trials where **M3** and **M4** vectors may be combined with other recombinant vaccine vectors such as simian adenoviruses.

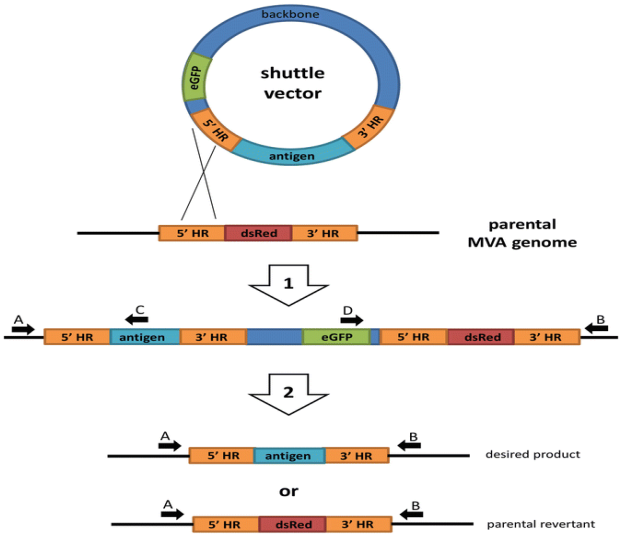
Table 2 Order of Mosaic Regions of tHIVconsv3 and tHIVconsv4

Insert	Immunogen	Region Order[1] tHIVconsvX
tHIVconsv3	Mosaic-1	3-6-2-5-1-4
tHIVconsv4	Mosaic-2	4-1-5-2-6-3

[1]Numbers correspond to the number shown in Figure 1and the region number (protein-X) listed as part of the sequence identifier for each region in Figure 2.

The human tissue plasminogen activator sequence (tPA-LS, Genbank sequence ID: AAA61213.1,) has been incorporated as a leader sequence to enhance expression of the tHIVconsvX immunogens (49-51). Recombinant MVA vaccines containing tPA-LS have been tested without report of serious adverse events (28, 30, 52).

Construction of the M3 and M4 vaccines: The parental non-replicating MVA originates directly from Professor Anton Mayr, passage 575 dated 14 December, 1983 (53) and was modified to contain red fluorescent protein (RFP)(54) (Figure 3). The tHIVconsv3 and tHIVconsv4 genes were first cloned into transfer plasmid p856MVA containing the green fluorescent protein (GFP) gene under control of the modified H5 promoter (p856MVA-GFP-TD-mH5). Recombinant MVAs were made as follows. SPF chicken embryo fibroblast (CEF) cells were infected with parental MVA.RED at a multiplicity of infection (MOI) of 1 and transfected with p856MVA-GFP-TD-mH5.tHIVconsv3 or p856MVA-GFP-TD-mH5.tHIVconsv4. The cell lysate from this recombination was harvested and used to infect CEF. RFP and GFP double positive cells were MoFlo single-cell sorted into 96-well plates and these were used to culture recombinant virus following addition of fresh CEF. Multiple passages gives rise to spontaneous intramolecular recombination between homology regions resulting in one of two possible products: antigen insertion (containing vaccine immunogen) or parental revertant (Figure 3). Those wells containing suitably infected (RFP-) cells were harvested and screened by PCR to confirm identity and test purity. Plaque picking was performed until the culture was free of parental virus, as determined by PCR.



Red- and Green fluorescent protein expression is used to detect parental (RFP+) and intermediate (GRP+RFP+) recombinant MVAs. The *markerless* MVA containing the vaccine immunogen/ antigen is purified following serial passage in CEFs and identity and purity confirmed by PCR. *HR*=homology region. Black arrows indicate primer location. Schematic from Pavot et al (54)

Figure 3 Generation of Markerless Recombinant MVA Vectors

Table 3 Clinical Testing of MVAs in HIV Seropositive Individuals

Study	MVA	Indication	N=	Route	Dose (pfu)	# doses	Treatment related SAE	Summary of Treatment-related SAEs
BCN02-ROMI[1]	MVA.HIVconsv	HIV	15	IM	2×10^8	2	0	None
River[2]	MVA.HIVconsv	HIV	30	IM	2×10^8		0	None
BCN01[3]	MVA.HIVconsv	HIV	24	IM	2×10^8	1	0	None
HIV-CORE 001 (41)	MVA.HIVconsv		11	IM	$2.2\text{-}5\times10^8$	2	0	None
GV-TH-01 (55)	MVA62B	HIV	9	IM	1×10^8	2	0	None
RisVac 03 (21)	MVA-B	HIV	14		1×10^8	3-4	0	None
C-030-485 (19)	MVA85A	TB	257				0	None
TB011 (56)	MVA85A	TB	12	IM	5×10^7		0	None
Greenough (57)	MVA.envgag MVA.tatrevnef-RT	HIV	20	IM	5×10^7	2	2	Classified possibly but probably not related to vaccination. Multiple cutaneous bullous lesions, none at immunization sites (developed from a papular lesion at time of immunization) Grade 4 CPK elevation (unexplained CPK elevations occurred prior to study participation)
Dorrell (58)	MVA. HIVA	HIV	16	ID	5×10^7	2	0	None
Cosma (59)	MVA. HIVnef	HIV	10	SC	5×10^8	3	0	None
Harrer (60)	MVA. HIVnef	HIV	50	IM	1 or 5×10^8	3	0	None
TOTAL			126				2	

[1] ClinicalTrials.gov NCT02616874 [2] ClinicalTrials.gov NCT02336074 [3] ClinicalTrials.gov NCT01712425 [4]ClinicalTrials.gov NCT00189930

2.2.3 Pre-Clinical Experience with MVA-Vectored Vaccines Expressing HIV Immunogens

Toxicity: A formal animal toxicity study of **M3** and **M4** has not been performed. We consider the more informative safety profile is the extensive clinical experience with similar MVA vectors. These studies, in part summarized in Table 1 and Table 3, have consistently shown MVA vaccination is safe and well tolerated at the dose and IM route proposed in this trial, and in both HIV positive and seronegative men and women.

A pre-clinical GLP toxicology study of MVA.HIVconsv (UNO 0011) assessed the systemic toxic potential of 2 x 10^7 pfu/dose of MVA.HIVconsv administered IM to groups of 10 male and 10 female BALB/c mice on days 1, 15 and 29. Vaccinations were not associated with any systemic toxicological changes.

Pre-clinical immunogenicity. In pre-clinical studies in BALB/c mice, the second generation core immunogens **M3+M4** together showed equivalent immunogenicity to the first generation MVA.HIVconsv. A single **M3** immunization induced an average response of almost 4000 SFU/10^6 splenocytes. HIV-specific T-cell responses were detected against 6 of 10 peptide pools containing overlapping peptides spanning the tHIVconsvX immunogen, indicating breadth of T cell responses. When tested in a combination prime-boost regimen with recombinant simian adenoviruses also expressing tHIVconsvX immunogens, no T-cell responses were detected against junctional sequences in BALB/c and C57BL mouse strains (47).

2.2.4 Clinical Experience with MVA-Vectored Vaccines in HIV Positive Populations

Recombinant MVAs expressing both HIV and non-HIV immunogens (TB) have been tested in 12 studies in the proposed study population of HIV-infected durably ART suppressed participants, in part summarized in **Error! Reference source not found.**.

MVA.HIVA. The HIVA immunogen was also designed at the University of Oxford, and comprises a consensus clade A gag p24/p17 and a string of clade A-derived CTL epitopes (61). MVA.HIVA has been given to both HIV seronegative and positive individuals. In HIV-uninfected volunteers, MVA.HIVA was shown to be safe in > 400 healthy HIV-uninfected volunteers across different trials (62). Vaccinations were administered ID, IM and subcutaneous (SC) routes at doses ranging from 5 x 10^7- 2.5 x 10^8 pfu (63). No SAEs attributed to the vaccine were observed (reviewed in (62)).

The safety and tolerability of MVAs expressing HIV and non-HIV antigens has also been demonstrated in HIV-1-infected individuals (**Error! Reference source not found.**). Among 16 HIV-1-infected persons receiving ART for at least 12 months with a CD4 count >300 cells/µl and plasma viral load <50 copies/ml who received two ID injections of

MVA.HIVA (5 x 10^7 pfu) four weeks apart, there were no SAEs in 192 person months of follow-up (58, 64). MVA.HIVA was also safely administered to 20-week-old infants born to HIV-uninfected and HIV-infected mothers in Kenya (24).

MVA.tHIVconsv. In the HIV-CORE 001 study of MVA.HIVconsv only (**Error! Reference source not found.**) in which HIV-1-infected participants on ART were randomized to receive 3 doses of MVA.HIVconsv ($5x10^7$ pfu or $2x10^8$ pfu) or placebo, there were no treatment-related SAEs or SUSARs. In this study, injection site reactions/AEs were the most commonly observed local AE and all were graded as mild or moderate. Systemic AEs observed were all mild or moderate, with malaise the most commonly reported in 7 of 15 vaccinated participants (IB, MVA.tHIVconsv3 version 2.0; IB, MVA.tHIVconsv4 version 2.0).

MVA expressing HIVconsv has been given to 213 participants (269 doses) ranging from $5.5x10^7$ pfu to $2.2x10^8$ PFU all given IM. The participant groups included HIV-1-seropositive adults, including recently infected individuals with early virus suppression after initiation of ART. There have been no SAEs considered to be possibly or likely related to MVA.tHIVconsv. In sum, clinical trial data with MVA.HIVconsv administered by IM injection with $2x10^8$ pfu indicates the vaccine is safe and well-tolerated (MVA.HIVconsv Investigator's Brochure Version 7.0, October 10, 2017, Table 1). The majority of observed AEs (approximately 90%) were mild or moderate in severity and resolved in 24-48 hours following vaccination, with Grade 3 events reported in less than 5% of vaccinees.

Based on these collective data, we consider the risked associated with **M3**, **M4** or **M3**+**M4** vaccination to be minimal.

2.3 Risk/Benefit Assessment

2.3.1 Known Potential Risks

Risks of M3 and M4 vaccines

Based on safety data from studies with earlier generations of the MVA.HIVconsv vaccines and other MVA-vectored vaccines, adverse reactions (AEs) to **M3** and **M4** vaccine are anticipated to involve transient Grade 1 and 2 local reactions such as pain or erythema at the injection site in addition to headache, malaise, and fever (IB, MVA.tHIVconsv3 version 2.0; IB, MVA.tHIVconsv4 version 2.0). There have been no SAEs or SUSARs in several studies of MVA.HIVconsv administered in combination with other vaccines (IB, MVA.tHIVconsv3 version 2.0; IB, MVA.tHIVconsv4 version 2.0).

Clinical trial data with MVA.HIVconsv administered by IM injection with $2x10^8$ pfu indicates that the following AEs may occur in some participants: injection site tenderness, erythema, swelling, pruritus, induration, myalgia, headaches, dizziness, fatigue, fever,

malaise, nausea, chills, vomiting, flu-like symptoms, diarrhea, sweating, anorexia, abdominal pain, and/or syncope.

Standard local (**Error! Reference source not found.**) and systemic reactions (Table 5) can occur, with most appearing within the first 24 hours and usually subsiding within 48 – 72 hours.

Table 4 Local Reactions

Injection site erythema (redness)	Injection site pruritus (itching)
Injection site tenderness	Injection site swelling
Warmth at injection site	Skin discoloration
Injection site pain	Skin damage (vesiculation or ulceration)
Induration (hardening or formation of a crust or scab)	

Table 5 Systemic Reactions

Vomiting	Fatigue (extreme tiredness)
Malaise	Headache
Flu like symptoms	Temperature (fever >37.7°C or 99.9°F)
Sweating	Chills
Diarrhea	Abdominal pain
Dizziness	Myalgia (muscle pain)
Anorexia	Nausea
Syncope	

The expectation is that these AEs will be primarily mild in severity; however, occasional moderate or severe AEs (local pain, headache, malaise, diarrhea, myalgia) have occurred in other vaccine studies with MVA.HIVconsv.

Due to lack of experience in humans, there is no information available about the relationship of AEs to the administration of **M3** or **M4** vaccines.

A potential concern regarding the safety of live attenuated viral vaccines is the possibility of pathogenesis, caused by viral mutation and replication. Any potential risk in this respect arising from the administration of **M3** or **M4** is thought to be very small, due to the avirulence and restricted replication of the parental virus MVA and the recombinant vaccine viruses MVA.tHIVcons3 and MVA.tHIVcons4 in human cells.

Animal reproduction studies have not been performed and human data on use during pregnancy are not available. Therefore, the vaccine should not be administered to pregnant women or women with the intention to become pregnant.

As with any other vaccine, Guillain-Barré Syndrome (GBS) or immune-mediated reactions may occur that can lead to organ damage. These problems, however, are very rare events and have never occurred with recombinant MVA-vectored vaccines to date.

Risk of Syncope

Syncope (fainting) can occur before or following any vaccination as a stress response to the needle injection.

Risks of Blood Drawing

Blood drawing may cause pain and bruising and may infrequently cause a feeling of lightheadedness or fainting. Rarely, it may cause infection at the site where the blood was drawn. Problems from use of an intravenous (IV) for blood drawing are generally mild and may include pain, bruising, minor swelling or bleeding at the IV site, and rarely, infection, vein, or blood clot (phlebitis). We minimize risk by using sterile technique and universal precautions.

Overdose

Overdose of **M3** and **M4** is not addressed given all doses will be provided and administered by licensed study staff only and as prepared by the manufacturing facilities.

Risks Associated with Leukapheresis

These include common side effects similar to blood drawing. However, more serious side effects could occur such as flushing, infection due to contaminated equipment, and damage to red blood cells. These serious side effects occur in less than 1 in 10,000 procedures. No such serious adverse events have occurred in our prior studies, in now more the 400 cell collections from HIV+ donors at UNC.

The following are the most common:

- Pain or bruising at the site of needle sticks,
- Phlebitis - Formation of a blood clot at the blood draw site,
- Citrate toxicity (10%),
- Oral paresthesia (tingling around the mouth),
- Paresthesia (tingling/cramping in hands, fingertips, and feet),
- Stiffness in the arms due to the immobilization during donation,
- Fatigue or tiredness, and

- Temporary fluctuations in blood pressure or heart rate.

Less common:

- Infiltration – the needle dislodges and comes out of the vein causing the fluid to go into the tissue,
- Muscle aches or cramps,
- Chills,
- Fever,
- Nausea or vomiting,
- Lightheadedness or headache, and
- Vasovagal reaction resulting in decrease in blood pressure and heart rate.

Rare:

- Seizures or fainting,
- Transient weight gain, ankle swelling, or increased urination for 24 hours due to fluid retention,
- Infection due to contamination of equipment,
- Skin rashes, flushing, or other allergic responses,
- Damage to or loss of red blood cells due to machine malfunction, and
- Remote possibility of air entering the vein and causing chest pains, shortness of breath or shock or death.

Risks Associated with Lymph Node Biopsy Procedure

- Serious complications after fine needle aspirations (FNA) or lymph node biopsies are rare.
- Minor bleeding under the skin at the biopsy site can occur. This can result in a tender, swollen area called a hematoma.
- Infection at the biopsy site is rare because of the use of sterile techniques and equipment.
- Common Local Reactions
    - Swelling,
    - Soreness, and
    - Pain - typically treated with acetaminophen (Tylenol).

Unknown Risks

New therapies can lead to unexpected, incidental finding that could have a potential effect on the participant's health. Upon confirmation of a potential health or reproductive effect, the study team will notify participants impacted by the new information and will advise

proper medical follow-up when indicated. If findings require more immediate medical attention, the study PI in conjunction with the study coordinators will assist participants in getting an appropriate care appointment.

2.3.2 Known Potential Benefits

The addition of the **M3**, **M4,** or combined **M3**+**M4** to a person's ART regimen and the donation of one's blood cells to this research study provides no direct medical benefits to participants. However, participation contributes to ongoing HIV research, potentially resulting in new treatments for HIV infection.

2.3.3 Assessment of Potential Risks and Benefits

Given that many HIV-1-infected patients on effective ART have persistent low-level viremia that can be detected by ultrasensitive research assays and exhibit impaired HIV-1 specific immune responses, interventions with the potential to improve HIV-specific T-cell responses and limit viremia should be tested. Therapies that improve clearance of persistently HIV-1-infected cells will likely be a necessary component of any HIV remission or eradication strategy. Based on the data outlined above, there is sufficient expectation that the proposed treatment interventions will be safe and well tolerated. Although participants in this early phase study will receive no direct benefit for their participation in this study, there remains a strong desire among HIV-infected individuals, and the HIV community at-large, to pursue HIV preventative and therapeutic vaccine strategies. The potential adverse effects, stigmatization, and financial costs encountered by HIV-infected persons receiving life-long antiretroviral therapy along with the potential harm of persistent immune activation/inflammation are strong reasons to pursue HIV vaccine research. In short, an HIV cure or sustained remission in the absence of ART remain desirable goals that would have substantial benefits for many individuals if achieved. An effective HIV vaccine represents a key strategy for both HIV prevention and cure.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
Evaluate the safety of vaccination with **M3** or **M4** or **M3**+**M4** in HIV-1 infected participants on ART with plasma HIV-1 RNA <50 copies/mL.	Safety Occurrence of at least one ≥ Grade 3 Adverse Event (AE) including signs/symptoms, lab toxicities, and/or clinical events, that is possibly or	*The study will use standard safety grades used in HIV clinical trials. If exceeded, it is currently felt that such risks would be*

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
	definitely related to study treatment any time from the first day of treatment through 28 days following vaccination. Safety data will include local and systemic signs and symptoms, laboratory measures of safety/toxicity, and all adverse and serious adverse events. Safety data will be routinely collected throughout the duration of the study. **NOTE**: The occurrence of a Grade 3 elevated blood pressure during a study-related leukapheresis that resolves following completion of the procedure will not be included as a primary safety endpoint.	*unacceptable in a research study of this nature.*

OBJECTIVES	**ENDPOINTS**	**JUSTIFICATION FOR ENDPOINTS**
Secondary		
Evaluate the safety of vaccination with **M3** or **M4** or **M3**+**M4** in HIV-1 infected participants on ART with plasma HIV-1 RNA <50 copies/mL.	Occurrence of any ≥ Grade 1 AE including signs/symptoms, lab toxicities, and/or clinical events, that is possibly, probably, or definitely related to study treatment any time from the first day of treatment through Day 168 (Week 24) following vaccination.	
Compare the within-participant relative change in magnitude of HIV-1-specific T cell responses in participants following vaccination with either **M3** or **M4** or **M3**+**M4**.	Relative changes in magnitude of T cell responses to HIV-1 conserved regions measured by *ex vivo* IFN-γ ELISpot from pre-vaccination on Day 0 to post-vaccination on Day 7 and 14.	*Previous studies indicate that the rMVA -induced T cell response, typically measured by* ex vivo *IFN-γ ELISpot, peaks between day 7-14 days following vaccination.*
Compare the between-arm change in breadth of HIV-1-specific T cell responses in participants from pre-vaccination to post-vaccination with **M3** or **M4** or **M3**+**M4.**	Change in breadth of T cell responses targeting HIV conserved regions from pre-vaccination (Day 0) to post-vaccination at Day 28.	*We hypothesize that the greater sequence coverage conferred by **M3+M4** vaccination will breadth of HIV-specific T cells that will remain detectable over time.*
Tertiary/Exploratory		
1. Compare the change in function and phenotype of HIV-1-specific T cell responses in participants from pre- to post-vaccination with either M3 or M4 or M3+M4.	1. Relative change in magnitude and phenotype of HIV-1-specific $CD8^+$ T cell populations, including tetramer reactivity, from pre-vaccination (Day 0) to	*Tertiary endpoints 1-3 will explore how MVA vaccination impacts T cell function and phenotype and whether this manifests as increased in vitro*

2. Evaluate the kinetics of immunologic responses in participants' pre- and post-vaccination with either **M3** or **M4** or **M3**+**M4**.	post-vaccination at Day 7 and 14 by flow cytometry. 2. Describe the magnitude and phenotype of HIV-1-specific $CD8^+$ T cell populations at multiple pre- and post-vaccination time points by flow cytometry.	*clearance of HIV-infected $CD4^+$ T cells.*
3. Evaluate the kinetics of $CD8^+$ T cell mediated HIV inhibition in participants pre- and post-vaccination with either **M3** or **M4** or **M3**+**M4**.	3. Relative change in HIV p24 levels in $CD4^+$ T cells at multiple pre- and post-vaccination time points by virus inhibition assay.	
4. Explore cellular activation status of total $CD4^+$ and $CD8^+$ T cells pre- and post-vaccination with either **M3** or **M4** or **M3**+**M4**.	4. Describe the cellular activation status of $CD4^+$ and $CD8^+$ T cell subsets at multiple pre- and post-vaccination time points by flow cytometry.	*Exploratory endpoint 4 examines whether MVA vaccination induces general activation of T cell subsets. This will inform the timing of treatments in future combination studies with other immunotherapies or LRAs.*
5. Explore the impact of vaccination with either **M3** or **M4** or **M3**+**M4** on low-level plasma viremia.	5. The proportion of participants in whom HIV-1 plasma RNA decreases from pre-vaccination (geometric mean of Day -60 and 0) to post-vaccination (geometric mean of Day 56 and 70) as measured by single copy assay.	*Exploratory endpoints 5– 7 examines whether MVA vaccination has an in vivo effect on persistent HIV infection.*

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Tertiary/Exploratory		
6. Explore the impact of vaccination with either M3 or M4 or M3+M4 on cell-associated HIV RNA in CD4+ T cells.	6. The proportion of participants in whom cell-associated RNA in total CD4+ T cells decreases post-vaccination (geometric mean of Day 28 and Day 56) compared to pre-vaccination (geometric mean of Day -45 and Day 30.)	*Exploratory endpoints 5– 7 examine whether MVA vaccination has an in vivo effect on persistent HIV infection.*
7. Explore the impact of vaccination with either M3 or M4 or M3+M4 on total viral DNA in CD4+ T cell.	7. Proportion of participants in whom total HIV DNA in total CD4+ T cells decreases post-vaccination (geometric mean of Day 28 and Day 56) compared to pre-vaccination (geometric mean of Day -45 and Day 30.)	

4 STUDY DESIGN

4.1 Overall Design

This is a Phase 1, single site, pilot study to evaluate the safety and immunogenicity of the **M3** and **M4** vaccines administered alone or in combination in HIV-infected participants suppressed on ART.

This is a double blind, randomized, placebo-controlled, parallel design study to evaluate the safety and immunogenicity of viral-vector, MVA, expressing immunogens, tHIVconsv3 (**M3**) and tHIVconsv4 (**M4**), derived from conserved yet immunogenic regions of HIV-1. The participant population is HIV-1 infected adults suppressed on ART with plasma HIV-1 RNA <50 copies/mL.

We hypothesize that the administration of **M3** or **M4** or **M3+M4** together will be safe in HIV-1-infected participants suppressed on ART. We hypothesize that the simultaneous administration of **M3** with **M4** (**M3+M4**) will result in both an increase in total magnitude

of HIV-1-specific T cell responses and increase the breadth of T cells targeting conserved regions of HIV-1 compared with either **M3** or **M4** vaccination alone.

We will administer vaccine and placebo doses to all participants as an IM injection in the deltoid muscle of the non-dominant arm, unless a participant requests vaccination in their dominant arm. Participants continue their baseline ART regimen throughout the study.

Randomized assignment 7:7:7:3 occurs at Day 0 to one of four arms as provided in Table 6.

Table 6 Randomized Assignment

Arm	N	Day	Treatment	Dose (pfu)	Route
1	7	0	M3	2×10^8	IM
2	7	0	M4	2×10^8	IM
3	7	0	M3+M4	1×10^8, each vaccine	IM
4	3	0	Placebo/saline	-	IM

M3 = MVA.tHIVconsv3
M4 = MVA.tHIVconsv4
pfu = plaque forming units
IM = intramuscular

The primary safety outcome is the occurrence of at least one ≥ Grade 3 Adverse Event (AE) including signs/symptoms, lab toxicities, and/or clinical events that are possibly, probably, or definitely related to study treatment through 28 days following vaccination. The primary safety analysis will be blinded through Day 28 after the last dose of vaccine/placebo and the second leukapheresis is completed by the last participant.

Screening, Enrollment, and Leukapheresis. The participant reviews and signs the informed consent (ICF). Participants meeting eligibility requirements enroll and undergo one leukapheresis procedure between Day -60 and Day 0. The leukapheresis procedure collects white blood cells allowing for completion of detailed immunologic and virologic assays with minimal blood loss. Participants have the option to consent to a lymph node FNA; participants choosing this option will complete the pre-vaccine FNA between Day -60 and Day 0 and a post-vaccine FNA between Day 7 and Day 21. The post-vaccine FNA should be collected even if the pre-vaccine collection attempt is unsuccessful.

Randomization, Study Treatment, Follow-up Assessment, and Leukapheresis. Randomization occurs at Day 0 when a randomization identification number (RID) will be assigned. All participants receive a vaccine or placebo dose as an IM injection. Post

vaccination safety assessments occur via clinical evaluations, and lab testing/evaluations. The study will collect research assays at designated visits.

At Day 28, all participants will undergo their 2nd leukapheresis. This procedure can be completed between Day 21 through Day 35. The leukapheresis product will be used for immunologic and virologic research assays post vaccine/placebo. Participants will be followed for immunogenicity assessments through Day 70 and safety assessments through Day 168 (Week 24) following the administration of vaccine/placebo at Day 0.

Note: The post-vaccine leukapheresis must be done as close to Day 28 as possible. There may be a rare situation where the completion of the procedure in the 2-week visit window will not be possible. As soon as the study coordinator becomes aware of this scenario, they should notify the study PI (or designee) to schedule the procedure outside the study window, preferably earlier (between Days 14 and 28). If the procedure can only be done after Day 35, participants should complete the Day 28 visit with collection of a 42.5 mL ACD sample at that visit.

4.2 Scientific Rationale for Study Design

As detailed above, MVA-vectored vaccines have excellent safety profiles in clinical studies, including in HIV-infected individuals and infants (39, 40, 47, 60-62). MVA vectored vaccines have also been equally immunogenic in men and women (34, 48, 63, 64). The dosing, route of immunization and scheduling for this study have all been previously tested using very similar vaccines and all parameters have been well tolerated. Accordingly, in our target population for HIV curative strategies of HIV-1 infected, durably suppressed male or female participants, this is a low risk study. The potential benefit provided in the induction of HIV-specific $CD8^+$ T cell responses has been associated with increased HIV control and may increase participants' anti-HIV immunity.

Individuals who were chronically infected prior to ART treatment will be prioritized for enrollment. Results from this study will inform the next study combining the ChAdOx vaccine, a novel vaccine incorporating a recombinant, attenuated, replication-defective Chimpadenovirus vector, with the MVA vaccine strategy determined to be the most immunogenic and safe in this study. The key hypothesis is that **M3** and **M4** vaccination will shift T cell dominance away from variable epitopes to immunogenic, but more conserved epitopes that are less subject to escape. Therefore, for this first proof-of-principle study we are seeking chronically HIV-infected participants who harbor a detectable and complex reservoir containing escape viruses.

We established inclusion criterion of $\geq$ 2 years of durable suppression for two reasons. Firstly, one of our endpoint virologic measures is change in total HIV DNA. Total HIV DNA levels do not stabilize until a year following ART (65). Secondly, HIV infection produces progressive immune dysregulation including effects on T cell proliferative

capacity and oligofunctionality. ART suppression over years, helps restore some, though not all, immune dysfunction (66).

4.3 Justification for Dose

Route of Immunization: *Intramuscular injection*

MVA vaccines have been given successfully using both IM and ID injections. MVA has proven to be safe and tolerated when delivered as an IM injection (61-64). In a study of MVA expressing influenza antigens delivered as an IM injection (n=8), fewer local adverse events were observed than with ID injection (n=12) (65). Systemic adverse events (AEs) were similar for both IM and ID injection (65).

Total Dosing: *2 x 10^8 pfu*

Multiple studies were conducted that used the 2 X 10^8 pfu MVA dose which reported the dose as safe and well tolerated ((23, 69), The AEs reported in the studies were mild (Grade 1) and moderate (Grade 2) local and systemic reactions. Most reactions were reactogenic, involving pain at the injection site (local) and malaise and myalgia (systemic). There were very few treatment-related SAEs reported (19).

By way of comparison, there were vaccine-related SAEs observed in a study of rMVA expressing malarial antigens conducted in healthy, malaria-naïve participants, dosed at 5 x 10^8pfu (28). The majority (75%) of participants reported moderate (Grade 2) to severe (Grade 3 or more) pain at the vaccination site and 25% reported a systemic reaction. However, the participants tolerated the injections when the MVA given in combination with a recombinant simian adenovirus primer administered at 1-2 x 10^8 pfu (same dose as proposed in this study). At both doses, rMVA significantly boosted vaccine-specific T cell responses.

Dosing Regimen: *Single vaccination in HIV-pre-immune individuals*

4.4 End of Study Definition

A participant is considered to have completed the study if they have completed all study-required visits including the last visit in the Schedule of Events (SOE), Section 1.3.

5 STUDY POPULATION

5.1 Inclusion Criteria

1. HIV 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 assay.

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.

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.

2. Ages ≥ 18 to ≤ 65 years old

3. Able and willing to give written informed consent.

4. Able and willing to provide adequate locator information.

5. Able and willing to comply with time requirements for protocol-specified visits and evaluations.

6. Able and willing to commit to all study visits including follow-up through Day 168 (Week 24).

7. Continuous ART prior to screening, defined as not missing more than 9 total days and never more than 4 consecutive days in the last 3 months.

8. On a stable ART regimen defined as no changes in any ART medication within the 30 days prior to screening.

9. Permitted ART regimens include:

    1) At least 3 ART agents (not counting ritonavir or cobicistat as one of the agents if less than a 200mg total daily dose). One of the agents must include an integrase inhibitor, NNRTI (Non-Nucleoside Reverse Transcriptase Inhibitors), or a boosted-PI (protease inhibitor).

    OR

    2) Two (2) ART agents in which one of the agents is either a boosted protease inhibitor or an integrase inhibitor.

NOTE: Other potent fully suppressive antiretroviral combinations will be considered on a case-by-case basis.

NOTE: Changes in drug formulation or dose are allowed (e g, TDF to TAF, ritonavir to cobicistat or separate ART agent dosing to fixed-dose combination), but none within 30 days prior to screening.

NOTE: Prior changes in, or elimination of, medications for easier dosing schedule, intolerance, toxicity, an improved side effect profile or within a drug class are permitted if an alternative suppressive regimen was maintained, but not within 30 days prior to screening.

10. Ability and willingness of participant to continue ART throughout the study.

11. Plasma HIV-1 RNA <50 copies/mL at 3 time points in the previous 24 months prior to screening and never ≥50 copies/mL on 2 consecutive time points in the last 24 months.

    NOTE: The documented plasma HIV-1 RNA must be performed by any US laboratory that has a Clinical Laboratory Improvement Amendments (CLIA) certification or its equivalent.

12. At least 1 documented HIV-1 RNA result <50 copies/mL ≥24 months but ≤ 36 months prior to screening.

13. Plasma HIV-1 RNA level <50 copies/mL on an FDA-approved HIV RNA assay performed at a US CLIA Certified Laboratory (or its equivalent) at screening.

14. CD4 cell count ≥ 350 cells/mm^3, performed at any US laboratory that has a CLIA certification or its equivalent at screening.

15. Hepatitis C (HCV) antibody negative result at screening or, if the participant is HCV antibody positive, a negative HCV RNA at screening.

16. Hepatitis B surface antigen (HBsAg) negative at screening.

17. Adequate vascular access for leukapheresis.

18. Able and willing to receive IM injections without difficulty.

19. All women must have a negative serum pregnancy test at screening regardless of reproductive potential.

Note: The serum pregnancy test must have a sensitivity of at least 25 mIU/mL.

20. All participants must agree not to participate in a conception process (e.g., active attempt to become pregnant or to impregnate, sperm donation, in vitro fertilization, egg donation) while on study and for 4 months after their vaccination.

    Note: Women of child bearing potential is defined as women who have not been post-menopausal for at least 24 consecutive months, i.e., who have had menses within the preceding 24 months, or women who have not undergone surgical sterilization, specifically hysterectomy and/or bilateral oophorectomy or bilateral salpingectomy

21. All men and women participating in sexual activity that could lead to pregnancy must agree to consistently use at least one of the following forms of birth control for at least 21 days prior to Visit 4 (Day 0) and for 4 months after their vaccination:

    - Condoms (male or female) with or without a spermicidal agent
    - Diaphragm or cervical cap with spermicide
    - Intrauterine device (IUD)
    - Tubal ligation
    - Hormone-based contraceptive

    NOTE: For female participants receiving ritonavir or cobicistat, estrogen-based contraceptives are not reliable and an alternative method should be suggested.

22. Men and women who are not of reproductive potential are eligible without requiring the use of contraceptives. Acceptable documentation detailing sterilization and menopause are specified below.

    Note: Men who have sex with men only will not be required to use contraception.

    Written or oral documentation communicated by clinician or clinician's staff of one of the following:

    - Physician report/letter
    - Operative report or other source documentation in the patient record (a laboratory report of azoospermia is required to document successful vasectomy)
    - Discharge summary
    - Follicle stimulating hormone-release factor (FSH) measurement elevated into the menopausal range as established by the reporting laboratory

23. Agrees not to enroll on another study of an investigational research agent during the study period.

NOTE: Investigational research agent is defined as any unlicensed investigational drug not yet approved for use in humans.

24. Willingness to defer routine vaccination except for influenza and COVID-19 from within the previous 28 days of screening ~~enrollment~~ through 28 days after vaccination at Day 0.

NOTE: Potential participants should delay enrollment on study until 14 days after receiving the influenza and/or COVID-19 vaccine.

25. Adequate organ function as indicated by the laboratory values provided in **Error! Reference source not found.**.

Table 7 Adequate Organ Function Values for Inclusion

System	Laboratory Value
Hematological	
Absolute neutrophil count	≥ 1,000 /mcL
Platelets	≥ 100,000 / mcL
Hemoglobin	≥ 12 g/dL (male) and ≥ 11.5 g/dL (females)
Coagulation	
Prothrombin Time or INR	<1.1 x ULN
Chemistry	
Serum potassium levels	Within normal limits
Serum magnesium levels[1]	WNL
Glucose	Screening serum glucose ≤ Grade 1 (fasting or non-fasting)
Renal	
Creatinine clearance determined by the CKD-Epi equation found at: https://www.qxmd.com/calculate/calculator_251/egfr-using-ckd-epi	eGFR > 60mL/min
Hepatic	
Serum total bilirubin	Total bilirubin <1.1 x ULN. If total bilirubin is elevated, direct bilirubin must be <2 x ULN If the participant is on an atazanavir –containing therapy then a direct bilirubin should be measured instead of the total bilirubin and must be ≤1.0mg/dL.
AST (SGOT) and ALT (SGPT)	<1.25 X ULN
Alkaline Phosphatase	<1.25 X ULN
Urinalysis	
Protein	< 2+
Blood	< 2+ (for women, before or after menses)

[1] LLN for Mg++ per the clinical laboratory's normal range used for this study is a grade 1 event per the DAIDS Toxicity Table and is allowed for eligibility.

LLN = lower limit of normal ULN = upper limit of normal WNL = within limit of normal

5.2 Exclusion Criteria

1. If the HIV provider or study investigator is unable, as assessed by the study PI or protocol team, to construct a fully active alternative regimen based on previous resistance testing and/or treatment history.

2. Women of childbearing age/potential must not be breast feeding, pregnant, or planning pregnancy any time from enrollment to 4 months after vaccination at Day 0.

3. Body Mass Index (BMI) ≥40 kg/m^2

4. Untreated syphilis infection (defined as a positive rapid plasma reagin (RPR) without clear documentation of treatment).

   NOTE: In cases of untreated syphilis, participant may rescreen following documentation of adequate treatment of syphilis.

5. Current treatment for HCV with antiviral therapy or participants who have received HCV treatment within 6 months prior to screening.

6. HIV RNA ≥150 copies/mL in the 6 months prior to screening.

7. Received any infusion blood product, immune globulin, or hematopoetic growth factors within 6 months prior to screening.

8. Use of any of the following within 90 days prior to screening: immunomodulatory, cytokine, or growth stimulating factors such as systemic cytotoxic chemotherapy, immune globulin, interferon, cyclosporine, methotrexate, azathioprine, anti-CD25 antibody, IFN, interleukins, interleukin-2 (IL-2), hydroxyurea, thalidomide, sargramostim (granulocyte macrophage-colony stimulating factor [GM-CSF]), growth factors, dinitrochlorobenzene (DNCB), thymosin alpha, thymopentin, inosiplex, polyribonucleoside, or diticarb sodium, coumadin, warfarin, or other Coumadin derivative anticoagulants.

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

10. Use of systemic corticosteroids or topical steroids over a total area exceeding 15 cm^2 within 30 days prior to screening, or anticipated need for periodic use of corticosteroids during the study.

NOTE: For participants receiving ritonavir or cobicistat, (either as a booster or protease inhibitor [PI]) as part of their ART regimen, the concomitant use of oral/systemic/topical/inhaled/intranasal corticosteroids is prohibited.

11. Use of any prior HIV vaccine (prophylactic and/or therapeutic) or HIV immunotherapy.

    Note: exceptions allowed for antibody therapies per PI review and approval.

12. Any experimental non-HIV vaccination within 1 year prior to screening.

    NOTE: the receipt of an FDA Emergency Use Authorization (EUA) sanctioned COVID-19 vaccine is not considered exclusionary and should be reviewed with the protocol PI on a case-by-case basis.

13. Prior immunization with a recombinant Adenovirus or MVA vaccine

    Note: prior immunization with smallpox vaccine is not exclusionary.

    NOTE: This exclusion INCLUDES COVID-19 vaccines with adenovirus vector (i.e., Janssen and AstraZeneca).

14. Live attenuated vaccines received within 60 days prior to screening (i.e., varicella; measles, mumps, rubella [MMR]; yellow fever, oral polio, shingles).

    NOTE: Individuals who require vaccination will delay screening for the study until 60 days after receiving the injection.

15. History of prior IgG therapy or immunization with any experimental immunogens (antibodies) within 6 months of screening.

16. Use of any investigational treatment within 6 months prior to screening, with the exception of Phase II studies of antiretroviral agents.

    NOTE: Co-enrollment with other studies under an IND using an FDA approved medication that are not otherwise listed as prohibited will be considered on a case-by-case basis.

    NOTE: The receipt of an FDA Emergency Use Authorization (EUA) sanctioned COVID-19 vaccine or treatment will be reviewed with the protocol PI and will be considered on a case-by-case basis.

17. For any serious illness requiring systemic treatment or hospitalization, the participant must either complete therapy or be clinically stable on therapy, in the opinion of the site investigator, for at least 90 days prior to screening.

18. Treatment for an active HIV-related opportunistic infection within 90 days prior to screening.

19. History of malignancy within the last 5 years.

    NOTE: A history of non-melanoma skin cancer (e.g., basal cell carcinoma or squamous cell skin cancer) is not exclusionary with documentation of topical treatment or of complete resection at least 3 months prior to screening).

20. Immune deficiency other than that caused by HIV infection.

21. Any medical, psychiatric, occupational or other condition that, in the judgment of the investigator, would interfere with, or serve as a contraindication to, protocol adherence or assessment of safety.

22. Hypertension - Exclude for blood pressure consistently > 150 mm Hg systolic and >100 mm Hg diastolic.

    Note: Elevated BP occurring during research leukapheresis procedures completed within the past 12 months are excluded from this requirement. Isolated elevations must be noted as acceptable and signed by study PI or designee.

23. History of auto-immune disease, including Type I diabetes mellitus, with specific exception of:

    - Vitiligo
    - Resolved childhood atopic dermatitis
    - Psoriasis (with the exception of psoriatic arthritis) not requiring systemic treatment (within the past 2 years).
    - Grave's disease with subsequent return to a euthyroid state (clinically and by laboratory testing).

24. Seizure disorder: History of seizure(s) within the past 3 years. Also exclude if participant has used medications in order to prevent or treat seizure(s) at any time within the past 3 years.

25. History of unexplained syncope or fainting episodes within 12 months of study screening.

26. History of Asplenia – absence of normal spleen function as indicated by:
    - Splenectomy
    - Sickle cell disease
27. Bleeding disorder including factor deficiency, coagulopathy or platelet disorder that requires special precautions (easy bruising without a formal diagnosis is not exclusionary).
28. Allergy to eggs and/or egg products.
29. History of anaphylaxis or severe adverse reaction to vaccines including symptoms such as hives, respiratory difficulty, angioedema, and/or abdominal pain.
30. History of hereditary angioedema, acquired angioedema or idiopathic angioedema.
31. Known or suspected hypersensitivity to any vaccine component.
32. Unstable asthma (e.g. sudden acute attacks occurring without an obvious trigger) or asthma requiring:
    - Daily steroid or long acting beta-agonist prevention
    - Hospitalization in the last two years
33. Depo-provera injection at the site of administration (upper left or right medial deltoid muscles) within the 3 months prior to screening, if other deltoid is not an option.
34. History of allergy to latex.
35. Active chronic skin problems such as eczema or psoriasis.
36. Known psychiatric or substance abuse disorder/dependence that, in the opinion of the site investigator, would interfere with cooperation with the requirements of the trial.
37. Compulsorily detained (involuntarily incarcerated) for treatment of either a psychiatric illness or a physical illness, e.g., infectious disease.
38. Prisoner recruitment and participation is not permitted.
39. Inability to communicate effectively with study personnel.

5.3 Screening

5.3.1 Failures

Screening evaluations must occur prior to the participant starting any medications, treatments, or interventions. Screening visit can occur up to 60 days prior to the Enrollment/Baseline Visit.

Demographic, clinical, and laboratory data is collected on persons at screening. The information collected on persons who fail screening and do not enroll will be retained in the screen failure section of the study file.

Labs and/or procedures completed within the 14 days preceding the screening visit can be used to qualify the participant upon approval of the study PI (or designee).

5.3.2 Re-screening

Potential participants who are unable to meet protocol-defined eligibility criteria at the Screening Visit may be eligible to re-screen again at the investigator's (or designee's) discretion.

If a screen failure or the failure to enroll or proceed to Day 0 is due to the inability to meet one of the laboratory parameters (hematology, chemistry, HIV RNA level, or CD4+ T cell count), a retest of the failed criteria may be performed one time only.

5.4 Strategies for Recruitment and Retention

5.4.1 Recruitment

There are several venues for recruitment available to the study team:

- In the UNC ID Clinic and/or other local HIV clinics, there is a large pool of patients with long-term viral suppression on ART interested in participating in research. Many have previously participated in clinical research studies. These individuals will be provided with the opportunity to discuss this study with their provider and the study coordinator.
- Individuals who signed the UNC CFAR database consent, as well as those who signed the consent for the UNC Cure and PHI studies/database, will be identified and approached about the study. Primary care providers or the study coordinator, after consultation with their primary care provider, will provide information about the study and participation.
- Advertisements and flyers in local clinics and with UNC HIV Community Outreach Organizations

5.4.2 Co-enrollment Guidelines

Co-enrollment on other studies will be addressed on a case-by-case basis with the study team. Due the potential interference with monitoring for the effects of this study's treatments, participants will not be able to participate on other studies that provide medications, with the exception of Phase 2 or greater ARV studies.

Co-enrollment in the ACTG 5332 REPRIEVE study (NCT023442900) using FDA approved pitavastatin is permitted provided the participant has taken the study provided medication ≥ 4 months.

Although not a study, FDA EUA sanctioned COVID-19 treatments and vaccines are permitted and must be addressed on a case-by-case basis with the study team.

6 STUDY INTERVENTION

6.1 Study Intervention(s) Administration

6.1.1 Study Intervention Description

MVA.tHIVconsv3 (**M3**) and MVA.tHIVconsv4 (**M4**) have been manufactured in accordance with Good Manufacturing Process (GMP) by IDT Biologika, Am Pharmapark, 06861 Dessau-Rosslau, Germany.

Description of MVA.tHIVconsv3 (M3)

The MVA.tHIVconsv3 vaccine is a second-generation HIV-1 conserved-region multi-component vaccine employing tHIVconsv3 immunogens comprised of Mosaic-1 regions. MVA.tHIVconsv3 is a recombinant, non-replicating MVA expressing six conserved sub-protein regions (regions 1-6) of HIV-1 as one chimeric protein designated tHIVconsv3.

Description of MVA.tHIVcons4 (M4)

The MVA.tHIVconsv4 vaccine is a second-generation HIV-1 conserved-region multi-component immunization regimen employing tHIVconsv4 immunogens comprised of Mosaic-2 regions. MVA.tHIVconsv4 is a recombinant, non-replicating MVA expressing six conserved sub-protein regions (regions 1-6) of HIV-1 as one chimeric protein designated tHIVconsv4.

6.1.2 Dosing and Administration

Dosing

All participants will receive a single vaccine/placebo administration on Day 0. Participants randomized to Arms 1 and 2 will receive 2×10^8 pfu of **M3** or **M4**, respectively.

Participants randomized to Arm 3 will receive a 1×10^8 pfu dose of both **M3** and **M4** (total dose $2x10^8$ pfu **M3+M4**). Participants randomized to Arm 4 will receive placebo (Table 7).

Table 7 Arms in the Clinical Study

Arm	N	Day	Treatment	Dose (pfu)	Volume (mL)	Route
1	7	0	M3	2×10^8	.59	IM
2	7	0	M4	2×10^8	1.11	IM
3	7	0	M3+M4	1×10^8, each vaccine	.29 + .56 (total 0.85)	IM
4	3	0	Placebo/saline	-	1.0	IM

M3 = MVA.tHIVconsv3 **M4** = MVA.tHIVconsv4 pfu = plaque forming units IM = intramuscular

For the purpose of this study and safety management, a maximum of 2 study participants will be vaccinated each week.

Study Treatment Administration

Both M3 and M4 vaccines should be thawed to room temperature and administered within 60 minutes of removal from the freezer.

The combination dose of M3 and M4 (Arm 3) will be delivered as a single injection, with both products drawn up into a single syringe for administration in IDS.

Administer all vaccines/placebo doses as a single injection at Day 0.

Administer all vaccine/placebo doses as an IM injection in the deltoid muscle of the non-dominant arm, unless a participant requests vaccination in their dominant arm.

The study DOES NOT ALLOW any modifications to any vaccine doses.

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Acquisition and accountability

The **M3** and **M4** vaccines will be manufactured and provided by IDT Biologika, Germany. Study products will be provided directly to the UNC Investigational Drug Services (IDS), and dispensed and administered according to site and study-specific SOPs.

Both investigational products will be used as supplied by the manufacturer.

The study site will supply the normal saline used for the placebo injections.

The primary IDS pharmacist will be responsible to the Principal Investigator (PI) for maintaining study drug accountability, reconciliation, preparation, distribution, and record maintenance during the study, including documentation of the amount of both study treatments (**M3** and **M4**) received in IDS and the amount administered to each participant.

The cleanup and disposal of spilled, wasted, or unused medication and used syringes must be documented appropriately (i.e., witnessed) in accordance with applicable federal regulations, Good Clinical Practice (GCP) procedures, and the procedures for handling biohazardous substances. Any incidents related to **M3** and **M4** vaccines and employee/participant exposure or employee needle sticks must be reported immediately to the UNC Institutional Biosafety Committee (IBC) and NIH Office of Science Policy (OSP) as per the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules dated April 2016.

An accountability form and dispensing log, documenting vial allocation number and date, will be kept during the study and will be available for monitoring and inspection/auditing as required. All unused vials will be stored in a designated local freezer at the end of each vaccination visit. Any used vials and expired pre-filled syringes should be disposed of in accordance with institutional or pharmacy policy.

6.2.2 Formulation, Appearance, Packaging, and Labeling

MVA.tHIVconsv3 (M3) vaccine is a white cloudy solution, formulated in Tris-saline buffer (10 mM Tris HCl, 140 mM NaCl, pH 7.7) at a concentration of $3.4x10^8$ pfu/ml. The extractable fill volume is 500 µl. The product is supplied in sterile rubber-stopped glass vials.

MVA.tHIVconsv4 (M4) vaccine is a white cloudy solution, formulated in Tris-saline buffer (10 mM Tris HCl, 140 mM NaCl, pH 7.7) at a concentration of $1.8x10^8$ pfu/ml. The extractable fill volume is 500 µl. The product is supplied in sterile rubber-stopped glass vials.

M3 and M4 vaccines will be packaged and labeled at the manufacturing facilities.

The vaccines will be labelled or have accompanying documentation indicating compliance with GMP and other regulatory requirements. The minimum information provided by the manufacturer includes:

- Study product name and concentration
- Manufacturer
- Date of manufacture
- Lot number
- Volume
- Expiration date (or re-test date)

Investigational vaccine release will comply with procedures required by International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use Good Clinical Practice (ICH)-GCP. The study PI provides written authorization for the shipment of the **M3** and **M4** vaccines to UNC after confirmation that all critical documents required for shipment authorization are completed. The investigational vaccines are shipped to UNC in cryoshippers with temperature monitoring capacity.

6.2.3 Product Storage and Stability

M3 and M4

The **M3** and **M4** vaccines are stored frozen at ≤ -65°C (nominal temperature) in a locked freezer at IDT Biologika according to good manufacturing practice (GMP). Upon QP certification to trial and associated labelling to trial, the vaccine will be transferred to the UNC IDS.

Once received in the IDS at UNC, the study vaccines are transferred to temperature-monitored storage at ≤ -65°C in a locked freezer (IB, MVA.tHIVconsv3 version 2.0; IB, MVA.tHIVconsv4 version 2.0). The freezers must be temperature monitored and if temperature goes outside the normal range, appropriate action is required in accordance with previously agreed SOPs. The placebo (normal saline) will be stored at room temperature.

A vaccine accountability log will be used to record the removal, including the time of removal, and use of each vaccine vial. Vaccine accountability, storage, shipment, and handling will be in accordance with the relevant IDS SOPs and forms.

Preparation

Dispensing and Handling

UNC IDS will prepare and dispense all study product according to UNC and/or study-specific SOPs.

M3 and M4 vaccines and placebo will be dispensed from the UNC IDS Pharmacy, where only the pharmacist(s) will be un-blinded.

Following randomization in IDS, the designated vaccine product(s) will be thawed, without shaking, until completely liquid by holding the vial in a gloved hand.

The vials will not be shaken.

All syringes (vaccine and placebo) will be covered with opaque tape and a label in the UNC IDS Pharmacy to maintain blinding.

Arm 1 - Using aseptic technique, withdraw the needed volume of M3 from the vial into a syringe. Affix a needle suitable for IM injection for administration.

Arm 2 - Using aseptic technique, withdraw the needed volume of M4 from the vial into a syringe. Affix a needle suitable for IM injection for administration.

Arm 3 - Using aseptic technique, withdraw the needed volume of M3 and M4 from each vial into a single syringe. Affix a needle suitable for IM injection for administration.

Arm 4 - Using aseptic technique, for placebo draw the appropriate amount of Sodium Chloride for Injection USP, 0.9% into a syringe. Affix a needle suitable for IM injection for administration.

Following study product preparation, all vaccine/placebo study product should be kept at room temperature until administered. Vaccines must be administered within 60 minutes (1 hour) of removal from the freezer.

Any unused portion of entered vials and expired pre-filled syringes should be disposed of in accordance with institutional or pharmacy policy.

6.3 Measures to Minimize Bias: Randomization and Blinding

Randomization

The 24 study participants will be randomly assigned to receive one vaccination with **M3** (n=7) or **M4** (n= 7) or **M3** + **M4** (n=7) or placebo (n=3) through a blinded randomization schedule. When the participant's eligibility is confirmed, the study specific identification numbers (SIDs) will be assigned sequentially as each participant enrolls at the study at the Enrollment Visit. Vaccine and placebo product will be indistinguishable. Site and laboratory personnel and participants will be blinded with respect to the allocation of

vaccine or placebo. A double-blind placebo-controlled design has been chosen to minimize bias in the reporting of safety and immunological data.

Participants will be randomized and assigned an RID on Day 0 in the IDS pharmacy after receipt of vaccine/placebo prescription. The randomization plan will be generated centrally by the study statisticians at the UNC CFAR using computer software, and will be kept concealed from study personnel.

Un-blinding

Un-blinding of an individual participant is indicated in the event of a medical emergency where the clinical management would be altered by knowledge of the group assignment. The decision to un-blind will be taken in conjunction with the independent members of the Study Monitoring Committee (SMC). Procedures and contact details for un-blinding procedures will specified in a site-specific SOP. The site personnel will ensure that the reasons for un-blinding are documented in the clinical research chart.

6.4 Study Intervention Compliance

Participants will keep a daily diary of solicited AEs for both local and systemic symptoms for 3 days after the vaccine/placebo administration.

6.5 Concomitant Medications

Whenever a concomitant medication is initiated or the dose is changed for all participants after receipt of vaccine/placebo at Day 0 through Day 28 of the study, the PI (or designees) must review the concomitant medications' most recent package inserts, investigator's brochures, or updated information from DAIDS to obtain the most current information on drug interactions, contraindications, and precautions.

Required Medications

1. All participants will be required to have durable HIV suppression on ART for ≥ 24 months prior to study screening and maintain ART therapy throughout the entire study. The study will not provide ART medications.

   The study team will consider alternate ART regimens on a case-by-case basis prior to enrollment per eligibility criteria. The study permits the use of all approved PIs, NNRTIs, NRTIs, and fusion and integrase inhibitors. Also permitted are changes to the participants' ART during the study for dosing simplification, tolerability issues, and improved side effect profile or at the investigator's discretion. If a participant develops toxicity related to his/her previously stable ART, consult the protocol team (preferably before appropriate therapy modification).

Prohibited Medications

1. Ongoing use of investigational ART with the exception of Phase II studies of antiretroviral therapy.

2. Concomitant use with oral or parental corticosteroids, immunosuppressive agents (including but not limited to chronic topical steroids or steroid containing inhalers, azathioprine, and cyclosporine) or any immunotherapy or immunomodulatory agents;

3. Antihistamine from 7 days prior to vaccination through Day 14;

4. Standard vaccines (e.g., pneumococcal, Hepatitis A, Hepatitis B) and live vaccinations (e.g., varicella, measles, mumps, rubella, MMR; yellow fever, oral polio) except influenza and COVID-19 are prohibited from 28 days prior to screening through Day 28 (reference Sections 5.1 and 5.2);

5. Any agent that suppresses lymphocytes or monocyte function;

6. Chemotherapeutic agents, growth factors, cytokines, or chemokines, white lineage colony stimulating factors (e.g., granulocyte-colony stimulating factor [G-CSF] and GM-CSF);

7. Chronic use of topical corticosteroids applied to large areas of the skin (exceeding the cumulative area of the palm of the participant's hand);

8. Sporadic topical use of corticosteroids (e.g. creams) to small areas of the skin (<15 cm^2) for participants who are receiving ritonavir or cobicistat as part of their current ART regimen;

9. Any form of corticosteroid or antihistamine medications at or near the injection site, including for treatment of injection site reactions;

10. Refer to exclusion criteria in Section 5.2.

Rescue Medicine

Not applicable.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 Discontinuation of Study Intervention

Premature Treatment Discontinuation Evaluations

There is only one study treatment intervention visit at Day 0. The collection of safety labs, virologic, immunologic, SCA, and the 2nd leukapheresis will continue to be collected per SOE, section 1.3.

Participants who complete the vaccine/placebo injection but do not complete the Day 7 study visit will be replaced, provided the inability to complete the Day 7 visit is not due to a study-related AE.

Participants who complete the vaccine/placebo injection but do not complete the Day 28 study visit and 2nd leukapheresis procedure will be replaced, provided the inability to complete the Day 28 visit is not due to a study-related AE.

Participants who receive vaccine/placebo but are unable to complete the Day 7 or 2nd leukapheresis should be encouraged to continue on study for safety assessments per the SOE.

Discontinuation of Antiretroviral Therapy

If the participant discontinues ART prior to DAY 0, DO NOT give the vaccine/placebo injection. DO NOT collect research labs for the time points after the participant discontinued ART. This participant will be replaced.

If the participant discontinues ART after the vaccine or placebo administration (Day 0), the participant should continue on study for safety follow-up as noted in the SOE. Collection of virologic, immunologic, SCA, and the 2nd leukapheresis samples will be determined on a case-by-case basis in discussion with the protocol team. This participant may be replaced.

7.2 Participant Discontinuation/Withdrawal from the Study

1. Enrolled Participants, who are not Randomized and Do Not Start Study Treatment

    a. Participants who withdraw or who are withdrawn from the study after the Enrollment Visit but prior to receipt of vaccine/placebo:
        - DO NOT re-use the SID.
        - REPLACE the participant.

    b. A participant who is unable to complete the leukapheresis procedure or from whom < 40 ml is collected prior to Day 0 will be terminated from the study.
        - DO NOT re-use the SID.
        - REPLACE the participant.
        - Termination can occur due to complications at the leukapheresis procedure as determined by the Clinical PI (or designee) and the Apheresis Medical Director and can include contraindications for further leukapheresis procedures.

c. Participants who complete the first leukapheresis procedure pre-vaccine but do not want to do the second leukapheresis procedure will be withdrawn from the study.
   - DO NOT re-use the SID.
   - REPLACE the participant.

2. Evaluations for Randomized Participants Who Do Not Start Study Treatment

   a. Participants who withdraw or are withdrawn after randomization but prior to starting study treatment (vaccine or placebo) will have no further evaluations or follow-up.
      - Notify IDS of the withdrawal and the randomization assignment.
      - RE-ASSIGN the Randomization Identification (RID).
      - DO NOT re-use the SID.
      - REPLACE the participant.

3. Evaluations for Randomized Participants Who Received Study Product and are Discontinued or Withdrawn from Study Participation.

   a. Participants who withdraw or are withdrawn after randomization and prior to Day 28, will have no further research evaluations or follow-up (based on review by PIs) but will be encouraged to complete EOS safety evaluation visit.
      - Notify IDS of the withdrawal and the randomization assignment.
      - REASSIGN the RID.
      - DO NOT re-use the SID.

4. A participant may withdraw or be withdrawn from the study if any of the following occurs:

   - Request by the participant to withdraw from the study and study procedures.
   - Development of an illness that requires treatment with medications prohibited in this study.
   - Change in the participant's medical condition that might make continuation in the study harmful to participant.
   - Participant does not continue to meet eligibility requirements.
   - Participant does not complete Day 7 research assays.
   - Request of the participant's primary care provider if she/he thinks the study is no longer in the best interest of the participant.
   - Participant judged by the study Principal Investigator (or designee) to be at significant risk of failing to comply with the provisions of the protocol, as to cause harm to self or seriously interfere with the validity of the study results.
   - At the discretion of the IRB/Ethics Committee, FDA, NIH, and other government agencies as part of their duties, Principal Investigator (designee), or vaccine supplier.

Premature Study Discontinuation (D/C) Evaluations

Participants who prematurely discontinue study participation after completing the vaccine/placebo at Day 0 but before Day 70 will have premature study discontinuation (D/C visit) evaluations performed per the SOE for Day 70. Participants who discontinue the study prior to the 2nd leukapheresis without having met the primary safety outcome measure will be replaced, provided the discontinuation from study was not due to a study-related AE.

7.3 Lost to Follow-Up

Participants classified as lost to follow-up (LTFU) need to meet both of the following criteria:

- Failure to respond or reply to 3 documented phone contact attempts, followed by
- Failure to respond to a certified letter sent to the address provided by the participant.

Only after documentation of these failed attempts to connect with the participant, will they be determined as LTFU. In scenarios in which a participant is relocated after 3 failed attempts, the study PI will address continued participation on a case-by-case basis.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 Efficacy Assessments

Remote Data Collection

Study visits may be conducted remotely (e.g., telephone, facetime) in the following situations:

- a participant is unable to attend a visit because of a debilitating illness; the site must inform the protocol team in advance
- the site is temporarily unable to conduct non-essential visits in the clinic per University policies at the time of the visit; the site must inform the protocol team in advance
- at the discretion of the protocol team if the risk of an on-site visit is felt to pose more risk than potential benefit to the participant; a message from the team will be sent to the study team

Regardless of the situation, the study team should designate which visits were conducted remotely and attempt to obtain as much of the visit-specific required information, based on the Schedule of Events, and record it on the relevant study specific checklist. The reason for conducting the visit remotely must also be recorded and documented in the participant's study file.

Visit Windows:

- Perform visits scheduled ≤ 7 days apart per window listed in SOE. There is no window around Day 7 visit. There is a ± 2 days window around the Day 14 Visit.

- All windows after Day 14 visit are +/- 7 days (6 in the case of overlapping visits). Please reference SOE. The protocol allows scheduling the Day 28 Leukapheresis outside the parameters of the visit window. Please reference SOE and sections 4.1 and 8.2.3.

Medical History:

The medical history must include all signs and symptoms and all diagnoses regardless of grade within the past 30 days prior to entry. Assessment and documentation of the medical history evaluation occurs at the screening visit. Update to medical history will occur at all clinical visits.

Document:

- All allergies to any medications and their formulations
- Date of birth, gender, race and ethnicity of participant
- Nadir CD4 and pre-ART HIV-1 RNA level, if available (if nadir documentation is not available, collect and record participant recall)
- Targeted Reproductive Assessment includes:
  - For women and men participating in activities that could lead to pregnancy, verify use of birth control for a minimum of 21 days prior to vaccination date (see Section 5.1) through the 4 months following vaccination.
  - Assess the date of the last menstrual period (LMP) for all women of childbearing potential (WOCBP) at all study visits as part of the complete PE or the targeted assessment.

Medication History:

Complete a medication history, include medication start and stop dates. Document the vaccine/placebo administration as in Table 8.

Table 8 Medication Complete History or Timeframe

Medication Category	Complete History or Timeframe
Antiretroviral Therapy	Complete History
Immune-based Therapy	Current & within 105 days prior to enrollment

HCV antiviral therapy	Complete history
Prescription Drugs	Within 30 days prior to enrollment
Systemic Corticosteroids	Within 30 days prior to enrollment
HIV-1 related vaccines	Complete History
Experimental non-HIV vaccine	Within the 12 months prior to enrollment
Live attenuated vaccines	Within 4 weeks prior to enrollment
IgG therapy or immunization w/experimental Abs	Within 6 months prior to enrollment
Non-prescription drugs (OTC)	Within 30 days of enrollment

Clinical Assessments

Complete Physical Exam (PE): A complete PE will be performed at screening and Day 28. This complete PE 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 extremity for edema. The complete PE will also include signs and symptoms, diagnosis, weight (kg), temperature (T°C, oral), respiratory rate (RR), pulse (P), and blood pressure (BP).

Targeted Physical Assessment: A targeted physical assessment is done at all other visits and includes vital signs. The targeted or directed physical assessment addresses any previously identified or new event that the participant experiences since the last study visit or any unresolved signs or symptoms previously experienced. In addition to the vital signs, this assessment includes updates to signs and symptoms, and clinical assessment of HIV disease.

Vital Signs (VS): Perform VS at all clinical visits. VS include P, BP, RR, T, and weight.

Height: Measurement of height (cm) that is required at the screening and baseline visits should be performed with shoes off.

BMI Assessment: Performed at screening for eligibility and again at Day -7 for dosing purposes.

EKG: Perform an EKG at screening for baseline assessment. The study PI (or designee) reviews the results documented in the participant's chart.

ART Adherence Assessment: This protocol requires participants take all required doses of ART. Document all missed doses, even if the reason/s for missing the doses are outside the participant's control. Report missed doses to study PI (or designee). Study participation can be terminated if HIV medication is missed.

For the purpose of this study, non-adherence is defined as missing doses for more than two (2) consecutive days or more than four (4) cumulative days in the prior 12 weeks (3 months) prior to screening visit. Assess and document any missed doses while on study and discuss missed ART doses with study PI (or designee). Continuation on study will be contingent on adherence.

Antiretroviral Medication Assessments: During the study, all modifications to the participant's ART regimen, including any ARV interruptions, dose modifications, formulations modifications, starts, and permanent discontinuations since the last study visit or at the study visit must be recorded.

Signs and Symptoms Assessments: At entry, all signs and symptoms, regardless of grade, that occurred within the 30 days before entry must be recorded.

Active solicitation of AEs will be done at every study visit. Post-entry signs and symptoms, Grade ≥ 2, will be recorded. All signs or symptom, definitely, possibly, or probably related to study interventions will be recorded, regardless of grade. Additionally, all signs and symptoms that lead to a change in study treatment or change in ART or discontinuance of the leukapheresis procedure, regardless of grade, must be recorded.

Diagnoses: After entry, record all diagnoses identified.

Concomitant Medication Assessments: At screening and entry, record all medications taken per medical history guidelines. Thereafter, record all new or discontinued concomitant medications, including prescription, dietary supplements, and over-the-counter medications taken or stopped since the last visit.

Laboratory Studies

$CD4^+/CD8^+$: All study required absolute $CD4^+/CD8^+$ count and percentages must be obtained from a laboratory that possesses a CLIA certification or equivalent. Eligibility will be determined based on a $CD4^+ \geq 350$ at screening.

Plasma HIV-1 RNA: All study required HIV-1 RNA assays must be performed by a laboratory that possesses a CLIA certification or equivalent. Eligibility will be determined based on an HIV-1 RNA value <50 at screening.

Research Assays

Blood will be collected for the following research laboratory evaluations:

FNA and Matching Blood Sample

Analyzed for T-cell specificity, phenotype and function, and virologic measurements per the SOE. Performance of assays is dependent on cell yield and viability. Specific testing may include the following:

- Quantification of T cell memory frequencies and activation pre- and post-treatment
- Cell-associated viral RNA in CD4+ T cells pre- and post-treatment

Measurements of HIV specific T cell responses pre- and post-treatment Human Leukocyte Antigen (HLA) Typing

HLA testing will be performed at one time point in the study; however, if HLA type is already available in the medical record, it does not need to be repeated. The result will be used for research purposes.

Stored Plasma/PBMC for Immunology Studies: Plasma and cryopreserved PBMC will be stored for analysis of low level viremia, T-cell specificity, phenotype and function, and virologic measurements per the SOE. Specific testing will include the following:

- Low-level HIV-1 RNA assays pre- and post-treatment
- Measurement of T cell responses pre- and post-treatment
- Measurement of T cell virus inhibition pre- and post-treatment
- Phenotyping of both total and specific $CD4^+$ and $CD8^+$ T cell activation, homing and memory differentiation using flow cytometry pre- and post- treatment
- Functional analysis (e.g., anti-viral cytokine and lytic molecule release) of both total and specific T cells pre- and post-treatment using flow cytometry
- Cell-associated viral RNA in $CD4^+$ T cells pre- and post-treatment

Site laboratory standardized protocols will guide the performance of all research assays.

NOTE: See Section 7.2.6 regarding collection of stored Plasma/PBMC for Immunology Studies for participants who discontinue ART or do not complete study treatment.

8.2 Safety and Other Assessments

8.2.1 Screening

Screening takes place in a stepwise manner and includes the completion of the following prior to enrollment:

- Informed consent (completed prior to screening assessments)
- Determination of HIV infection status
- Documentation of stable continuous ART
- Documentation of HIV RNA values that are <50 copies/mL for eligibility
- Participant locator information
- Assessment of baseline laboratory testing for continued safety monitoring

8.2.2 Participant Enrollment

Enrollment occurs within the 60 days following screening. Participants not meeting eligibility requirements will not continue on study. The completion of the following occurs prior to enrollment:

- Inclusion/exclusion criteria met

Once eligibility is confirmed, participant study identification numbers (SIDs) will be assigned sequentially as each participant enters the study.

8.2.3 Leukapheresis

Participants undergo 2 leukapheresis procedures. The first study-required leukapheresis procedure occurs at or after Enrollment/Baseline (Visit 2) but prior to Day 0. This leukapheresis provides the cells needed to perform baseline research assays and measurements required to establish pre-treatment immune responses.

NOTE: Aspirin and nonsteroidal anti-inflammatory drugs (NSAIDS) do not need to be held prior to the procedure.

<u>The Baseline Leukapheresis</u>

This procedure can occur at any time point within the 60 days following the Screening visit. It can occur at or after the Enrollment/Baseline (Visit 2). 0.

- If Leukapheresis procedure is scheduled on a date other than the Enrollment/Baseline visit,
  - The procedure should be scheduled after the Enrollment/Baseline visit.
  - Safety labs will be performed with the leukapheresis and not at the Enrollment/Baseline visit.
  - Complete VS and BMI assessment

- The assessments listed under the Enrollment/Baseline Visit will be done at Visit 2. Leukapheresis procedures done at UNC will have a CBC with differential done prior to the procedure (unless result available within the past 24 hours) as this is required for the Apheresis Lab.

If the leukapheresis procedure is done on the Enrollment/Baseline visit, collect the leukapheresis product in addition to the research labs. (Reference the Study Specific Lab Manual.)

The Day 28 Leukapheresis

- If Leukapheresis cannot be done within the Day 28 Visit window due to scheduling conflicts or unforeseen issues, please contact study PI for option to see person outside the visit window, either 1 week earlier or up to 2 weeks after the Day 28 Visit window.

8.2.4 Randomization

Participants are randomized to the study Arms, assigned a RID, and receive vaccine/placebo at Day 0. Study Arm assignment is double-blinded and all randomized participants will receive either vaccine or placebo. The administration of the vaccination or placebo designates Day 0 and the calculation of the remaining visits are identified by consecutive days starting with Day 0.

Vaccine and placebo product will be indistinguishable. Site and laboratory personnel and study participants will be blinded with respect to the allocation of vaccine or placebo. The randomization plan will be generated centrally by the study statisticians at the UNC CFAR using computer software, and will be kept concealed from the study personnel.

8.2.5 Participants randomized to receive study product and do not receive the vaccine/placebo or are withdrawn from the study after receipt of the vaccine (reference protocol sections 7.2 and 7.3) will be replaced and the RID re-assigned at the time of next eligible participant randomization. Study Un-blinding

Study un-blinding will occur following an independent SMC review commencing approximately 28 days after the last participant receives their vaccine/placebo injection and completes the 2nd leukapheresis.

8.2.6 Unscheduled Visits

Visit windows are defined in the protocol and SOE. For a visit not performed within the window period, the interim visit will be completed and documented as a protocol deviation. If there is a missed visit that requires safety assessments or local safety labs,

study staff should attempt to bring the participant in for an interim visit as soon as possible. Safety evaluations should be completed per SOE for the missed visit.

Should a participant require an unscheduled visit between visits, procedures performed at these visits are usually toxicity/safety assessments (including local safety labs). The procedures completed at the unscheduled visit should be performed only if clinically indicated.

8.2.7 Vaccine or Placebo Administration Visit:

Vaccine (**M3** or **M4** or **M3**+**M4**) or placebo injections are given at Day 0.

1. Complete required pre-vaccine/placebo assessments and blood draws (per SOE) prior to the administration of the vaccine/placebo.

2. Record the RID and the vaccine/placebo injection, including dose, and time of administration. Record where the injection was given (location). If unable to administer the entire dose for any reason, notify the study PI (or designee) and document the reason.

3. Complete the following:

    - During administration of the vaccines, medicines and resuscitation equipment will be immediately available for the management of anaphylaxis.

    - Two licensed staff will verify and confirm the vaccine/placebo for administration.

    - Two licensed staff will confirm the research chart PID /SID to the PID/SID on the blinded study treatment label.

    - Document the verification process.

    - Have participant seated in a secure chair prior to injection to avoid the risk of fall due to possible dizziness/lightheadedness, or in rare cases, syncope.

    - Administer vaccine/placebo as a single IM injection in the deltoid muscle, preferably in the non-dominant arm, unless a participant requests vaccination in their dominant arm.

    - The study DOES NOT ALLOW any modifications to any vaccine doses.

    - Study staff will wear gloves and eye protection when handling the vaccine/placebo product.

8.2.8 Post Vaccination/Injection Management:

1. Observe participant and injection site for 60 minutes following each vaccine/placebo injection for clinical AEs.
    - Assess for injection site reaction every 15 minutes × 4, document observed reactions
    - Observe and assist participant when coming to a standing position for the first time following each administration of vaccine/placebo
    - Obtain vital signs (BP, P, RR, and T) prior to discharge home
2. Instruct participant to contact the study coordinator immediately with any concerns about the injection site after leaving the clinic.
3. Provide post vaccination symptom log and instructions for completion prior to leaving the clinic.
    - Review symptom log at the Day 2 and Day 7 visits following the vaccination
    - Symptom logs are collected at the Day 7 visit
4. Contact participant the day after the injection in the manner pre-determined by the study coordinator and participant.
    - Assess for signs and symptoms resulting from vaccine or placebo administration
5. Following receipt of vaccine/placebo, participants will be followed with study visits through Day 70 and the Days 84 and 168 visits will be completed via phone contact.
6. Participants will be contacted by phone at Days 84 and 168 to assess for and document new adverse events.
7. If the participant reports any AEs possibly related to treatment at either the Day 84 or Day 168 assessments, an additional unscheduled visit may be needed to further evaluate the participant and attribution to treatment.

8.2.9 Post Vaccination Symptom Log

Participants will be given a “Post vaccination symptom log” to use as a memory aid for tracking adverse events that occur during the 3 days following the injection. Participants will record daily temperature and systemic symptoms.

1. For this study, solicited AEs occurring during the 3 days after the vaccine/placebo injection will include:
   - unusually tired/feeling unwell
   - muscles aches
   - headache
   - chills
   - nausea
   - joint pain

2. Instruct participants to take their temperature at least once daily, starting with the day of injection (Day 0), after leaving the clinic and through Day 3 post-injection.

3. Temperature should be taken orally in the evening whenever possible. If more than one measurement is made in a day (for example due to feeling unwell), then the highest temperature taken that day should be recorded.

4. The symptoms and assignment of grades will be reviewed with participants to assure an understanding of how to use and evaluate each symptom during these first 3 days.

The study coordinator will discuss the symptom logs for accuracy and completeness at Day 2 and review and collect the completed symptom log at the Day 7 Visit. The study coordinator will review symptoms with participants for each identified symptom and clarify the grading assigned at the time. Additionally, active solicitation of AEs will be done at every visit. Participants will also be asked to contact the study coordinator, study PI, or designee should they experience any adverse events at any time points during the study.

8.2.10 Solicited AE Assessments:

The study coordinator will assess for Solicited AEs (expected) symptoms at Day 2 and Day 7 following each injection.

Assessments will consist of a standard series of questions administered at approximately 48 hours post vaccination (Day 2) and at the Day 7 Visit. The following local reaction evaluations (Table 9) and systemic reaction evaluations (Table 10) are part of each assessment.

Table 9 Local Reaction Solicited AE Assessments

Injection site erythema (redness)	Injection site pruritus (itching)
Injection site tenderness	Injection site swelling

Warmth at injection site	Skin discoloration
Injection site pain	Skin damage (vesiculation or ulceration)
Induration (hardening or formation of a crust or scab)	

Table 10 Systemic Reaction Solicited AE Assessments

Vomiting	Fatigue (extreme tiredness)
Malaise	Headache
Flu like symptoms	Temperature (fever >37.7°C or 99.9°F)
Sweating	Chills
Diarrhea	Nausea
Dizziness	Myalgia (muscle pain)
Anorexia	Abdominal pain
Syncope	

Study staff will follow new or unresolved vaccine-related AEs to resolution. In general, participants self-reporting any post-injection reaction greater than mild are to be evaluated by the study PI (or designee) within 72 hours after onset, unless the reaction is improving and/or has completely resolved.

Institute appropriate countermeasures, including medical intervention or procedures, if clinically indicated.

8.2.11 Laboratory Evaluations

Details of specimen collection are found in the Lab Procedures Manual for this study.

Labs utilized for processing and resulting the clinical evaluations are the UNC McLendon Lab and LabCorp. The study PI (or designee) assesses the results of all clinical laboratory tests to determine continuing eligibility or AE relatedness at specified visits. Lab results or values outside the normal reference range require the investigator (or designee) to determine if the abnormal value is clinically significant and/or related to a study intervention. All confirmed abnormal laboratory values that the investigator deems clinically significant and/or related to a study intervention must be reported as AEs, regardless of grade. Post-entry laboratory values, Grade ≥2, will be recorded. Participants and/or their primary care provider will be informed of any clinically significant laboratory test result or clinical event that occurs throughout the study. Participants will be referred for care when appropriate.

Hematology: Perform hemoglobin, hematocrit, white blood cell count (WBC), differential WBC, absolute neutrophil count (ANC), and platelet count in real time at the local laboratory.

Note: CBC with differential for Leukapheresis procedures:

- A STAT CBC with differential is required prior to each leukapheresis performed at the UNC Apheresis Lab unless results completed within 24 hours of the procedure are available to the Apheresis Lab performing the procedure.

- Leukapheresis procedures performed at a local apheresis collection center require a CBC within 30 days of procedure.

Liver Function Tests: Perform total bilirubin, AST (SGOT), ALT (SGPT), and alkaline phosphatase in real time at the local laboratory.

NOTE: For participants on ritonavir boosted atazanavir, total and direct bilirubin should be measured.

Blood Chemistries

Screening lab evaluation includes electrolytes (sodium, chloride, potassium, CO2/bicarbonate), glucose, blood urea nitrogen (BUN), creatinine, calcium, phosphate, albumin, magnesium, LDH, and total protein in real time at the local laboratory.

Safety lab evaluations throughout the study include electrolytes (sodium, potassium, chloride, CO2/bicarbonate), BUN, creatinine, calcium, magnesium, and glucose (also reference Pre-Vaccine Safety Lab Assessment).

Creatinine and Creatinine Clearance

Creatinine clearance (eGFR) calculations will use the CKD-EPI equation. This calculation can be found at https://www.qxmd.com/calculate/calculator_251/egfr-using-ckd-epi

The creatinine clearance (eGFR) will be required for study enrollment eligibility. The study assesses serum creatinine level at all the safety lab checks and uses the serum creatinine value for toxicity grading and as confirmation to receive vaccine or placebo doses.

The creatinine clearance (eGFR) value reported as part of the lab report of serum creatinine level will be re-assessed and re-calculated if incidental finding indicates a value ≥ Grade 3. The re-calculated value using the CKD-EPI equation will be used to determine the severity of the lab abnormality.

Pre-Vaccine Safety Lab Assessments

Participants will have a Pre-Dose Safety Lab Assessment completed at either Visit 2 or the leukapheresis and within the 10 days prior to the vaccine/placebo dosing visit. These safety lab results must be within the eligibility criteria parameters established for this study. Labs that fall outside the parameters can be repeated one time only to qualify the participant to proceed on study.

After repeat testing, any lab value that remains outside the guidelines but considered clinically non-significant and documented as such by the study clinical PI (or designee), must be reviewed and approved by the protocol and/or safety committee prior to the vaccination.

Serum and Point of Care (POCT) Urine Pregnancy Test: A negative serum pregnancy test (to rule out pregnancy) is required on all women at screening, and a negative urine pregnancy test on Day 0, regardless of documented procedures that prohibit pregnancy.

The use of the POCT urine pregnancy test as confirmation of negative pregnancy status is acceptable for vaccination visit at Day 0, unless pregnancy is suspected. If pregnancy is suspected, a serum pregnancy test must be done to rule out pregnancy. The test must be negative within 48 hours of the dose.

In addition, a POCT pregnancy test should be performed at any visit post vaccination, if pregnancy is suspected. Because the study has no direct clinical benefit, this added protection is warranted.

The study will use the date of LMP to rule out suspected pregnancy. Pregnancy will be suspected if >35 days since first day of last menstrual period in pre-menopausal women has elapsed. **Hepatitis Screen**: Both hepatitis tests (HCV AB and HBsAg) must be negative or non-detected to be included on the study. A positive HCV AB test reflexed to Hepatitis C RNA revealing a negative result is acceptable.

Eligibility will be determined based on negative testing, per above definition at screening.

RPR: Complete at screening to rule out clinically active untreated syphilis.

Participant may participate on study following documentation of adequate treatment of syphilis at screening (Visit 1). Participants diagnosed with syphilis at screening, will be referred for and require documentation of treatment. The participant may rescreen following a minimum of 14 days post syphilis treatment.

HIV Ag/Ab Test: Complete HIV testing at screening if documentation of HIV infection is not available from prior records.

Fasting Lipid Panel: Obtain total cholesterol, HDL, LDL calculation, VLDL calculations, and triglycerides at screening.

Prothrombin time (PT), INR, and APTT: Evaluate at the Screening Visit.

Urinalysis: Do dipstick testing (including protein, glucose, hemoglobin, pH, and ketone) at select visits. Do microscopic analysis only in the event of abnormal results from dipstick testing.

Estimated Blood Volumes Associated with Study Visits: Estimated blood volumes associated with each study visit is provided in Table 11.

Table 11 Blood Volumes Associated with Study Visits

Day #	Approximate Blood Volume (mL)	Day #	Approximate Blood Volume (mL)
Screen	100	Day 7	115
Enrollment/Baseline	75	FNA (optional)	3
Leukapheresis	20	Day 14	110
		Day 28	75
		Day 56	100
FNA (optional)	3	Day 70	130
Day 0	85	Day 84	0
Day 2	0	Day 168	0

8.3 Adverse Events and Serious Adverse Events

8.3.1 Definition of Adverse Events (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition, or abnormal results of diagnostic procedures, including laboratory test abnormalities.

Definition of Serious Adverse Events (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- death,
- a life-threatening adverse event,
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias, or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.3.2 Classification of an Adverse Event

8.3.2.1 Severity of Event

Event severity will be assigned according to the Investigator's (or designee's) assessment.

Severity Grade for Parameters Not Identified in the Grading Table:

The functional table (

Table 12) should be used to grade the severity of an AE that is not specifically identified in the grading table. In addition, all deaths related to an AE are to be classified as Grade 5.

Table 12 Severity Grade for Parameters

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING

Clinical adverse event NOT identified elsewhere in the grading table	Mild symptoms causing no or minimal interference with usual social & functional activities with intervention not indicated	Moderate symptoms causing greater than minimal interference with usual social & functional activities with intervention indicated	Severe symptoms causing inability to perform usual social & functional activities with intervention or hospitalization indicated	Potentially life-threatening symptoms causing inability to perform basic self-care functions with intervention indicated to prevent permanent impairment, persistent disability, or death

8.3.2.2 Relationship to Study INTERVENTION

Attribution/Assessment of Causality is a determination that describes the relationship or association of the study product with an adverse event.

This assessment of causality or relationship of AEs to the study product is provided by the Investigator and is determined by 1) temporal relationship of the event to the administration of study product, 2) whether an alternative etiology has been identified, and 3) biological plausibility. Causality must be assessed separately for each study product.

The causality assessment categories that will be used for this study are described below.

1. Causality assessments that are considered not related to study product:

    a. Not related:

    The event is related to an etiology other than the study product (the alternative etiology must be documented in the participant's medical record).

    If an SAE is considered "unrelated" to study product, the Investigator should offer his/her clinical opinion as to what factor(s), agent(s), or process(s) were the likely causative mechanism for the event.

2. Causality assessments that are considered related to study product:

    a. Possible:

    There is an association between the event and the administration of the study product and there is a plausible mechanism for the event to be related to study product; but there may also be alternative etiology, such as characteristics of the participant's clinical status or underlying disease.

    b. Probable:

There is an association between the event and the administration of study product, a plausible mechanism for the event to be related to the study product, and the event could not be reasonably explained by known characteristics of the participant's clinical status or an alternative etiology is not apparent.

c. Definite:

There is an association between the event and the administration of study product; a plausible mechanism for the event to be related to the study product, causes other than the study product have been ruled out, and/or the event re-appeared on re-exposure to the study product.

8.3.2.3 Expectedness

The study PI (or designee) will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention or is not listed in the most current IBs for the study intervention.

8.3.3 Time Period and Frequency for Event Assessment and Follow-Up

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review of medical record.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured in the research record on the appropriate form. Information collected includes:

- Event description,
- Time of onset,
- Study PI's (or designee's) assessment of severity, relationship to study product and expectedness,
- Time of resolution/stabilization of the event.

All AEs occurring while on study must be documented appropriately regardless of relationship. All study- related AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The study research coordinator will record all reportable events with start dates occurring any time on or after the enrollment visit until 7 days (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the research coordinator will inquire about the occurrence of AE/SAEs since the last visit. Vaccine-related and/or reportable events will be followed for outcome information until resolution or stabilization.

Clinical Laboratory Changes:

Safety laboratory assessments will be carried out locally at UNC and evaluated by the Investigator (or designee) to ensure participant safety. The Investigator (or designee) is responsible for reviewing the results of all laboratory tests as they become available.

- Laboratory values that fall outside of a clinically accepted reference range or differ significantly from previous values must be evaluated for clinical significance by the Investigator (or designee). The Investigator (or designee) may repeat the laboratory test or request additional tests to verify the results of the original laboratory tests.

- If the Investigator (or designee) determines the laboratory value is an abnormal change from baseline and is of clinical significance for that participant, it is considered an AE.

- Generally, Grade 1 and Grade 2 laboratory findings need not be reported as AEs unless deemed clinically significant by the Investigator (or designee).

- Consistent with the DAIDS designation of Grade 3 events as severe or medically significant and Grade 4 events as life-threatening, Grade 3 and Grade 4 laboratory findings should be reported as AEs or SAEs, as appropriate.

- The test result or finding should be reported as the AE. Such laboratory values should generally be recorded as “increased” or “decreased” (e.g., change from baseline potassium of 5.0 to 3.5 mEq/L = potassium decreased).

The study PI (or designee) is responsible for appropriate reporting of AEs to the regulatory authorities. The study PI (or designee) will also report all suspected unexpected serious adverse reactions. The study PI (or designee) must report suspected unexpected serious adverse reactions to the FDA if applicable and to the UNC IRB, per the UNC IRB reporting requirements.

8.3.4 Adverse Event Reporting

Adverse events will be graded according to the DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), corrected Version 2.1, July 2017, on the DAIDS RSC website at http://rsc.tech-res.com/clinical-research-sites/safety-reporting/daids-grading-tables.

The study will monitor participants for adverse events. Toxicities will be characterized in terms including duration, intensity, and time to onset. Safety endpoints will include all adverse experiences, in addition to laboratory safety assessments, and vital signs. All AEs will be recorded on the appropriate study form.

AEs will be recorded in the database if any of the following criteria have been met:

- Study-related Grade ≥ 1 AEs
- Grade ≥ 3 AEs
- Autoimmune-related AEs regardless of grade
- HIV viral loads ≥ 50 copies/mL
- Injection reactions regardless of grade
- AEs that led to a change in study intervention regardless of grade
- AEs meeting SAE definition or EAE (Expedited Adverse Event) reporting requirement

Grade 1 or 2 Toxicity

Participants who develop a Grade 1 or 2 AE or lab toxicity that occurs following a vaccine/placebo injection that is thought to be possibly, probably, or definitely related to the study treatment should be discussed with the study team. Grade 1 or 2 AEs that may be related to the vaccine/placebo injection will be handled according to standard clinical practice and documented.

Grade 3 Toxicity

For participants who develop a Grade 3 AE following the administration of vaccine/placebo judged by the study PI (or designee) to be at least possibly study treatment-related, the protocol team must be notified within 24 hours. Participants experiencing Grade 3 AEs should be followed closely and if the AE does not return to

Grade ≤ 2 within 2 weeks, the study team should again be notified within 24 hours and the SMC will be notified.

For participants who experience a Grade 3 AE judged not related to the study treatment by the investigator, continued study participation is at the discretion of the study PI (or designee) in consultation with the protocol team and SMC.

Grade 4 Toxicity

For participants who develop a Grade 4 AE following administration of vaccine/placebo judged by the study PI (or designee) to be at least possibly study treatment-related, the protocol team and SMC must be notified within 24 hours. Participants experiencing Grade 4 AEs should be followed closely with additional clinical assessments and laboratory testing as clinically indicated. If the AE does not return to Grade ≤ 2 within 2 weeks, the protocol team and SMC should again be notified within 24 hours.

All study treatments will be discontinued in the event of Grade 4 AEs possibly related to study treatment.

Local or Systemic Reactions to Vaccine/Placebo Injections

Grade 1 or 2

Local reactions of mild (Grade 1) or moderate (Grade 2) severity will usually resolve spontaneously. If needed, they may be managed with local application of cold packs, oral acetaminophen, oral non-steroidal anti-inflammatory agents, or a combination of these measures as appropriate.

NOTE: Topical steroids should not be applied to the injection site.

Grade 3 or 4

For severe (Grade 3) or potentially life-threatening (Grade 4) local reactions, the protocol team must be notified within 24 hours. For Grade 4 local reactions, definitive medical and/or surgical intervention should be undertaken as appropriate.

Systemic Reactions

The protocol team must be contacted within 24 hours for any non-local Grade 3 or 4 systemic reactions thought definitely, possibly, or probably related to the vaccine/placebo injections.

The common systemic adverse events usually seen with MVA vectored vaccines, **M3** and **M4**, include headache, feverishness, myalgia, arthralgia, fatigue, malaise, nausea, transient

flu like illness within 24 hours of vaccination, which usually resolves within 48 hours. It is anticipated that the majority of systemic adverse events post vaccination will be mild in intensity. However, there is a possibility of moderate or severe headache or malaise.

If an adverse event of special interest (AESI) occurs (see section 8.3.8), the study coordinator, in collaboration with the study PI (or designee), will evaluate the severity and seriousness of the AE and the relationship to the study treatment, and will document the findings. The protocol team should be contacted within 24 hours of notification of any reactions thought definitely, possibly, or probably related to the vaccine/placebo injections.

Injection Site Reaction

Injection Site Erythema or Redness and Injection Site Induration or Swelling will not be considered an AE if there is no interference with usual social and functional activities such that:

- Grade 1 is: 2.5 to < 5 cm in diameter OR 6.25 to < 25 cm2 surface area;
- Grade 2 is: ≥ 5 to < 10 cm in diameter OR ≥ 25 to < 100 cm2 surface area;
- Grade 3 is: ≥ 10 cm in diameter OR ≥ 100 cm2 surface area OR Ulceration OR Secondary infection OR Sterile abscess OR Drainage;
- Grade 4 is: Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue)

Allergic or Hypersensitivity Reactions

Record all allergic and hypersensitivity reactions with both grade severity and attribution. Serious allergic or hypersensitivity reactions (≥ Grade 3) that are deemed possibly related to the study treatment by the study PI (or designee) will be reported to the protocol team and SMC within 24 hours of notification and to the NIH/DAIDS, UNC IRB, FDA, and study product sponsors within 48 hours per their reporting requirements.

Dose Limiting Toxicity (DLT)

- Hematologic dose-limiting toxicity will be defined as any confirmed toxicity ≥ Grade 2, that cannot clearly be attributed to another reversible cause.
- Follow participants per protocol schedule of events until the lab abnormality or toxicity resolves to ≤ Grade 1 or the participant's baseline.

- Non-hematologic dose-limiting toxicity will be defined as any confirmed symptomatic ≥ Grade 3, if related (definitely, probably, or possibly) to vaccine/placebo.

Monitoring HIV RNA levels

In the event of viremia of ≥ 50 copies/mL the following should occur per standard of care:

- Adherence to ART should be carefully assessed and documented.
- A standard HIV RNA assay should be repeated within 1-4 weeks.
- HIV resistance testing will be performed at the time of drawing a confirmatory sample and as indicated for persistent viremia.
- For HIV RNA confirmed >200 copies/mL on repeat testing, stored samples may be tested for the presence of one or more ARV drugs in the participant's regimen.
- HIV RNA will be repeated every 2 weeks, or sooner as clinically indicated, until <50 copies/mL and the participant can continue on study.
- In the event of confirmed viremia and documented adherence to ART, ART should be managed by the primary care provider in discussion with the study team. The results of the HIV resistance tests will be shared with the participants and their care providers.

The Protocol Core Team will monitor the conduct and safety of the study via monthly meetings and regular summaries. Accrual, baseline characteristics, conduct of the study (including premature study discontinuations), any interruptions of ART, virologic failures, and all reported toxicities and events will be monitored during the study and discussed with the protocol team on a monthly basis or more frequently, if needed.

The study protocol team will review the individual safety data monthly to assess relation of all reported toxicities and AEs to the study treatment. A study unique independent Safety Monitoring Committee (SMC) will receive monthly study progress and safety monitoring reports. Study feasibility and the achievement of study milestones will be assessed in these reports.

The DAIDS program and medical officers will review and assess the monthly safety reports, as well as EAE reports for potential impact on the study participant safety and protocol conduct as per DAIDS policies, guidance documents, and SOPs, as applicable.

8.3.5 Serious Adverse Event Reporting

All SAEs at least probably related to **M3** or **M4** will be considered unexpected and be reported as SUSARs within the regulatory timelines.

All SAEs occurring during the study must be reported to the UNC IRB per the UNC IRB reporting requirements. A written report (Serious Adverse Event Report Form) will be submitted to DAIDS and DAIDS MO within 24 hours of site becoming aware of the SAE. Additional information will be supplied as requested. All SAEs occurring during the study will be reported to DAIDS following guidelines for expedited AE reporting and to the FDA according to their regulatory guidelines.

After 30 days following the last dose of vaccine/placebo, only SAEs the study PI (or designee) considers related to study product or a protocol procedure, should be reported. Information regarding SAEs will be submitted to the DAIDS on required SAE reporting forms, which must be completed and signed by the study PI or designee, and sent within 24 hours of the site becoming aware of the SAE.

All Grade 3 or Grade 4 AE/SAEs considered related to study product must be followed until recovery to baseline or Grade 1 with the date of resolution recorded in the source documents. In addition, the investigator should report all follow-up for reportable Grade 3 or Grade 4 AE/SAEs to the UNC IRB. Resolution of an event is defined as the return to pre-treatment status or stabilization of the condition with the expectation that it will remain chronic.

All SAEs that have not resolved by the end of the study, or that have not resolved upon discontinuation of the participant's participation in the study, must be followed until any of the following occurs:

- The event resolves
- The event stabilizes
- The event returns to baseline, if a baseline value/status is available
- The event can be attributed to etiology other than the vaccine/placebo or to factors unrelated to study conduct
- It becomes unlikely that any additional information can be obtained (participant’s or health care practitioner’s refusal to provide additional information, or lost to follow-up after demonstration of due diligence with follow-up efforts)

Any event requiring hospitalization (or prolongation of hospitalization) that occurs during the course of a participant's participation in a study must be reported as an SAE, except hospitalizations for the following:

- Hospitalizations not intended to treat an acute illness or adverse event (e.g., social reasons such as pending placement in long-term facility)
- Surgery or procedure planned before entry into the study (must be documented in the source document)

The study PI (or designee) will be responsible for notifying the UNC IRB, FDA, the University of Oxford, and DAIDS of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the information. In addition, the sponsor must notify FDA in an IND safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days.

8.3.6 Reporting Events to Participants

We are engaged in a number of similar studies of novel immunotherapies for HIV, persistent HIV infection, monoclonal antibodies, latency reversing agents, and other interventions. We report the scientific findings of our work in the literature, and have regular (annual or more frequent) presentations with the local and national HIV-infected and affected community. Due to the lack of evidence thus far that our studies have a clinical impact, we discuss the scientific findings in a general way with each participant after EOS visits. Our studies have extremely stringent stopping criteria, and thus far, we have had no relevant or study-related SAEs to report. We would inform all study participants by letter or electronic messaging of any AEs determined by the External Study Safety Monitoring Committee to merit such notice.

8.3.7 Events of Special Interest

An adverse event of special interest (AESI; serious or non-serious) is one of scientific and medical concern specific to the protocol team, for which ongoing monitoring and rapid communication to the protocol team is required. These events might warrant further investigation in order to characterize and understand it. Depending on the nature of the event, rapid communication by the protocol team to the UNC IRB, FDA, NIH, and product manufacturers (i.e., regulators) might also be warranted.

AESI for this protocol include those listed below.

- Grade 3 or higher Injection site reactions
- Immune-related AEs attributed to study treatment of any grade

8.3.8 Reporting of Pregnancy

If the participant becomes pregnant during the study, vaccine/placebo will not be administered. Although not considered an adverse experience, it is the responsibility of investigators or their designees to report any pregnancy in a participant or participant's partner (spontaneously reported to them) that occurs during the study or within 4 months after vaccination. All participants or their partners who become pregnant must be followed to the completion/termination of the pregnancy. If the pregnancy continues to term, the outcome (health of infant) must also be reported the UNC IRB, the DAIDS Medical Officer, and the FDA (US Food and Drug Administration).

If a male participant impregnates his partner while he is participating in this study, the pregnancy must be reported and the study PI (or designee) should make a concerted effort to follow the pregnancy and outcome. The study PI (or designee) will make every effort to obtain a medical release and a separate pregnancy outcome consent from the pregnant partner granting permission to follow the health of both the pregnant partner and her unborn child to the UNC IRB, DAIDS, study product sponsor, and FDA without delay and within 24 hours if the outcome is an SAE (e.g. death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn).

Pregnancy and pregnancy outcome will be recorded. 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 woman has completed the study or chooses to discontinue from the study before the end of the pregnancy, then site study staff should request permission via a separate pregnancy outcome consent to contact her regarding pregnancy outcomes at the end of pregnancy. If the information is obtained, pregnancy outcomes will be submitted at the end of the pregnancy.

Pregnant women will discontinue study treatment and will be encouraged to continue on study and complete the evaluations included in the post-treatment evaluation section. At the end of the pregnancy, outcome and adverse events for participant and infant will be recorded. If pregnancy is suspected in a woman on study after study treatment, then a pregnancy test should be obtained. If pregnancy is confirmed, then further study treatment will be discontinued and the woman should continue on study (off study product) safety follow-up visits as noted in the SOE. Stored plasma/PBMC for stored plasma for virologic studies should not be obtained to minimize blood volume.

The site study staff should request permission via a separate pregnancy outcome consent to contact her regarding pregnancy outcomes at the end of pregnancy. A visit 6 months

following the end of pregnancy will be conducted to assess for evidence of adverse events (AEs) in the participant and infant and documented.

8.4 New Safety Information

8.4.1 Definition of New Safety Information (NSI)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others as NSI and this includes, in general, any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures/study treatment that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol, the informed consent document, and Investigator's Brochure (IB); and (b) the characteristics of the participant population being studied;

- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures/treatment involved in the research); and

- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.4.2 NSI Reporting

The investigator will report NSI to the UNC IRB. The NSI report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an NSI;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the NSI.

To satisfy the requirement for prompt reporting, NSI reports will be reported using the following timeline:

- NSI that are serious adverse events (SAEs) will be reported to the IRB and the DAIDS MO within 3 business days of the investigator becoming aware of the event.

- Any other NSI will be reported to the IRB and to the DAIDS MO within 7 business days of the investigator becoming aware of the event.

- All NSI should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the NIH MO (or designee), and the Office for Human Research Protections (OHRP) within 30 business days of the IRB's review and determination that the report of the problem from the investigator imposed a safety risk.

8.4.3 Reporting NSI to Participants

We would inform all study participants by letter or electronic messaging of any NSI if it is determined by the UNC IRB to merit such notice.

9 STATISTICAL CONSIDERATIONS

This is a phase I, single-site, pilot study to evaluate the safety of **M3** and **M4** given individually or in combination to HIV-infected individuals suppressed on ART. There are two secondary analyses and multiple exploratory objectives to measure the effect of this therapy on HIV-specific T cell responses and on persistent HIV infection.

9.1 Statistical Hypotheses

Hypothesis

Intramuscular (IM) vaccination with **M3** or **M4**, given individually or in combination (**M3+M4**) in adult HIV-infected participants on suppressive combination antiretroviral therapy (ART) will be safe and increase HIV-1-specific T cell responses targeting conserved regions of HIV-1.

The simultaneous administration of **M3** with **M4** (**M3+M4**) will result in a greater increase in the breadth of HIV-1-specific T cells targeting conserved regions of HIV-1 than with the individual administration of **M3** or **M4** alone.

9.2 Sample Size Determination

9.2.1 Primary Sample Size Consideration

From previous publications employing MVA- vectored vaccines, the proposed number of participants (7 vaccinees per arm and 3 placebo controls) is sufficient to evaluate the risk

of SAEs or severe local or systemic reactions in the study population. Descriptive analyses will be used to summarize AEs or SAEs in each arm as well as combined MVA arms.

Enrollment into the trial will be suspended if two participants experience a study treatment-related toxicity of Grade 3 or higher or if one or more participants experience an SAE possibly related to study treatment (See Section 10.1.6). If zero of 7 participants experience a safety event, a 95% 1-sided exact binomial upper confidence limit for the probability of a safety event will be 35%. If one participant experiences a safety event, the corresponding 95% 1-sided upper confidence limit will be 52%. Each safety endpoint will be estimated with an exact binomial 95% 1-sided upper confidence limit. If zero safety events are observed in the three vaccine arms and data are pooled across arms (n=24), then the upper limit of the exact, 1-sided 95% confidence interval (CI) will be 13%.

9.2.1.1 Procedure for accounting for missing and unused data

Based on previous experience with similar trials, we anticipate that the amount of missing or invalid data will be small. All participants who are evaluable for adverse event assessment will be included in the safety analyses, regardless of whether they are replaced or missing for additional endpoints. Participants who do not start study treatment or are unable to provide the first or second leukapheresis will be replaced to achieve the planned evaluable sample size. Given the small size of this study, formal imputation methods will not be used. To assess whether key findings are sensitive to missing data, missing values can be replaced with worst possible outcomes as a sensitivity analysis (e.g., by replacing missing values in the M3 and M4 only arms with the best rank and replacing missing values in the M3+M4 arm with the worst rank, to assess if a finding regarding the M3+M4 arm is sensitive to missing data).

9.2.1.2 Study Duration and Accrual

Participants will be screened approximately 75 days prior to vaccination and will be followed for 168 days after vaccination. The total study duration from screening to completion of follow-up per participant will be approximately 33.5 weeks (~8.4 months). We will recruit and screen up to 40 participants to obtain 24 participants with evaluable data to study. Given the focus of this study is on safety and potential adverse events, accrual will be staggered such that enrollment and treatment administration will include a maximum of 1 participant per week.

9.2.1.3 Randomization

Following enrollment, the 24 study participants will be randomly assigned to receive one vaccination with either **M3** (n=7), **M4** (n=7), **M3**+**M4** (n=7) or placebo (n=3) through a blinded randomization schedule.

9.2.1.4 Un-blinding procedures

Un-blinding of an individual participant is indicated in the event of a medical emergency where the clinical management would be altered by knowledge of the group assignment. The decision to un-blind will be taken in conjunction with the independent members of the SMC. Procedures and contact details for un-blinding procedures will be held in a site-specific SOP. The site study personnel will ensure that the reasons for un-blinding are documented in the CRF.

All study participants will be un-blinded when the last vaccinated participant has completed the Day 28 Visit and the second leukapheresis.

9.3 Statistical Analyses

9.3.1 General Approach

We plan a double blind, randomized, placebo-controlled, parallel design study to evaluate the safety and immunogenicity of viral-vector, MVA, expressing immunogens, tHIVconsv3 (**M3**) and tHIVconsv4 (**M4**), derived from conserved yet immunogenic regions of HIV-1. The study population is HIV-1 infected adults on c-ART with plasma HIV-1 RNA <50 copies/mL.

Participants will be randomized to receive **M3** or **M4** alone, **M3+M4** together, or placebo at Day 0 and continue baseline ART. At Day 28, all participants undergo a 2nd leukapheresis to evaluate for an increase in the breadth of T cells targeting conserved regions of HIV-1. Unblinding will occur after the last participant has completed the Day 28 visit and leukapheresis. Participants will be followed for safety assessments through Day 168.

9.3.2 Immunological and Virologic Analysis

Given the small sample size in this study, exact methods that do not rely on large sample assumptions will be used for statistical inference and will always be presented with corresponding descriptive statistics and/or data visualization. Because this is a Phase 1 study with a small sample size and exploratory endpoints, results will be presented without adjustment for multiple hypothesis testing; a statistical significance level of 0.05 will be used. Whenever feasible, data visualization will present individual-level data points as well as summary statistics. Emphasis will be placed on estimated effect sizes and descriptive summaries. The sample size was chosen to evaluate safety and the primary efficacy outcome; this study includes a small total number of participants and thus may not detect all scientifically meaningful effects at a .05 statistical significance level. All study results should be interpreted within the context of the study eligibility criteria and the population available and willing to participate in this double-blinded, placebo-controlled randomized clinical trial.

9.3.3 Analysis of the Primary Endpoint(s)

The probability of a primary safety event through Day 28 following vaccination will be estimated with a proportion and a corresponding 95% 1-sided exact binomial upper confidence limit.

9.3.4 Analysis of the Secondary Endpoint(s)

9.3.5 Immunological and Virologic Analysis

To evaluate changes in HIV-1 epitope-specific $CD8^+$ T cell responses detected in IFN-γ ELISpot assays before and after vaccination, the relative change within participants (from Day 0 to both Day 7 and Day 14) will be estimated using a geometric mean ratio and evaluated with an exact 2-sided Wilcoxon signed-rank test. If the symmetry assumption for change in natural log-transformed summed HIV-1 specific T cell response appears violated, a sign test will be conducted as a sensitivity analysis. Efficacy and exploratory endpoints defined as a proportion of participants will be analyzed with a 2-sided binomial 95% CI. Within a vaccination arm, changes in continuous measurements over time (e.g., change in an immune parameter from baseline to post-vaccination) will be summarized graphically and evaluated with an exact 2-sided Wilcoxon signed-rank test, or a sign test for endpoints that appear to violate assumptions of the Wilcoxon test.

Observed immunologic measurements, such as the change in breadth following vaccination, may not be normally distributed or may be partially censored due to assay limits of quantification, and thus, an exact Wilcoxon rank-sum test will be used for between-arm comparison.

The focus of our **secondary** objectives is to examine the effect of vaccination on within-participant T cell magnitude and between-arm breadth of HIV-specific T cell responses.

9.3.6 T cell Magnitude:

The primary *within arm* outcome will be the summed HIV-specific T cell response, either to peptides pools spanning the immunogen or pools of optimal HIV $CD8^+$ epitopes. HIV specific T cell responses will be measured at baseline, Day 7, and Day 14 (following **M3**, **M4,** or **M3**+**M4** vaccination). An average fold change of at least 2 (comparing T cell responses within participant) was anticipated to be scientifically meaningful. The specific alternative hypothesis is a geometric mean ratio (GMR) of 2. The assumed between-participant standard deviation (SD) for natural log-transformed HIV-specific T cell response was estimated from previous baseline data derived from HIV-infected participants durably suppressed by ART sampled weekly or monthly. Data were provided by Dr Goonetilleke (66). Empirical power for the exact Wilcoxon signed-rank test was calculated using 100,000 simulated datasets assuming natural log-transformed HIV-specific T cell

response follows a normal distribution, and the effect size under the alternative hypothesis was a GMR of 2 on the raw HIV-specific T cell response measures. A between-participant SD of 0.8 at baseline was observed using log-transformed HIV-specific T cell response from previous baseline data; the same SD for both pre-vaccination and post-vaccination measurements was assumed to calculate power. The estimated correlation within a participant was >0.9 between paired log-transformed HIV-specific T cell response measures. Using a 2-sided exact Wilcoxon signed-rank test and a 0.05 significance level, n=7 vaccinated participants provides >95% power to detect a GMR of 2 or greater for a pre- and post-vaccine paired measurement correlation of 0.9 and a between-individual SD of 0.8. For a smaller within-individual correlation of 0.8 and the same assumptions otherwise, the estimated power is 82%.

Comparison of peak HIV specific T cell responses *between* arms (**M3**+**M4** vs **M3**, **M3**+**M4** vs **M4**) will be compared using a 2-sided exact Wilcoxon rank-sum test. With n=7 per vaccination group it is possible to achieve an exact p-value <0.05. Assuming a 90% probability that the peak HIV specific T cell responses for any given participant in the **M3**+**M4** arm is higher than for any given individual in the **M3** arm, there is >80% power to detect a difference between arms. This same power calculation applies for the **M3**+**M4** vs **M4** comparison. Power for the exact Wilcoxon rank-sum test was calculated in R version 3.4.3 using the wmwpow package.

9.3.7 T cell Breadth:

Observed immunologic measurements, such as the change in breadth following vaccination, may not be normally distributed, and thus, a 2-sided exact Wilcoxon rank-sum test will be used for comparisons between arms.

We anticipate zero change in T-cell breadth for the **M3** and **M4** arms. For the **M3**+**M4** arm, we anticipate change of breadth ranging from 0-6 epitopes (Table 13). Data in Table 13 describes the distribution of HIV-specific T-cell responses identified in 20 HIV-infected, durably suppressed participants provided by Dr Goonetilleke.

Table 13 Distribution of Regions of HIV Epitopes Targeted by T-Cells in HIV-1 Infected Participants Durably Suppressed with ART

# Epitopes found in tHIVconsvX	% distribution
0	15
1	15
2	30

3	20
4	5
5	10
6	5

Given the assumptions in Table 13 and above (zero change in T cell breadth for the M3 and M4 only arms), n=7 evaluable participants per arm provides >90% power to detect a difference between arms (M3+M4 vs M3, M3+M4 vs M4) using a 2-sided exact Wilcoxon rank-sum test and a 0.05 significance level.

9.3.8 Safety Analyses

For the safety endpoints, we will describe all study treatment-related AEs through Day 28 (primary endpoint) and the end of study (secondary endpoint). AEs will be coded per the Medical Dictionary for Regulatory Activities (MedDRA). Events prior to treatment (e.g., due to study-related procedure) will be listed separately in an appendix to the final clinical study report. The following tables of AE data will be created to summarize the number and percent of participants who experience at least one event of each of the following types:

- Study treatment-related AEs by severity grade
- All SAEs (this may be a listing if there are few events)
- Study treatment-related SAEs
- Fatal AEs (this may be a listing if there are few events)
- AEs that result in study discontinuation or study treatment discontinuation
- AEs categorized as AESI
- AEs with severity Grade 3 or greater
- Study treatment-related AEs with severity Grade 3 or greater
- All treatment-related AEs with severity Grade 1 or greater

All of these tables will display the number and percent of participants that experience the given event and will display events by System Organ Class (SOC) and Preferred Term (PT). Events will be displayed alphabetically for SOC and in descending order of overall PT incidence within each SOC.

9.3.9 Tabulation of Individual Participant Data

Individual participant data will be listed by measure and time point.

9.3.10 Exploratory Analyses

Other objectives or exploratory analysis can be grouped into additional immunological measures and virologic measures.

Immunologic measures: Kinetic data will be analyzed by descriptive statistics and graphical displays. Other observed immunologic measurements such as virus inhibition may not be normally distributed or may be partially censored due to assay limits of quantification, and thus, an exact Wilcoxon signed-rank test will be used for within-participant comparisons.

Virologic measures: Virologic measures such as cell-associated HIV-1 RNA will be log-transformed prior to analyses. Appropriate analysis method will be used for plasma HIV-1 RNA by SCA depending on the amount of results below assay limit. For example, if a substantial fraction (~50%) are below the assay limit, the analysis will focus on estimating the proportion of participants with detectable SCA at various time points and comparison between arms will use Fisher's exact test; otherwise, changes within participants will be summarized and compared between arms using Wilcoxon rank-sum tests.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 Regulatory, Ethical, and Study Oversight Considerations

10.1.1 Informed Consent Process

10.1.1.1 Consent/assent and Other Informational Documents Provided to Participants

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to any screening procedures and prior to starting study intervention/administering study intervention.

10.1.1.2 Consent Procedures and Documentation

Informed consent is a process initiated prior to individuals agreeing to participate in the study and continues throughout the individual's study participation. The consent forms are UNC IRB -approved and the participant will be asked to read and review the document. The study investigator (or designee) and/or study coordinator will explain the research

study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants are provided the opportunity to discuss the study with their family, primary care provider, or significant other or to just think about the study and its requirements prior to agreeing to participate. The participant signs the informed consent document prior to any procedures being done specifically for the study. Participants are informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants.

The informed consent process will be conducted and documented in the source document, and the consent form signed, before the participant undergoes any study-specific procedures. The study PI (or designee) or the research coordinator will inform participants that the quality of their medical care will not be adversely affected if they decline to participate in this study, thus emphasizing the protection of the rights and welfare of the participants.

10.1.2 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, funding agency (NIH), the FDA, the University of Oxford, and the UNC IRB. If the study is prematurely terminated or suspended, the study PI (or designee) will promptly inform study participants, the UNC IRB, and DAIDS and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to the study visit schedule.

Circumstances that may warrant termination or suspension include but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, the UNC IRB, DAIDS, and/or the Food and Drug Administration (FDA).

10.1.3 Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their study staff, and the sponsor(s)/funding source(s) and their representatives. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the UNC IRB, regulatory agencies, or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the study PI, including but not limited to, medical records and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participants' contact information will be securely stored at the clinical study site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be stored at the UNC HIV Cure Center. This will not include the participants' contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the clinical staff of the UNC HIV Cure Center and by research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived.

10.1.3.1 Certificate of Confidentiality

To further protect the privacy of study participants, a Certificate of Confidentiality will be issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of

Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10.1.4 Future Use of Stored Specimens and Data

Data collected for this study will be analyzed and stored at the Goonetilleke Laboratory in the UNC HIV Cure Center. After the study is completed, the de-identified, archived data will be stored in the UNC HIV Cure Center Database, for use by the Goonetilleke Research Laboratories and their collaborators. Permission to transmit data to researchers outside the Goonetilleke Collaboration will require review and approval by the UNC IRB. In some circumstances, we will need to obtain additional consent from participants to share samples collected for the purpose of this study.

With the participants' approval and as approved by the UNC IRB, de-identified biological samples will be stored at the Goonetilleke and Archin/Margolis Laboratories both in the UNC HIV Cure Center and/or the UNC Chapel Hill HIV/STD Laboratory Core. These samples could be used to research the causes of HIV cure for which individuals with HIV infection can greatly benefit. These samples may also be used for genetic testing. The Goonetilleke Research Laboratory will also be provided with a code-link that will allow linking the biological specimens with the phenotypic data from each participant, maintaining the blinding of the identity of the participant.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to biosample storage may not be possible after the study is completed.

When the study is completed, access to study data and/or samples will be provided through the Goonetilleke Laboratory in the UNC HIV Cure Center Laboratory.

10.1.5 Key Roles and Study Governance

Key roles and study governance will be provided as in

Table 14.

Table 14 Key Roles

Protocol Principal Investigator	**Grant Principal Investigator**
Cynthia Gay, MD, MPH	Nilu Goonetilleke, PhD
Associate Professor, Medical Director HIV Cure Center	Assistant Professor, Dept. of Microbiology & Immunology/Medicine
University of North Carolina at Chapel Hill	University of North Carolina at Chapel Hill

130 Mason Farm Road, Suite 2112 Bioinformatics Building, Campus Box 7030	120 Mason Farm Road, 2017 Genetic Medicine Building, Campus Box 7042
Chapel Hill, NC 27599-7030	Chapel Hill, NC 27599-7042

10.1.6 Safety Oversight

Safety oversight will be under the direction of a Safety Monitoring Committee (SMC) composed of individuals with the appropriate expertise. Members of the SMC are independent from the study conduct and free of conflict of interest.

The SMC advises the study PI and protocol team for this First in Man, Phase I study. The primary responsibility of the SMC is to monitor human subject safety. The SMC considers study-specific data as well as relevant background information about the disease, test agents, and target population under study.

During the trial, the SMC will review:

- Real-time and cumulative safety data for evidence of study-related adverse events;
- Adherence to the protocol;
- Factors that might affect the study outcome or compromise the trial data (such as protocol violations, losses to follow-up, etc.).
- Review the achievement of enrollment benchmarks

The SMC will receive monthly reports via email for review and comments. The SMC will be contacted directly via email (and possibly via teleconferencing) for any event or situation that impacts participant safety throughout the study and specified interventions or study participation will be suspended or terminated dependent on the response of the SMC.

The study will undergo review at least annually by the SMC. The SMC will review information on accrual, baseline characteristics, conduct of the study (including premature study discontinuations and premature study treatment discontinuations), AEs by treatment arm (including protocol team assessment of relationship to study treatment), virologic failures, and HIV-1 RNA levels over time, and completeness of follow-up.

<u>Safety Pause</u>
Enrollment into the study and treatment injections will be temporarily suspended and the Study Monitoring Committee (SMC), unblinded to treatment assignment, will be asked to review all safety data. Review will include the relation to study treatment of the event(s) thought by the blinded core team to be a primary safety outcome, if any of the following occur:

a) Two or more participants experience a primary safety outcome measure that is a Grade ≥3 AE possibly related to study treatment (as judged by the core team, blinded to treatment arm); or

b) One or more participants experience an SAE possibly related to study treatment (as judged by the core team, blinded to treatment arm).

Following the review, the SMC will recommend if and how the study should proceed with respect to resuming enrollment and continuing study treatment.

10.1.7 Clinical Monitoring

To ensure the safety of participants in the study, compliance with applicable regulations, and to ensure accurate, complete, and reliable data, the study PI will keep records of laboratory tests, clinical notes, and participant medical records in the participant files as source documents for the study.

An independent study monitor will monitor the study on a regular basis throughout the study period according to the study monitoring plan. The study PI (or designee) will allocate adequate time for such monitoring activities. The study monitor periodically will conduct a review of a sample of the participant data recorded on source documents at the study site. The study PI (or designee) will also ensure that the monitor is given access to all the above noted study-related documents, source documents (regardless of media), and study-related facilities (e.g., IDS pharmacy, etc.), and has adequate space to conduct the monitoring visit. Queries may be raised if any datum is unclear or contradictory. The study PI and site study personnel must address all queries in a timely manner.

Participation as an Investigator in this study implies acceptance of the potential for inspection by the study Funder and its Representatives, US or non-US government regulatory authorities, IRB, and applicable compliance and quality assurance offices. The study PI (or designee) will permit study-related audits and inspections and will provide access to all study-related documents (e.g., source documents, regulatory documents, data collection instruments, study data, etc.). The study PI will ensure the capability for inspections of applicable study-related facilities (e.g., IDS pharmacy, CTRC, etc.).

10.1.7.1 Minimizing risk to participants

Procedures to minimize risk to participants in the conduct of this study include:

- Informing participants about risks so they can recognize and report harms in partnership with the study team;
- respecting local/national blood draw limits;

- direct observation of participants after vaccine/placebo administration and collection of information regarding side effects for several days post product administration;

- having study staff properly trained in administering study procedures that may cause physical harm or psychologic distress, such as blood draws and injections; and

- providing study monitoring.

10.1.8 Quality Assurance and Quality Control

UNC will perform internal quality management of study conduct, data and biological specimen collection, documentation, and completion. A quality management plan was developed to describe the quality management program.

UNC follows Standard Operating Procedures (SOPs) for quality management. Clinical research files verify and insure that the clinical trial is conducted per protocol and that data is generated and biological specimens collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonization Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)). Data Quality is monitored per the data quality management plan with routine and specific research chart review.

The study staff will be educated on the protocol and training will be provided as needed to implement protocol procedures. The study data management team will be responsible for addressing QA issues (e.g., correcting procedures that are not in compliance with the protocol) and QC issues (e.g., correcting errors in data entry). Documentation, as required, will be maintained in the regulatory files.

UNC will provide direct access to all trial-related sites, source data/documents, and reports for the purpose of monitoring and auditing by the funding sponsor, and inspection by local and regulatory authorities.

10.1.9 Data Handling and Record Keeping

10.1.9.1 Data Collection and Management Responsibilities

The clinical research staff is responsible for data collection under the supervision of the site study PI (or designee). The study PI (or designee) is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Hard copies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into a UNC School of Medicine Database. The data system includes password protection. Clinical data will be entered directly from the source documents.

10.1.9.2 Study Records Retention

Per ICH guidelines, all essential documents, including source documents (regardless of media), signed ICFs, and laboratory test results, should be retained by the study PI (or designee) for at least 2 years after last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since formal discontinuation of clinical development of the investigational products.

10.1.10 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, International Conference on Harmonization Good Clinical Practice (ICH GCP), or Manual of Procedures (MOP) requirements. The noncompliance may be on the part of the participant, the investigators, or the study site staff. As a result of deviations, corrective actions will be developed and implemented promptly, when necessary.

These practices are consistent with ICH GCP:

It is the responsibility of the site PI (or designee) to use continuous vigilance to identify and report deviations at the annual renewal of the protocol, provided there is no impact on participant safety as a result of the deviation. All deviations must be addressed in study source documents. Protocol deviations are sent to the UNC IRB per their policies. The site PI is responsible for knowing and adhering to reviewing the IRB requirements. Further details about the handling of protocol deviations will be included in the SOP.

10.1.11 Publication and Data Sharing Policy

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

- National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH-funded research. It requires

scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

- NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov, if required.

- NIH Genomic Data Sharing Policy, which applies to all NIH-funded research that generates large-scale human or non-human genomic data, as well as the use of these data for subsequent research. Large-scale data include genome-wide association studies (GWAS), single nucleotide polymorphisms (SNP) arrays, and genome sequence, transcriptomic, epigenomic, and gene expression data.

10.1.12 Conflict of Interest Policy

The University of North Carolina at Chapel Hill recognizes that conflicts of interest will arise from the research enterprise, from technology transfer activities, and from the many facets of our investigators' professional activities. UNC seeks to identify and manage these conflicting relationships, restricting activities where necessary, to preserve transparency, independent decision-making, protection of research participants, and integrity of the educational experience. UNC's Conflict of Interest Program will have oversight over this study.

10.2 Abbreviations

4A4OA	4-anilino-4oxobutanoic acid
ACD	Acid Citrate Dextrose
AE	Adverse Event
AESI	Adverse Event of Special Interest
AHA	Autoimmune Hemolytic Anemia
AHI	Acute HIV Infection
Alk Phos	Alkaline Phosphatase
ALT	Alanine Transaminase
ANA	Antinuclear Antibody
ANC	Absolute Neutrophil Count
ANCOVA	Analysis of Covariance
Anti-dsDNA	Anti-double-stranded DNA, IgG

APTT	Activated Partial Thromboplastin Time
ART	Antiretroviral Therapy
AST	Aspartate Transaminase
ATI	Analytic Treatment Interruption
AUC	Area Under the Curve
BMI	Body Mass Index
BP	Blood Pressure
BUN	Blood Urea Nitrogen
CBF	Clinical Biomanufacturing Facility
CBC	Complete Blood Count
CEF	Chicken Embryo Fibroblast
CFAR	Center for AIDS Research
CFR	Code of Federal Regulations
ChAd	Simian Adenovirus
CLIA	Clinical Laboratory Improvement Amendments
CM	Clarification Memo
CMP	Clinical Monitoring Plan
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
COVID-19	SARS-CoV-2 virus
CPK	Creatine Phosphokinase or Creatine Kinase (CK)
CRF	Case Report Form
CSRC	(DAIDS) Clinical Science Review Committee
CTRC	Clinical and Translational Research Center
DAIDS	Division of AIDS
D/C	Discontinue
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DNCB	Dinitrochlorobenzene
DSMB	Data Safety Monitoring Board
DRE	Disease-Related Event
E/CIA	Enzyme or Chemiluminescence Immunoassay

EC	Ethics Committee
ECG	Electrocardiogram
eCRF	Electronic Case Report Forms
eGFR	Estimated Glomerular Filtration Rate
EOS	End of Study
EUA	Emergency Use Authorization
FNA	Fine Needle Aspiration
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FFR	Federal Financial Report
FSH	Follicle Stimulating Hormone
GBS	Guillian-Barré Syndrome
G-CSF	Granulocyte-Colony Stimulating Factor
GM-CSF	Granulocyte Macrophage-Colony Stimulating Factor
GCP	Good Clinical Practice
GFP	Green Fluorescent Protein
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GWAS	Genome-Wide Association Studies
HBsAg	Hepatitis B Surface Antigen
HCV	Hepatitis C Virus
HCVAb	Hepatitis C Virus Antibody
HIPAA	Health Insurance Portability and Accountability Act
HLA	Human Leukocyte Antigen
IB	Investigator's Brochure
IBC	Institutional Biosafety Committee
ICF	Informed Consent Form
ICH	International Conference on Harmonization
ICMJE	International Committee of Medical Journal Editors
ID	Intradermal
ID Clinic	Infectious Diseases Clinic
IFNγ	Interferon gamma

IgG	Immunoglobulin G
IDS	Investigational Drug Services
IL-1	Interleukin - 2
IL-12	Interleukin - 12
IM	Intramuscular
IND	Investigational New Drug Application
INR	International Normalized Ratio
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ISO	International Organization for Standardization
ITT	Intention-To-Treat
IUD	Intrauterine device
IV	Intravenous
LAN-HSD	Los Alamos National Laboratory HIV Sequence Database
LDH	Lactate Dehydrogenase
LDL	Low Density Lipoprotein
LMP	Last Menstrual Period
LOA	Letter of Amendment
LRA	Latency Reversing Agent
LSMEANS	Least-Squares Means
LTFU	Lost to Follow-up
M3	MVA.tHIVconsv3
M4	MVA.tHIVconsv4
MedDRA	Medical Dictionary for Regulatory Activities
mg/dL	milligram per deciliter
ml/min	milliliter per minute
mm Hg	millimeters of mercury
MMR	Measles, Mumps, Rubella
MO	Medical Officer
MOI	Multiplicity of Infection
MOP	Manual of Procedures
MSDS	Material Safety Data Sheet

MVA	Modified Vaccinia virus (Ankara strain)
MVA.HIVA	MVA expressing the HIVA immunogen, comprising p24 and HIV epitope string
MVA.HIVconsv	MVA expressing the 1st generation conserved HIV immunogen, HIVconsv
MVA.tHIVconsv3	MVA expressing a 2nd generation conserved HIV immunogen, tHIVconsv3
MVA.tHIVconsv4	MVA expressing a 2nd generation conserved HIV immunogen, tHIVconsv4
NCT	National Clinical Trial
NIH	National Institutes of Health
NIH IC	NIH Institute or Center
NRTI	Nucleoside Reverse Transcriptase Inhibitor
NNRTI	Non-nucleoside Reverse Transcriptase Inhibitor
NSI	New Safety Information
OHRP	Office for Human Research Protections
OSP	Office of Science Policy
OTC	Over the Counter
P	Pulse
PBMC	Peripheral Blood Mononuclear Cells
PE	Physical Exam
pfu	Plaque-forming units
PHI	Primary HIV Infection
PI	Principal Investigator
PID	Participant Identification Number
PI	Protease Inhibitor
POCT	Point of Care Test
PT	Preferred Term
PT	Prothrombin time
QA	Quality Assurance
QC	Quality Control
RID	Randomization Identification Number
rMVA	Recombinant MVA
RFP	Red Fluorescent Protein
RPR	Rapid Plasma Reagent
RR	Respiratory Rate

SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SFU	Spot-forming units
SID	Study Identification Number
SMC	Safety Monitoring Committee
SNP	Single Nucleotide Polymorphism
SOE	Schedule of Events
SOC	System Organ Class
SOP	Standard Operating Procedure
SUSAR	Serious unexpected serious adverse reaction
T	Temperature
TAF	Tenofovir Alafenamide
TDF	Tenofovir
TNF	Tumor Necrosis Factor
tPA-LS	Human tissue activator sequence
ULN	Upper Limit of Normal
UNC	University of North Carolina
UP	Unanticipated Problem
US	United States
VS	Vital Signs
WHO	World Health Organization
WOCBP	Women of Child Bearing Potential

10.3 Protocol Amendment History

Version	**Date**	**Description of Change**	**Brief Rationale**
Draft	20 April 2018	Original version	To DAIDS CSRC
1.0	10 Sept 2018	Incorporation of DAIDS CSRC and FDA suggested changes	DAIDS CSRC and FDA review
1.1	14 Nov 2018	Incorporation of UNC IRB and DAIDS RSC suggested changes and UNC Protocol Team changes	DAIDS RSC and UNC IRB and UNC Protocol Team review

2.0	02 July 2019	Incorporated CM#1 & CM#2; decreased number of participants to 24; decreasing each active arm (Arm 1, 2 & 3) to n=7. Modifications to Inclusion and Exclusion Criteria and terms regarding the re-assignment of randomization ID	To DAIDS MO and RSC Review
3.0	06 January 2021	Incorporated CMs #1 – 5 and LOAs #1 and 2 into this amendment. Additional information added to protocol to address COVID-19 vaccines and treatments received by potential participants. Also modified exclusion criteria to allow topical treatment of non-melanoma cancer; to remove exclusion of tattoos on deltoid area of arms; and modified text pertaining to the receipt of depo-provera injections. Modified Dosing and Administration section to allow administration of 2 vaccines per week.	To DAIDS MO and RSC Review

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