IGHID 12107 - A Phase I Study to Evaluate the Safety and Immunogenicity of The ChAdOx1.HIVconsv62 - MVA.tHIVconsv4 (C62-M4) or, ChAdOx1.tHIVconsv1+C62 - MVA.tHIVconsv3+M4 (C1C62-M3m4) PRIME-BOOST REGIMENS in persons with HIV-1 Suppressed on Antiretroviral Therapy – THE CM (HIV-CORE 008) STUDY

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THE CM (HIV-CORE 008) STUDY

DAIDS ES-ID Protocol Number: 38832

National Clinical Trial (NCT) Identified Number: TBD

Grant Principal Investigator: Nilu Goonetilleke, PhD

Protocol Principal Investigator: Cynthia Gay, MD, MPH

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23 October 2023

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THE CM (HIV- CORE 008) STUDY

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SIGNATURE PAGE

I will conduct the study in accordance with the provisions of this protocol and all applicable protocol-related documents. I agree to conduct this study in compliance with United States (US) Health and Human Service regulations (45 CFR 46); applicable 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: _____

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TABLE OF CONTENTS

TABLE OF TABLES

TABLE OF FIGURES

TABLE OF APPENDICES

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.

1 PROTOCOL SUMMARY

1.1 Synopsis

Title: IGHID 12107 - A Phase I Study to Evaluate the Safety and Immunogenicity of the ChAdOx1.HIVconsv62 - MVA.tHIVconsv4 (C62-M4) or, ChAdOx1.tHIVconsv1+C62 - MVA.tHIVconsv3+M4 (C1C62-M3M4) Prime-Boost Regimens in Persons with HIV-1 Suppressed on Antiretroviral Therapy – The CM (HIV-CORE 008) Study

Study Description: Phase 1, double blind, randomized, placebo-controlled, parallel design study to evaluate the safety and immunogenicity of vaccines **C62** followed by **M4** or **C62** and **C1** together (**C1C62**) followed by **M4** and **M3** together (**M3M4**). Serial vaccination will be denoted as **C62-M4** or **C1C62-M3M4.**

Hypotheses: Intramuscular (IM) vaccination with **C62-M4** or **C1C62-M3M4** in persons with HIV-1 (PWH) on suppressive antiretroviral therapy (ART) will be safe and increase HIV-1-specific T cell responses targeting conserved regions of HIV-1.

Administration of **C1C62-M3M4** will result in a greater increase in the breadth of HIV-1-specific T cells targeting conserved regions of HIV-1 than **C62-M4**.

Objectives: *Primary Objective:*

Evaluate the safety of vaccination with i) **C62-M4** and ii) **C1C62-M3M4** in PWH on ART through 28 days after the last vaccination or placebo.

Secondary Objectives:

1. Evaluate the safety of vaccination through the end of study
2. Compare the within-participant relative change in magnitude of HIV-1-specific T cell responses following vaccination with **C62-M4** or **C1C62-M3M4.**
3. Compare the between-arm change in breadth of HIV-1-specific T cell responses following vaccination **C62-M4** or **C1C62-M3M4**.

Other Objectives:

1. Compare the change in function and phenotype of HIV-1-specific T cell responses in participants from pre-vaccination to post-vaccination with **C62-M4** or **C1C62-M3M4**.

2. Evaluate the kinetics of immunologic responses in participants post-vaccination with **C62-M4** or **C1C62-M3M4.**

3. Evaluate the kinetics of $CD8^+$ T cell mediated HIV inhibition pre- and post-vaccination following vaccination with **C62-M4** or **C1C62-M3M4**.

4. Explore cellular activation of total $CD4^+$ and $CD8^+$ T cells pre- and post-vaccination with **C62-M4** or **C1C62-M3M4.**

5. Explore the impact of vaccination with **C62-M4** or **C1C62-M3M4** on low-level plasma viremia.

6. Explore the impact of vaccination with **C62-M4** or **C1C62-M3M4** on cell-associated HIV RNA in $CD4^+$ T cells.

7. Explore the impact of vaccination with **C62-M4** or **C1C62-M3M4** on total HIV DNA in $CD4^+$ T cells

8. Assess the long-term safety and tolerability of vaccination with **C62-M4** and **C1C62-M3M4**

Study Population:	PWH, inclusive of men and women ≥ 18 and ≤ 70 years of age and all ethnicities, with viral suppression on ART and a CD4 cell count ≥ 350 cells/mm^3 at screening.
Phase:	Phase 1
Description of Sites Enrolling Participants:	The University of North Carolina, Chapel Hill, North Carolina, USA Duke University Health System, Durham, North Carolina, USA

Description of Study Intervention

Double blind, randomized, placebo-controlled, parallel design study in which 18 participants with durable viral suppression are randomly assigned to receive vaccination with:

1. **C62** followed by **M4**
2. **C1C62** combined followed by **M3M4** combined, or
3. Placebo (normal saline)

Participants will be randomized 8:8:2 to one of three study arms, and receive study treatment or placebo at Days 0 and 28.

A maximum of 2 participants will receive the Day 0 vaccination per week.

Study Schema

Arm	N	Day	Treatment	Total Dose (vp)	Route	Day	Treatment	Total Dose (pfu)	Route
1	8	0	**C62**	5×10^{10}	IM	28	**M4**	1.8×10^{8}	IM
2	8	0	**C1C62***	5×10^{10}	IM	28	**M3M4**[#]	1.9×10^{8}	IM
3	2	0	Placebo[◊]	-	IM	28	Placebo[◊]	-	IM

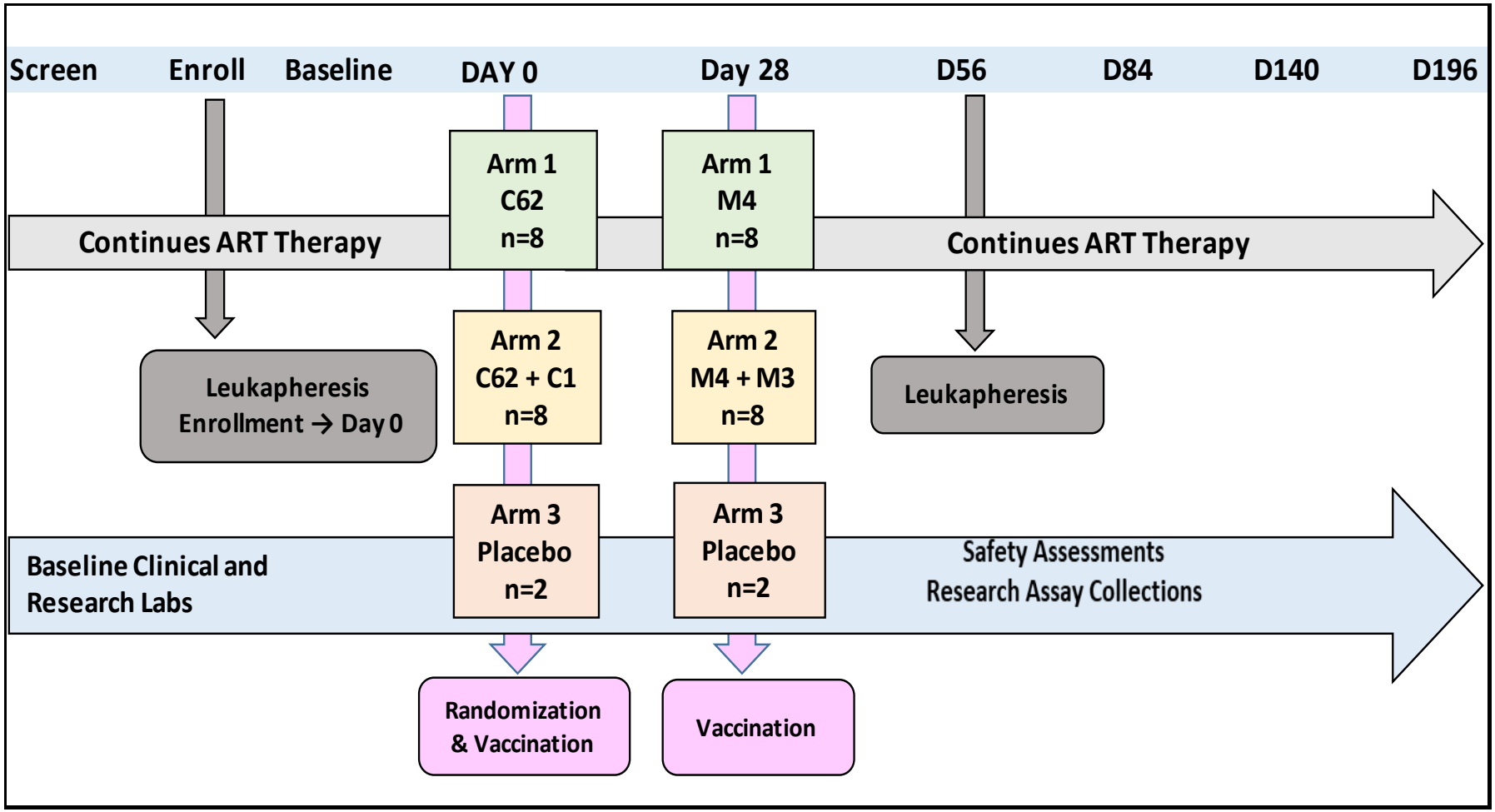
C6= ChAdOx1.HIVconsv62 (mosaic 2)
C1 = ChAdOx1.tHIVconsv1 (mosaic 1)
M4 = MVA.tHIVconsv4 (mosaic 2)
M3 = MVA.tHIVconsv3 (mosaic 1)
vp = virus particles
pfu = plaque forming units
IM = intramuscular
*2.5×10^{10} vp, each vaccine
[#] 0.9×10^{8} pfu M4; 1.0×10^{8} pfu M3
[◊] normal saline

Primary Endpoint Statistics

If zero of 8 participants experience a safety event, a 95% 1-sided exact binomial upper confidence limit for the probability of a safety event will be 31%. If one participant experiences a safety event, the corresponding 95% 1-sided upper confidence limit will be 47%. Each safety endpoint will be estimated with an exact binomial 95% 1-sided upper confidence limit. If no safety events are observed in the two vaccine arms and data is pooled across arms (n=16), then the upper limit of the exact, 1-sided 95% confidence interval will be 0.17.

Study Duration	The study will take approximately 2.0 years from the time the study opens to enrollment until completion of data analyses.
Participant Duration:	Each participant will complete the study in approximately 36 weeks (9 months).

1.2 Schema


Screen
Enroll
Baseline
DAY 0
Day 28
D56
D84
D140
D196
Arm 1
C62
n=8
Arm 1
M4
n=8
Continues ART Therapy
Continues ART Therapy
Leukapheresis
Enrollment → Day 0
Arm 2
C62 + C1
n=8
Arm 2
M4 + M3
n=8
Leukapheresis
Arm 3
Placebo
n=2
Arm 3
Placebo
n=2
Baseline Clinical and
Research Labs
Safety Assessments
Research Assay Collections
Randomization
& Vaccination
Vaccination

1.3 Schedule of Activities (SoA)

	Screening	Enrollment and Baseline	Day 0 Vaccination	Day 2	Day 7	Day 14	Day 28 Vaccine Booster	Day 30	Day 35	Day 42	Day 56	Day 84	Day 140	Day 196 EOS
D = days W = weeks	D-60		0		W1	W2	W4		W5	W6	W8	W12	W20	W28
Visit window		≤ 60 D Visit 1	≤ 10 D Visit 2			+/- 2 D	+/- 2 D			+/- 2 D	+/- 2 D	+/- 7 D	+/- 7 D	+/- 14 D
Visit	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Clinical Procedures														
Informed consent	X													
Eligibility and Demographics	X													
Locator Information	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PID Assignment	X													
SID Assignment		X												
RID Assignment			X											
Medical and Medication History	X													
Complete PE	X													X
Targeted PE			X		X	X	X		X	X	X	X	X	
Vital Signs (VS)	X	X	X		X	X	X		X	X	X	X	X	X
Height	X													
Review ART Adherence	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Targeted Reproductive History	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Updated Medical History		X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medication review and update		X	X	X	X	X	X	X	X	X	X	X	X	X

	Screening	Enrollment and Baseline	Day 0 Vaccination	Day 2	Day 7	Day 14	Day 28 Vaccine Booster	Day 30	Day 35	Day 42	Day 56	Day 84	Day 140	Day 196 EOS
Assess AEs		X	X	X	X	X	X	X	X	X	X	X	X	X
Assess SAEs, MAAEs and AESIs			X	X	X	X	X	X	X	X	X	X	X	X
Study Treatment or Placebo			X				X							
Post-vaccine assessment			X	X	X	X	X	X	X	X	X			
Complete post-vaccine symptom diary				X	X			X	X					
Telephone Assessment[1]				X				X						
Leukapheresis		X[2]									X[3]			
Clinical Laboratory														
CBC with differential	X	X			X	X			X		X	X	X	X
Chemistries	X	X			X	X			X		X		X	X
Serum Pregnancy test	X													
Urine Pregnancy – POCT - WOCBP only			X				X							
Coagulation Tests (PT/INR/ APTT)	X													
HIV Ag/Ab Test	X													
CD4+/CD8+ T Cell Differential Panel	X												X	X
HIV-1 RNA PCR	X				X		X		X					X
RPR	X													
HBsAg & HCV Ab	X													
PF4 antibody ELISA (serum)		X[4]												
COVID-19 RT-PCR (nasal swab)		X	X				X							
Research Laboratory														
HLA Typing		X												

	Screening	Enrollment and Baseline	Day 0 Vaccination	Day 2	Day 7	Day 14	Day 28 Vaccine Booster	Day 30	Day 35	Day 42	Day 56	Day 84	Day 140	Day 196 EOS
CMV and EBV Test		X												
COVID-19 Antibody Test		X[5]	X[5]		X[5]	X[5]	X[5]		X[5]	X[5]	X[5]		X[5]	
Plasma and PBMC for Immunologic and Virologic Studies	X	X[6]	X		X	X	X		X	X	X	X[7]	X	X

Table Legend

[1] See Appendix 2 detailing additional telephone assessments at Days 5, 10, 18 and 22.
[2] Leukapheresis should be scheduled as close to the screening visit as feasible.
[3] See Section 8.1.2.
[4] Collect and store sample at enrollment. All other testing (if required) will be performed in real time.
[5] Test only retrospectively from stored plasma if immune response data suggest need.
[6] Collect separate research PBMCs samples at Enrollment.
[7] Collect sample only if participant completed Day 56 leukapheresis. If participant completed large blood draw instead of leukapheresis, do not collect sample.
AE=adverse events; AESI = AE of special interest; EOS = end of study; MAAE = medically attended AE; PE=physical exam; PID=participant ID; POCT=point of care test; RID=randomization ID; SAE=serious AE; SID=study ID; WOCBP=woman of childbearing potential

2 INTRODUCTION

2.1 Study Rationale

ART has transformed human immunodeficiency virus type 1 (HIV) from a fatal disease to a chronic condition for the 37 million PWH. However, ART must be taken life-long as interruption of therapy in PWH mostly results in viral rebound within weeks (1-4). This rebound results from cells harboring HIV proviral DNA that is integrated into the host genome. While >95% of proviral DNA is replication-incompetent, the remaining fraction that we define as the 'HIV reservoir', retains the capacity to produce infectious virus particles, either stochastically (5-8), or through interventions to induce coordinated reactivation (9-12). The HIV reservoir is highly stable, with an estimated half-life of 44 months (13, 14). Natural decay of the HIV reservoir would take >70 years. Therefore, to enable PWH to cease ART, additional strategies are required.

Current strategies permitting PWH to stop ART without viral rebound aim to either eliminate all HIV-1 infected cells harboring persistent, replication-competent virus (HIV eradication), or to achieve a state of durable HIV-1 suppression without rebound (HIV remission). Both approaches could harness $CD8^+$ T cell immunity to achieve reservoir reduction or elimination. Clearance of reactivated, HIV infected cells by $CD8^+$ T cells has been demonstrated with in vitro studies (15, 16). These observations are consistent with the extensively documented role of $CD8^+$ T cells in control of HIV in untreated infection (17-24), as well as more recent studies suggesting $CD8^+$ T cells contribute to control of HIV reactivation and/or virus rebound (25-27).

The frequency and quality of memory T-cells induced by vaccination are to a large extent determined by the vaccine vectors and modalities employed to deliver the HIV-derived immunogens (28-31). While live, replication-competent virus vectors elicit more vigorous immune responses (32), their immunogenicity has to be balanced with safety. Like replicating vaccines, non-replicating vaccines deliver the immunogen to the major histocompatibility complex (MHC) class I presentation pathway either directly or by cross-presentation, (33-35) however, no infectious vaccine progeny are produced (36). This increase in safety does lessen immunogenicity (37, 38) that cannot be easily enhanced by repeated boosts with the same vaccine, because of a build-up of anti-vector immunity, particularly humoral immunity, which dampens insert-specific T-cell induction (39, 40).

Serial vaccinations are an established approach to increase the frequency of vaccine-induced immune responses. In both pre-clinical and clinical testing, heterologous vaccination with different viral vectors expressing common or highly related immunogens has been consistently demonstrated to induce higher frequencies of T cells against the immunogen than homologous vaccination with the same viral vector (41-46). Previous studies have shown that vaccination with rChAdV followed by rMVA consistently induces high frequencies of T cells against the vaccine immunogen; this includes studies in HIV seropositive and seronegative participants.

The goal of this clinical study is to increase the frequency of circulating $CD8^+$ T cell responses against HIV in PWH on ART. This will be achieved by vaccination with heterologous,

attenuated viral vectors expressing highly related HIV immunogens spanning conserved regions of the virus.

2.2 Background

2.2.1 T Cell Correlates and HIV Control

HIV-specific $CD8^+$ T cells contribute to control of HIV in untreated PWH, however the level of contribution across individuals is highly variable. A small minority of PWH are able to maintain long-term control of HIV (<50 copies/ml) without ART (47). Certain MHC Class I alleles such as B*27:05, B*57:01 and B*81:01 are highly overrepresented in these 'elite-controllers'. HIV elite controllers maintain high frequencies of HIV-specific $CD8^+$ T cells that are broadly functional, including mediating strong in vitro suppression of HIV-infected $CD4^+$ T cells. Large cohort studies have consistently observed an inverse correlation between higher breadth (number of epitopes targeted) of IFN-γ producing $CD8^+$ T cells targeting the HIV Gag protein measured by ELISpot assay and virus load in untreated individuals (48). These studies in humans are supported by studies of SIV in non-human primates in which $CD8^+$ T cell depletion have defined a clear contribution of $CD8^+$ T cells, particularly those targeting Gag, against HIV.

Virus escape from $CD8^+$ T-cell immune pressure is a major and highly successful immune evasion mechanism for HIV-1 and the resulting HIV-1 genetic variability constitutes the biggest roadblock for HIV vaccine development. In natural infection, mutations in and around T cell epitopes emerge within weeks of primary T cell responses, limiting $CD8^+$ T cell detection and clearance of virus-infected cells (21). Virus escape occurs are different rates and at different regions across the HIV proteome. This is largely because different HIV proteins have different levels of tolerance of mutations. Envelope and regions of Nef tolerate higher levels of virus mutation than Gag and Pol proteins. HIV fitness is associated with sequence variation (22). More conserved mutations in Gag and Pol are more likely to induce fitness costs in the virus (49). The higher level of Gag conservation is consistent with the observed association between T cell targeting of Gag and lower HIV viremia.

An additional challenge is that T cell responses targeting structurally/functionally conserved regions of HIV-1 proteins are often subdominant relative to T cells targeting highly variable regions such as Env. Circulating T cells in PWH on ART are no exception. Mapping of HIV-specific T cell responses in PWH on ART by our group and others found very similar patterns of T cell targeting to untreated infection (50). Specifically, $CD8^+$ T cells consistently target Gag but also highly variable regions in Env and Nef. Of note, in these studies, we also observed that PWH on ART had a higher proportion T cells targeting Pol.

While ART prevents virus escape, the persistent HIV reservoir in PWH 'archives' previous virus escape mutations (50, 51). Deep sequencing of replication-competent HIV-1 recovered from resting $CD4^+$ T cells in durably suppressed individuals found that, similar to untreated infection, approximately one in three circulating T cells harbor escape variants in the HIV reservoir (52). Following virus reactivation (e.g. after treatment interruption), these escape mutations likely limit the effectiveness of circulating HIV-specific T cell responses. An important observation

from these studies was that virus escape occurred less frequently in low entropy or more conserved regions of HIV (50, 51). These observations indicate that, while T cell escape occurs in the replication competent HIV reservoir, it is not ubiquitous and some $CD8^+$ T cell populations still effectively detect and clear virus infected cells, consistent with the sustained functionality of HIV-specific $CD8^+$ T cells observed over time (53). The implications of these data for HIV-1 cure strategies is an optimal vaccine regimen will need to preferentially boost T-cell responses from which there is no pre-existing escape and/or induce strong de novo $CD8^+$ T-cell responses.

Altogether, these data indicate HIV vaccine regimens in PWH should (re)focus T-cell responses on the protective, structurally/functionally conserved regions of HIV-1 proteins, which are often subdominant.

2.2.2 Overview of the Vaccines

Simian adenovirus-vectored ChAdOx1.tHIVconsv1 (**C1**) and ChAdOx1.HIVconsv62 (**C62**) vaccines (Table 1) have been designed to be given serially with replication-deficient poxvirus Modified vaccinia virus Ankara (MVA)-vectored vaccines MVA.tHIVconsv3 (**M3**) and MVA.tHIVconsv4 (**M4**) vaccines (Table 2). All vaccines express highly-related HIV-derived conserved immunogens designed to maximize vaccine immunogenicity, maximize coverage of HIV viral diversity and minimize the effects of viral escape.

Table 1 Vaccines Vectored by Engineered Simian Adenovirus ChAdOx1

Vaccine	Manufacturer	Description
ChAdOx1.tHIVconsv1 (**C1**)	ADVAXIA BIOLOGICS SRL[1]	The ChAdOx1.tHIVconsv1 vaccine Drug Product has a genome size of 32,906 bp and is a slightly opaque frozen liquid, essentially free from visible particulates. The appearance is dependent upon the concentration of the virus.
ChAdOx1.HIVconsv62 (**C62**)	ADVAXIA BIOLOGICS SRL[1]	The ChAdOx1.HIVconsv62 vaccine Drug Product has a genome size of 32,846 bp and is a slightly opaque frozen liquid, essentially free from visible particulates. The appearance is dependent upon the concentration of the virus.

[1]Manufacturing authorization MIA aM42/2018, ADVAXIA BIOLOGICS SRL, Via Pontina Km 30.600-00071 Pomezia, Italy

Table 2 Vaccines Vectored by Poxvirus Modified Vaccinia Virus Ankara

Vaccine	Manufacturer	Description
MVA.tHIVconsv3 (**M3**)	IDT Biologika GmbH (IDT)[1]	The MVA.tHIVconsv3 vaccine Drug Product a genome size of approximately 181, 000 bp and is a white to light brownish opaque homogeneous suspension.
MVA.tHIVconsv4 (**M4**)	IDT Biologika GmbH (IDT)[1]	The MVA.tHIVconsv4 vaccine Drug Product a genome size of approximately 181, 000 bp and is a white to light brownish opaque homogeneous suspension.

[1]Manufacturing authorization DE-ST-01_MIA_2016_0017/604.41501.A.18 Dessau-Rosslau, Germany

2.2.3 The Immunogens

2.2.3.1 Rationale for Design of HIVconsvX

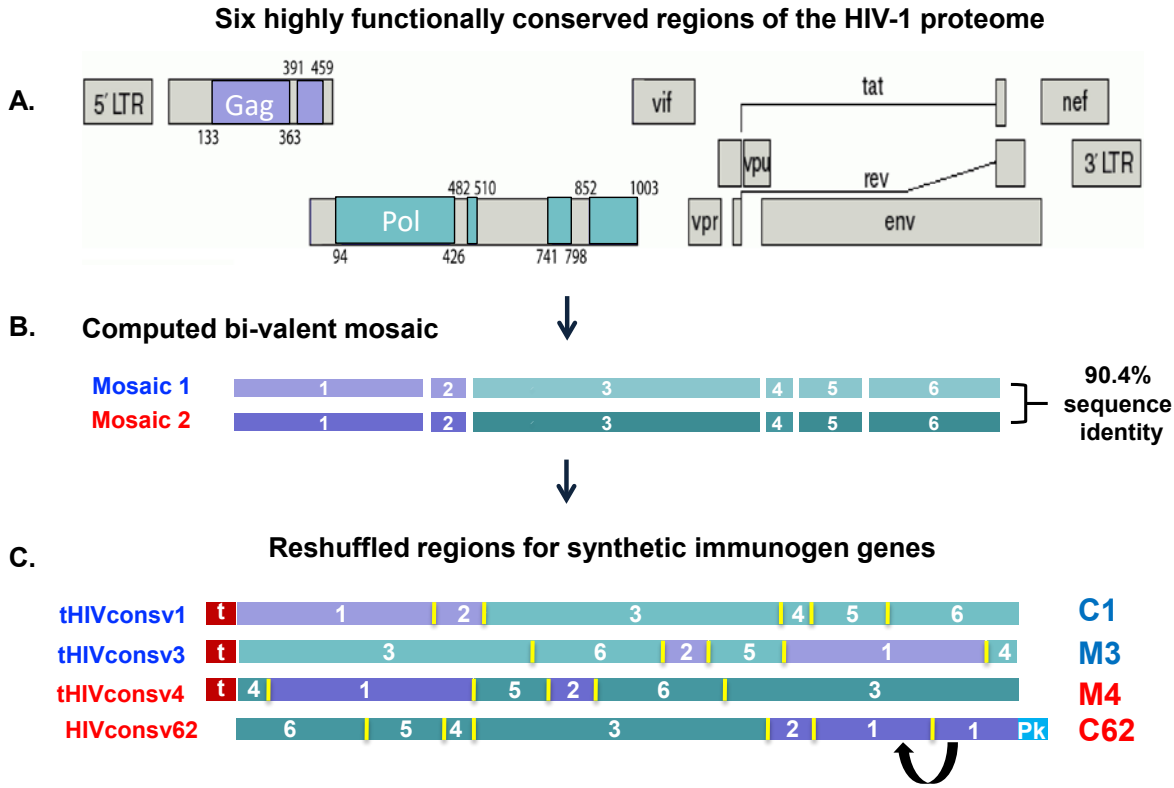
HIVconsvX is was designed in silico using full-genome sequences of group M HIVs in the Los Alamos National Laboratory HIV Sequence Database (LANL-HSD), as of September 2013 (54). HIVconsvX is comprised of six conserved regions of the Gag (region 1 and 2) and Pol (regions 3-6) of HIV computed into two complementary amino acid sequences, Mosaic-1 and Mosaic-2 (Figure 1A). Mosaic-1 and -2 differ in about 10% of amino acids and between them, maximize the match of the vaccine to the global circulating HIV-1 isolates (Figure 1B). Each of the four vaccines in this study uses a different order of the six conserved regions to minimize induction of T cell responses to potential newly formed and, therefore, irrelevant non-HIV epitopes generated by joining two adjacent regions together (Figure 1C).

The six regions in Gag and Pol in HIVconsvX contain a high number of previously identified $CD8^+$ T cell epitopes against HIV, restricted by 84 different HLA I alleles (54). These regions maximize epitopes associated with control of HIV viremia and minimize epitopes associated with poor HIV control (55). The conserved nature of these regions not only increase the likelihood of a match between the immunogens and infecting virus/es but also decreases the effects of virus escape, including pre-existing escape in the HIV reservoir (52).

The potential immunogenicity and efficacy of HIVconsvX has been tested in cohort studies. First in a cohort of treatment naïve PWH, higher magnitude and breadth of $CD8^+$ T-cell responses reactive to HIVconsvX-derived peptides correlated with higher $CD4^+$ T-cell count and lower viral load (54). More recently, our group used empirical methods to identify whether virus variants in the HIV reservoir could escape circulating T cell responses in PWH on ART. Epitope mapping and HIV sequencing of a cohort of 25 PWH on ART, found that 32% of T cell epitopes harbored escape variants in the HIV reservoir (50). On average, participants's T cells targeted 3 epitopes within HIVconsvX (0-6), suggesting that therapeutic vaccination of HIVconsvX will successfully increase circulating HIV-specific T cell responses (50). Importantly, T cell escape in regions corresponding to HIVconsvX was 2.5-fold lower than escape outside of the immunogen (15.3% vs 43.5%) (50).

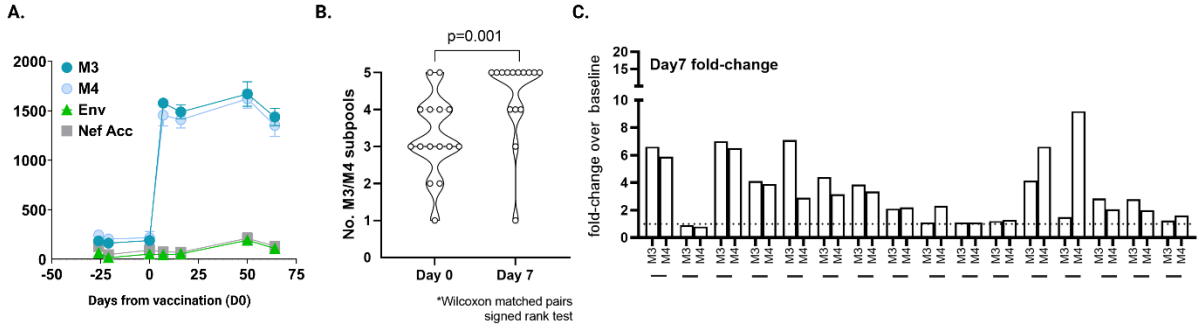
These data are consistent with observations to date in the ongoing Phase I M&M Study examining the safety and immunogenicity of **M3** and **M4** vaccines given alone or in combination in PWH on ART (NCT03844386). The M&M study closed to enrolment in December 2021 and blinded safety data are summarized in Section 2.2.6.2. T cell responses to HIVconsvX, mosaic-1 and mosaic-2 immunogens have been measured in 16 participants to date. Blinded results consistent increase in T cell breadth to peptides spanning either the Mosaic-1 or Mosaic-1 immunogens following vaccination. In 12 of 16 participants, T cell magnitude also increased ≥ 2-fold following vaccination when compared to baseline (average of pre-vaccination visits) (Figure 2).

Figure 1 Overview of HIVconsvX Vaccine Immunogens



(A) The HIVconsvX immunogens are comprised of six (1-6) conserved regions of HIV. (B) The vaccine design is bivalent, consisting of Mosaic-1 (872aa) and Mosaic-2 (872aa) immunogens that together increase the vaccine match to all the circulating HIV strains globally. (C) The regions in each Mosaic were re-ordered to generate four immunogens that were inserted into vaccines, **C1**, **C62**, **M3** and **M4**. The goal of the re-ordering is to minimize exposure of participants to non-natural junctions between HIV regions. t= tPA-leader sequence in **C1, M3** and **M4**, Pk= a C-terminal mAb tag, which facilitates protein detection in C62, black arrow= inversion of sections 1-103 aa and 104-231 aa of region 1(p24 Gag) in HIVconsv62

Figure 2 Immunogenicity Data from the M&M Study



PWH on ART received a single vaccination of **M3, M4, M3+M4,** or placebo in a 7:7:7:2 distribution. (A) T cell responses to the HIV immunogens, Mosaic-1 (**M3**) and Mosaic-2 (**M4**) and to Env, Nef and Acc (Rev, Vif, Vpr, Vpu, Tat) proteins not found in the vaccine immunogen in one participant. (B) T cell responses to peptides spanning Mosaic-1 (**M3**) or Mosaic-2 (**M4**) were measured pre- and post- the vaccination visit. Analysis of unblinded datas suggests that vaccination increased in T cell breadth to vaccine immunogens (C) Twelve of 16 participants show a ≥2 increase in T cell response to either or both mosaic immunogens at D7 post-vaccination.

2.2.3.2 Individual Immunogens

tHIVconsv1 was inserted into ChAdOx1 to generate the **C1** vaccine. The tHIVconsv1 immunogen uses Mosaic-1 and the regions are ordered as 1-2-3-4-5-6. tHIVconsv1 does not harbor a Pk tag described in (Table 3). tHIVconsv3 contains a human tissue plasminogen activator leader sequence (tPA-LS) described in Section 2.2.3.3.

tHIVconsv3 was inserted into MVA to generate the **M3** vaccine. The **tHIVconsv3** immunogen was generated using Mosaic-1 sequences. **tHIVconsv3** differs from **tHIVconsv1** only in the order of its 6 conserved regions which are 3-6-2-5-1-4 (Table 3). tHIVconsv3 does not harbor a Pk tag described in Section 2.2.3.3. tHIVconsv3 contains a tPA-LS described in Section 2.2.3.3.

HIVconsv62 was inserted into ChAdOx1 to generate the **C62** vaccine. The HIVconsv62 immunogen uses Mosaic-2 and the regions are ordered as 6-5-4-3-2-1/1. It uses the same amino acids and is immunologically equivalent to a previous design HIVconsv6, but uses different codons and has a rearranged region 1 (p24 Gag) (Table 3). Also, region 1 (p24, 231 amino acids) has been inverted, with sub-region 104-231 now followed by amino acids 1-103. The inverted region 1 is identified as 1/1. These changes increased the stability of the immunogen during virus propagation (ChAdOx1.HIVconsv62 IB). HIVconsv62 harbors a Pk tag but does not contain a tPA-LS.

The **tHIVconsv4** immunogen was generated using Mosaic-2 sequences. The amino acid sequence of **tHIVconsv4** differs from **HIVconsv62** only in the order of its 6 conserved regions which are 4-1-5-2-6-3 (Table 3). Region 1 in **tHIVconsv4** is not inverted (see Figure 1).

tHIVconsv4 does not harbor a Pk tag described in Section 2.2.3.3. tHIVconsv4 contains a tPA-LS described in Section 2.2.3.3.

Table 3 Characteristics of individual HIVconsvX immunogens

Immunogen	Vaccine	Parent Mosaic (M)	Region Order	Pk Tag[1]	tPA-leader sequence[1]
tHIVconsv1	C1	M-1	1-2-3-4-5-6	-	+
tHIVconsv3	M3	M-1	3-6-2-5-1-4	-	+
HIVconsv62	C62	M-2	6-5-4-3-2-1/1	+	-
tHIVconsv4	M4	M-2	4-1-5-2-6-3	-	+

1 +, in vaccine construct; -, not in vaccine construct

HIVconsv62 differs from the other 3 immunogens in that it is flanked by a C-terminal monoclonal antibody epitope Pk (IPNPLLGLD) derived from the P and V proteins of the paramyxovirus of simian virus 5 (SV5) family (56). The Pk tag was included to facilitate detection of the full-size HIVconsv62 protein expression from the vaccine during manufacture and vaccine quality control.

HIV-MVA and HIV-DNA vaccines harboring the Pk tag were safely administered to 492 HIV-negative adults, 97 HIV-negative 20-week-old infants and 30 HIV-positive adults (42, 57-59). In these studies, no SAEs related to vaccine products were observed in any study participants.

2.2.3.3 tPA-LS

C62 differs from the other 3 immunogens in that it does not contain a human tPA-LS; the ‘t’ in the tHIVconsvX nomenclature (Figure 1). The tPA-LS was included in the first three immunogens because it had been shown to increase *in vitro* expression of the vaccine immunogen (60). The presumption was that increased expression of the immunogen would enhance the immune response following vaccination. However, subsequent work found that the tPA-LS only enhances T cell induction for recombinant DNA vaccines (54, 60), not viral-vectored vaccines (54), and therefore is not included in the generation of **C62**.

Note, the prior eight Phase1/2a clinical studies using vector derived from simian adenovirus serotype 63 (ChAdV63) and MVA vectors expressing first-generation conserved HIV immunogens did not include tPA-LS (reviewed in (61). These studies were conducted in HIV seropositive and seronegative participants in Europe and Africa (62-65). In these clinical trials, ChAdV63/MVA vaccination induced consistent and strong T-cell responses in people, including in PWH on ART (61, 63-70). Murine studies also demonstrated that the inclusion of tPA-LS in viral vectors did not lessen immunogenicity in mouse models (54).

2.2.4 The Viral Vectors

Prime-boost regimens describe sequential vaccination with two different recombinant vaccine vectors expressing a common immunogen, an approach which focuses the immune response on the common immunogen (71). Prime-boost heterologous regimens have consistently induced higher frequencies of T cells against the immunogen than homologous vaccine boosting. This is likely because homologous vaccination induces anti-vector immunity, limiting the immunogenicity of the boosting vaccination. One of the most promising prime boost combinations is a recombinant adenovirus vector (rAdV) prime followed by MVA boost. These regimens have demonstrated immunogenicity that has translated across mice, non-human primates and humans. Both clinical and animal data clearly show that rAdV/MVA prime-boost is more immunogenic than either vaccine alone (64). One limitation with adenoviral vectored vaccination is high, pre-existing immunity for some serotypes. Approximately 40% of the US population are seropositive for the HAdV-5 serotype, and seropositivity is substantially higher in sub-Saharan Africa (72). In response, two approaches have been taken. The first approach involves the development of human adenoviral vectors (HAdVs) which are genetically distinct from HAdV-5 and against which the prevalence of pre-existing immunity is considerably lower. The second approach employed herein, uses recombinant simian adenoviruses (SAdV) against which pre-existing human adenovirus immunity has no impact. Animal testing and subsequent clinical studies demonstrate that SAdV vectors are almost, if not equally, immunogenic as HAdVs (73).

2.2.5 Simian adenovirus viral vectors

Chimpadenoviruses (ChAdV) were developed as vaccine vectors following concerns that pre-existing immunity to HAdv serotypes could limit future widespread use of these vaccine vectors. ChAdV and HAdV are not phylogenetically distinguishable and fall into the same eight adenovirus groups. ChAdOx1 is replication-deficient in vaccinated animals or humans due to deletion of the essential E1 gene region. ChAdOx1 can only propagate in cells complementing the E1 functions that are used for manufacture (ChAdOx1.HIVconsv62 IB Section 2.1). ChAdOx1 is closely related to ChAd63 which has been extensively tested as a vaccine vector including as a HIV vaccine in PWH (74).

Like all adenoviruses, ChAdVs possess a stable genome such that inserts of foreign genes are not deleted. ChAdVs and the transgene information remains extra-chromosomal, thus avoiding any potential for insertional mutagenesis.

2.2.5.1 Generation of C1 and C62 vectors

Full details of the generation of the **C1** and **C62** vaccine vectors are provided in ChAdOx1.tHIVconsv1 IB, and ChAdOx1.HIVconsv62 IB.

Description of ChAdOx1.tHIVcons1 (C1) ChAdOx1.tHIVconsv1 vaccine consists of the replication-deficient simian adenovirus vector ChAdOx1, which carries a transgene coding for the leader sequence of tPA-LS fused to a chimeric protein assembled from 6 conserved regions

of the HIV-1 proteome expressed under the control of the CMV promoter. These regions have no biological or enzymatic activities and are delivered solely for the purpose of inducing T-cell responses.

Description of ChAdOx1.HIVconsv62 (C62): ChAdOx1.HIVconsv62 vaccine consists of the replication-deficient simian adenovirus vector ChAdOx1, which carries a transgene coding for a chimeric protein assembled from 6 conserved regions of the HIV-1 proteome expressed under the control of the CMV promoter. These regions have no biological or enzymatic activities and are delivered solely for the purpose of inducing T-cell responses.

2.2.5.2 Clinical Experience with ChAdV-Vectored Vaccines

Sixteen clinical trials testing nine different ChAdOx1 vaccines have been or are in clinical testing (n> 22,000 participants) (ChAdOx1.HIVconsv62 IB Section 6, (75)). This includes peer-reviewed data from 12,082 healthy volunteers who received at least one dose of either low dose or standard dose ChAdOx1 nCoV-19 vaccine in the COV001 and COV002 clinical trial in the United Kingdom (UK) (EudraCT 2020-001228-32), the COV003 trial conducted in Brazil (ISRCTN89951424), and the COV005 trial conducted in South Africa reported in (75). Across the four clinical trials with 12,021 participants receiving ChAdOx1 nCoV-19 were included in safety analyses. Across this group, there were 79 SAEs (0.7%) (Table S6, supplement, (75), <1% occurrence of anaphylactic reactions. Only two events were considered possibly related to vaccination with ChAdOx1 nCoV-19, including a case of transverse myelitis reported 14 days after ChAdOx1 nCoV-19 booster vaccination, which an independent neurological committee determined was most likely due to an idiopathic, short segment, spinal cord demyelination. A potentially vaccine-related SAE related to fever to 40°C was reported 2 days post-vaccination in a participant in South Africa who recovered rapidly without an alternative diagnosis and was not admitted to hospital; this participant remains blinded and received a second dose of allocated vaccine without a similar reaction. There were two additional cases of transverse myelitis originally reported as potentially related but later determined unlikely to be related to vaccination by an independent committee of neurological experts. There was one non-COVID-19 death reported across the studies in the ChAdOx1 nCoV-19 arm that was considered unrelated to the vaccine.

Safety and some immunogenicity data for recombinant ChAdOx1 vaccines have been reported across several clinical trials for 6,596 participants with detailed safety data available for 755 study participants receiving at least one IM dose of 5×10^{10} virus particles (vp); the same dose and route of vaccination proposed for this CM Study (Section 1.1). Data are summarized in Table 4 (76). In these studies, solicited AEs have mostly been mild or moderate with unsolicited AEs mostly classed as mild/Grade 1.

As noted above, ChAdOx1 is highly related to the ChAd63 (=ChAdV63) vector which has also undergone extensive clinical testing. Participants (n=1,367) received recombinant ChAd63 vaccines given IM at a dose of $5x10^{10}$ vp, mostly in prime-boost combination with MVA-vectored vaccines. Vaccination has been safe and well tolerated with no SAEs reported (ChAdOx.tHIVconsv1 IB Section 6.5, ChAdOx1.HIVconsv62 IB Section 6).

In summary, recombinant ChAdOx1 vaccines have an established and good safety record in the vast majority of humans. Based on this information the Sponsor does not consider toxicology testing of **C1** or **C62** will generate novel safety or reactogenicity to further inform this proposed vaccination regimen.

Table 4 Summary of Safety Data for Recombinant ChAdOx1 Studies at 5 x 10^{10} Virus Particles

Country	Trial	Vaccine	Age	n	Summary of Results
UK[1]	FLU004	ChAdOx1 NP+M1	18-50	6	AEs probably associated with vaccination were experienced by most vaccinees in the 7 days following vaccination. AEs were mostly mild and all were self-limiting. No SAEs were reported.
UK[1]	CHIK001	ChAdOx1Chik	18-50	9	The majority of local and systemic solicited AEs were mild or moderate in nature. No SAEs were reported. All solicited local and systemic AEs resolved within 1-6 days.
UK[1]	MERS001	ChAdOx1 MERS	18-50	9	AEs probably associated with vaccination were all experienced by vaccinees in the 7 days following vaccination. Local and systemic AEs were mostly moderate and all were self-limiting. Unsolicited AEs in the 28 days following vaccination considered related to ChAdOx1 MERS were predominantly mild and resolved. No SAEs were reported.
UK[1]	COV001	ChAdOx1 nCoV-19	18-55	543	AEs probably associated with vaccination were experienced by the majority of vaccinees in the 7 days following vaccination and were mostly mild and all self-limiting. Prophylactic paracetamol decreased AEs. Unsolicited AEs in the 28 days following vaccination considered related to ChAdOx1 nCoV-19 were predominantly mild/moderate and resolved. No SAEs related to ChAdOx1 nCoV-19 were recorded. 10 participants received a second ChAdOx1 nCoV-19 booster. AEs related to vaccination were in the 7 days following booster vaccination and were mostly mild and all self-limiting. No SAEs related to ChAdOx1nCoV-19 recorded.
UK[2, 3]	COV002	ChAdOx1 nCoV-19 ($2{\cdot}2 \times 10^{10}$ vp followed by $3{\cdot}5–6{\cdot}5 \times 10^{10}$ vp or MenACWY)	18-55 56-69 ≥70	50 30 46	No severe local symptoms were reported. No SAEs related to ChAdOx1nCoV-19 recorded.
UK[2, 3]	COV002	ChAdOx1 nCoV-19 (2 doses of $3{\cdot}5–6{\cdot}5 \times 10^{10}$ vp or MenACWY)	18-55 56-69 ≥70	49 30 49	No severe local symptoms were reported. No SAEs related to ChAdOx1 nCoV-19 recorded.
			Total	**821**	

[1] Source: ChAdOx.tHIVconsv1 IB Section 6 and ChAdOx1.HIVconsv62 IB Section 6,
[2] Source: Voysey et al Lancet 2021 (75), Ramasamy et al Lancet 2021 (76)
[3] Post emergency use authorization clinical data relating to ChAdOx1 nCoV-19 are detailed in Table 5.

2.2.5.3 COVID-19 Adenovirus Vectors

The COVID-19 pandemic resulted in emergency use authorization from the FDA for several COVID-19 vaccines, including the Janssen/J&J Ad26COVS1 vaccine as a single dose recombinant HAd26 vector (77). Other adenovirus-vectored COVID-19 vaccines are authorized for use in other countries, including AZD1222-ChAdOx1 nCoV-19 (75) and the Russian Gam COVID-Vacc, a heterologous HAd26-HAd5 prime-boost (78). Overall, there is increased likelihood that some potential study participants may have received an adenovirus-based vaccine.

Pre-existing humoral immunity has been shown to interfere with vaccine-induced immunity, including the induction of T cell immunity to autologous adenoviruses. The best example includes Ad5 which is a group C virus. Pre-existing Ad5-specific NAb titers impaired the Merck Ad5 vector expressing HIV immunogens (72, 79, 80). Similarly, autologous antibodies to rhesus adenoviruses were shown to interfere with T cell immunity in mice models (81). By contrast, studies in humans, animal models and in vitro cell models, have shown that pre-existing Ab titers to adenoviruses from phylogenetically different groups do not impair induction of immunity to adenovirus vaccination (82). For example, high Ad5 antibody titers did not impair T cell responses following vaccination of macaques with Ad26 (group D)-RhAd prime-boost regimens in NHP (81). Lastly, sera from ChAd63 (Group E) vaccinated individuals did not neutralize ChAdOx1 (Group E) infection (74); though no data are available regarding impact on immune responses. Pre-existing Ad26 and Ad35 (Group B) antibodies did not impact the induction of vaccine-specific immune responses following Ad26-Ad35 heterologous vaccination in healthy adults in the US, East and South Africa (83). Altogether, these studies suggest that adenoviral vaccination with phylogenetically different groups will not impede the induction of T cell responses to C1 and or C62 vaccines.

More data are needed on interactions between adenoviruses within the same phylogenetic group. As such, prior vaccination with Group E vaccine vectors, including the COVID-19 vaccine AZD1222, will be an exclusion criterium for this study. By contrast, prior vaccination with the Janssen Ad26COVS1 COVID-19 vaccine (and Gam COVID-Vacc) will not be exclusionary. Conversely, C62 and, or C1C62 vaccination could impede subsequent vaccination with AZD1222.

2.2.5.4 Post-authorization experience with Adenovirus Vectored COVID-19 vaccines

As of July 2021, over 110 million doses of AZD1222-ChAdOx1 nCoV-19 had been given across Europe, including the UK. Post-authorization experience identified new possible AEs. Given these AEs are reported voluntarily, it is not possible to estimate frequency or as yet establish a causal relationship following vaccine exposure. These AEs are listed in the AZD122 and Ad26COVS1 packet inserts as either very rare frequency (< 1in 10,000) (Table 5, ChAdOx.tHIVconsv1 IB Section 6.2, ChAdOx.HIVconsv62 IB Section 6.2) . Post-authorization clinical reporting is addressed in the **Risk/Benefit Assessment** in Section 2.3.

Blood and lymphatic system disorders: Review of clinical data in the 4 week window following the first AZ1222 vaccination in the UK and European Union (EU) AZ1222 programs (>49 million Europeans, 09 April 2021) identified rare cases of unusual thromboses associated with thrombocytopenia in people without previous history of prothrombotic medical conditions (84, 85), https://www.ema.europa.eu/en/news/astrazeneca-covid-19-vaccine-review-very-rare-cases-unusual-blood-clots-continues, ChAdOx.tHIVconsv1 IB Section 7.2, ChAdOx.HIVconsv62 IB Section 7.2, Table 5). Specifically thromboses with thrombocytopenia occurring in the first 3.5 weeks of vaccination, median of < 2 weeks post-vaccination (84, 85). Thromboses with thrombocytopenia were also reported in a smaller number of individuals vaccinated with the Janssen Ad26COVS1 COVID-19 vaccine. The majority of patients tested to date harbored antibodies against platelet factor 4 suggesting immune-related events, independent of heparin-therapy (84-86). At present, no specific risk factors, such as age, gender or a previous medical history of clotting disorders have been identified for these very rare events. A summary of findings reported to the European Medicines Agency (EMA) are reported in Table 5.

Immune system disorders: Events of anaphylaxis following AZ1222 vaccination have been reported (https://www.ema.europa.eu/en/documents/prac-recommendation/prac-recommendations-signals-adopted-8-11-march-2021-prac-meeting_en.pdf, Table 5).

Vascular Disorders: Very rare cases of capillary leak syndrome (CLS) have been reported in the first days after vaccination with AZD1222 and the Janssen Ad26COVS1 vaccine. A history of CLS was apparent in some of the cases. Fatal outcome has been reported. CLS is a rare disorder characterized by acute episodes of edema mainly affecting the limbs, hypotension, hemoconcentration, and hypoalbuminemia (ChAdOx.tHIVconsv1 IB Section 7.2, ChAdOx.HIVconsv62 IB Section 7.2, Table 5).

Nervous System Disorders: Very rare events of neuroinflammatory disorders have been reported following vaccination with AZ1222/Vaxzevria. A causal relationship has not been established (ChAdOx.tHIVconsv1 IB Section 7.1, ChAdOx.HIVconsv62 IB Section 7.1, Table 5).

Table 5 Adverse reactions reported following vaccination with AZD1222 COVID-19 Vaccine[1]

	Very Common ≥1 per 10	**Common** ≥1 per 100 to <1 per 10	**Uncommon** ≥1 per 1,000 to <1 per 100	**Rare** ≥1 per 10,000 to <1 per 1000	**Very Rare** < 1 per 10,000	**Not Known (cannot be estimated from post-authorization data)**
Blood and lymphatic system disorders		thrombocytopenia	lymphadenopathy		Thrombosis in combination with thrombocytopenia[2]	
Metabolic and Nutrition system disorders			Decreased appetite			
Nervous system disorders	Headache		Dizziness, somnolence			Guillian-Barré syndrome
Vascular system disorders					Thrombosis in combination with thrombocytopenia[2]	Capillary Leak Syndrome
Gastrointestinal disorders	Nausea	Vomiting, diarrhea				
Skin and subcutaneous tissue disorders			Hyperhidrosis, pruritus, rash			
Musculoskeletal and connective tissue disorders	Myalgia arthralgia					
Immune System Disorders						Allergic reactions including anaphylaxis
General disorders and administration site conditions	Fatigue, malaise, feverishness, chills, injections site tenderness, pain, warmth, pruritus, bruising	Fever [3], injection site erythema, injection site swelling,	Injection site hematoma			

[1]Source: Product Information Vaxzevria, COVID-19 Vaccine ANNEX 1, https://www.ema.europa.eu/ https://www.ema.europa.eu/en/documents/product-information/vaxzevria-previously-covid-19-vaccine-astrazeneca-epar-product-information_en.pdf, Table 1 and EMA EPITT no.19683

[2] Severe and very rare cases of thrombosis in combination with thrombocytopenia have been reported post-marketing. These included venous thrombosis such as cerebral venous sinus thrombosis (CVST), splanchnic vein thrombosis, as well as arterial thrombosis.
[3] Measured fever > 38°C

2.2.5.5 Pre-Clinical Experience with ChAdV-Vectored Vaccines

Eight ChAdOx1-vectored vaccines have been examined in seven Good Laboratory Practice (GLP) toxicology studies in BALB/c mice conducted at ENVIGO (formerly Huntingdon Life Science) (ChAdOx.tHIVconsv1 IB 5.3 and ChAdOx1.HIVconsv62 IB 5.3)

As an overview, adult mice (male and female) typically received two doses of the recombinant ChAdOx1 vaccine 14 days apart with up to a 14-day observation window. Across toxicology studies, IM doses calculated to be >500-fold the proposed clinical dose of ChAdOx1 (5×10^{10} vp) were tested. All of these studies uniformly concluded IM delivery of the ChAdOx1-vectored vaccines was well tolerated and was not associated with any adverse effects. Non-adverse effects observed were attributable to inflammatory and immune responses at the IM injection site and induction of immune responses often reflected as changes in the spleen, drainage and/or increased cellularity in lymph nodes, and slight increases in white blood cell count and/or protein concentrations. Of note, one GLP toxicity study (Study TX05CW) described in ChAdOx1.HIVconsv62 IB Section 5.3.2, tested ChAdOx1.HTI and another experimental rChAdOx1 vaccine with very similar HIV transgene called HTI (87). For the purposes of toxicity, the HIV-1-derived vaccine inserts (=immunogens=transgene products) of HIVconsv, tHIVconsv1, tHIVconsv3, tHIVconsv4, HIVconsv62 and HTI employed by experimental vaccines are very similar and the regions partially overlap among the different immunogen designs. They are assembled from small regions of HIV-1 proteins, have no known biological or enzymatic activity, and are joined into a chimeric protein of 872 amino acids for the sole purpose of inducing $CD4^+$ and $CD8^+$ T-cell responses.

In two of the seven GLP toxicology studies described, ChAdOx1 vaccinations were boosted with MVA vaccines. Both of these studies tested two IM doses with one study testing a third IV dose of either ChAdOx1 and/or MVA. In both combination studies, >500-fold the proposed clinical dose of ChAdOx1 were tested. Study GG05TY used a combination regimen similar to the proposed **C62-M4** and **C1C62-M3M4** regimens. Similar to the toxicity studies testing ChAdOx1 alone, both studies with ChAdOx1 plus MVA booster concluded that ChAdOx1 and MVA were well tolerated and not associated with any adverse effects. Non-adverse effects included local inflammatory responses at the IM injection site and induction of immune responses.

2.2.6 MVA Vaccine Vectors

MVA is a vaccinia virus strain which was attenuated by serial passage in chick embryo fibroblasts (CEF). It has lost 15% of the parental genome, including cytokine and chemokine receptor genes (88). It replicates well in CEFs and baby hamster cells but is unable to replicate efficiently in human and most other mammalian cells (89). The replication defect occurs at a late

stage of virion assembly such that the early viral and transgene expression is unimpaired. This makes MVA an efficient single-round-expression vaccine vector that is itself incapable of replication and spread in most mammalian cells. The DNA sequence of the entire MVA genome has been determined (90).

*2.2.6.1 Generation of **M3** and **M4** vectors*

Full details of the generation of the **M3** and **M4** vaccine vectors are provided in IND 18368, Investigator Brochures.

MVA.tHIVconsv3 (M3): The M3 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 (Table 2).

MVA.tHIVcons4 (M4): The M4 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 (Table 2).

*2.2.6.2 Clinical Experience with MVA-Vectored Vaccines including **M3** and **M4** Vaccines*

Despite the high level of attenuation, MVA has been shown to induce strong cellular immune responses. MVA was administered to more than 120,000 previously unvaccinated individuals as part of the smallpox eradication campaign in a field study in Germany in the 1970s. MVA-vectored candidate vaccines for AIDS, malaria, tuberculosis, influenza, hepatitis C, cancer and Ebola have been tested extensively in previous clinical trials by The Jenner Institute, University of Oxford (UOXF) and other laboratories resulting in considerable cumulative safety data on recombinant MVAs over the last four decades (42, 58, 88, 91-117) (See IND 18368, Investigator Brochures).

Over 4000 people have received this MVA vector, which has been shown to be safe and immunogenic. The first MVA vaccine from the University of Oxford, MVA.HIVA was administered to over 400 healthy seronegative and PWH volunteers in the UK, Europe and Africa, including 60 twenty-week-old African infants in clinical trials. The first generation conserved-region MVA.HIVconsv was administered to over 224 participants. The candidate vaccine against TB, MVA85A, was safely injected into 2,400 participants including adults, adolescents, children and 1500 infants. See Table 6.

Table 6 Safety Data for selected Recombinant MVA Studies expressing HIV immunogens, given IM at 1-2 x 10^8 vpu

Site	Trial	Vaccine	Age	n	Summary of Results
Spain	BCN01ROMI NCT02616874	MVA.HIVconsv (2 doses)	18-65yr	15	No SAE relative to vaccination. Grade 4 SAE: CPK elevation; total of 10 Grade 3 AEs: local pain at injection site, headache, fever myalgia, anorexia, abdominal pain
Spain	BCN01 NCT01712425	MVA.HIVconsv	18-65yr	24	No SAE relative to vaccination. 4 Grade 3 SAE: pain at site of injection, 3x intense tiredness myalgia
US	GV-TH-01 NCT01378156	MVA62B (2 doses)	18-50yr	9	No SAE relative to vaccination. No SAE reported
Spain	RisVac 03 NCT01571466	MVAHIV-B (3 doses)	18-65yr	14	No SAE relative to vaccination. 4 Grade 3 AEs: hyperglycemia, pneumonia, influenza, renal colic

Twenty-two participants have been randomized to receive **M3, M4, M3M4** or placebo given IM into the arm in a 7:7:7:3 ratio at final dose of 2×10^8 pfu in the M&M Study (NCT03844386; IND 18368) conducted at the University of North Carolina at Chapel Hill (UNC). Safety data for 24 participants are summarized in Table 7. Consistent with the broad literature summarizing MVA clinical safety profiles, data to date indicate vaccination with **M3** and **M4,** given alone or in combination, is safe and well tolerated with vaccine-related AEs resolving within 48 hours.

Table 7 Summary of M&M Trial Safety data

Site	Trial	Vaccine	Age	n	Summary of Results
UNC-Chapel Hill	M&M	M3, M4, M3M4, placebo	21-60	24[1]	• 192 solicited AEs at least probably associated with vaccination were experienced by 23 participants in the 28 days following vaccination. • 91% were Grade 1-2 and 9% were Grade 3and all resolved within 48 hours. • 4 participants reported 17 Grade 3AEs (Section 2.3.2) • 65 unsolicited AEs were experienced by 22 participants. No unsolicited Grade 3AEs were related to study product.

[1] Blinded data summary of 24 participants who have progressed to D28 post-vaccination or beyond. 18 participants have completed the study through D168 post-vaccination.

2.2.6.3 Pre-Clinical Experience with MVA-Vectored Vaccines

Formal GLP-compliant acute toxicity and biodistribution studies of **M3** and **M4** was not required for Phase I testing of **M3** and **M4** vaccines. Both vaccines demonstrated to be safe, non-toxic and immunogenic in over 1,000 mice during routine experimental immunizations (representative immunogenicity data published in (54, 118), MVA.tHIVconsv3 IB Section 2.3, MVA.tHIVconsv4 IB Section 2.3).

Toxicity studies have been performed by the early generation HIV MVA vaccine, MVA.HIVA also generated by the UOXF. No significant toxic effects, either local or systemic, were seen in SCID mice receiving two doses of MVA.HIVA by the intradermal (ID)route in study MRC/015 or in BALB/c mice receiving two doses of MVA.HIVA by IM or the subcutaneous route in study MRC/018 (119, 120). The toxicity and persistence of MVA.HIVA in macaques infected with pathogenic strains of SIV (SIVmac) was evaluated in a GLP safety and biological clearance study. In SIV-infected macaques, no MVA.HIVA DNA was detected in any organ examined (testes, epididymis, ovary, blood, brain, heart, spleen, kidney, liver, mesenteric lymph node, draining axillary lymph node, skin and muscle at the site of injection) up to 9 weeks after ID immunization. SIV infection did not enhance the risk of target organ toxicity or persistence of MVA.HIVA or transfer of vaccine genomic DNA into germ cells in these animals (120).

2.2.7 Simian Adenovirus- and MVA Prime Boost Vaccination

2.2.7.1 Clinical Experience with Chimpadenovirus OX1 AND MVA-Vectored Vaccines

Heterologous vaccination with simian adenovirus- and MVA-vectors has been tested in multiple humans studies (91, 92, 121-126), including PWH on ART (64, 65). In these trials, MVA has been mostly delivered as boosting vaccination 2 or 4 weeks after ChAd-vaccination. In all studies, combination vaccination was well-tolerated with few SAEs reported. Table 8 summarizes safety data of ChAd0x1/MVA vaccine regimens.

Table 8 Summary of Safety Data for vaccination with a ChAdOx1[1] prime followed by MVA boost[2]

Site	Trial	Vaccine	Age	n	Summary of Results
UK	NCT02390063	ChAdOx1 5T4-MVA-5T4	≥18	40	No SAE related to vaccination.
UK	NCT01818362	ChAdOx1NP+M1 -MVA NP+M1	18->50	73	No SAE related to vaccination.
UK	NCT01829490	ChAdOx185A-MVA85A	18-55	24	No SAE related to vaccination.

[1] ChAdOx1 dose $2.5x10^{10}$vp given IM
[2] MVA dose 1.0-2.0 $x10^{8}$pfu given IM

2.2.7.2 Clinical Experience with Simian Adenovirus 63 AND MVA-vectored Vaccines

Over 1,200 participants have received rChAd63/rMVA expressing a range of immunogens, including vaccination of 700 infants (5-17 months) (66, 92, 103, 124, 126-130). All clinical studies, including those expressing conserved HIV immunogens, have shown that heterologous ChAdV/MVA vaccination is safe and well tolerated. Heterologous vector delivery with rChAdV (ChAdv63) and rMVA vaccines expressing first generation conserved HIV immunogens has been tested in 154 humans, including PWH on ART. Consistent with all other ChAdV/MVA studies, vaccination was safe and well-tolerated (ChAdOx1.HIVconsv62 IB Section 6.5).

2.2.7.3 Pre-clinical Experience with Simian Adenovirus 63 AND MVA-Vectored Vaccines

Two additional GLP toxicity studies were performed using first generation HIVconsv vaccines using boosting regimens of ChAdV63 or MVA. In both studies, there was 14 days between ChAd63 and MVA boosts. The HIVconsv vaccines were well-tolerated and not associated with any adverse effects. The only effects of treatment seen were those attributable to inflammatory and immune responses at the IM injection site, lymph nodes, and the spleen (ChAdOx1.HIVconsv62 IB and ChAdOx1.tHIVconsv1 IB).

2.3 Risk/Benefit Assessment

2.3.1 Known Potential Risks of C1 and C62 Vaccines

Previous experience with other simian adenovirus viral vectored vaccines suggests AEs provided in Table 9 and Table 10 are expected to occur in some participants after vaccination with **C1**, **C62** or **C1C62** vaccines.

Table 9 Common Local Reactions

Injection site erythema	Injection site pruritus
Injection site pain	Injection site swelling
Injection site warmth	

Table 10 Common Systemic Reactions

Arthralgia	Fatigue
Malaise	Headache
Nausea	Fever (>37.7°C or 99.9°F)
Myalgia	Feverishness

These AEs are primarily mild in severity, however occasionally moderate or severe events have been reported. These events usually occur within 24 hours of vaccination and resolve within 24 to 48 hours. Given existing data for ChAdOx1-vectored vaccines, it is anticipated that the majority of systemic AEs post **C1**, **C62,** or **C1C62** will be mild in intensity (ChAdOx1.tHIVconsv1 IB and ChAdOx1.HIVconsv62 IB).

Transient lymphopenia has been observed in the recent HIVCORE 0052 trial and previously in trials of other ChAdOx1-vectored vaccines. This is asymptomatic and resolves spontaneously within a week (ChAdOx1.tHIVconsv1 IB and ChAdOx1.HIVconsv62 IB). Complete blood cell counts will be monitored throughout the study.

Severe allergic reactions including anaphylaxis may occur, as with receipt of any vaccine or medication. Participants will be vaccinated in a clinical area equipped to provide immediate advanced life support. **Rare Side-effects**: Some rare side effects have been observed following vaccination with AztraZeneca COVID-19 vaccine (AZD1222) (Table 5) and the Janssen vaccine (Ad26COVS1) COVID-19 vaccine with an estimated 1 case per 100,000 – 250,000 vaccine recipients. These side effects are:

1. A combination of thrombosis and thrombocytopenia known as Thrombosis with Thrombocytopenia Syndrome (TTS), also termed vaccine-induced prothrombotic immune thrombocytopenia (VIPIT)

2. New onset neurological disease (i.e. Guillain-Barre syndrome or GBS)

3. Systemic CLS. Cases of TTS have occurred within the first twenty-four days (range 5-42 days) following vaccination and occurred mostly in women under 60 years of age (84). This includes rare severe cases presenting as venous thrombosis, including unusual sites such as CVST, splanchnic vein thrombosis, deep vein thrombosis and pulmonary embolism, as well as arterial thrombosis, concomitant with thrombocytopenia with some cases resulting in death. Participants to date have mostly harboured antibodies against the Platelet Factor 4 which is released by activated platelets (84, 85).

There have been very rare cases of CLS reported in the first days after vaccination. CLS is a rare disorder characterised by acute episodes of edema mainly affecting the limbs, hypotension, concentrated blood and hypoalbuminemia. Investigation of some these cases indicated a history of CLS. Some cases were fatal.

Participants in this study will be followed using a combination of phone calls (n=5) and in-person visits (n=2) for the development of symptoms associated with the development of new onset blood clots and neurological deficits (Appendix 2) for the 28 days following C62 or C1C62 vaccination. All participants will be advised to seek immediate medical attention should they develop symptoms. CBC will be performed to examine platelet levels at Day 7 and 14 post-vaccination. Participants will be provided with a vaccination information card (Section 8.2.13), describing the study vaccine and the need to consider TTS if clinically warranted.

As with any other vaccine, rare events of GBS or immune mediated reactions that can lead to organ damage may occur. Rare cases of GBS have been reported in individuals who received the AstraZeneca and the Johnson and Johnson COVID-19 vaccines. In a few individuals who received the AstraZeneca COVID-19 vaccine (ChAdOx1), a variant of GBS with significant facial weakness has been described.

2.3.2 Known Potential Risks of M3 and M4 vaccines

The M&M study conducted at UNC is the first human clinical trial using **M3** and **M4.** To date, there have been no SAEs among 23 PWH on ART who received either **M3, M4, M3M4** or placebo (Table 7). Ninety-one percent of AEs reported as related to the study product were grade 1 or 2 (solicited). Four participants reported 17 Grade 3 events (solicited) and included arthralgia (1), headache (1), unexplained diaphoresis (1), myalgia (2), chills (3), fatigue (3), flu-like symptoms (3), malaise (3)felt to be treatment related, which resolved in 24 hours without intervention. All AEs resolved within 24 – 48 hours of vaccination.

The safety profile of **M3, M4** or **M3M4** in the M&M Study is consistent with safety data from other MVA-vectored vaccines, including those given to PWH. Local and systemic reactions are summarized in Table 11 and Table 12 respectively (See IND 18368, Serial 0004).

Altogether we anticipate AEs to **M4** and **M3M4** in this study will be mild in severity involving transient Grade 1 and 2 local reactions such as pain or erythema at the injection site in addition to headache, malaise, and fever (See IND 18368 Serial Number 0004).

Standard local (Table 11) and systemic reactions (Table 12) can occur, with most appearing within the first 24 hours and usually subsiding within 48 – 72 hours.

Table 11 Local Reactions to MVA vaccine

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

Table 12 Systemic Reactions to MVA vaccine

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

2.3.3 Known Potential Risks Common to Both ChAdOx1-vectored and MVA-vectored Vaccines

Viral Reversion: 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 **M3** and **M4** in human cells.

Pregnancy: 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.

Allergy: Serious allergic reactions including anaphylaxis may occur as with any vaccine. The incidence of this is unknown but is estimated at one per 10^5 to 10^6 vaccinations. Participants will be vaccinated in a clinical area that has the capacity to administer medications and utilize emergency equipment for the management of serious adverse reactions.

2.3.4 Known Potential Risks of Overdose

A drug overdose is defined as the accidental or intentional use of a drug or medicine or an administration error in an amount that is higher than is normally used. Given all doses of **C1, C62, M3** and **M4** will be provided and administered by licensed study staff only and as prepared by the manufacturing facilities, overdose is not anticipated.

Overdose in this study is specifically defined as any dose greater than the intended protocol dose. In the unlikely event of overdose, it is recommended that the participant be monitored for any signs or symptoms of adverse reactions or effects and appropriate symptomatic treatment be administered immediately. Note that administration of the “wrong” vaccine is a protocol deviation, but not, in the absence of associated AE, an SAE.

Any overdose must be reported to the protocol team and sponsor within 24 hours of awareness. Only overdoses associated with a clinical SAE needs to be reported as an SAE. The quantity and duration of the excess dose should be documented.

2.3.5 Known Potential Risks of Syncope

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

2.3.6 Known Potential 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. We minimize risk by using sterile technique and universal precautions.

2.3.7 Known Potential Risks of Leukapheresis

These include common side effects similar to blood draws. 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 SAEs have occurred in our prior studies, including more the 500 cell collections from PWH as donors at UNC.

The following are the most common risks:

- Pain or bruising at the site of needle sticks,
- Phlebitis,
- Citrate toxicity (10%),
- Oral paresthesia,
- Paresthesia,
- Stiffness in the arms due to the immobilization during donation,
- Fatigue,
- Temporary fluctuations in heart rate, and
- Temporary increases in blood pressure.

Less common:

- Infiltration the tissue,
- Muscle aches or cramps,
- Chills,
- Fever,
- Nausea or vomiting,
- Lightheadedness or headache, and
- Vasovagal reaction.

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.

2.3.8 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.

2.3.9 Known Potential Benefits

The addition of the **C62** and **C1** vaccines boosted by **M4**, or combined **M3M4** 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.

2.3.10 Assessment of Potential Risks and Benefits

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 vaccines will be safe and well tolerated. Although participants in this early phase study will receive no direct benefit for their participation, there remains a strong desire among PWH, and the HIV community at-large, to pursue HIV preventative and therapeutic vaccine strategies. The potential adverse effects, stigmatization, and financial costs encountered by PWH receiving life-long ART along with the potential harm of persistent immune activation are strong reasons to pursue HIV vaccine research. In short, an HIV cure or sustained remission in the absence of ART would have substantial benefits for many individuals if achieved. An effective HIV vaccine represents a key strategy for both HIV prevention and cure.

Participants will be informed of potential adverse events associated with study products at the time of informed consent by a study investigator and will be encouraged to talk with other providers and family/significant others as needed prior to signing the informed consent.

3 OBJECTIVES AND ENDPOINTS

The objectives and endpoints for the clinical study are provided in Table 13.

Table 13 Objectives and Endpoints for the Clinical Study

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
Evaluate the safety of vaccination through 28 days after the last vaccination or placebo with: **C62-M4** **C1C62-M3M4** in PWH on ART	Occurrence of at least one ≥ Grade 3 AE including signs/symptoms, lab toxicities, and/or clinical events, that is possibly or definitely related to study treatment from the first day of treatment (D0) through 28 days following the boost vaccination or D 56. 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.	In previous clinical studies of ChAdOx1 and MVA modalities, given alone or in combination, all observed vaccine related AEs occurred within 28 days of vaccination. The study will use standard safety grades used in HIV clinical trials. Should these grades be exceeded, it is currently felt that such risks would be unacceptable in a research study of this nature.
Secondary		
Evaluate the safety of vaccination through the end of study	Occurrence of any ≥ Grade 1 AE including signs/symptoms, lab toxicities, and/or clinical events, that is possibly, or definitely related to study treatment any time from the first day of treatment (D0) through D196 (Week 28). NOTE: The occurrence of a Grade 3 elevated blood pressure during leukapheresis that resolves following completion of the procedure will not be included as a secondary safety endpoint. 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 study.	The study will use standard safety grades used in HIV clinical trials. Should these grades be exceeded, it is currently felt that such risks would be unacceptable in a research study of this nature.
Compare the within-participant relative change in magnitude of HIV-1-specific T cell responses following vaccination with **C62-M4** or **C1C62-M3M4**.	Relative change in magnitude of T cell responses to HIV-1 conserved regions from baseline (before **C62 or C1C62** vaccination) to post-boost vaccination at D35 and D42.	Vaccination of PWH on ART with MVA vaccines expressing HIV immunogens, given alone or as a boosting vaccination, induced the peak HIV-specific T cell responses at day 7 or day 14 post-vaccination

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Compare the between-arm change in breadth of HIV-1-specific T cell responses following vaccination **C62-M4** or **C1C62-M3M4**.	Change in breadth of T cell responses targeting HIV conserved regions from baseline (before **C62 or C2C62** vaccination) to post-boost vaccination measured at D56.	The mosaic immunogen design is predicted to broaden the number reactive HIV epitope variants (i.e. breadth) detected by $CD8^+$ T cells in vaccinees. T cell breadth is maintained over time, enabling measurement at D56 providing economical use of participant samples.
Assess the long-term safety and tolerability of study vaccines	Incidence and severity of SAEs, MAAEs, and AESIs through EOS.	Multiple post-vaccination timepoints needed to evaluate the longer-term safety of the C1 and/or C62 vector.
Tertiary/Exploratory		
Compare the change in function and phenotype of HIV-1-specific T cell responses in participants from pre- to post-vaccination with **C62-M4** or **C1C62-M3M4**.	Change in function of T cell responses targeting HIV conserved regions from baseline (before **C62 or C1C62** vaccination) to post-boost vaccination at D35 or D42.	Vaccination is expected to induce temporal changes in memory phenotype and breadth of function
Evaluate the kinetics of immunologic responses in participants pre- and post-vaccination with **C62-M4** or **C1C62-M3M4.**	Relative change in magnitude of T cell responses to HIV-1 conserved regions at multiple pre- (minimum 2) and post-vaccination (D35 or D42) timepoints.	Multiple pre- and post-vaccination timepoints are needed to evaluate the stability of any change observed.
Evaluate the kinetics of $CD8^+$ T cell mediated HIV inhibition pre- and post-vaccination with **C62-M4** or **C1C62-M3M4**.	Relative change in magnitude of T cell responses to HIV-1 conserved regions at multiple pre- (minimum 2) and post-vaccination (D35 or D42) timepoints.	Anticipate greatest change in T cell virus inhibition will occur at peak-magnitude of T cell response.
Explore cellular activation status of total $CD4^+$ and $CD8^+$ T cells pre- and post-vaccination with **C62-M4** or **C1C62-M3M4**.	Relative change in magnitude of T cell responses to HIV-1 conserved regions at multiple pre- (minimum 2) and post-vaccination (include D35, D42) timepoints.	Anticipate greatest change in T cell activation status to occur at peak-magnitude of T cell response.
Explore the impact of vaccination with **C62-M4** or **C1C62-M3M4** on low-level plasma viremia.	Relative change in low-level viremia at multiple pre- (minimum 2) and post-vaccination (minimum 2) timepoints.	Multiple pre- and post-vaccination timepoints are needed to evaluate the stability of any change observed.
Explore the impact of vaccination with **C62-M4** or **C1C62-M3M4** on cell-associated HIV RNA in $CD4^+$ T cells.	Relative change in cell-associated RNA at multiple pre- (minimum 2) and post-vaccination (minimum 2) timepoints.	Multiple pre- and post-vaccination timepoints needed to evaluate the stability of any change observed.
Explore the impact of vaccination with **C62-M4** or **C1C62-M3M4** on total HIV DNA in $CD4^+$ T cells.	Relative change in cell-associated RNA at multiple pre- (minimum 2) and post-vaccination (minimum 2) timepoints.	Multiple pre- and post-vaccination timepoints needed to evaluate the stability of any change observed.

4 STUDY DESIGN

4.1 Overall Design

This is a Phase 1, pilot study with a double blind, randomized, placebo-controlled, parallel design to evaluate safety and immunogenicity of **C62** followed by **M4** or, **C1C62** given in combination followed with **M3M4** given in combination.

The participants will be PWH suppressed on ART with plasma HIV-1 RNA <50 copies/mL.

We hypothesize that **C62** followed by **M4** or, **C1C62** given in combination followed with **M3M4** given in combination will be safe and increase HIV-1-specific T cell responses targeting conserved regions of HIV-1. We also hypothesize that administration of **C1C62-M3M4** will result in a greater increase in the breadth of HIV-1-specific T cells targeting conserved regions of HIV-1 than **C62-M4** vaccination.

Vaccine and placebo doses will be administered to all participants as 2 separate IM injections in the deltoid muscle of each arm. Participants continue baseline ART regimen throughout the study.

Participants will be randomized 8:8:2 to one of three study arms and receive study treatment or placebo at D0 and D28 as in Table 14.

Table 14 Study Randomization

Arm	N	Day	Treatment	Total Dose (vp)	Route	Day	Treatment	Total Dose (pfu)	Route
1	8	0	**C62**	5×10^{10}	IM	28	**M4**	1.8×10^{8}	IM
2	8	0	**C1C62**	5×10^{10}	IM	28	**M3M4**	1.9×10^{8}	IM
3	2	0	Placebo (saline)	-	IM	28	Placebo (saline)	-	IM

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, or definitely related to study treatment. The period of observation is from the day of ChAdV vaccination at D0 through to D56 which is 28 days following the MVA vaccination or placebo. The primary safety analysis will be blinded until D56 or 28 days following the MVA vaccination or placebo in the last study participant.

Participants will be followed and monitored for clinical events that are possibly, or definitely related to study treatment per the SoA. All participants will be informed about potential contraindications and risks associated with the study vaccines.

Safety will be assessed for the duration of the study. Solicited AEs will be recorded for 28 days after each dose of study intervention (through Day 57), and SAEs, MAAEs, and AESIs will be recorded through the EOS.

Study analysis plans are discussed in the Statistical Analyses Section 10.7.

4.2 Scientific Rationale for Study Design

The scientific rationale of this study is that vaccination that shifts $CD8^+$ T cell targeting (breadth and magnitude) away from variable HIV epitopes and towards conserved HIV regions enabling better T cell mediated control of HIV-reactivation.

Vaccine Vectors: Immunogenicity - We are testing serial vaccination with two live-attenuated vaccine modalities ChAdOx1 and MVA expressing complementary immunogens. This heterologous vector approach is often termed, prime-boost vaccination. Vaccination with heterologous viral vectors consistently elicits higher frequencies of immune responses against the vaccine immunogen than homologous vaccination regimens (131). Safety: As detailed above, Both ChAdOx1 and MVA vectored vaccines given IM either alone or serially have been safe and well tolerated. Altogether, the immunogenicity and safety profiles of these vaccines support their choice in this study.

Immunogens: The HIVconsvX design targets regions of HIV that are both conserved and harbor high numbers of T cell epitopes associated with control of HIV viremia. T-cell epitopes in the HIVconsvX immunogen are also significantly less-likely to harbor virus escape variants in either vRNA or the latent HIV reservoir. We therefore hypothesize these conserved immunogens will induce consistent and significant increases in circulating T cell-mediated immunity in participants. We will compare bivalent mosaic (**C1C62-M3M4**) and monovalent mosaic (**C62-M4**) immunogens. We hypothesize that following vaccination, the bivalent mosaic immunogens will induce a greater breadth of T-cell response than the monovalent immunogen.

Cohort: Individuals chronically infected prior to ART treatment will be prioritized for enrollment.

We and others have documented that individuals treated in chronic infection have sustained and detectable HIV specific T-cell responses (132). This compares with ART initiated in acute infection where treatment may blunt the induction of T cell responses against HIV. Studies in individuals treated in chronic infection will allow us to better address our study hypothesis which focuses on shifting T cell dominance away from variable epitopes to immunogenic, but more conserved epitopes that are less subject to escape. Accordingly, our endpoint assays are designed to also examine de novo induction of HIV-specific T cell responses.

Our exploratory objectives will examine whether vaccination decreases the size of the HIV reservoir. Therefore, we are targeting chronically HIV-infected participants who harbor a detectable, complex and relatively stable reservoir.

Durable Suppression: The inclusion criterion of > 2 years of durable suppression has been included for two reasons. First, we have shown that HIV T cell responses in durably suppressed participants are highly stable, providing a reliable baseline to examine changes in T cell frequencies (132). Second, the size of the HIV reservoir stabilizes after 2 years providing a stable baseline to examine change in virologic measurements (65).

Placebo Controls: Two placebo controls are included in this study. Powering calculations described Section 10.3 are independent of a placebo arm. Placebos have been included to ensure real-time objectivity in recording study AEs. Placebo-associated safety and immunogenicity data in this study will be combined with data collected from placebos (n=3) from our current trial IGHID 11810 (The M&M Study), giving a final n=5 of placebo controls.

4.3 Justification for Dose

The vaccine doses to be used in the clinical study are provided in Table 15.

Table 15 Vaccine Doses

Group	Vaccine	Dose	Route
1	C62	C62 = 5 x 10^{10} virus particles (vp)	IM
	M4	M4 = MVA.tHIVconsv4 = 1.8 x 10^{8} plaque-forming units (pfu)	IM
2	C1C62	C1 = 2.5 x 10^{10} vp C62 = 2.5 x 10^{10} vp	IM
	M3M4	M4 = MVA.tHIVconsv4 = 0.9 x 10^{8} pfu M3 = MVA.tHIVconsv3 = 1 x 10^{8} pfu	IM
3	Placebo	Normal saline injection	IM

Justification for ChAdOx1 vaccine dose: Fourteen clinical trials testing 10 different ChAdOx1 vaccines have been or are in clinical testing (n=872 participants) (ChAdOx.tHIVconsv1 IB Section 6, ChAdOx1.HIVconsv62 IB Section 6). Safety and immunogenicity data are available for 789 participants with 567 study participants receiving an IM dose of 5×10^{10} vp; the same dose and route of vaccination proposed for this CM Study. The safety profiles of ChAdOx1 vectors have been well tolerated to date. Solicited AEs have mostly been mild or moderate with unsolicited AEs mostly classed as mild/Grade 1 (ChAdOx.tHIVconsv1 IB Section 6, ChAdOx1.HIVconsv62 IB Section 6).

ChAdOx1 is highly related to the ChAd63 vector which has also undergone extensive clinical testing. Participants (n=1,367) have received recombinant ChAd63 vaccines given IM at a dose of 5×10^{10} vp, mostly in prime-boost combination with MVA-vectored vaccines. Vaccination has been safe and well tolerated (ChAdOx.tHIVconsv1 Section 7, ChAdOx1.HIVconsv62 IB Section 7). This dose has also been consistently immunogenic, either inducing or increasing the frequency of circulating T cells against the vaccine immunogen.

Justification of the MVA vaccine dose: **M4** vaccination will be given at a dose of 1.8×10^8 pfu and **M3M4** given at a final dose of 1.9×10^8 pfu. We anticipate dosing to be immunogenic, well tolerated but also provide economical use of **M4** Good Manufacturing Practice (GMP) vaccine.

In the ongoing UNC Study NCT03844386 (IND 18368), **M3** and **M4** given IM to PLW on ART at a final dose of 2×10^8 pfu has been safe and well tolerated in 21 participants (Table 7). These data are consistent with other studies in which recombinant MVA has proven to be safe and tolerated when delivered IM at a dose of 2×10^8 pfu (Table 6) (92, 97). The AEs reported in prior 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) (See IND 18368, Investigator Brochures).

Based on data from all studies, there were very few treatment-related SAEs reported following MVA vaccination (See IND 18368, Investigator Brochures) (93). In a study of MVA expressing influenza antigens delivered as an IM injection (n=8), fewer local adverse events were observed than with intradermal (ID) injection (n=12) (133). Systemic adverse events (AEs) were similar for both IM and ID injection.

Blinded data to date also suggests that both the **M3** and **M4** vaccines given at similar doses are likely to be immunogenic (Figure 2). These immunogenicity data (NCT03844386; IND 18368) are supported by other studies in which doses of MVA from 5×10^7 to 2×10^8 have been immunogenic, including in PWH (66, 97, 102, 134, 135). Across those studies stronger immunogenicity has been observed at doses between $1\text{-}2\times10^8$ pfu (66, 97, 102).

4.4 End of Study Definition

A participant is considered to have completed the study if he or she has completed all study required visit including the last visit per 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 ≤ 70 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 all study requirements through D196/Week 28.

6. Continuous ART prior to screening, defined as not missing more than 14 total days and never more than 7 consecutive days in the last 3 months prior to screening.

7. No change in any ART medication in the 30 days prior to screening.

   Permitted ART regimens include:

   a. At least 3 ART agents (not counting ritonavir or cobicistat as one of the agents if less than a 200 mg 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

   b. 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.

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

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

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

10. Documented plasma HIV-1 RNA result <50 copies/mL ≥24 months but ≤ 36 months prior to screening.

11. 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.

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

13. Completion of COVID-19 vaccine dosing at least 14 days prior to enrollment (see exclusion criterion regarding COVID-19 vaccination).

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

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

16. Adequate vascular access for leukapheresis.

17. Able and willing to receive IM injections in both arms without difficulty.

18. All women must have a negative serum pregnancy test with a sensitivity of a least 25 U/mL at screening regardless of reproductive potential.

19. 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.

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

20. 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 3 (D0) and for 4 months after their last vaccination:
    a. Condoms (male or female) with or without a spermicidal agent
    b. Diaphragm or cervical cap with spermicide
    c. Intrauterine device (IUD)
    d. Tubal ligation
    e. Hormone-based contraceptive

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

21. Men and women 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.

NOTE: Women who have sex with women only will not be required to use contraception.

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

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

22. Agrees not to enroll on another study of an investigational agent during the study period, defined as any unlicensed investigational drug not yet approved for use in humans.

23. Willingness to defer routine vaccination, including influenza, from 14 days prior to enrollment through Day 56 of the study.

    NOTE: Individuals who require vaccination will delay enrollment until 14 days after vaccination.

24. Agrees to refrain from blood donation during the course of the study.

25. Participants with Type 2 diabetes must have a hemaglobin A1C <8 within 12 months prior to screening.

26. Adequate organ function as indicated by the laboratory values provided in Table 16.

Table 16 Adequate Organ Function Values for Inclusion

System	Laboratory Value
Hematological	
Absolute neutrophil count	≥ 1,000 /mcL
Platelets	≥ 150,000 / 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	WNL
Serum magnesium levels	>LLN[1]
Glucose	Screening serum glucose ≤ Grade 1 (fasting or non-fasting)
Renal	
Creatinine clearance determined by the 2021 CKD-Epi equation found at: https://www.mdcalc.com/calc/3939/ckd-epi-equations-glomerular-filtration-rate-gfr	eGFR > 60mL/min
Hepatic	
Serum total bilirubin	Total bilirubin <1.5 x ULN. If elevated, direct bilirubin must be <2 x ULN If on atazanavir –containing therapy, a direct bilirubin should be measured instead and must be ≤1.0mg/dL.
AST (SGOT) and ALT (SGPT)	<1.5 X ULN
Alkaline Phosphatase	<1.5X ULN

[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.

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

5.2 Exclusion Criteria

1. Women of childbearing age/potential who are breast feeding, pregnant, or planning pregnancy from enrollment to 4 months after the last vaccination.

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

NOTE: Potential participants may rescreen with documentation of adequate treatment. Participants reporting symptoms consistent with syphilis infection between the screening visit

and the vaccination visit should be assessed to determine the need for repeat testing prior to vaccination.

3. Current treatment for HCV or HCV treatment within 6 months prior to enrollment.

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

5. Received any infusion blood product or hematopoetic growth factors within 6 months prior to enrollment.

NOTE: receipt of COVID convalescent plasma ≥ 90 days prior to enrollment is not exclusionary.

6. Use of any of the following agents within 90 days prior to enrollment: immunomodulatory, cytokine, or growth stimulating factors such as systemic cytotoxic chemotherapy or immune globulin.

7. Intent to use immunomodulatory treatment during the study.

8. Use of systemic corticosteroids or topical steroids over a total area exceeding 225 cm^2 within 30 days prior to enrollment, or anticipated need for periodic use of systemic corticosteroids during the study.

NOTE: Participants receiving stable physiologic doses of glucocorticoids, defined as the equivalent of prednisone ≤10 mg/day, will not be excluded. Participants receiving inhaled, intranasal, topical (as defined), intermittent intra-articular corticosteroids, or topical imiquimod will not be excluded.

NOTE: Concomitant use of oral/systemic /intra-articular/inhaled/intranasal corticosteroids is prohibited for participants receiving ritonavir or cobicistat.

9. Use of any investigational HIV vaccine or HIV immunotherapy.

NOTE: Exceptions allowed per PI review and approval.

10. Any experimental non-HIV vaccination within the 6 months prior to enrollment.

NOTE: receipt of FDA or EUA approved or licensed COVID-19 vaccines is not exclusionary if ≥ 14 days prior to enrollment on a case by case review by PI or designee.

11. Prior receipt of any adenovirus Group E-vectored vaccines including those for COVID-19 (i.e. AZD1222/AstraZeneca).

NOTE: Prior immunization with smallpox vaccine is not exclusionary.

NOTE: Janssen Ad26COVS1 COVID-19 vaccine is not exclusionary.

12. Prior receipt of a live vaccine within 60 days prior to enrollment (i.e. varicella, measles, mumps, rubella (MMR), and yellow fever).

NOTE: Individuals who require live vaccination will delay enrollment until 60 days after vaccination.

NOTE: Receipt of Mpox vaccine is not exclusionary as long as the last Mpox vaccination is received at least 60 days prior to enrollment.

13. Use of any other investigational treatment within 6 months prior to enrollment, with the exception of Phase II or higher 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.

14. 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 enrollment.

15. History of inflammatory diseases involving the peripheral or central nervous system, including but not limited to GBS, myasthenia gravis, optic neuritis, multiple sclerosis, transverse myelitis, neuromyelitis optica spectrum disorder (NMOSD) and chronic inflammatory demyelinating polyneuropathy (CIDP), that in the opinion of the investigator would preclude participation.

16. History of pregnancy, head trauma or major surgery within 90 days prior to enrollment, that in the opinion of the investigator would preclude participation.

NOTE: Minor surgery within 90 days prior to enrollment and not resulting in reduced mobility will be assessed on a case-by-case basis.

17. Any clinically significant acute or chronic medical condition, other than HIV infection, that in the opinion of the investigator would preclude participation.

18. History of malignancy within the last 3 years.

NOTE: History of non-melanoma skin cancer (e.g., basal cell carcinoma or squamous cell skin cancer) is not exclusionary with documentation of resolution per topical treatment or complete resection as determined by a dermatologist at least 3 months prior to enrollment.

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

20. Any medical, psychiatric, substance abuse, 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.

21. Blood pressure consistently > 150 mm Hg systolic and >100 mm Hg diastolic.

NOTE: Elevated BP during leukapheresis procedures is not exclusionary. Isolated elevations must be noted as acceptable and signed by study PI or designee.

22. History of seizure(s) within the past 3 years.

23. History of splenectomy.

24. Bleeding disorder including factor deficiency, coagulopathy or platelet disorder that requires special precautions (easy bruising without a formal diagnosis is not exclusionary) or on chronic anticoagulation

25. Allergy to eggs and/or egg products.

26. History of anaphylaxis or severe adverse reaction to prior vaccines including symptoms such as hives, respiratory difficulty, angioedema, and/or abdominal pain.

27. History of hereditary angioedema, acquired angioedema or idiopathic angioedema.

28. Known allergy/sensitivity or hypersensitivity to components of study vaccines.

29. Unstable asthma (e.g. sudden acute attacks occurring without an obvious trigger) or asthma requiring:
    a. Daily steroid or long-acting beta-agonist prevention
    b. Hospitalization in the last two years

30. Active chronic skin problems such as eczema or psoriasis not controlled with topical treatments.

31. Inability to communicate effectively with study personnel.

5.3 Screening

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).

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

5.3.1 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.

5.3.2 Screen Failures

Screen failures are defined as participants who consent to participate in this study but are not subsequently randomly assigned to the study intervention or entered in the study.

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.

5.4 Strategies for Recruitment and Retention

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

- In the UNC and Duke ID Clinics 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 and Duke HIV Community Outreach Organizations.

5.4.1 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, some FDA or EUA sanctioned COVID-19 treatments and vaccines are permitted (see eligibility criteria) but 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

Double blind, randomized, placebo-controlled, parallel design, study in which 18 participants with durable viral suppression are randomly assigned to receive vaccination with C62-M4, C1C62-M3M4, or placebo.

Participants will be randomized 8:8:2 to one of three study arms and receive study treatment or placebo at D0 and D28.

6.1.1.1 Manufacture of C62 AND C1

The **ChAdOx1.tHIVconsv1 (C1)** drug substance has a genome size of 32,906 bp and is supplied as a slightly opaque frozen liquid, essentially free from visible particulates. The appearance is dependent upon the concentration of the virus (Table 1). **C1** was manufactured in accordance with GMP by ADVAXIA BIOLOGICS S.r.l., Via Pontina km 30.600, 00071 Pomezia, Rome, Italy. The code name for the Drug Substance is ChAdOx1.tHIVconsv1. There is no recommended International Non-proprietary Name (INN).

The **ChAdOx1.HIVconsv62** drug substance has a genome size of 32,846 bp and is supplied as a slightly opaque frozen liquid, essentially free from visible particulates. The appearance is dependent upon the concentration of the virus (Table 1). **C62** was manufactured accordance with GMP by ADVAXIA BIOLOGICS S.r.l., Via Pontina km 30.600, 00071 Pomezia, Rome, Italy. The code name for the Drug Substance is ChAdOx1.HIVconsv62. There is no recommended INN.

6.1.1.2 Manufacture of M3 + M4

The drug substance/investigational medicinal product for **M3** and **M4** was manufactured in accordance with GMP by IDT Biologika GmbH (IDT), Am Pharmapark, 06861, Dessau-Rosslau, Germany.

6.1.2 Dosing and Administration

For further information on Dosing and Administration please see Section 4.1.

6.1.2.1 Dosing

All participants will receive vaccine/placebo administered on D0 and a booster vaccine/placebo administered at D28. Participants will be randomized to Arms 1, 2 or 3, respectively.

For the purpose of this study and safety management, a maximum of 2 study participants can be vaccinated in one week.

6.1.2.2 Study Treatment Administration

C1, **C62**, **M3** and **M4** vaccines should be thawed to room temperature and administered within 60 minutes of removal from the freezer. Vaccination will be performed, and the study product handled according to the relevant SOPs.

Administer all vaccines/placebo doses at D0 and D28.

Administer all vaccine/placebo doses as an IM injection in the deltoid muscle (Table 17).

Table 17 Study Treatment Administration

STUDY ARM	DAY	Deltoid - 1	Deltoid - 2
1. C62-M4	0	**C62** (2.5×10^{10} vp)	**C62** (2.5×10^{10}vp)
	28	**M4** (0.9×10^{8} pfu)	**M4** (0.9×10^{8} pfu)
2. C1C62-M3M4	0	**C62** (2.5×10^{10} vp)	**C1** (2.5×10^{10} vp)
	28	**M4** (0.9×10^{8} pfu)	**M3** (1.0×10^{8} pfu)
3. PLACEBO	0	Saline	Saline
	28	Saline	Saline

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

6.1.2.2.1 C1 and C62

Study Arm 1: Half of the **C62** vaccine will be administered into the deltoid muscle of each arm, with the dose prepared for administration by the site Investigational Drug Service (IDS). The IDS will direct which arm (left or right) each syringe will go into as assigned by the randomization schedule.

Study Arm 2: **C1** will be delivered in the deltoid of one arm and **C62** will be delivered to the other arm. The dose of both products will be prepared for administration by the site IDS. The IDS will direct which arm (left or right) each vaccine will go into as defined by the randomization schedule.

6.1.2.2.2 M3 and M4

Study Arm 1: Half of the **M4** vaccine will be administered into the deltoid muscle of each arm, with the dose prepared for administration by the site IDS. The IDS will direct which arm (left or right) each syringe will go into as assigned by the randomization schedule.

Study Arm 2: **M3** will be delivered in the deltoid of one arm and **M4** will be delivered to the other arm. The dose of both products will be prepared for administration by the site IDS. The IDS will direct which arm (left or right) each syringe will go into as defined by the randomization schedule.

6.1.2.2.3 Saline Placebo

Study Arm 3: Saline will be administered into the deltoid muscle of each arm, prepared for by the site IDS. The IDS will direct which arm (left or right) each syringe will go into as assigned by the randomization schedule.

6.1.2.2.4 Rescheduled Booster Vaccination

Participants whose D28 vaccination is delayed due to toxicity or other unanticipated event; that has been reviewed and approved by the PI (or designee) will have post D28 visits at the timepoints per the original schedule of events. This means booster vaccination will remain Visit 7 but will be re-named to represent the number of days post prime vaccination (D0). Consequently, Visit 8 will occur 2 days after booster vaccination, Visit 9 - 7 days, Visit 10 – 14 days, and so on. Post D28 procedures will take place per schedule of events based on the date of D28. The events will be renamed to match actual day. (i.e. D28 will remain as Visit 7 but will become actual day booster vaccine given post D0).

New day numbers and timelines will be documented appropriately in study records.

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Acquisition and Accountability

The **C1**, **C62**, **M3** and **M4** vaccines will be used as supplied by the manufacturer. It will be prepared, dispensed and administered according to Pharmacy Manual and site-specific SOPs.

Normal saline use for placebo will be obtained by the site, supplied by site IDS.

The **C1**, **C62**, **M3** and **M4** vaccines will be stored, distributed from, and accountability maintained in the site IDS at UNC and Duke.

The primary IDS pharmacist at each site will be responsible to the Protocol Principal Investigator for maintaining study drug accountability, reconciliation, and record maintenance during the study, including documentation of the amount of both study treatments (**C1**, **C62**, **M3** and **M4**) received in IDS and the amount administered to each participant.

6.2.2 Formulation, Appearance, Packaging, and Labeling

ChAdOx1.tHIVconsv1 vaccine (C1) was manufactured in formulation buffer at a target concentration of >1.1x10^{11} vp/mL. It is supplied at a concentration of 1.3 x10^{11} vp/mL. The fill volume is 0.65 mL.

ChAdOx1.HIVconsv62 vaccine (C62) was manufactured in formulation buffer at a target concentration of >1.1x10^{11} vp/mL. It is supplied at a concentration of 1.6 x10^{11} vp/mL. The fill volume is 0.65 mL.

MVA.tHIVconsv3 vaccine (M3) is a white cloudy solution, formulated in Tris-saline buffer 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 vaccine (M4) is a white cloudy solution, formulated in Tris-saline buffer 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.

C1, C62, 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:

1. Study product name
2. Concentration
3. Manufacturer
4. Date of manufacture
5. Lot number
6. Volume

6.2.3 Product Storage and Stability

The investigational vaccines are shipped from manufacturing site to study site IDS pharmacy with temperature monitoring capacity. Once received at the IDS Pharmacy, the study vaccines are transferred to temperature-monitored storage.

Vaccine accountability, storage and shipment will be in accordance with Pharmacy Manual, site IDS SOPs (as applicable) and DAIDS requirements.

6.2.3.1 C1 and C62

The vaccines are supplied as frozen liquids in glass vials for IM administration and are stored at -75°C ± 15°C in a secure freezer. The freezers are temperature monitored and if outside

designated range appropriate action is taken in accordance with Pharmacy Manual, site IDS SOPs (as applicable) and DAIDS requirements.

6.2.3.2 M3 and M4

The study vaccines are kept in temperature-monitored storage at -75 °C ± 15°C in a locked freezer (See IND 18368, Investigator Brochures). The freezers are temperature monitored and if outside designated range appropriate action is taken in accordance with Pharmacy Manual, site IDS SOPs (as applicable) and DAIDS requirements.

6.2.3.3 Saline

The placebo (normal saline) will be stored at room temperature.

6.2.4 Preparation

6.2.4.1 Dilution of C1 and C62

Refer to Pharmacy Manual.

6.2.4.2 Dispensing and Handling

1. The vaccine will be dispensed according to Study Specific Pharmacy Manual and/or study-specific SOPs.

2. All vaccines will be used as supplied by the manufacturer and prepared per pharmacy manual guidelines.

3. The vaccines will be thawed to room temperature.

4. The required dose volume will be drawn into the appropriate syringe and C1, C62, M3 and M4 vaccines will be administered within 1 hour of thawing. C1, C62, M3 and M4 vaccines will be dispensed from the UNC or Duke IDS Pharmacy, where only the pharmacist(s) will be un-blinded.

5. All syringes (vaccine and placebo) will prepared per research site's IDS Pharmacy procedures to maintain blinding.

   NOTE: When preparing the placebo, sodium chloride for injection 0.9% should be used in place of C1, C62, M3 or M4.

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

6.2.4.3 Labeling

Label the prepared study product or placebo with the following to maintain blinding:

1. Participant identifier(s)
2. Study Product Name
3. Route
4. Site (L or R deltoid) to be administered.
5. Expiration time or beyond use date and time.
6. Any additional information required by IDS.
7. Caution: New Drug—Limited by Federal (or United States) law to investigational use

6.3 Measures to Minimize Bias: Randomization and Blinding

6.3.1 Randomization

The 18 study participants will be randomly assigned to receive one vaccination with **C62-M4** (n=8) or **C1C62-M3M4** (n= 8) or placebo (n=2) through a blinded randomization schedule as further described in Section 8.2.5. When participant eligibility is confirmed, study specific identification numbers (SIDs) are assigned sequentially for each participant at the Enrollment Visit. Vaccine and placebo product will be indistinguishable and clearly labeled with the administration site: left deltoid or right deltoid muscle. Site and laboratory personnel and participants will be blinded with respect to the allocation of vaccine or placebo. A double-blind placebo-controlled, permuted-block design has been chosen to minimize bias in the reporting of safety and immunological data.

Participants will be randomized and assigned a Randomization ID (RID) on D0 by study data staff prior to vaccine/placebo administration. The institutional or site IDS will be provided a clearly decoded key of the three study arms in a separate file that will be maintained in a secure location. The randomization schedule will be generated centrally by the study statisticians at the UNC CFAR using computer software and will be kept concealed from study personnel.

6.3.2 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. Procedures and contact details for un-blinding procedures will specified in a unit SOP. Reference Section 8.2.5.

Unblinding may occur in response to a specific request from any regulatory agency to unblind the study. Blinding information will be shared with study staff who will then contact participants to inform them of the treatment they received.

6.4 Study Intervention Compliance

Participants will keep a daily diary of solicited AEs for local and systemic symptoms for 7 days after both vaccine/placebo administrations.

6.5 Concomitant Therapy

Whenever a concomitant medication is initiated or the dose is changed for all participants after receipt of vaccine/placebo at D0 through D56 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.

6.5.1 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.

2. 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)

6.5.2 Prohibited Medications

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

2. Concomitant use with oral or parental corticosteroids, immunosuppressive agents (including but not limited to azathioprine, and cyclosporine) or any immunotherapy or immunomodulatory agents.

3. Inhalers or nasal sprays containing steroids from 14 days prior to first vaccination through Day 42, 14 days after the second vaccination.

4. Antihistamines from 7 days prior to vaccination through Day 35.

5. Standard vaccines (e.g., pneumococcal, Hepatitis A, Hepatitis B, influenza) are prohibited 14 days prior to enrollment through Day 56.

6. Available COVID-19 vaccines are prohibited 14 days prior to enrollment through Day 56. NOTE: receipt of adenoviral vectored vaccines (i.e. AstraZeneca, Janssen) should be deferred until 3 months after the last dose of the ChAdOx1.tHIVconsv1 vaccine (Day 90).

7. Live vaccinations (e.g., varicella, measles, mumps, rubella, MMR, yellow fever, oral polio) are prohibited from 60 days prior to enrollment through Day 56, reference Sections 5.1 and Section 5.2;

8. Mpox vaccinations are prohibited from 60 days prior to enrollment through D56.

   NOTE: Administration of live vaccine for Mpox is allowed at any time after enrollment if indicated for post-exposure prophylaxis.

9. Any agent that suppresses lymphocytes or monocyte function.

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

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

12. Sporadic topical use of corticosteroids (e.g. creams) to small areas of the skin (<225 cm^2) for participants who are receiving ritonavir or cobicistat as part of their current ART regimen.

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

14. Refer to exclusion criteria in Section 5.2.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 Discontinuation of Study Intervention

7.1.1 Premature Treatment Discontinuation Evaluations

Study treatment intervention will occur at D0 and D28. The collection of safety labs, virologic, immunologic, SCA, and the 2nd leukapheresis will continue to be collected per SoA, Section 1.3.

Participants who do not complete both vaccine/placebo D0 and D28 injections will be replaced, provided the inability to complete the D28 vaccination visit is not due to a study-related AE.

Participants who complete both vaccine/placebo D0 and D28 injections but do not complete the D35 study visit will be replaced, provided the inability to complete the D35 visit is not due to a study-related AE.

Participants who receive at least one dose of vaccine/placebo but are unable to complete either the D28 vaccination, the D35 visit, the 2nd leukapheresis or the D56 visit should be encouraged to continue on study for safety assessments per the SoA, Section 1.3.

7.1.2 Discontinuation of Antiretroviral Therapy

If the participant discontinues ART prior to D0, DO NOT give the vaccine/placebo injection.

If the participant discontinues ART at any time after the first vaccine or placebo administration, the participant should continue on study for safety follow-up as noted in the SoA, Section 1.3. 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

7.2.1 Enrolled Participants, who are Not Randomized and Do Not Start Study Treatment

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.

A participant who is unable to complete the leukapheresis procedure or has less than 20 ml of leukapheresis product collected prior to D0 may be terminated from the study as determined by the PI.

If terminated:

DO NOT re-use the SID.

REPLACE the participant.

Termination can also 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.

Participants who complete the first leukapheresis procedure pre-vaccine but do not want to do the second leukapheresis procedure may be withdrawn from the study as determined by the PI.

If terminated:

DO NOT re-use the SID.

REPLACE the participant.

7.2.2 Evaluations for Randomized Participants Who Do Not Start Study Treatment

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.

- DO NOT re-use the RID.
- DO NOT re-use the SID.
- REPLACE the participant.

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

Participants who withdraw or are withdrawn after randomization and prior to D28, will have no further research evaluations. Continue follow-up for safety through D28 (based on review by PIs) will be encouraged and if participant is unable to continue for visits through D28, they will be asked to complete EOS safety evaluation visit.

- DO NOT re-use RID.
- DO NOT re-use the SID.
- REPLACE the participant.

7.2.4 Participant May Withdraw or be Withdrawn from the Study

- Request by the participant to withdraw from the study and study procedures.
- Development of an illness that requires treatment with medications prohibited in the 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 D7 or D35 visit.
- 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 PI (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.

7.3 Premature Study Discontinuation (D/C) Evaluations

Participants who prematurely discontinue study participation after completing the vaccine/placebo prime (D0) and boost (D28) but before D196 will have premature study discontinuation (D/C visit) evaluations performed per the SoA for D196.

7.4 Lost to Follow-Up

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

1. Failure to respond or reply to 3 documented phone contact attempts, followed by

2. Failure to respond to a certified letter sent to the address provided by the participant.

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 Exclusion Efficacy Assessments

8.1.1 Remote Data Collection

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

a. a participant is unable to attend a visit because of a debilitating illness; the site must inform the protocol team in advance.

b. 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.

c. 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.

8.1.2 Visit windows

a. Perform visits per window listed in SoA, Section 1.3. There is no window around the D2, D7, D30, and D35 visits. There is a ± 2 days window around the visits at Days 14, 28, 42 and 56.

b. There is a ± 1 day window around the telephone call assessments at Days 5, 10, 18 and 22. See Appendix 2.

c. There is a ± 7 days window around the visits at Days 84 and 140. D196 has a ± 14 days window. Please reference SoA, Section 1.3 and Sections 4.1 and 8.2.3.

d. Post D28 visits are scheduled based on the date of D28 booster vaccination. Consequently, D30 will occur exactly 2 days after booster vaccination, D35 will occur exactly 7 days after, D42 will occur 14 days (+/- 2 days) after, and so on.

e. Window for D56 leukapheresis is – 7/+21 days with preference for the procedure to be completed as close to Day 56 as possible. If unable to complete the D56 leukapheresis and upon review and approval of the protocol PI, a large blood draw can be completed between 14 – 21 days after D56 (Days 70-77). This large blood draw will be completed as close to 21 days after D56 as possible (Section 8.2.3)

8.1.3 Medical History

The medical history includes all signs, symptoms and diagnoses regardless of grade within the 30 days prior to entry and any significant medical conditions noted in medical records (e.g., hospitalizations, surgeries, prior medical history). Assessment and documentation of the medical history evaluation occurs at the screening visit. Update to medical history will occur at all clinical visits.

Document:

a. All allergies to any medications and their formulations

b. Date of birth, gender, race and ethnicity of participant

c. HIV history, including acute status at ART initiation and HIV-1 RNA suppression, if available

d. Nadir CD4 and pre-ART/peak HIV-1 RNA level, if available (if documentation is not available, collect and record participant recall)

e. 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 first vaccination date (see Section 5.1) through the 4 months following the last vaccination date.

- 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.

8.1.4 Medication History

Complete a medication history, include medication start and stop dates. Document the vaccine/placebo administration as indicated Table 18.

Table 18 Medication Complete History or Timeframe

Medication Category	Complete History or Timeframe
Antiretroviral Therapy	Complete History
Immune-based Therapy	Current & within 90 days prior to screening.
HCV antiviral therapy	Complete history
Prescription Drugs	Within 30 days prior to screening
Systemic Corticosteroids	Within 30 days prior to screening
HIV-1 related vaccines	Complete History
Experimental non-HIV vaccine	Within the 6 months prior to screening
Vaccines	Within 60 days prior to screening (For Covid-19 and Mpox vaccines, complete history)
Experimental treatment for COVID-19 illness	Within the 90 days prior to screening
IgG therapy or immunization w/experimental Abs	Within 90 days prior to screening
Non-prescription drugs (OTC)	Within 30 days of enrollment

8.1.5 Clinical Assessments

a. Complete Physical Exam (PE): A complete PE will be performed at screening and D196. 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).

b. Targeted Physical Assessment: A targeted physical assessment is done at all identified 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.

c. Vital Signs (VS): Perform VS at all clinical visits. VS include P, BP, RR, T, and weight. Measurement of weight is required at check in only.

On vaccination days, vital sign measurements will be collected before vaccination. If individual vital sign measurements are considered clinically significant by the investigator, vaccination may be withheld that day (or, as noted for BP, delayed briefly pending a calming period), and participants may return on a subsequent day for re-evaluation and vaccination, ideally, within the allowed visit window (SoA, Section 1.3).

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

e. 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.

f. 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.

g. 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. This will be checked along with the concomitant medications at each visit.

h. Post Vaccine Assessment: Assess for any injection site reactions and systemic reactions. Record any reportable events.

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

j. Solicited Adverse Events

Local and systemic predefined solicited AEs for reactogenicity assessment (Section 8.2.10) will be collected in a Participant Symptom Diary for 7 days following the administration of each dose of study product. In addition, assessments of solicited AEs will occur at study visits through 28 days following administration of each dose of study product.

Solicited AEs should not be reported as unsolicited AEs (see Section 8.3). However, solicited AEs should be reported as SAEs, MAAEs or AESIs if they meet criteria (Sections 8.3.2, 8.3.3 and 8.3.4 respectively).

k. Unsolicited Adverse Events

Active solicitation of AEs will be done at every study visit. Post-entry signs and symptoms, Grade ≥ 2, will be recorded. All signs or symptoms, definitely or possibly 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.

l. Diagnoses: After entry, record all diagnoses identified.

m. Concomitant Medication Assessments: At screening, 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.

8.1.6 Laboratory Studies

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

b. 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.

c. Heparin Platelet Factor (PF) 4 Antibody: A test for heparin-induced thrombocytopenia (HIT) antibody, also called heparin-PF4 antibody, is performed to detect antibodies that develop in some people who have been treated with heparin. It is used to help establish a diagnosis of immune-mediated heparin-induced thrombocytopenia (HIT type II) when you have a low platelet count (thrombocytopenia) and excessive clotting (thrombosis).

This serum sample will be collected at enrollment and processed and stored in the research lab for testing should participant develop a clotting adverse event.

8.1.7 Research Assays

Blood will be collected for the following research laboratory evaluations:

a. Human Leukocyte Antigen (HLA) Typing: HLA testing will be performed; however, if HLA type is available in the medical record, it does not need to be repeated. The result will be used for research purposes.

b. CMV Testing: Cytomegalovirus (CMV) is a common virus that usually causes no symptoms or only mild illness. CMV testing detects antibodies in the blood that the body produces in response to the infection or detects CMV directly. In the United States, as many as 60% of people have been exposed to CMV at some point in their life. If positive CMV antibody results are available in the medical record, it does not need to be repeated.

c. EBV Testing: The Epstein-Barr virus (EBV), also known as human herpesvirus 4, is a gamma herpes virus that occurs only in humans. Laboratory testing can help distinguish whether someone is susceptible to EBV infection or has a recent or past infection.

The Anti-VCA IgG appears in the acute phase of EBV infection, peaks at two to four weeks after onset, declines slightly then persists for the rest of a person's life. If positive EBV antibody results are available in the medical record, it does not need to be repeated.

d. COVID-19 RT-PCR Testing: The COVID-19 RT-PCR test is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test for the qualitative detection of nucleic acid from SARS-CoV-2 collected as upper respiratory specimens (such as nasopharyngeal or oropharyngeal swabs, nasal swabs, or mid-turbinate swabs). Participants testing positive by RT-PCR at enrollment would delay progression on study until 30 days from date of diagnosis if asymptomatic or 30 days from resolution of mild to moderate symptoms related to COVID-19.

e. COVID-19 Antibody Testing: COVID-19 nucleocapsid antibody testing on stored plasma as indicated per the SoA, Section 1.3.

f. Plasma/PBMC for Immunology Studies: Plasma and cryopreserved PBMC will be stored for analysis of low-level viremia, T-cell specificity, phenotype and function of immune cells, and virologic measurements per the SoA, Section 1.3. Specific testing will include the following:

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

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

NOTE: See Section 7.1 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 inclusions and exclusion prior to enrollment.

8.2.2 Participant Enrollment

Enrollment occurs within the 60 days following screening. Participants not meeting eligibility requirements will not continue on study.

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

Participants testing RT-PCR positive for COVID-19 at enrollment, will delay vaccination following enrollment (See Section 8.1.7D above), determined by PI (or designee) on a case by case basis. Labs obtained at the original enrollment will not need to be repeated unless requested by PI after review.

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. Safety labs (chemistry, hematology, and COVID-19 RT-PCR) and vital signs assessment will be done at the last visit prior to vaccination and D0 will be scheduled within the 10 days of that visit.

8.2.3 Leukapheresis

Participants undergo 2 leukapheresis procedures. The first leukapheresis occurs at or after Enrollment/Baseline (Visit 2) but prior to D0. This leukapheresis provides cells needed to perform baseline research assays and measurements required to establish pre-treatment immune responses.

Participants will be provided with institutional instructions to prepare for the procedure.

1. **Baseline Leukapheresis**

   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).

   If leukapheresis is scheduled on a date other than the Enrollment/Baseline visit:

   - The procedure should be scheduled after the Enrollment/Baseline visit.
   - If the leukapheresis is the last procedure done within the 60 days of screening and prior to the vaccination, perform safety labs (chemistry, hematology, and COVID-19 RT-PCR) and vital signs assessment and schedule the D0 visit within 10 days of the leukapheresis.
   - CBC with differential is required for the completion of the leukapheresis facility and will be obtained per facility policies and procedures.
   - If the leukapheresis procedure is done at the Enrollment visit, collect the leukapheresis product in addition to the research labs. (See Study Specific Lab Manual.)

2. **Day 56 (Visit 8) Leukapheresis**

If cannot be done within the D56 Visit window due to scheduling conflicts or unforeseen issues, reference procedure window in Section 8.1.2.

The preference would be the completion of a second leukapheresis, however, if unable to perform leukapheresis, after review and approval by PI, a large blood draw can be completed. The amount to be drawn will be determined by total blood volume required by the study and will not exceed blood volumes allowed per site SOP. Total volume will not exceed 150 mL for this collection.

8.2.4 Randomization

The 18 study participants will be randomly assigned to **C62-M4** (n=8) or **C1C62-M3M4** (n=8) or placebo (n=2) through a blinded randomization schedule. The randomization schedule will be generated in SAS version 9.4 or higher (Cary, NC) using a permuted-block design to ensure balance cumulatively. Each assignment will be given a consecutive randomization ID (RID: 1001-1018).

The administration of the first vaccination or placebo designates D0, and the remaining visits are calculated by consecutive days starting with D0. Participants will be randomized to Study Arm, and will receive secondary randomization with respect to vaccine administration sites (left and right deltoid muscles). The randomization to vaccine administration sites that takes place at D0 will dictate the vaccine administration sites at D28.

Within **C1C62-M3M4**, exactly half of the participants will be randomized to receive **C62** in the left deltoid and **C1** in the right deltoid muscle at D0, followed by **M4** in the left deltoid and **M3** in the right deltoid muscle at D28. The remaining half of participants will receive **C62** in the right deltoid and **C1** in the left deltoid muscle at D0, followed by **M4** in the right deltoid and **M3** in the left deltoid muscle at D28. Although no randomization to administration site is required for Study Arms **C62-M4** or Placebo since the vaccine/placebo will be split between the left and right deltoid muscles, an administration site (right or left deltoid) will be designated on these prepared syringes to guarantee blinding.

8.2.4.1 Participants Randomized and Do Not Receive Study Product

Participants randomized to receive study product but who do not receive the vaccine/placebo are withdrawn from the study (Sections 7.2 and Section 7.3) and will be replaced. A new randomization ID (RID) will be generated and appended to the end of the randomization schedule, so that any replacements are randomized at the end of the study.

8.2.5 Study Unblinding

Study un-blinding of the research team can occur 28 days after the last participant receives their vaccine/placebo booster injection and following completion of an independent SMC review.

Unblinding of study participants can commence days after all participants have completed the last study visit and all queries and outstanding protocol implementation requirements have been resolved.

8.2.5.1 Emergency Unblinding

If, in the judgment of the protocol PI or in the judgment of the participant's medical provider and the site PI, a medical event is of sufficient extreme severity that it requires the immediate unblinding of a participant, the site PI may proceed with unblinding a participant per the instructions in the study specific SOP. The decision to un-blind will be taken in conjunction with the independent members of the Study Monitoring Committee (SMC). Emergency unblinding is expected to be extremely rare. It should only occur in the setting of a potentially life-threatening clinical event, and if knowing the participant's treatment assignment would affect decisions regarding the participant's immediate medical management. Both conditions must be satisfied. The site personnel will ensure that the reasons for un-blinding are documented in the clinical research chart.

8.2.6 Unscheduled Visits

Visit windows are defined in the protocol (Section 8.1.2) and SoA, Section 1.3. 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 SoA, Section 1.3 for the missed visit.

8.2.7 Vaccine and Placebo Administration Visit

First vaccine (**C62** or **C1C62**) or placebo injections are given at D0 and second vaccine (**M4** or **M3M4**) or placebo injections at D28.

1. Complete required pre-vaccine/placebo assessments and blood draws (Section 1.3) prior to the administration of the vaccine/placebo.

2. At D0 and D28, 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.

8.2.8 Administration of study vaccine/Post Vaccine/Injection management

1. Observe participant and injection site for 30 minutes following each vaccine/placebo injection for clinical AEs per SOP.

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 diary and instructions for completion prior to leaving the clinic per SOP: Completion of Post Vaccination Symptom Diary.

4. Contact participant two days after the injection in the manner pre-determined by the study coordinator and participant for AE assessment.

5. Following receipt of vaccine/placebo, participants will be followed with study visits through D196 after D0

8.2.9 Post Vaccination Symptom Diary

Solicited AEs will be captured in the post vaccination symptom diary given to each participant following each vaccination. Reference Table 9, Table 10, Table 11, and Table 12 for a complete list of the predefined solicited AEs for reactogenicity assessment.

8.2.10 Solicited AE Assessments

Participants will be given a Post Vaccination Symptom Diary, an oral thermometer and tape measure along with an emergency 24-hour telephone number to contact the on-call study physician, if needed.

Following administration of each dose of study product, participants will be instructed to record for 7 days the timing and severity of local and systemic solicited AEs, if applicable and whether medication was taken to relieve the symptoms.

Participants will be instructed to take their temperature once daily, starting with the day of both injections (D0 and D28), after leaving the clinic and through D7 post-injection. 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. Study staff will follow new or unresolved solicited AEs to resolution. In general, participants reporting any post-injection reaction greater than mild should to be evaluated by the study PI (or designee) within 72 hours after onset, unless the reaction is improving and/or has completely resolved.

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

8.2.11 Severity Assessment of Solicited AEs

Severity will be assessed for solicited AEs by the participant according to toxicity grading scales provided in the Post Vaccination Symptom Diary.

Participants experiencing any severe symptoms, will be instructed to call study coordinator or on call study physician for evaluation.

Following the administration of the first vaccine/placebo, the study coordinator will contact the participant on D2, D5, D10, D18, D22 and D28 to review symptoms and any issues they may be experiencing. The Post Vaccination Symptom Diary is reviewed with the participant for accuracy and completeness on D7 and D35. Assessment of local and systemic unsolicited AEs will be done at every visit. Participants will be asked to contact the study team should they

experience AEs, especially those listed as emergent on their Vaccine Information Card and in the Post Vaccination Symptom Diary, at any time point during the study.

8.2.12 End of Study

The End of Study (EOS) Visit will occur 6 months following the last vaccination. Throughout the protocol and study documents, this last required visit will be referred to as the EOS visit, EOS, Day 196 or D196.

8.2.13 Vaccination Information Card

Participants will be provided with a vaccination information card listing the signs and symptoms associated with TTS and general instructions for care should they need to seek care outside the study for the development of AEs associated with clotting disorders, neurological disorders or other AESIs.

8.2.14 Laboratory Evaluations

Details of specimen collection are in the Lab Procedures Manual.

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.

1. **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.

    a. CBC with differential for leukapheresis procedures per collection agency policy:

        1. 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.

        2. Leukapheresis procedures performed at a local apheresis collection center require a CBC within 30 days of procedure, neutrophil count (ANC), and platelet count in real time at the local laboratory.

2. **Blood Chemistries:** Perform the following:

a. Electrolytes (sodium, chloride, potassium, CO2/bicarbonate), glucose, blood urea nitrogen (BUN), creatinine, calcium, and magnesium, in real time at the local laboratory.

b. Liver function test: (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.

3. **Creatinine and Creatinine Clearance**: 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.

   Creatinine clearance (eGFR) calculations will use the 2021 CKD-EPI equation. This calculation can be found at https://www.mdcalc.com/calc/3939/ckd-epi-equations-glomerular-filtration-rate-gfr

   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 2021 CKD-EPI equation will be used to determine the severity of the lab abnormality.

4. **Pregnancy Test - Serum or Urine beta-HCG per SOE:** A negative serum pregnancy test (to rule out pregnancy) is required on all women at screening. A negative urine POCT pregnancy test on D0 and D28 will be completed on all women of childbearing potential. Urine test must have a sensitivity of <25 mIU/mL.

   NOTE: Confirmation of a negative urine POCT pregnancy test within 7 days of a leukapheresis at the American Red Cross is required on all women of childbearing potential.

   The use of the POCT urine pregnancy test as confirmation of negative pregnancy status is acceptable for vaccination visit at D0 and D28, 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.

5. **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. If participant has history of Hepatitis C with prior positive HCV AB, perform RNA test only.

6. **RPR**: Participants diagnosed with syphilis at screening may rescreen following a minimum of 14 days following documentation of adequate treatment of syphilis.

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

8. **Prothrombin time (PT), INR, and APTT**: Evaluate at the Screening Visit.
9. **PF4 Antibody ELISA:** A sample will be collected and stored at enrollment and collected per clinical management of suspected clotting AESI. (Section 8.1.6)

10. **Estimated Blood Volumes**: The estimated blood volumes associated with each study visit are provided Table 19.

Table 19 Blood Volumes Associated with Study Visits

Day #	Approximate Blood Volume (mL)	Day #	Approximate Blood Volume (mL)
Screen	103	Day 35	88
Enrollment/Baseline	78	Day 42	60
Baseline Leukapheresis	20	Day 56	42
Day 0	77	D56 Leukapheresis	20
Day 7	88	Large Blood Draw [1]	150
Day 14	60	Day 84 [2]	88
Day 28	57	Day 140	99
Day 30	0	Day 196	105

[1] Large blood to be completed only on approval of PI if unable to complete D56 leukapheresis (Section 8.1.2)
[2] If leukapheresis at D56 occurs, blood is drawn at D84. If large blood draw is scheduled, then no D84 research blood draw is performed.

8.3 Adverse Events and Serious Adverse Events

The PI is responsible for ensuring that all staff involved in the study are familiar with the content of this section.

All AEs are considered to be unsolicited AEs (collected by 'open question' at study visits) unless categorized as solicited AEs (Reference Table 9, Table 10, Table 11, and Table 12).

Solicited AEs are local or systemic predefined events for assessment of reactogenicity. Solicited AEs will be reported by the participant and collected in a Post Vaccination Symptom Diary

(Section 8.2.9). Solicited AEs will be assessed separately from the unsolicited AEs collected during the study visits or telephone assessments.

General information for AEs in this protocol excludes solicited AEs.

The PI and any designees are responsible for detecting, documenting, and recording events that meet the definition of an AE.

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.

8.3.2 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:

1. Results in death – the cause of death is the AE; death is an outcome.

2. A life-threatening adverse event – The term ‘life-threatening’ in the definition of ‘serious’ refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

3. Inpatient hospitalization or prolongation of existing hospitalization - In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician’s office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfils any other serious criteria, the event is serious. When in doubt as to whether ‘hospitalization’ occurred or was necessary, the AE should be considered serious. Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an SAE.

4. A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions - The term disability means a substantial disruption of a person’s ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting,

diarrhea, influenza, and accidental trauma (e.g., sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

5. A congenital anomaly/birth defect.

6. Important medical events – 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.

8.3.3 Definition of Medically Attended Adverse Events (MAAEs)

MAAEs are defined as AEs leading to medically-attended visits that were not routine visits for physical examination or vaccinations, such as an emergency room visit, or an otherwise unscheduled visit to from medical personnel (medical doctor) for any reason. AEs, including abnormal vital signs, identified on a routine study visit or during the scheduled illness visit will not be considered MAAEs.

8.3.4 Definition of Adverse Events of Special Interest (AESIs)

An AESI (serious or non-serious) is one of scientific and medical concern specific to the sponsor's product or program, for which ongoing monitoring and rapid communication by the investigator to the sponsor can be appropriate. Such an event might warrant further investigation in order to characterize and understand it. Depending on the nature of the event, rapid communication by the trial sponsor to other parties (e.g., regulators) might also be warranted.

AESIs will be collected according to the timepoints in the SoA (Section 1.3).

An AESI can be serious or non-serious. All AESIs will be recorded in the participant chart and the database.

Serious AESIs will be recorded and reported as per Section 9.5.

AESI for this protocol include those listed below.

1. Grade 3 or greater Injection Site Reactions

2. Immune-related AEs of any grade

3. Blood clot related events any grade (as per Section 9.2.1)

4. New onset neurological diseases of any grade (as per Section 9.2.2)

NOTE: Participants with an ongoing AESI at EOS should be followed until the AESI has resolved or stabilized in the opinion of the study team.

8.4 Time Period and Frequency for Collecting AE and SAE Information

8.4.1 Classification of an Adverse Event

8.4.1.1 Severity of Event

Event severity will be assigned according to the PI's (or designee's) assessment. The grading system for drug toxicities is located in the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), corrected Version 2.1, July 2017, which can be found on https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables

Severity Grade for Parameters Not Identified in the Grading Table:

Table 20 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 20 Criteria for Grading the Severity of an AE

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

8.4.1.2 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.

The PI or designee must assess the relationship between vaccine/placebo and each occurrence of each AE/SAE using their clinical judgement. This assessment of causality or relationship of AEs to the study product 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.

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

- Causality assessments that are considered not related to study product:

  1. **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.

- Causality assessments that are considered related to study product:

  1. **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.

  2. **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.

8.4.1.3 Expectedness

The 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.

8.4.2 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 by a study monitor.

AEs will be assessed and recorded from the time the participant is enrolled on the study through the date of the last participant required contact. Reference SoA, Section 1.3 and Appendices for enhanced assessments that will be done during the 28 days following administration of study product.

Solicited AEs will be assessed and recorded for 28 days following each dose of study product.

SAEs will be assessed and recorded from the time the participant is enrolled on the study through the date of the last participant required contact

MAAEs and AESIs will be assessed and recorded starting on Day 0 (vaccine/placebo administration), through all post treatment visits, and through the date of last participant required contact.

All unsolicited AEs not meeting the criteria for SAEs will be captured in the research record on the appropriate form. Information collected includes:

1. event description,
2. time of onset,
3. PI's (or designees) assessment of:
    - severity,
    - relationship to study product,
    - expectedness,
4. time of resolution/stabilization of the event.

All AEs occurring while on study must be documented appropriately regardless of relationship.

Any study related AE that is unresolved at the participant's last study visit should be followed up by the investigator or designee for as long as medically indicated, but without further recording in the database. All study-product related AEs should be followed to adequate resolution unless in the investigator's opinion, the AE is unlikely to resolve and has become a chronic underlying disease. AE outcomes will be documented as one of the following:

1. Recovered/resolved.
2. Recovering/resolving
3. Not recovered/not resolved
4. Recover with sequelae/resolved with sequelae.
5. Fatal
6. Unknown

The action taken with the vaccine/placebo due to the AE should be recorded using one of the following:

1. No action taken.

2. Next dose delayed.

3. Permanently discontinued/withdrawn from further study vaccination (with date).

4. Not applicable.

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 research coordinator will record all reportable AEs with start dates occurring any time on or after the enrollment visit through the last participant required contact. At each study visit, the research coordinator will inquire about the occurrence of AEs and SAEs since the last visit. Starting with Day 0, in addition to AEs and SAEs evaluations, the study coordinate will additionally assess for MAAEs and AESIs.

If the investigator becomes aware of an SAE or AESI with a suspected causal relationship to the study intervention that occurs after the end of the clinical study in a treated participant, the investigator shall, without undue delay as described in Table 21.

8.4.3 Clinical Laboratory Assessments:

Safety laboratory assessments will be carried out at UNC, Duke, or local Lab Corp and evaluated by the investigator to ensure participant safety. The investigator is responsible for reviewing the results of all laboratory tests as they become available.

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

b. If the PI (or designee) determines the laboratory value to be of clinical significance for that participant, it is considered an AE.

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

d. 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.

e. 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).

9 CLINICAL MANAGEMENT

9.1 Toxicities

Adverse events will be graded according to the DAIDS AE Grading Table. The PI (or designee) is responsible for appropriate reporting of AEs to the regulatory authorities.

Table 21 Expedited Reporting Guidelines

Entity (Notification Method)	**Protocol Team (Email[1])**	**SMC (Email)**	**NIH DAIDS and DAIDS MO**	**University of Oxford (Email)**	**UNC IRB (PRI)**	**FDA[2]**
Personnel Responsible for Reporting	**Site PI**	**Protocol PI**	**Site PI**	**Protocol PI**	**Protocol PI**	**Protocol PI**
Grade 3 AEs (including local or systemic reactions)	Within 24 hours of site awareness *(report again within 24 hours if AE does not return to ≤G2 after 14 days)*	As determined per protocol team *(report again within 24 hours if AE does not return to ≤G2 after 14 days)*				
Grade 4 AEs (including local or systemic reactions)	Within 24 hours of site awareness *(report again within 24 hours if AE does not return to ≤G2 after 14 days)*	Within 24 hours of protocol team awareness *(report again within 24 hours if AE does not return to ≤G2 after 14 days)*	Within 3 days of site awareness	Within 3 days of site awareness	Report according to UNC IRB Promptly Reportable Information SOPs (SOPs 1401, 1402)	Report according to FDA requirements for IND reporting
Grade 3 or 4 allergic or hypersensitivity reactions at least possibly related to study treatment	Within 24 hours of site awareness	Within 24 hours of protocol team awareness	Within 3 days of site awareness	Within 3 days of site awareness	Report according to UNC IRB Promptly Reportable Information SOPs (SOPs 1401, 1402)	Report according to FDA requirements for IND reporting
AESIs	Within 24 hours of site awareness *(AESI Clotting Form)*	**Within 24 hours of site awareness**	**Within 24 hours of site awareness**	Within 3 days of site awareness	Report according to UNC IRB Promptly Reportable Information SOPs (SOPs 1401, 1402)	Report according to FDA requirements for IND reporting
SAEs NOT related to study treatment	Within 24 hours of site awareness	As determined per protocol team	Within 3 days of site awareness	Within 3 days of site awareness		Report according to FDA requirements for IND reporting
SUSARs (SAEs at least possibly related to study treatment)	Within 24 hours of site awareness	Within 24 hours of protocol team awareness	Within 3 days of site awareness	Within 3 days of site awareness	Report according to UNC IRB Promptly Reportable Information SOPs (SOPs 1401, 1402)	Report according to FDA requirements for IND reporting

[1] Email the protocol team at UNC_IGHID12107@med.unc.edu.
[2] Reference IGHID 12107 CQMP for additional details. Report according to FDA requirements for IND reporting: https://www.fda.gov/drugs/investigational-new-drug-ind-application/ind-application-reporting-safety-reports

9.1.1 Grade 1 and Grade 2 Toxicities

Participants who develop a Grade 1 or Grade 2 AE or regardless of relationship to study product may complete study treatment at the discretion of the site PI. Grade 1 or Grade 2 AEs that may be related to the vaccine/placebo injection will be handled according to standard clinical practice and documented. The adverse event will be monitored closely.

9.1.2 Grade 3 and Grade 4 Toxicities

For participants who develop a Grade 3 or Grade 4 AE following administration of vaccine/placebo, the site PI or designee must be notified within 24 hours. Grade 3 or Grade 4 AEs should be reported as described in Table 21.

Grade 3 events

If a participant develops a Grade 3 AE, the participant may not receive further study product until:

- Written consultation and approval from the study PI (and SMC, as needed)
- The AE has returned to baseline or ≤ Grade 2 and determined clinically stable by the site PI (or designee).

If any grade 3 AE has not returned to baseline or ≤ Grade 2 within the D28 window, the vaccine can only proceed after consultation with the protocol team. Ideally, the booster vaccination is administered within 30 days of the Day 28 visit window but can be delayed up to 60 days after Day 28 visit window.

Grade 4 events

Grade 4 events at least possibly related to study treatment will result in permanent discontinuation of study product for the participant.

If a participant develops a Grade 4 AE not related to study treatment, the participant may not receive further study product until:

- Written consultation and approval from the study PI (and SMC, as needed)
- The AE has returned to baseline or ≤ Grade 2 and determined clinically stable by the site PI (or designee).

Ideally, the booster vaccination is administered within 30 days of the Day 28 visit window but can be delayed up to 60 days after Day 28 visit window

9.2 AESI

9.2.1 Potential blood clots AESIs and Thrombosis with Thrombocytopenia Syndrome (TTS)

A high index of suspicion for blood clots should be maintained in participants who develop symptoms or diagnoses associated Thrombosis with Thrombocytopenia Syndrome (TTS), after receiving vaccine/placebo on study. The PI and study team must be notified immediately of all suspected cases. Signs and symptoms include: shortness of breath; chest pain; blurred vision or other vision changes; swelling, pain or erythema in a limb; severe or persistent abdominal pain; nausea or vomiting; dizziness; mental status changes; seizure, severe or persistent headache; easy bruising and/or bleeding; microhemorrhage beside the site of vaccination. If a participant experiences new onset (acute or subacute) symptoms listed above, there should be prompt evaluation, and consideration of prompt imaging to evaluate for the diagnoses listed below and hematology consultation. Associated diagnoses include:

- Deep vein thrombosis (DVT) – symptoms depend on location of thrombosis such as: swelling, pain, warmth of an extremity; headache, visual disturbances, seizures for sinus vein thrombosis; abdominal pain for intraabdominal thrombosis
- Pulmonary thromboembolism – sudden onset of shortness of breath, pleuritic chest pain, sudden death/pulseless electrical activity arrest
- Stroke or cerebral venous sinus thrombosis
- Myocardial infarction
- Arterial thrombosis

Initial evaluation should include the following:

- CBC with manual count
- D-dimer
- PT/INR
- PF4 antibody ELISA
- Fibrinogen

For any suspected TTS, WITHOLD further doses of vaccine/placebo until diagnostic evaluation has been completed and etiology of the signs and symptoms has been determined.

In cases of concern for TTS, see Appendix 5 for a recommended testing algorithm.

For this study, Vaccine-induced immune thrombotic thrombocytopenia (VITT) will be defined as the following:

- Platelet count <150 x 10^9/L with a confirmatory peripheral smear showing reduced platelets with no evidence of clumping (that could indicate falsely low platelet count)

- Presence of thrombosis/thromboembolism (refer to the list of MedDRA Preferred Terms proposed by the CDC; See Appendix 6) confirmed by:

1) Imaging (i.e. ultrasound, CT scan, magnetic resonance imaging or venography or arteriography, echocardiogram, perfusion V/Q scan, conventional angiography, etc)
2) Surgical Procedure (confirming presence of thrombus), i.e. thrombectomy

Pathologic examination, i.e. biopsy or autopsy

PFA antibody positive

Study staff will complete an AESI clotting form using the list of MedDRA Preferred Terms (Appendix 6) to ensure complete documentation of the event.

If evaluation provides evidence that TTS is unlikely, it is important to document the scientific evidence for and treat per standard clinical practice. Participants may continue study procedures unless clinically contraindicated.

If TTS is confirmed or probable, the participant should not receive additional vaccine/placebo, but should be followed for safety per the SOA and as clinically indicated. See Section 9.1 for expedited reporting as an AESI.

9.2.2 Potential Neurological AESIs

The PI and study team must be notified immediately of all suspected cases of neurological diseases. Neurological diseases are most commonly based on clinical symptoms.

If a participant experiences new onset (acute or subacute) motor or sensory disturbances (i.e., weakness, numbness, dysarthria, dysphagia, paresthesias, hypoesthesia, hyperesthesia, dysesthesias), bowel/bladder dysfunction, gait impairment, visual disturbance, or any event of myelitis, encephalomyelitis, transverse myelitis, or other sudden neurological deficit, there should be prompt neurological evaluation, including referral to a neurology specialist for further evaluation, testing and treatment, as clinically indicated. In cases of concern for spinal cord disease, see Appendix 4 for a recommended testing algorithm which would be shared with providers caring for the participant.

For new onset neurological symptoms, proceed with the following:

- WITHHOLD further doses of vaccine/placebo until clinical and laboratory evaluation are completed and etiology of the symptoms or laboratory abnormalities are defined

- Consultation with neurology immediately

If neurological disease is confirmed or probable, the participant should not receive additional vaccine/placebo, but should be followed for safety per the SOA and as clinically indicated. See Section 9.5 for expedited reporting as an AESI.

9.2.3 Delayed Dose

In the event of acute illness or inability to return for Day 28, the booster vaccination can be delayed. The booster vaccine should ideally be given within 30 days after the Day 28 visit (booster vaccine dose) but can be extended up to 60 days, if needed. See Section 9.1.2 for additional instructions regarding delayed doses for Grade 3 or Grade 4 AEs.

9.2.4 Consideration for SARS-CoV-2 Pandemic

See Appendix 3 for guidance about participant follow-up during periods of restrictions of in-person study visits due to local SARS-CoV-2 transmission. Participants should be tested for SARS-CoV-2 infection at the discretion of the site investigator according to any applicable local guidelines or SOC.

9.3 Adverse Event Reporting

The study will monitor participants for adverse events. 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:

1. Study treatment related Grade ≥ 1 AE

2. Grade ≥ 2 AEs

3. Autoimmune-related AEs regardless of grade

4. MAAEs regardless of grade

5. AESIs regardless of grade

6. HIV viral loads ≥ 50 copies/mL

7. Injection Reactions regardless of grade

8. AEs that led to a change in study treatment/intervention regardless of grade

9. AEs meeting SAE definition

9.3.1 Local or Systemic Reactions to Vaccine/Placebo Injections

9.3.1.1 Grade 1 or 2

Local reactions of Grade 1 or 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.

9.3.1.2 *Grade 3 or 4*

Grade 3 or Grade 4 local reactions (Table 9 and Table 11) should be reported as described in Table 21 (AESI per definition in Section 8.3.4). For Grade 4 local reactions, notify site PI or designee immediately (or Emergency response team, as appropriate) as definitive medical and/or surgical intervention should be undertaken as appropriate.

Grade 3 and Grade 4 systemic reactions (Table 10 and Table 12) should be reported as described in Table 21. The common systemic adverse events usually seen with ChAdV-vectored vaccines and MVA-vectored vaccines are listed in Section 2.3.2 (Table 9, Table 10, Table 11, and Table 12). These events usually occur within 24 hours of vaccination and will resolve within the following 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.

9.3.1.3 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:

1. Grade 1: 2.5 to < 5 cm in diameter OR 6.25 to < 25 cm^2 surface area;

2. Grade 2: ≥ 5 to < 10 cm in diameter OR ≥ 25 to < 100 cm^2 surface area;

3. Grade 3: ≥ 10 cm in diameter OR ≥ 100 cm^2 surface area OR Ulceration OR Secondary infection OR Sterile abscess OR Drainage;

4. Grade 4: Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue)

9.3.2 *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 PI (or designee) will be reported as described in Table 21 and as detailed at https://www.fda.gov/drugs/investigational-new-drug-ind-application/ind-application-reporting-safety-reports.

9.3.3 *Monitoring HIV RNA levels*

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

a. Adherence to ART should be carefully assessed and documented.

b. A standard HIV RNA assay should be repeated within 1-4 weeks.

c. HIV resistance testing will be performed at the time of drawing a confirmatory sample and as indicated for persistent viremia.

d. 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.

e. HIV RNA will be repeated every 2 weeks, or sooner as clinically indicated, until <50 copies/mL and the participant can continue on study.

f. 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 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 monthly with the protocol team or more frequently, if needed.

The 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 quarterly 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 SAE reports for potential impact on the study participant safety and protocol conduct as per DAIDS policies, guidance documents, and SOPs, as applicable.

9.4 Serious Adverse Event Reporting

All SAEs at least possibly related to study treatment will be considered unexpected and be reported as SUSARs within the regulatory timelines outlined in Table 21.

All SAEs at least possibly related to study treatment occurring during the study must be reported to the UNC IRB per the UNC IRB reporting requirements. A written report will be submitted to the FDA as outlined in Table 21. Additional information will be supplied as requested.

All SAEs occurring during the study will be reported to DAIDS as outlined in Table 21.

All SAEs considered possibly related to study product must be followed until recovery to baseline or stabilization with the date of resolution or stabilization recorded in the source documents. In addition, the investigator should report all follow-up for reportable 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 determined as possibly related to the vaccine/placebo 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:

1. The event resolves.

2. The event stabilizes.

3. The event returns to baseline, if a baseline value/status is available.

4. The event can be attributed to etiology other than the vaccine/placebo or to factors unrelated to study conduct.

5. 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:

1. Hospitalizations not intended to treat an acute illness or adverse event (e.g., social reasons such as pending placement in long-term facility)

2. Surgery or procedure planned before entry into the study (must be documented in the source document)

The protocol 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 SAEs as soon as possible, but in no case later than the timelines described in Table 21. In addition, the PI must notify the 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 after the sponsor determines that the information qualifies for reporting.

9.5 AESI reporting

If an AESI occurs, the study coordinator, in collaboration with the site 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 AESIs (reference Section 8.3.4).

All AESIs occurring during the study must be reported to the UNC IRB per the UNC IRB reporting requirements. **A written report will be submitted to the DAIDS MO and the IRB within 24 hours of site becoming aware of the AESI**. Additional information will be supplied as requested.

All AESIs, serious and non-serious, must be reported using the same reporting process as for SAE reporting.

9.6 Other Reporting Situations

9.6.1 Reporting to DAIDS

The Protocol Team will be responsible for reporting to DAIDS the following information via email to DAIDSRSCSafetyOffice@tech-res.com.

a. Monthly listing of all AEs within 5 business days of the 1st of each month.

b. Monthly Line listing of SAEs including suspected unexpected serious adverse reactions (SUSARs) within 5 business days of the 1st of each month.

c. All safety reports submitted to the FDA within 48 hours of submission.

d. All IND submissions within 5 business days of submission.

e. All FDA communications within 5 business days of the communication.

f. Notification that the study is halted or put on clinical hold by the PI, IRB, FDA or other regulatory entity within 24 hours of notification that the study was halted or put on hold.

g. All annual reports to FDA within 5 business days of the submission.

h. Open SMC or DSMC summary reports within 5 business days of open report finalization.

9.6.2 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 SMC to merit such notice.

9.6.3 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.

9.6.4 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.

9.6.5 Product Quality

Any suspected transmission of an infectious agent via a medicinal product or other product quality issue that results in an event of clinical consequence are AEs. The product quality issue must be reported within 1 business days after being made aware of the event.

A product quality complaint (PQC) is any written, electronic or oral communication that states possible deficiencies of a study product related to manufacturing, labeling, or packaging, i.e., any dissatisfaction relative to the identity, quality, durability, or reliability of a product, including its labeling or package integrity. A PQC may have an impact on the safety and efficacy of the product. Timely, accurate, and complete reporting and analysis of PQC information from studies is crucial for investigators, the manufacturer and IND sponsor UNC, and for the protection of the participants' rights, welfare and well-being; and is mandated by regulatory agencies worldwide. UNC has established procedures in conformity with regulatory requirements to ensure appropriate reporting of PQC information and all trials will be conducted in accordance with those procedures.

All initial PQCs must be reported to DAIDS by the study-site personnel within 1 business day after being made aware of the event.

If the complaint is combined with an SAE, the study-site personnel must report the PQC to DAIDS the sponsor according to the SAE reporting timelines (refer to Section 9.4, SAEs). A sample of the suspected product should be maintained under correct storage conditions for further investigation if requested by DAIDS.

UNC must report any self-identified or site personnel-reported PQC to DAIDS within one business days of being made aware of the complaint and include notifications regarding immediate action taken.

9.7 New Safety Information

9.7.1 Definition of New Safety Information (NSI)

The Office for Human Research Protections (OHRP) considers new safety Information 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:

1. 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 IRB-approved

research protocol, the informed consent document, and IB; and (b) the characteristics of the participant population being studied.

2. 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

3. 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.

4. Please reference the UNC Office for Human Research Ethics SOP 1401 to address Promptly Reportable Information. This SOP provides definitions and procedures for the reporting promptly reportable information to the UNC-Chapel Hill IRB.

9.8 NSI Reporting

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

1. Protocol identifying information: protocol title and number, PI's name, and the IRB project number.

2. A detailed description of the event, incident, experience, or outcome.

3. An explanation of the basis for determining that the event, incident, experience, or outcome represents an NSI;

4. 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:

1. NSI that are SAEs will be reported to the IRB and the DAIDS MO within 3 business days of the investigator becoming aware of the event.

2. 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.

3. 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 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.

9.9 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.

10 STATISTICAL CONSIDERATIONS

10.1 Statistical Hypotheses

1. Vaccination with either C62-M4 or C1C62-M3M4 will be safe and well tolerated

2. Both C62-M4 and C1C62-M3M4 vaccination will increase the frequency of circulating HIV-specific T cells

3. C1C62-M3M4 will induce a greater breadth of T cells targeting conserved regions of HIV than C62-M4

10.2 Endpoints

Primary Safety Endpoint:

- Occurrence of at least one ≥ Grade 3 Adverse Event (AE) including signs/symptoms, lab toxicities, and/or clinical events, that is possibly or definitely related to study treatment any time from the first day of treatment (D0) through 28 days following the boost vaccination or D56.

Secondary Efficacy Endpoint(s):

- Occurrence of any ≥ Grade 1 AE including signs/symptoms, lab toxicities, and/or clinical events, that is possibly or definitely related to study treatment any time from the first day of treatment (D0) through D196 (Week 28).

Secondary Immunogenicity Endpoint(s):

- Relative change in magnitude of T cell responses to HIV-1 conserved regions from baseline (before C62 or C1C62 vaccination) to post-boost vaccination at D35 and D42.

- Change in breadth of T cell responses targeting HIV conserved regions from baseline (before C62 or C1C62 vaccination) to post-boost vaccination at D56.

10.3 Sample Size Determination

The proposed number of 8 vaccinees per arm and 2 placebo controls was determined to be sufficient to evaluate the risk of common SAEs or severe local or systemic reactions based on

early phase trials of simian adenovirus- and MVA-vectored vaccines, given alone or serially (59, 64, 92-94, 97, 121, 125-128, 136-139).

Enrollment into the trial will be suspended if two or more participants experience a study treatment-related AE of Grade 3 or higher or if one or more participants experience an SAE possibly related to study treatment.

If zero of 8 participants experience a safety event, an exact one-sided binomial 95% upper confidence limit for the probability of a safety event will be 31%. If one participant experiences a safety event, the corresponding one-sided 95% upper confidence limit will be 47%. If no safety events are observed in the two vaccine arms and data is pooled across arms (n=16), then the exact one-sided 95% upper confidence limit will be 17%.

10.4 Procedure for missing and invalid data

There is very little missing or invalid data in our concurrent study using MVA-vectored vaccines (NCT03844386; IND 18368), so it is anticipated that this study will be similar. Due to the small sample size, formal imputation methods will not be used. However, if there is concern that key findings are sensitive to missing data, missing values can be replaced with the best (worst) rank in the **C62-M4** arm and the worst (best) rank in the **C1C62-M3M4** arm to determine if findings are sensitive to missingness.

Safety analyses will include all evaluable participants regardless of whether they are replaced or missing for additional endpoints. In order to achieve the planned evaluable sample size, participants who do not receive both sets of vaccines at D0 and D28 will be replaced.

10.5 Study Duration and Accrual

Participants will be screened approximately 60 days prior to vaccination and will be followed for 196 days after vaccination. The total study duration from screening to completion of follow-up per participant will be approximately 36 weeks (approximately 9 months). Given the focus of this study is on safety and potential adverse events, accrual will be staggered such that a maximum of up to 2 participants per week will receive the Day 0 vaccination.

10.6 Randomization

Following enrollment, the 18 study participants will be randomly assigned to receive vaccination with either **C62-M4** (n=8), **C1C62-M3M4** (n=8), or placebo (n=2) through a double-blinded, randomized block design. As detailed in (Section 7.2.1), participants who do not receive both sets of vaccines at D0 and D28 will be replaced. In cases such as these, the unblinded statistician will generate a new randomization ID that will serve as a replacement for the investigational product assigned to the dropout.

10.7 Statistical Analyses

10.7.1 General Approach

This is a Phase 1 study with a small sample size chosen to evaluate safety and the primary efficacy outcome. Exact methods that do not rely on large sample assumptions will be used for statistical inference and presented with descriptive statistics and/or data visualization. When feasible, graphs will present data at the participant-level with summary statistics. Emphasis will be placed on descriptive summaries and estimated effect sizes.

For inferential tests, a significance level of 0.05 will be employed. However, this study recruits a small number of participants and thus may not detect scientifically meaningful effects at a significance level of 0.05. Since this is a small study with multiple exploratory endpoints, adjustment for multiple hypothesis testing will not be performed. Results should be interpreted within the context of the protocol-defined eligibility criteria and the target population available and willing to participant in this double-blinded, placebo-controlled, randomized clinical trial.

10.7.2 Analysis of the Primary Safety Endpoint(s)

The probability of a primary safety event (≥ Grade 3 AE) from D0 through D56 (or 28 days following the second vaccination) will be estimated with a proportion and corresponding one-sided exact binomial 95% upper confidence limit. These estimates will be calculated separately for **C62** (D0) followed by **M4** (D28) and **C1C62** (D0) followed by **M3M4** (D28) in PWH on ART.

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.

10.7.3 Analysis of the Secondary Safety Endpoints

The probability of a secondary safety event (≥ Grade 1 AE) from D0 through D196 will be estimated with a proportion and corresponding one-sided exact binomial 95% upper confidence limit. These estimates will be calculated separately for **C62 (**D0) followed by **M4** (D28) and **C1C62** (D0) followed by **M3M4** (D28) in PWH on ART.

NOTE: Safety events include incidence and grade of all reportable unsolicited and solicited adverse events including SAEs, MAAEs, and AESIs post first dose of C1 and/or C62 through Day 196.

10.7.4 Analysis of Secondary Immunogenicity Endpoints

10.7.4.1 T cell magnitude

To evaluate changes in HIV-1 epitope-specific $CD8^+$ T cell responses detected in IFN-γ ELISpot assays from baseline (pre-vaccination) to post-boost vaccination (D35 and D42), the relative change within participants will be estimated using a geometric mean ratio (GMR, also known as fold change) and evaluated with an exact two-sided Wilcoxon signed-rank test for within-arm comparisons and an exact two-sided Wilcoxon rank-sum test for between-arm comparisons. The Wilcoxon signed-rank test assumes symmetry of the change in natural log-transformed summed HIV-1 specific T cell response (estimated by GMR) under the null. If this assumption is violated, then a sign test will be conducted as a sensitivity analysis.

10.7.4.2 Power Calculations

To compute power for a continuous covariate, such as T cell magnitude, a meaningful effect size must be determined ahead of time. Comparing T-cell responses within participants, an average fold change of at least 2 (GMR ≥ 2) is anticipated to be scientifically meaningful. Baseline HIV-specific T cell responses from HIV-infected durably-suppressed PWH (53) were used to inform power calculations. A between-participant standard deviation of 0.8 was estimated using this data and the same standard deviation is assumed pre- and post-vaccination.

Assuming the natural, log-transformed, HIV-specific T cell responses follow a normal distribution with between-participant standard deviation of 0.8 and a within-participant Pearson correlation of 0.9 for pre- and post-vaccine measurements, using 10,000 simulated datasets for n=8 participants provides 87% power to detect a GMR of 2 or greater. For a smaller within-participant correlation of 0.8, there is >95% power to detect a GMR of 2 or greater.

10.7.4.3 T cell Breadth

To evaluate change in breadth in the number of T cell responses targeting HIV-conserved regions from baseline (pre-vaccination) to post-vaccination (D56),
an exact two-sided Wilcoxon signed-rank test will be used for within-arm comparisons and an exact two-sided Wilcoxon rank-sum test will be used for between-arm comparisons.

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10.7.4.4 Power Calculations for T cell Breath

Baseline HIV-specific T cell breadth from durably-suppressed PWH were used to inform power calculations (50). In this study, reactive HIV T cell epitopes in the HIV proteome (Clade B consensus and autologous reservoir virus) were defined in 25 PWH on ART.

For the **C62-M4** and **C1C62-M3M4** arms, we anticipate that vaccination will induce a change of breadth (the detection of a new reactive T cell epitope) ranging from 0-3 epitopes and 2-6 epitopes, respectively.

- We anticipate that **C62-M4** will induce a change in breadth with the following frequency distribution: 0 (20% of participants), 1 (50%), 2 (20%), and 3 (10%).

- We anticipate that **C1C62-M3M4** will induce a change in breadth with the following frequency distribution: 2 (30% of participants), 3 (40%), 4 (15%), 5 (10%), and 6 (5%).

Given these assumptions, n=8 evaluable participants per treatment arm provides 95% power to detect a difference between arms using an exact two-sided Wilcoxon rank-sum test (at a 0.05 significance level).

10.7.5 Safety Analyses

For the safety endpoints, we will describe all study treatment-related AEs through D56 (primary endpoint) and the end of study at D196 or 24 weeks following the second vaccination (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

- AESIs

- MAAEs

- 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.

10.7.6 Baseline Descriptive Statistics

Participant data will be summarized using the following baseline characteristics: gender, ethnicity, race (% Hispanic or Latino), onset of ART (number of acute versus chronic), age, CD4 nadir, and years of ART suppression.

10.7.7 Planned Interim Analyses

There are no planned formal interim analyses.

10.7.8 Tabulation of Individual participant Data

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

10.7.9 Exploratory Analysis

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

10.7.9.1 Immunologic Measures

Kinetic data will be analyzed by descriptive statistics and data visualization. Other observed immunologic measurements, such as virus inhibition, may not be normally distributed or may be partially censored due to assay limits of quantification (LoQ). Thus, an exact two-sided Wilcoxon signed-rank test will be used for within-participant comparisons. If the assumption of symmetry of differences (required for the Wilcoxon signed-rank test) is violated, then a sign test will be conducted as a sensitivity analysis. For censored data, we will impute half the limit of detection.

10.7.9.2 Virologic Measures

Virologic measures such as cell-associated HIV-1 RNA will be log-transformed prior to analyses. The analysis method chosen for plasma HIV-1 RNA SCA will depend on the number of results that fall below the assay limit of quantification. For example, if a substantial fraction (~50%) are below the assay limit of quantification, the analysis will focus on estimating the proportion of participants with SCA above the LoQ 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.

11 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

11.1 Regulatory, Ethical, and Study Oversight Considerations

11.1.1 Informed Consent Process

11.1.1.1 Consent 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.

11.1.1.2 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.

11.1.2 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:

1. Determination of unexpected, significant, or unacceptable risk to participants
2. Demonstration of efficacy that would warrant stopping
3. Insufficient compliance to protocol requirements
4. Data that are not sufficiently complete and/or evaluable
5. Determination that the primary endpoint has been met
6. 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).

11.2 Confidentiality

11.2.1 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 IDS 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 Goonetilleke Laboratory located on the campus of UNC in Chapel Hill. 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.

11.2.2 Certificate of Confidentiality

To further protect the privacy of study participants, a Certificate of Confidentiality will be issued by the 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.

11.3 Future Use of Stored Specimens and Data

Data collected for this study will be analyzed and stored at the Goonetilleke Laboratory at UNC, Chapel Hill. After the study is completed, the de-identified, archived data will be stored in the UNC Database, for use by the Goonetilleke Research Laboratory and their collaborators. Permission to transmit data to researchers outside the study collaboratory team 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 Laboratory on the campus of UNC Chapel Hill. 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 biological sample 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.

11.4 Key Roles and Study Governance

Provided in Table 22 are the key roles for the study governance.

Table 22 Key Roles for Clinical Study

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

11.5 Safety Oversight

Safety oversight will be under the direction of a 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:

1. Real-time and cumulative safety data for evidence of study-related adverse events;

2. Adherence to the protocol;

3. Factors that might affect the study outcome or compromise the trial data (such as protocol violations, losses to follow-up, etc.).

4. Review the achievement of enrollment benchmarks

The SMC will receive quarterly 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.

11.5.1 Safety Pause

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:

1. 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

2. 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.

11.6 Clinical Monitoring

To ensure the safety of participants in the study, compliance with applicable regulations, and to ensure accurate, complete, and reliable data, the protocol 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 protocol 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 protocol 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 protocol 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 protocol 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 protocol PI will ensure the capability for inspections of applicable study-related facilities (e.g., IDS pharmacy, CTRC, etc.).

11.6.1 Clinical Monitoring Procedures

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

1. Informing participants about risks so they can recognize and report harms in partnership with the study team;
2. respecting local/national blood draw limits;
3. direct observation of participants after vaccine/placebo administration and collection of information regarding side effects for several days post product administration;
4. having study staff properly trained in administering study procedures that may cause physical harm or psychologic distress, such as blood draws and injections; and
5. providing study monitoring.

11.6.2 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., GLP, 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.

11.6.3 Data Handling and Record Keeping

11.6.3.1 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 housed in Advarra Electronic Data Capture (EDC). EDC includes password protection. Clinical data will be entered directly from the source documents.

11.6.3.2 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.

11.7 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.

11.8 Publication and Data Sharing Policy

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

1. 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.

2. 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.

3. 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.

11.9 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.

12 ABBREVIATIONS

4A4OA	4-anilino-4 oxobutanoic acid
Ab	Antibody
ACD	Acid Citrate Dextrose
ACE	Angiotension Converting Enzyme
ADC	Apparent Diffusion Coefficient
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
ANCA	Antineutrophil Cytoplasmic Antibodies
ANCOVA	Analysis of Covariance
Anti-dsDNA	Anti-double-stranded DNA, IgG
APTT	Activated Partial Thromboplastin Time
AQP4	Aquaporin 4
ART	Antiretroviral Therapy
AST	Aspartate Transaminase
ATI	Analytic Treatment Interruption
AUC	Area Under the Curve
BP	Blood Pressure
BUN	Blood Urea Nitrogen
C1	ChAdOx1.tHIVconsv1
C62	ChAdOx1.HIVconsv62
CBF	Clinical Biomanufacturing Facility
CBC	Complete Blood Count
CEF	Chicken Embryo Fibroblast
CFAR	Center for AIDS Research
CFR	Code of Federal Regulations
ChAdV	Simian Adenovirus of chimpanzee origin
CIDP	Chronic Inflammatory Demyelinating Polyneuropathy
CLIA	Clinical Laboratory Improvement Amendments
CLS	Capillary Leak Syndrome
CMP	Clinical Monitoring Plan
CMV	Cytomegalovirus
CNS	Central Nervous System
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CPK	Creatine Phosphokinase or Creatine Kinase (CK)
CRF	Case Report Form
CRP	C-Reactive Protein
CSF	Cerebral Spinal Fluid

CSRC	(DAIDS) Clinical Science Review Committee
CT	Computed Tomography
CTRC	Clinical and Translational Research Center
CVST	Cerebral Venous Sinus Thrombosis
D196	Day 196 or End of Study
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
DVT	Deep Vein Thrombosis
DWI	Diffusion Weighted Image
EBV	Epstein-Barr Virus
E/CIA	Enzyme or Chemiluminescence Immunoassay
EC	Ethics Committee
ECG	Electrocardiogram
eCRF	Electronic Case Report Forms
eGFR	Estimated Glomerular Filtration Rate
ED	Emergency Department
EMA	European Medicines Agency
ENA	Extractable Nuclear Antigen Antibodies
EOS	End of Study or Day 196
ESR	Erythrocyte Sedimentation Rate
EU	European Union
FLAIR	Fluid Attenuated Inversion Recovery
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	Guillain-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
GRE	Gradient Echo
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

HIV	Human Immunodeficiency Virus
HIVconsv	1st generation conserved-region immunogen
HLA	Human Leukocyte Antigen
HSV	Herpes Simplex Virus
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
IDS	Investigational Drug Service
IgG	Immunoglobulin G
IFN-γ	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
LANL-HSD	Los Alamos National Laboratory HIV Sequence Database
LDH	Lactate Dehydrogenase
LDL	Low Density Lipoprotein
LMP	Last Menstrual Period
LRA	Latency Reversing Agent
LSMEANS	Least-Squares Means
LTFU	Lost to Follow-up
M3	MVA.tHIVconsv3
M4	MVA.tHIVconsv4
MAAEs	Medically Attended Adverse Events
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
MOG	Myelinoligodendrocyte Glycoprotein
MOI	Multiplicity of Infection
MOP	Manual of Procedures

MSDS	Material Safety Data Sheet
MRI	Magnetic Resonance Image
MVA	Modified Vaccinia virus (Ankara strain)
MVA.HIVA	MVA expressing the HIVA immunogen, comprising clade A 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
NMOSD	Neuromyelitis Optica Spectrum Disorder
NNRTI	Non-nucleoside Reverse Transcriptase Inhibitor
NRTI	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
PCR	Polymerase Chain Reaction
PE	Physical Exam
PF4 antibody ELISA	Platelet Factor 4 antibody ELISA
pfu	Plaque-forming units
PHI	Primary HIV Infection
PI	Principal Investigator
PID	Participant Identification Number
PI	Protease Inhibitor
POCT	Point of Care Test
PQC	Product Quality Complaint
PT	Preferred Term
PT	Prothrombin time
QA	Quality Assurance
QC	Quality Control
RID	Randomization Identification Number
rMVA	Recombinant MVA
RF	Rheumatoid Factor
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
SoA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
STIR	Short T1 Inversion Recovery
SUSAR	Serious Unexpected Serious Adverse Reaction
SWI	Susceptibility-Weighted Imaging
T	Temperature
TAF	Tenofovir Alafenamide
TDF	Tenofovir
TM	Transverse Myelitis
TNF	Tumor Necrosis Factor
TSH	Thyroid Stimulating Hormone
TTS	Thrombosis with Thrombocytopenia Syndrome
tPA-LS	Human tissue activator sequence
UK	United Kingdom
ULN	Upper Limit of Normal
UNC	University of North Carolina
UOXF	University of Oxford
UP	Unanticipated Problem
US	United States
VDRL	Venereal Disease Research Laboratories
VITT	Vaccine-Induced Immune Thrombotic Thrombocytopenia
VS	Vital Signs
VZV	Varicella Zoster Virus
WHO	World Health Organization
WOCBP	Women of Child Bearing Potential

13 PROTOCOL AMENDMENT HISTORY

The table below is intended to capture changes of IRB-approved versions of the protocol, including a description of the change and rationale. A Summary of Changes table for the current amendment is located in Appendix 1.

Version	Date	Description of Change	Brief Rationale
1.0	14-Jan-2022	Original Version	Original Version
2.0	28-Oct-2022	Updated Protocol Team Roster, Section 1.3, Section 3, Section 5.1, Section 5.2, Section 6.1.2, Section 6.2.4, Section 6.3, Section 6.5.2, Section 7.2.2, Section 7.2.3, Section 8.1, Section 8.2, Section 8.4.3, Section 10.2, Section 10.7.4, Section 11.5, Section 11.6.3, Appendix 1, Appendix 2	Modified exclusion criterion #12 to adjust timing of live vaccines prior to enrollment and address monkeypox vaccines, provide clarification and resolve discrepancies.
3.0	12-Jun-2023	Updated Section 1.1, Section 2.2, Section 2.3, Section 5.1, Section 5.2, Section 6.1, Section 6.5.2, 8.1, Section 8.3, Section 8.4, Section 9.1, Section 9.2, Section 9.3, Section 9.4, Section 9.5, Section 10.5, Section 11.4, Appendix 2	Updated C1-C62 risks section per C1 IB v4 dated 09-Dec-2022 and C62 IB v3 dated 09-Dec-2022. Incorporated CM#1 dated 27-Feb-2023. Removed contradictory language for allowable Mpox vaccine window. Clarified AE and SAE reporting guidelines. Provide clarification and resolve discrepancies.

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Appendix 1

Rationale for Protocol Version 3.0

The rationale for this amendment is to update C1-C62 risks section per C1 IB v4 and C62 IB v3, incorporated clarification memo #1 dated 27-Feb-2023, to clarify AE/SAE reporting, to remove contradictory language for allowable Mpox vaccine window, and to resolve discrepancies and provide clarifications. The table below outlines substantive and non-substantive changes to the protocol. Other minor typographical and/or editorial changes have been made. A redline version showing detailed changes to the protocol is available on request.

Summary of Changes in IGHID 12107 Protocol Version 3.0

Section	Summary of changes and rationale
Section 1.1	**Description of Sites Enrolling Participants** Clarified Duke is also a study site.
Section 1.1 **Section 10.5**	**Description of Study Intervention** Clarified vaccination that a maximum of two participants per week will initiate study treatment at Day 0.
Section 1.1 **Section 10.5**	**Participant Study Duration** Corrected duration of participation to be approximately 36 weeks (9 months).
Section 2.2.2, **Section 6.1.1**	**C1-C62 Manufacturer** Updated the name of C1 and C62 manufacturer from Advent Srl to Advaxia Biologics Srl per Clarification Memo #1 dated February 17, 2023
Section 2.3.1	**Known Potential Risks of C1 and C62 Vaccines** Updated potential risks to include transient lymphopenia as reported in C1 IB v4 dated 09-Dec-2022 and C62 IB v3 dated 09-Dec-2022
Section 5.1, **Section 5.2**	**Inclusion and Exclusion Criteria** Adjusted inclusion and exclusion timelines for IC #13 and #23 as well as EC #3, #5, #6, #8, #10, #12, #13, #14, #16, and #18. Adjusted IC #26 to indicated Magnesium must be > LLN. Clarified which investigational ART is allowed in EC #13.
Section 6.5.2	**Prohibited Medications** Removed conflicting information regarding allowable window for Mpox vaccination. Adjusted prohibited medication windows for vaccine to align with eligibility criteria. Clarified allowed investigational ART.
Section 8.1.2, **Appendix 2**	**Visit windows** Clarified visit windows for telephone call visits (D2, D5, D10, D18, D22, and D30) per Clarification Memo #1 dated February 17, 2023
Section 8.1.3	**Medical History** Clarified medical history to include significant medical history condition in noted in medical records.

Section	Summary of changes and rationale
Section 8.1.5	**Clinical Assessments** Clarified vital sign measurements on vaccination days. Clarified post-vaccine assessment.
Section 8.2.14	**Laboratory Evaluations** Clarified Hepatitis C screening requirements.
Section 8.3, **Section 8.4,** **Section 9.1,** **Section 9.3,** **Section 9.4,** **Section 9.5**	**Adverse Event Reporting** Created Table 21 to summarize adverse event reporting requirements and timelines for Grade 3 or 4 AEs, hypersensitivity or allergic reactions, SAEs, and AESIs. Removed redundant reporting requirements consolidated in Table 21. Adjusted reporting timelines for UNC IRB and FDA in alignment with agency policies.
Section 9.1.1 **Section 9.1.2** **Section 9.3.1**	**Adverse Event Toxicity Management** Clarified Grade 1 and Grade 2 toxicity management for events related and unrelated to study treatment. Harmonized Grade 3 and Grade 4 AE toxicity management for AEs in Section 9.1.2 and solicited AEs (local or systemic reactions) in Section 9.3.1. Added note to reference Table 21 (AESI reporting) for Grade 3 or greater injection site reactions.
Section 8.4.1	**Relationship to Study Intervention** Removed instruction to assess causality separately for each study product.
Section 8.4.2	**Time Period and Frequency for Event Assessment and Follow-Up** Clarified solicited AEs will be followed through 28 days following each dose of study product. Removed references to Day 196.
Section 9.2.3	**Delayed Dose** Added reference to Section 9.1.2 for additional instructions regarding delayed booster dose in the event of a prior Grade 3 or 4 AE.
Section 11.4	**Table 22** Adjusted table numbering after adding Table 21.

Appendix 2 Assessment for Post C62 and C1C62 Vaccine Adverse Reactions

I. Telephone Assessment Schedule

	Day 2	Day 5	Day 10	Day 18	Day 22
Visit Window		± 1 D	± 1 D	± 1 D	± 1 D
Review Symptom Check List for Post Vaccine Local and Systemic Adverse Reactions	X				
Review Symptom Check List for Post vaccine for development of symptoms associated with clotting disorder	X	X	X	X	X

II. Telephone Assessment for Local and Systemic Reactions (Reference Participant Symptom Diary)

1. Review Solicited AE assessment diary on the phone with the participant.
   a. Observation of solicited AEs?
      - Systemic solicited AEs include increased body temperature, malaise, fatigue, myalgia, arthralgia, headache, nausea, and feverishness.
      - Local solicited AEs include erythema, swelling, pain, pruritus, and warmth at infusion site.
   b. Observation of other signs/symptoms

III. Telephone Assessment for Development of Symptoms Associated with Clotting Disorder

1. Assess for the following signs and symptoms
   a. Shortness of breath
   b. Chest pain
   c. Blurred vision or other vision changes
   d. Swelling, pain or erythema in a limb
   e. Severe or persistent abdominal pain
   f. Nausea or vomiting
   g. Dizziness
   h. Mental status changes
   i. Seizure
   j. Severe or persistent headaches
   k. Easy bruising and/or bleeding
   l. Microhemorrhage beside the site of vaccination
   m. Change in gait; problem with walking, balance or coordination
   n. Weakness
   o. Facial weakness
   p. Numbness or tingling
   q. Difficulty swallowing or slurring of your speech

Those vaccinated should be instructed to contact study staff immediately for clinical evaluation. If symptoms develop after clinical hours or on the weekend the participant should seek immediate medical attention if they develop any of the above.

Appendix 3 SARS-CoV-2 Pandemic Considerations

Sites should follow local guidelines regarding management of participants during periods of SARS-CoV-2 activity (or other public health emergencies), and all applicable DAIDS guidelines.

Participants diagnosed with or suspected of having SARS-CoV-2 infection

Participants with suspected SARS-CoV-2 infection should be offered testing using locally available, FDA-approved or FDA-EUA assays. Participants who become infected with SARS-CoV-2 may remain on study. Sites should follow local guidelines for management of participants in consultation with primary care providers, local specialists, and the protocol team.

Screening and Enrollment

Participants acquiring SARS-CoV-2 during the screening window may complete screening/enrollment, but no study visits should occur while the participant is deemed to be infectious using local guidelines. Screening and enrollment periods can be extended to allow for recovery. However, all entry criteria must be met as described in the protocol. The participant may be required to repeat screening labs and evaluations at a later time point, based on time of initial screening evaluations and recovery from illness, per site investigator discretion.

Intervention Period (Day 0 through Day 35)

Participants acquiring SARS-CoV-2 during study intervention period should not return for study visits until the participant is deemed to be no longer infectious. Participants should be followed using remote visits (e.g., telephone or video) at least weekly to follow the clinical course of disease and to monitor any ongoing AEs. No study treatment should be administered while the infection is thought to be ongoing. This may lead to missing the Day 28 vaccine, in this scenario the participant can receive the booster dose preferably within the 30 days after the Day 28 visit, but can be extended out 60 days, if needed.

Immunogenicity Follow up Period (Day 42 through Day 196)

Participants with suspected SARS-CoV-2 should not return for study visits until the participant is deemed no longer infectious. Participant will be asked to complete the post vaccine leukapheresis procedure when they are recovered from their illness per site investigator discretion. Participants should be followed using remote visits (e.g., telephone or video) at least weekly to follow the clinical course of disease and to monitor any ongoing AEs.

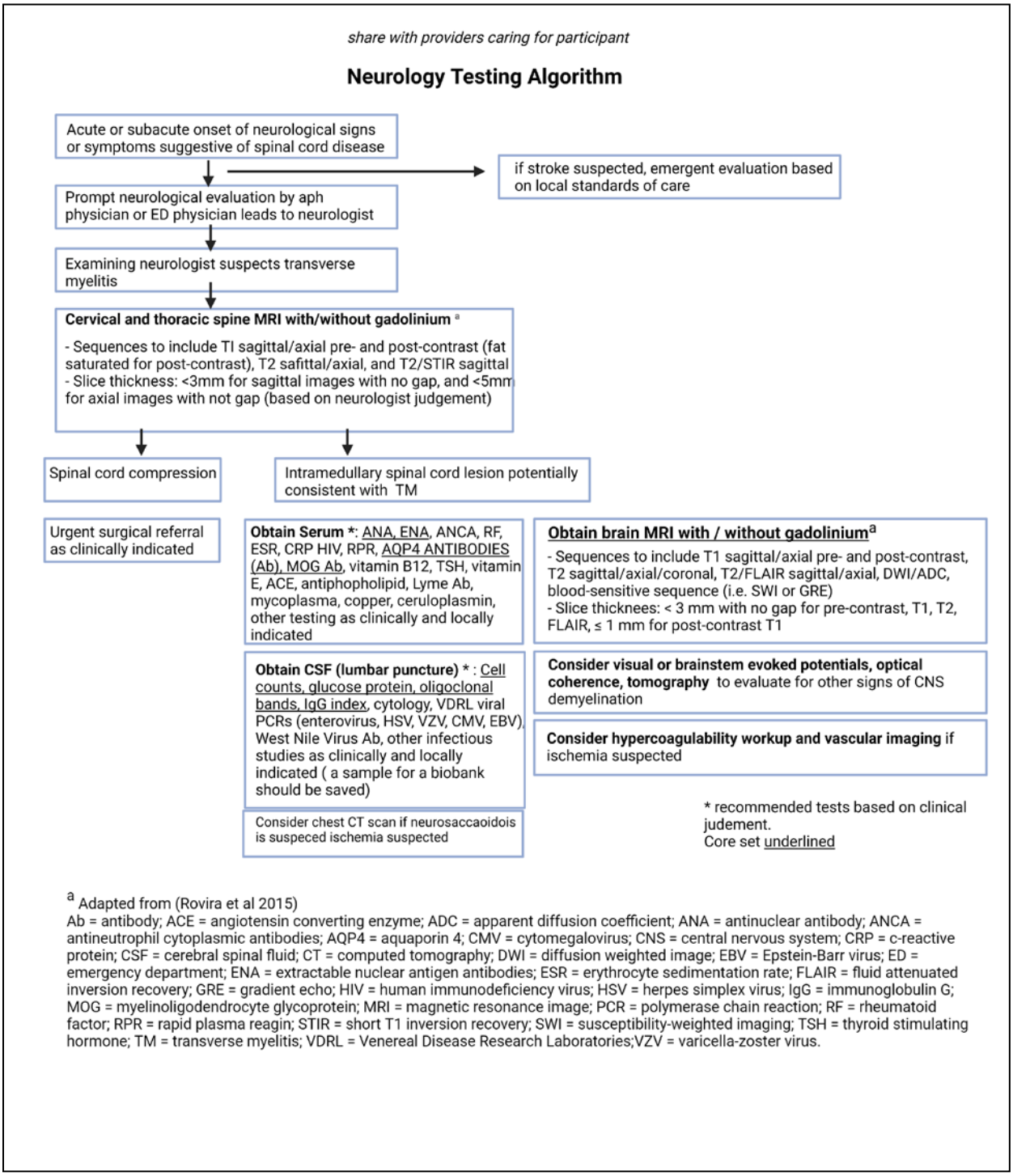
SARS-CoV-2 vaccination

Potential IGHID 12107 participants who are participating in a clinical trial with an acceptable experimental SARS-CoV-2 vaccine or who have been vaccinated with an acceptable SARS-CoV-2 vaccine are able to screen after 14 days since receiving the final dose of vaccine. Receipt of licensed SARS-CoV-2 vaccine is encouraged. Participants are allowed to co-enroll in clinical trials of acceptable investigational SARS-CoV-2 agent per PI approval and provided that limits for blood collection volumes can be maintained and vaccination follows eligibility criteria in regards to timing of vaccination.

Conduct of visits and assessments of study endpoints during periods of restricted travel or in-person visits

If in-person visits are restricted at the CRS, or if the site investigator feels that in-person visits pose a risk to the participant that alters the overall risk-benefit ratio, then reasonable efforts should be made to conduct study assessments remotely. Sites should follow FDA guidance (https://www.fda.gov/media/136238/download or use the more recent FDA updates) for conducting assessments of study endpoints during a public health emergency. Sites can obtain study-required plasma HIV-1 levels and safety laboratories through alternative sites such as a commercial laboratory near the participant's home. The decision to do the test at a commercial laboratory is made by the site based on the local situation. The site should take into account any change in blood volume required based on the laboratory selected.

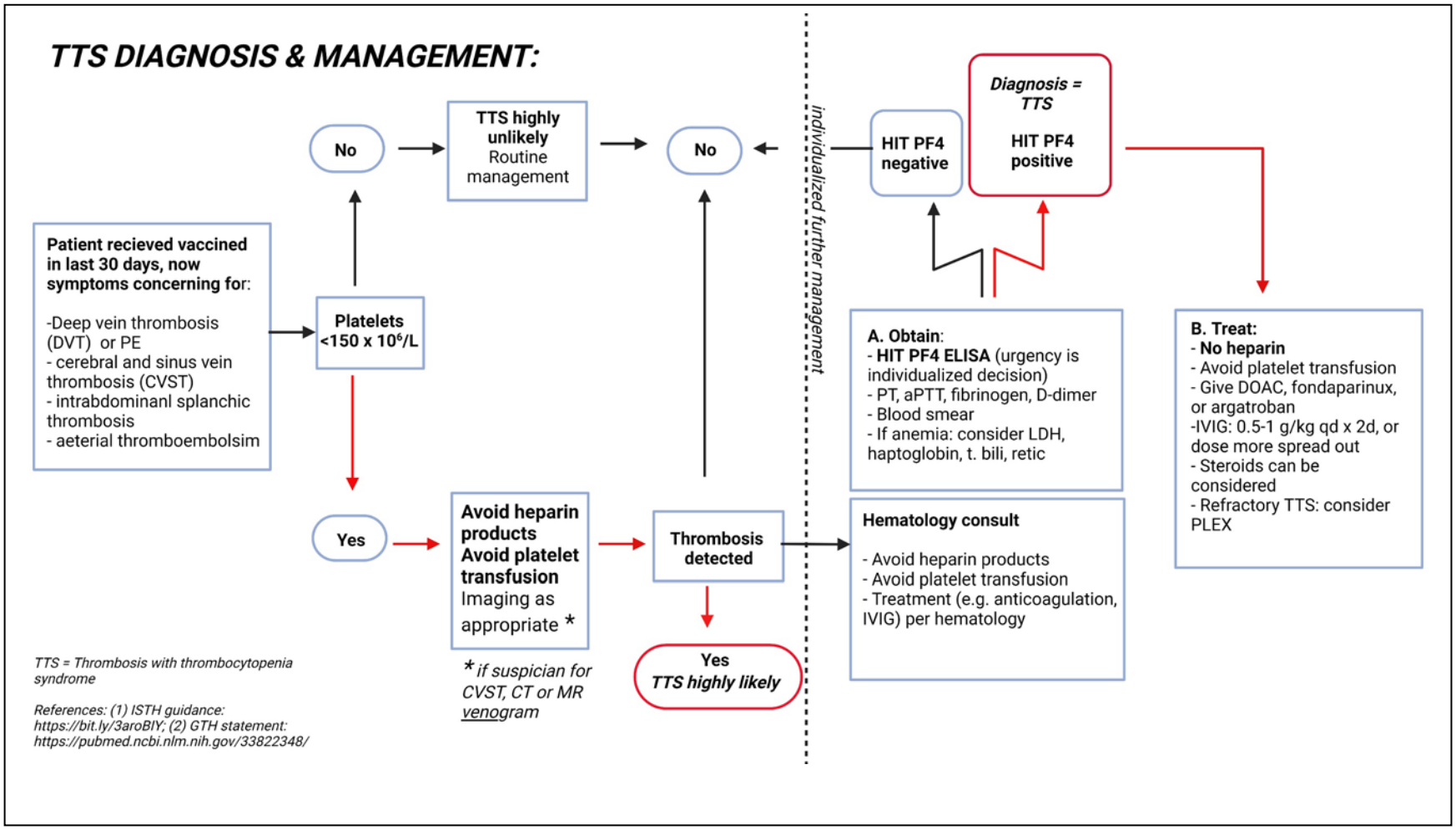
Appendix 4 Neurology Testing



[a] Adapted from (Rovira et al 2015)

Ab = antibody; ACE = angiotensin converting enzyme; ADC = apparent diffusion coefficient; ANA = antinuclear antibody; ANCA = antineutrophil cytoplasmic antibodies; AQP4 = aquaporin 4; CMV = cytomegalovirus; CNS = central nervous system; CRP = c-reactive protein; CSF = cerebral spinal fluid; CT = computed tomography; DWI = diffusion weighted image; EBV = Epstein-Barr virus; ED = emergency department; ENA = extractable nuclear antigen antibodies; ESR = erythrocyte sedimentation rate; FLAIR = fluid attenuated inversion recovery; GRE = gradient echo; HIV = human immunodeficiency virus; HSV = herpes simplex virus; IgG = immunoglobulin G; MOG = myelinoligodendrocyte glycoprotein; MRI = magnetic resonance image; PCR = polymerase chain reaction; RF = rheumatoid factor; RPR = rapid plasma reagin; STIR = short T1 inversion recovery; SWI = susceptibility-weighted imaging; TSH = thyroid stimulating hormone; TM = transverse myelitis; VDRL = Venereal Disease Research Laboratories;VZV = varicella-zoster virus.

Appendix 5 Diagnosis and Management



Appendix 6 MedDRA Preferred Terms (PTs) for TTS

I. MedDRA PTs for large vessel thrombosis and embolism in unusual locations

Aortic embolus, aortic thrombosis, aseptic cavernous sinus thrombosis, brain stem embolism, brain stem thrombosis, carotid arterial embolus, carotid artery thrombosis, cavernous sinus thrombosis, cerebral artery thrombosis, cerebral venous sinus thrombosis, cerebral venous thrombosis, superior sagittal sinus thrombosis, transverse sinus thrombosis, mesenteric artery embolism, mesenteric artery thrombosis, mesenteric vein thrombosis, splenic artery thrombosis, splenic embolism, splenic thrombosis, thrombosis mesenteric vessel, visceral venous thrombosis, hepatic artery embolism, hepatic artery thrombosis, hepatic vein embolism, hepatic vein thrombosis, portal vein embolism, portal vein thrombosis, portosplenomesenteric venous thrombosis, splenic vein thrombosis, spontaneous heparin-induced thrombocytopenia syndrome, femoral artery embolism, iliac artery embolism, jugular vein embolism, jugular vein thrombosis, subclavian artery embolism, subclavian vein thrombosis, obstetrical pulmonary embolism, pulmonary artery thrombosis, pulmonary thrombosis, pulmonary venous thrombosis, renal artery thrombosis, renal embolism, renal vein embolism, renal vein thrombosis, brachiocephalic vein thrombosis, vena cava embolism, vena cava thrombosis, truncus coeliacus thrombosis

II. MedDRA PTs for more common thrombotic events

Axillary vein thrombosis, deep vein thrombosis, pulmonary embolism

III. MedDRA PTs for thrombocytopenia

Autoimmune heparin-induced thrombocytopenia, Heparin-induced thrombocytopenia, Immune thrombocytopenia, Nonimmune heparin associated thrombocytopenia, Spontaneous heparin-induced thrombocytopenia syndrome, Thrombocytopenia, Thrombocytopenic purpura

IV. Text string for
"thrombocytopenia" or "low platelets" in symptom text

IGHID 12107 – THE CM (HIV-CORE 008) STUDY – PROTOCOL VERSION 3.0

CLARIFICATION MEMO

DATE: January 22, 2024

SUBJECT: Clarification Memo #1 for IGHID-12107 Protocol Version 3.0

This clarification memo (CM) does not result in a change in the protocol informed consent document. Each site should file a copy of this CM with the protocol for reference. The protocol clarification contained in this memo should be implemented immediately.

This clarification memo further clarifies adverse events are the defined reactogenicity adverse events beginning within 7-days following vaccination. In addition, it updates Appendix 6, MedDRA Preferred Terms (PTs) for Thrombosis with Thrombocytopenia Syndrome (TTS) events. These updates are inclusive of Coagulopathy PTs from CDC Vaccine Adverse Event Reporting System (VAERS) Standard Operating Procedures for COVID-19 (as of 02Feb2022) and Standardised MedDRA Query (SMQ) for embolic and thrombotic events.

Text noted below with a strikethrough represents deletion; text appearing below in bold represents an addition.

1. Section 4.1, Overall Design, page 46

   Safety will be assessed for the duration of the study. ~~Solicited AEs will be recorded for 28 days after each dose of study intervention (through Day 57), and~~ **AEs,** SAEs, MAAEs, and AESIs will be recorded through ~~the~~ EOS.

2. Section 8.1.5.j, Solicited Adverse Events, page 71:

   Local and systemic predefined solicited AEs for reactogenicity assessment (Section 8.2.10) will be collected in a Participant Symptom Diary for 7 days following the administration of each dose of study product. ~~In addition, assessments of solicited AEs will occur at study visits through 28 days following administration of each dose of study product.~~

3. Section 8.4.2, Time Period and Frequency for Event Assessment and Follow-Up, page 85:

   Solicited AEs will be assessed and recorded for **7** ~~28~~ days following each dose of study product.

4. Section 10.2, Endpoints, page 101:

   Secondary **Safety** ~~Efficacy~~ Endpoint(s):

   - Occurrence of any ≥ Grade 1 AE including signs/symptoms, lab toxicities, and/or clinical events, that is possibly or definitely related to study treatment any time from the first day of treatment (D0) through D196 (Week 28).

5. Appendix 6, MedDRA Preferred Terms (PTs) for Thrombosis with Thrombocytopenia Syndrome (TTS) events, page 143:

   I. MedDRA PTs for large vessel thrombosis and embolism in unusual locations

   Aortic embolus, aortic thrombosis, aseptic cavernous sinus thrombosis, brain stem embolism, brain stem thrombosis, carotid arterial embolus, carotid artery thrombosis, cavernous sinus thrombosis, cerebral artery thrombosis, cerebral venous sinus

thrombosis, cerebral venous thrombosis, superior sagittal sinus thrombosis, transverse sinus thrombosis, mesenteric artery embolism, mesenteric artery thrombosis, mesenteric vein thrombosis, splenic artery thrombosis, splenic embolism, splenic thrombosis, thrombosis mesenteric vessel, visceral venous thrombosis, hepatic artery embolism, hepatic artery thrombosis, hepatic vein embolism, hepatic vein thrombosis, portal vein embolism, portal vein thrombosis, portosplenomesenteric venous thrombosis, splenic vein thrombosis, spontaneous heparin-induced thrombocytopenia syndrome, femoral artery embolism, iliac artery embolism, jugular vein embolism, jugular vein thrombosis, subclavian artery embolism, subclavian vein thrombosis, obstetrical pulmonary embolism, pulmonary artery thrombosis, pulmonary thrombosis, pulmonary venous thrombosis, renal artery thrombosis, renal embolism, renal vein embolism, renal vein thrombosis, brachiocephalic vein thrombosis, vena cava embolism, vena cava thrombosis, truncus coeliacus thrombosis**, embolism venous, intracranial venous sinus thrombosis**

II. MedDRA PTs for more common thrombotic events

Axillary vein thrombosis, deep vein thrombosis, **disseminated intravascular coagulation,** pulmonary embolism**, thrombosis, venous thrombosis**

III. MedDRA PTs for thrombocytopenia

Acquired amegakaryocytic thrombocytopenia, Amegakaryocytic thrombocytopenia, Autoimmune heparin-induced thrombocytopenia, Heparin-induced thrombocytopenia, Immune thrombocytopenia, Nonimmune heparin associated thrombocytopenia, **Severe fever with thrombocytopenia syndrome,** Spontaneous heparin-induced thrombocytopenia syndrome, Thrombocytopenia, Thrombocytopenic purpura**, Thrombotic thrombocytopenic purpura**

IV. Text string for

"thrombocytopenia" or "low platelets" in symptom text

V. Reference additional PTs included in the Standardised MedDRA Query (SMQ) for Embolic and thrombotic events