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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STATISTICAL ANALYSIS PLAN

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Revision History

Version	Changes Made	Rationale	Effective Date
1.0	Adapted from IGHID 11810 The M&M Study SAP V1.3 Feb. 21, 2022		

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1 Introduction

This statistical analysis plan (SAP) details the statistical procedures that address the study objectives specified in Protocol version 3.0 of the 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. New versions of the SAP will be issued to document updates and changes in the plan. Any meaningful changes or additions to this SAP (e.g., in response to protocol amendments or violations of assumptions underlying pre-planned analyses), and the timing of such changes, will be documented in in the Revision History.

A DSMB will not be used for monitoring IGHID 12107. Instead, a study monitoring committee (SMC) will convene at least annually to monitor progress and participant safety, and safety data will be communicated to all SMC members on a monthly basis. This will include periodic assessments of recruitments, accrual, retention, and data quality, as well as a recommendation to the Principle Investigator (PI) concerning modifications of the trial based on the observed beneficial or adverse effects of the intervention. The responsibilities of the Safety Monitoring Committee are further described in the SMC Charter. Note, the SMC are not responsible for formal review of immunologic or virologic data. Review and finalization of these data are the responsibility of the Grant PI working with protocol biostatisticians.

1.1.1 Acronyms

AE	adverse event
AESI	adverse event of special interest
ARV	antiretroviral
cART	combination antiretroviral therapy
CFAR	Center for AIDS Research
C1	ChAdOx1.tHIVconsv1
C62	ChAdOx1.HIVconsv62
CI	confidence interval
CRF	case report form
DSMB	data and safety monitoring board
ECRF	electronic case report form

HIV	human immunodeficiency virus
IGHID	Institute for Global Health and Infectious Diseases
ITT	intention to treat
MedDRA	Medical Dictionary for Regulatory Activities
M3	MVA.tHIVconsv3
M4	MVA.tHIVconsv4
PI	principal investigator
PBMC	peripheral blood mononuclear cells
PT	preferred term
QC	quality control
REDCap	Research Electronic Data Capture
RNA	ribonucleic acid
SAE	serious adverse event
SAP	statistical analysis plan
SCA	single copy assay
SD	standard deviation
SFU	spot forming units
SIDs	study-specific identification numbers
SMC	study monitoring committee
SOC	system organ class
SOE	schedule of events
SOP	standard operating procedure
UNC	University of North Carolina

2 Study Objectives and Summary

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**.

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

3 Immunogenicity Overview

3.1 Hypothesis

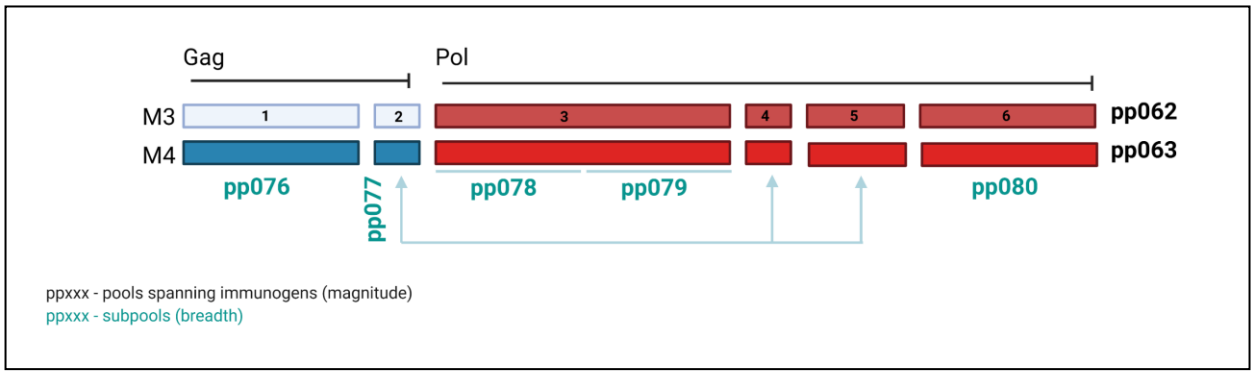
Hypothesis 1: Intramuscular (IM) immunization with **C62, C1, M3, M4** vaccines, given individually or in combination (**C62-M4, C1C62-M3M4**) in adult HIV-infected participants on suppressive combination antiretroviral therapy (ART) will be safe and increase HIV-1-specific T cell responses targeting conserved regions of HIV-1.

Hypothesis 2: The simultaneous administration of **vaccines** (**C1C62-M3M4**) will result in a greater increase in the breadth of HIV-1-specific T cells targeting conserved regions of HIV-1 than with the individual administration of **C62-M4**.

3.2 Approach to Immune Testing

The **Mos-1 and Mos-2** vaccine immunogens differ by 10% at the amino acid levels. This means that T cells targeting identical regions between **Mos-1 and Mos-2** are the 'same' population of T cells. We therefore do not then 'sum' **Mos-1**- and **Mos-2** specific T cell responses for analysis of any of the vaccine arms. Analyses of **Mos-1**- and **Mos-2**-specific T cell responses are separate.

Figure 1: Mos-1 and Mos-2 immunogens differ by ~ 10% of amino acids. Peptide pools (ppXXX) are indicated in either black (spanning whole immunogen) or teal text (subpools, spanning both immunogens).



Participants will be tested in ex vivo IFN-gamma ELISpot at available visits against peptides spanning the i) **Mos-1**/PP062 immunogen ii) **Mos-2**/PP063 immunogen iii) all peptides spanning the **Mos-1** and **Mos-2** immunogens divided into 5 subpools , PP076-PP080 iv) Clade B HIV Env/PP004 v) Clade B Nef and HIV accessory proteins/PP081 and vi) non-HIV junctions that connect the 6 sections across the **M3** and **M4** immunogens /PP082B (Table 1 / Figure 1).

Table 1: PPL021 – Peptide Pools tested in CM Study

Stimulant	Peptide Pool	Descriptor	[final assay]	No. Replicates	Reason for testing	Analysis
Mock	-	Culture media-only	-	6	Negative control to assess non-specific IFN-g production	All

M3	PP062	18mer peptides (119) overlapping by 11aa spanning Mosaic-1 immunogen	2ug/ml	4	Spans 6 highly conserved regions of HIV, all from Gag and Pol. Based on a M clade. We will examine if vaccination increases/induces responses to these conserved HIV regions. We expect that in placebo group that T cell responses will be stable.	Secondary: Magnitude (SFU/10^6 PBMC)
M4	PP063	18mer peptides overlapping (119) by 11aa spanning Mosaic-1 immunogen	2ug/ml	4	Spans 6 highly conserved regions of HIV, all from Gag and Pol. Based on a M clade. **Mos-1** differs from **Mos-2** at about 10% of aa We will examine if vaccination increases/induces responses to these conserved HIV regions. We expect that in placebo group that T cell responses will be stable.	Secondary: Magnitude (SFU/10^6 PBMC)
G1 (MOS1+MOS2)	PP076	18mer peptides overlapping (64) by 11aa spanning G1 of both Mosaic-1 and Mosaic-2	2ug/ml	4	Examining breadth at the subpool level. Also, a second an independent check of **Mos-1/Mos-2** data. Specifically, we expect any changes in **Mos-1**- or **Mos-2**-specific T cell responses to be reflected in changes at the sub-pool level.	Secondary: Breadth (0-5)
G2_P4 P5 (MOS1+MOS2)	PP077	18mer peptides overlapping (38) by 11aa spanning G2, P4 and P5 of both Mosaic-1 and Mosaic-2	2ug/ml	4	Examining breadth at the subpool level. Also, a second an independent check of **Mos-1/Mos-2** data.	Secondary: Breadth (0-5)
P3-A (MOS1+MOS2)	PP078	18mer peptides overlapping (42) by 11aa spanning the 5` half of P3 of both Mosaic-1 and Mosaci-2	2ug/ml	4	Examining breadth at the subpool level. Also, a second an independent check of **Mos-1/Mos-2** data.	Secondary: Breadth (0-5)

P3-B (MOS1+MOS2)	PP079	18mer peptides overlapping (54) by 11aa spanning the 5` half of P3 of both Mosaic-1 and Mosaic-2	2ug/ml	4	Examining breadth at the subpool level. Also, a second an independent check of **Mos-1/Mos-2** data.	Secondary: Breadth (0-5)
P6 (MOS1+MOS2)	PP080	18mer peptides overlapping (41) by 11aa spanning the 5` half of P3 of both Mosaic-1 and Mosaic-2	2ug/ml	4	Examining breadth at the subpool level. Also, a second an independent check of **Mos-1/Mos-2** data.	Secondary: Breadth (0-5)
Clade B Env	PP004	Overlapping 18mer peptides spanning Clade B Envelope (year)	2ug/ml	4	Not in vaccines. i) Descriptive analysis of whether vaccination has any non-specific effects on ENV-specific T cell responses in ELISpot ii) Immunodominance analysis	Exploratory: (SFU/10^6 PBMC)
Clade B Nef/Acc	PP081	Overlapping peptides spanning Nef, Rev, Tat, Vif, Vpr, Vpu	2ug/ml	4	Not in vaccines. i) Descriptive analysis of whether vaccination has any non-specific effects on ENV-specific T cell responses in ELISpot ii) Immunodominance analysis	Exploratory: (SFU/10^6 PBMC)
Junctions	PP082**B**	Overlapping peptides spanning junctional sequences between the 6 HIV regions of the vaccine immunogens across the four immunogens inC1, C62, M3,M4	2ug/ml	4	In the vaccine immunogen, but not HIV-specific. Descriptive analysis as to whether vaccination induced T cell response to these regions	Exploratory: (SFU/10^6 PBMC)
PHA	-	Phytohemagglutinin (mitogen)	5ug/ml	2	Positive control to confirm T cell functionality	Lab QC Control, data not submitted

4 Statistical Methods

4.1 General Analytic Considerations

4.1.1 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 (9 months). Given the focus of this study is on safety and potential adverse events, accrual will be staggered such that enrollment and treatment administration will include a maximum of 2 participants per week.

4.1.2 Randomization

Following enrollment, the 18 study participants will be randomly assigned to receive one vaccination with either **C62-M4** (n=8), **C1C62-M3M4** (n=8) or placebo (n=2) through a double-blind, placebo-controlled, block randomization design. This design has been chosen to minimize bias in the reporting of safety and immunological data. When the participant's eligibility is confirmed, the study-specific identification numbers (SIDs) will be assigned sequentially as each participant enrolls at the study. The arm assigned to the participant at randomization is summarized in Table 2.

Table 2: Randomized assignment

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

4.1.3 Blinding

Vaccine and placebo product will be indistinguishable. Site, laboratory personnel, and study participants will be blinded with respect to the allocation of vaccine or placebo. The randomization plan will be generated centrally by the study statisticians at the UNC CFAR using computer software and will be kept concealed from the study personnel. The study personnel will be made aware that the randomization will take place in blocks, but will be naïve to the block size used for randomization. As such, the specific block size is not addressed in this document.

4.1.4 Unblinding

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

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. Un-blinding 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.

4.1.5 Data Sources

The Data Management Plan is detailed in a separate, protocol-specific document. The Advarra Electronic Data Capture (EDC) clinical research management tool will be utilized as the clinical database for the IGHID 12107 clinical trial. Study Staff will be trained to enter all study-captured events and AE information in the appropriate electronic case report forms (eCRFs). QC reports from the data manager will be used to manage and resolve data inconsistencies, and AEs will be coded per the Medical Dictionary for Regulatory Activities (MedDRA). Upon completion of the interim and final databases, the statistician will be responsible for independent review before finalization of the database.

4.1.6 Baseline Data

Baseline demographic data, medication history, and laboratory data will be summarized using descriptive statistics (e.g. frequencies, percentages, means, medians, interquartile ranges, minima, and maxima). When continuous data has clinically meaningful cut-points (e.g. age distribution, laboratory data), the data may be summarized into categories for easy interpretation, providing that the analyses are not affected by this categorization. Summaries will be provided for the screened cohort and by randomized arms and placebo.

4.1.7 Missing Data

Based on previous experience with similar trials, we anticipate that the amount of missing or invalid data will be small. All participants who are evaluable for adverse event assessment will be included in the safety analyses, regardless of whether they are replaced or missing for additional endpoints. Participants who do not start study treatment or are unable to provide the first or second leukapheresis will be replaced to achieve the planned evaluable sample size. Given the small size of this study, formal imputation methods will not be used. To assess whether key findings are sensitive to missing data, missing values can be replaced with worst possible outcomes as a sensitivity analysis (e.g., by replacing missing values in the **C62-M4** only arms with the best rank and replacing missing values in the **C1C62-M3M4** arm with the worst rank, to assess if a finding regarding the **C62-M4** arm is sensitive to missing data).

4.1.8 Test Size and Confidence Levels

For all primary and secondary endpoints in this trial, 95% confidence intervals will be computed for all relevant estimates. Because this is a Phase I study with a small sample size and exploratory endpoints, results will be presented without adjustment for multiple comparisons or multiple testing of endpoints. Given the small sample size, exact methods that do not rely on large sample assumptions will be used for statistical inference and will always be presented with corresponding descriptive statistics and/or data visualization. Type-I error control (alpha) for all comparisons will be set to 0.05, and all tests will be two-sided.

4.2 Visit Schedule and Analysis Windows

Per protocol,

1) Perform visits per window list in the SoA, Section 1.3 of the protocol. There is no window around the Day 2, Day 7, Day 30, and Day35 visits. There is a ±2 days window around the visits at Days 14, 28, 42, and 56.

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

3) There is a ± 7 days window around the visits at Days 84 and 140. D196 has a ± 14 days window.

4) 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.

   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)

All measures on or before the randomization date are grouped and averaged. Analysis visit windows will be constructed around each study visit using the target windows given in Table 3. If there are multiple evaluations within the window for a given visit, the evaluation closest to the scheduled study day will be used.

Table 3: Target visit windows

Visit	Visit Type	Lower Target Window (-)	Target Day	Upper Target Window (+)
1.0	Screening	N/A	-60	N/A
2.0	Enrollment & Baseline	-60	-10	0

	Enrollment leukapheresis	After -60	-10	0
3.0	Day 0	-10	0	0
4.0	Day 2	N/A	2	N/A
5.0	Day 7	N/A	7	N/A
6.0	Day 14	12	14	16
7.0	Day 28	26	28	30
8.0	Day 30	N/A	30	N/A
9.0	Day 35	N/A	35	N/A
10.0	Day 42	40	42	44
11.0	Day 56	54	56	58
	Day 56 leukapheresis *	49	56	77
12.0	Day 84	77	84	91
13.0	Day 140	133	140	147
14.0	Day 196	182	196	210

*If no post-vaccination leukapheresis is performed (e.g. poor venous access) then a large blood draw will be collected within 21 days of Visit 11.

5 Study Size and Power

5.1 Primary Sample Size Determination

From previous publications employing ChAdOx1- and MVA- vectored vaccines, the proposed number of participants (8 vaccinees per arm and 2 placebo controls) is sufficient to evaluate the risk of SAEs or severe local or systemic reactions in the study population. Descriptive analyses will be used to summarize adverse or SAEs in each arm.

5.2 Upper Confidence Limits for Primary Safety Endpoint

The trial will be suspended if two participants experience a study treatment-related toxicity of Grade 3 or higher as defined in the primary safety endpoint. Safety events of grade three or higher will be estimated with an exact binomial 95% 1-sided upper confidence limit. 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%. If zero safety events are observed in the two vaccine arms and data are pooled across arms (n=16), then the upper limit of the exact, 1-sided 95% confidence interval (CI) will be 17%.

5.3 Power Calculations for Secondary Endpoints

Table 4 and Table 5 provide power calculations for both immunogenicity secondary endpoints. For the first of the secondary endpoints, the outcome will be the HIV-specific T cell response to peptides spanning either the **Mos-1** (PP062) or **Mos-2** (PP063) immunogens at baseline visits, Day 35, and Day 42 (following **C62-M4 or C1C62-M3M4** vaccination). For the within arm calculation, an average fold change of at least 2 (comparing T-cell responses within participants) is anticipated to be scientifically meaningful. For the between arm calculation, a relative change that is greater for any given participant in the **C1C62-M3M4** arm than in the **C62-M4** arms alone is anticipated to be scientifically meaningful if it occurs at least 90% of the time.

For the second of the secondary endpoints, the outcome will be breadth of T-cells targeting peptide subpools (n=5 subpools PP076, PP077, PP078, PP079, PP080) spanning the conserved immunogens of HIV-1. HIV-specific T-cell responses will be measured at pre-vaccination visits (1-3 visits/participant) and at post-vaccination (Day 56).

Table 4: Power for detecting differences in T-cell magnitude.

Within Arms	**Between Arms (C1C62-M3M4 vs C62-M4)**
Aim: Determine the statistical power necessary to detect a geometric mean ratio (GMR) of 2 for the within-participant HIV-specific T-cell response.	**Aim:** Assuming identical distributions of differences in both arms (i.e. assuming a location-shift-only alternative), determine the statistical power necessary to compare median differences in HIV-specific T-cell responses.
Data: Assume normally distributed natural, log-transformed, HIV-specific T cell responses from	**Data:** Assume normally distributed differences in HIV specific T-cell response

pre-vaccination (one or more of screening, enrolment, pre-vaccination leuk, Day 0) and post-vaccination (Day 35 and 42) for all arms.	between pre-vaccination (screening, enrolment, pre-vaccination leuk, Day 0) and post-vaccination (Day 35 and 42) for vaccine arms.
Design: Empirical power calculated by simulating 10,000 random samples, assuming log-transformed HIV-specific T-cell response follows a normal distribution. The effect under the alternative hypothesis was a GMR of 2 on the raw T-cell response measures. An SD of 0.8 for both pre- and post-vaccination measurements was assumed to calculate power. This assumption was based on previous, log-transformed HIV-specific T-cell response data, which gave a between-participant SD of 0.8 at baseline. A within-participant Pearson correlation of between r = 0.8 and r = 0.9 was assumed between paired log-transformed HIV-specific T-cell response measures.	**Design:** Empirical power calculated by simulating 10,000 random samples from a normal distribution. Assume a p=90% or p=87% probability that the difference in HIV-specific T-cell response for any given participant in the **C1C62-M3M4** arm is higher than any given individual in the **C62-M4** arm.
Statistical Test for Power: Two-Sided Exact Wilcoxon Signed-Rank Test to achieve exact p-value < 0.05	**Statistical Test for Power:** Two-Sided Exact Wilcoxon Rank-Sum Test to achieve exact p-value < 0.05
Sample Size: N=8 (per arm)	**Sample Size:** N=8 (per arm)
Empirical Power: >95% (within-individual r = 0.9) >80% (within-individual r = 0.8)	**Empirical Power:** 90% (p=90% effect size) >80% (p=87% effect size)

Table 5: Power for detecting differences in T-cell breadth between arms.

Aim: Determine the statistical power necessary to detect a change in T-cell breadth (T cell breadth is defined as the number of reactive HIV subpools as described in **Section 8.2**) between vaccine arms
Data: 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%). Note, that these estimates are based on previous empirical

mapping of HIV-specific T cells against 'individual' peptides spanning Gag/Pol regions corresponding to the HIVconsvX immunogens [1]. In the breadth analysis here, we first examine change of breadth to subpools (n=5) of peptides spanning the HIVconsvX immunogens. Future exploratory analyses will examine change in breadth at the individual peptide level.
Design: Calculate empirical power by simulating 10,000 random samples (as described in Data) from the **C1C62-M3M4** cohort. Compare this generated sample to **C62-M4** only, assuming zero change in T-cell breadth. Count the number of successes (p-values < 0.05).
Statistical Test for Power: Two-Sided Exact Wilcoxon Rank-Sum Test to achieve exact p-value < 0.05
Sample Size: N=8 (per arm)
Empirical Power: 95%

6 Primary Outcome: Safety

6.1 Objective

Evaluate the safety of vaccination through 28 days after the last vaccination with: **C1C62-M3M4 and C62-M4** in PWH on ART.

6.2 Endpoint Analysis

Outcome (Primary Safety Event): 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 from the first day of treatment (D0) through 28 days following the boost vaccination or D56.

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.

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.

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:

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

All of these tables will be generated for analysis reporting purposes.

7 Secondary Outcome: Safety

7.1 Objective

Evaluate the safety of vaccination through the end of study

7.2 Endpoint Analysis

Outcome (Secondary Safety Event): 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.

Analysis: 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.

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.

8 Secondary Outcomes: Immunological and Virologic Analysis

8.1 Objectives

Compare the within-participant relative change in magnitude of HIV-1-specific T cell responses following vaccination with **C62**-**M4** or **C1C62**-**M3M4**.

Compare the between-arm change in breadth of HIV-1-specific T cell responses following vaccination **C62**-**M4** or **C1C62**-**M3M4**.

8.2 Endpoint Analysis

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

Outcome 1 (T-Cell Magnitude): Relative changes in magnitude of T-cell responses to HIV-1 conserved regions measured by ex vivo IFN-γ ELISpot from pre-vaccination (one or more of

screening, enrolment, pre-vaccination leukapheresis or Day 0) to post-vaccination on Days 35 and 42.

This outcome consists of the following eight components:

Outcome 1.1: Relative change in magnitude of T-cell response to Mosaic-1 from pre-vaccination to Day 35 within each vaccinated arm.

Outcome 1.2: Relative change in magnitude of T-cell response to Mosaic-1 from pre-vaccination to Day 35 between vaccinated arms.

Outcome 1.3: Relative change in magnitude of T-cell response to Mosaic-2 from pre-vaccination to Day 35 within vaccinated arm.

Outcome 1.4: Relative change in magnitude of T-cell response to Mosaic-2 from pre-vaccination to Day 35 between vaccinated arms.

Outcome 1.5: Relative change in magnitude of T-cell response to Mosaic-1 from pre-vaccination to Day 42 within vaccinated arm.

Outcome 1.6: Relative change in magnitude of T-cell response to Mosaic-1 from pre-vaccination to Day 42 between vaccinated arms.

Outcome 1.7: Relative change in magnitude of T-cell response to Mosaic-2 from pre-vaccination to Day 42 within vaccinated arm.

Outcome 1.8: Relative change in magnitude of T-cell response to Mosaic-2 from pre-vaccination to Day 42 between vaccinated arms.

Analysis: Each of outcomes 1.1-1.8 will be analyzed separately. The magnitude of ex vivo ELISpot response will be examined using the log2 fold change of the quantified ELISpot response between the average of baseline visits and Day 35 and Day 42.To evaluate changes in magnitude 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.

Outcome 2 (T-Cell Breadth): Change in breadth (difference in the number of ex vivo reactive/positive P076, PP077, PP078, PP079, PP080 subpools pre- and post-vaccination) of T-cell responses targeting HIV-conserved regions from pre-vaccination (one or more of screening, enrolment, pre-vaccination leuk, Day 0) to post-vaccination (Day 56).

This outcome consists of the following two components:

Outcome 2.1: Change in breadth of T-cell responses from pre-vaccination to post-vaccination (Day 56) within vaccinated arm.

Outcome 2.2: Comparison of change in breadth of T-cell responses from pre-vaccination to post-vaccination (Day 56) between vaccinated arms.

Analysis: Breadth is measured as the number of ex vivo reactive subpools (P076, PP077, PP078, PP079, PP080). 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.

8.3 Endpoint Analysis Details

A. Preliminary calculations

a. Calculate mock-adjusted T cell responses for all participants at baseline (pre-vaccination) and Days 35, 42, and 56.

i. (arithmetic mean antigen wells – arithmetic mean mock), averaged across replicates.

ii. Report mock adjusted antigen response as spot-forming units (SFU) per 10^6 PBMC, typically extrapolated from an input cell number of 0.2M PBMC/well.

iii. Calculations require a minimum of 3 mock replicates.

iv. Calculations require a minimum of 2 antigen replicate wells.

Note I: Lower replicate numbers arise from i) insufficient cells or ii) well failure, mostly due to membrane issues.
Note II: The PHA positive control is a QC assessment at the laboratory level. Only passing data will be submitted for analysis (see also Table 1).

b. Define and report ex vivo *positive/reactive* T responses to antigen pools at each timepoint per participant:

i. Calculate ex vivo mock-adjusted T cell response with the following additional criteria:

1. Average (arithmetic) of ex vivo mock-adjusted replicate wells > 20 SFU/10^6 PBMCs. Otherwise, assign a value of 20 SFU/10^6 PBMCs.
2. Average (arithmetic) of ex vivo antigen replicate wells (not mock-adjusted) is ≥ four times the average (arithmetic) of mock wells. Otherwise, assign a value of 20 SFU/10^6 PBMCs.
3. Zero values are not accepted in any ex vivo replicate of antigen-stimulated wells. Otherwise, assign a value of 20 SFU/10^6 PBMCs.
4. For example, if replicates in peptide pool PP062 wells are 40, 0, 34, 10 and the average was 21 SFU/10^6, this would not be designated a positive/reactive response because of lack of certainty.
5. Summarize reactivity data per individual and per treatment arm.

B. Calculate fold-change in ex vivo ELISpot response to both vaccine and non-vaccine immunogens (Outcome 1 T-Cell Magnitude):

a. Analysis criteria:

i. The change in ex vivo ELISpot response will be examined using the log2 fold change of the quantified ELISpot response between the average of baseline visits and Day 35 and Day 42.

ii. No submitted data are to be excluded for fold-change analysis.

iii. All mock-adjusted averages (arithmetic) < 20 SFU/10^6 PBMCs or that do not meet the criteria of a 'positive/reactive' T cell response (described in C) will be assigned a value of 20 SFU/10^6 PBMCs.

b. Secondary analysis within vaccine arms (n=8/group):

i. Report results as log2 fold change to each antigen pool (**Mos-1**/PP062, **Mos-2**/PP063, Subpools PP076-080) for each vaccine arm.

ii. All log2 fold changes will be statistically assessed using a two-sided exact Wilcoxon signed-rank test. If a value in the dataset matches the hypothetical value, then the value will be ignored in the analysis (Wilcoxon method of handling ties).

c. Secondary analyses between vaccine arms (n=8/group):

i. Examine the between-arm difference in log2 fold change to each antigen pool **Mos-1**/PP062 and **Mos-2**/PP063 pre- and post-vaccination.

ii. Between-arm comparisons in log2 fold changes will be statistically assessed using a two-sided exact Wilcoxon rank-sum test. If a value in the

dataset matches the hypothetical value, then the value will be ignored in the analysis (Wilcoxon method of handling ties).

C. Calculate gain or loss of a positive T cell responses to vaccine immunogens to assess breadth (Outcome 2 T-Cell Breadth):

a. To analyze breadth, we are looking at the number of reactive subpools before (screening, enrollment, Day 0) and after vaccination (Day 56).

b. As per section 8.3.A.b, assess whether ex vivo T cell response is reactive/positive at each visit.

c. Analysis criteria:

   i. For breadth analysis, output should be binary: non-response or response
   ii. No. of positive HIV subpools 1-5 (PP076-PP080) pre-vaccination at baseline.
   iii. Pre-vaccination breadth: if a reactive/positive T cell response is observed at ***any*** pre-vaccination visit that the participant will be deemed to have a positive/reactive T cell response pre-vaccination.
   iv. No. of positive antigen subpools 1-5 (PP076-PP080) post-vaccination.
      1. Post vaccination breadth: ex vivo vaccine-induced T cell response must be detected at > 1 post-vaccination visit, Day 35 onward.
   v. Data was only submitted for analysis if it met biological criteria.

d. Secondary analysis across all vaccinees (n=16) & within arm (n=8/group):
   i. Change in number of positive HIV subpools (PP076-80) at Day 56 (post-vaccination) vs baseline (pre-vaccination) across all vaccinees (n=16).
   ii. Change in number of positive/reactive HIV subpools (PP076-80) at Day 56 (post-vaccination) vs baseline (pre-vaccination) for each vaccine arm.
   iii. The change in number of positive/reactive antigen subpools will be statistically assessed using a two-sided exact Wilcoxon signed-rank test. If

a value in the dataset matches the hypothetical value, then the value will be ignored in the analysis (Wilcoxon method of handling ties).

e. Secondary analysis between vaccine arms (n=8/group):
    i. Compare the change in number of positive/reactive antigen subpools at Day 56 vs baseline for study arm **C1C62-M3M4** to the change in number of positive/reactive antigen subpools at Day 56 vs baseline for study arm **C62-M4**.
    ii. Between-arm comparisons in breadth will be statistically assessed using a two-sided exact Wilcoxon rank-sum test. If a value in the dataset matches the hypothetical value, then the value will be ignored in the analysis (Wilcoxon method of handling ties).

9 Interim Analyses

During the SMC annual meeting and Closed Session Reports, the SMC will discuss the primary endpoint, participant safety through Day 28 following second vaccination with M3M4 or M4. This assessment will serve as a basis for allowing enrollment to continue and, at the request of the SMC, the statistician may be present during the closed session.

Safety Pause meetings will be called if:

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

This “ad hoc” report, typically prepared by the statistician or data manager, will include the relation of the AE or SAE to study treatment thought by the blinded core team to be a primary safety outcome. Following the review, the SMC will recommend if and how the

study should proceed with respect to resuming enrollment and continuing study treatment. To the extent possible, decisions regarding additions or modifications to planned analyses, inclusion/exclusion from analysis cohorts, and handling of missing or incomplete data will be made by the statisticians or other study staff prior to review of arm-specific results.

10 ELISPOT Data

10.1 UNC ELISpot assays

Peptides: A study-specific peptide plate (PPL039 – CM longitudinal) was made and QC'd in healthy donors prior to testing in CM participants . Stability the peptide plate was tested over the course of the study using reference PBMC. Each plate contains pools of overlapping peptides spanning i) Mosaic-1 (**Mos-1**) and ii) Mosaic-2 (**Mos-2**) immunogens designed, iii) Subpool G1 iv) Subpools G2, P4, P5 v) Subpool P3-A vi) Subpool P3-B vii) Subpool P6 viii) Clade B Env ix) Clade B Nef/Acc (Rev, Tat, Vif, Vpu, Vpr) and x) Junctions

ELISpot: ELISpots were performed as previously described [2]. 200,000 PBMC were added/well.

Spot counting: Peptide plates were counted using an AID ELISpot reader. Plates were first read using standardized settings with no changes made and counts stored (see 'Count Settings Intensity 10' below). Plates were then re-read using an administrator setting allowing minor changes to spot counts such as removal of dust, as per SOP G002B_V2. Any changes between first and admin reads were documented both as print-outs and exported text files.

Data Listings: The data listing comprises one worksheet per participant. ELISpot data were directly exported as text files (after the Admin Read) from the AID reader and imported into an Excel spreadsheet. All data were reviewed by technicians, N Goonetilleke and/or Yinyan Xu prior to submission.

10.2 Lab QC Controls

All technicians in the Goonetilleke laboratory have passed ELISpot proficiency testing using internal QC controls. Full training records are maintained. The Goonetilleke laboratory also participates in NIH-sponsored EQUAPOL ELISpot proficiency.

All cell cultures and peptide plates are routinely screened using light microscopy for evidence of infection.

Peptide plates have been tested for stability against reference PBMC over the course of the study.

Freezers, cryostores and incubators used in these experiments have been temperature-monitored and all key equipment such as biosafety hoods, cell counter and AID reader maintained under service contract.

- Only data that meets laboratory QC parameters will be submitted for analysis.
- Within participant, longitudinal timepoints may be performed on different days if standard QC measures (counting, controls) are met.
- If ELISpots (same visit) are repeated and results discordant, then no data will be submitted for secondary endpoint analysis.
- If ELISpots (same visits) are repeated and results concordant, then the first assay performed will be submitted for secondary endpoint analysis.
- Typically, 0.20 million PBMC/well were added. Other input values are accepted. All magnitude data will be graphed as SFU/million cells.

10.3 Reporting

Analysis of blinded study data and preparation of a study report for primary and secondary endpoints will be performed by UNC CFAR Biostatistician, Melissa Mischell under the supervision of Dr Michael Hudgens. Data will be submitted by .xls and .pdf (QC control).

11 References

1. Warren, J.A., et al., *The HIV-1 latent reservoir is largely sensitive to circulating T cells.* Elife, 2020. **9**.
2. Xu Y, et al., *HIV-specific T cell responses are highly stable on antiretroviral therapy.* Molecular Therapy Methods and Clinical Development, 2019.