NCT05423418

A Phase 1 Randomized Study to Evaluate the Safety, Tolerability, and Immunogenicity of Ranging Doses of ALFQ Adjuvant in a Candidate HIV Vaccine Containing A244 and B.65321

January 7, 2025

A Phase 1 Randomized Study to Evaluate the Safety, Tolerability, and Immunogenicity of Ranging Doses of ALFQ Adjuvant in a Candidate HIV Vaccine Containing A244 and B.63521

Protocol Number:	RV 575/WRAIR 2924
Compound/Product Number/Name:	A244, B.63521, and ALFQ Adjuvant
Sponsor:	The Surgeon General, Department of the Army
Sponsor's Representative: Senior Regulatory Advisor & IND Sponsor's Representative:	Emily D. Badraslioglu, MS, PMP, RAC US Army Medical Research and Development Command (USAMRDC) 1430 Veterans Drive Fort Detrick, MD 21702-5009 Tel: +1 301-619-0317 DSN: 343-0317 Fax Number: +1 301-619-0197 E-mail: usarmy.detrick.medcom-usamrmc.mbx.usamrmcregulatory-affairs@health.mil
Funding Source:	Division of AIDS, National Institute of Allergy and Infectious Disease, US National Institutes of Health and Cooperative Agreement (#W81XWH-18-2-0040)
Regulatory Agency Identifying Number:	IND 28352
Clinical Trial Site:	Clinical Trials Center, Room 2w78 Center for Enabling Capabilities (CEC) Walter Reed Army Institute of Research 503 Robert Grant Avenue Silver Spring, MD 20910 Phone Number: 301-319-9660
KEY ROLES	
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Research Laboratory:	US Military HIV Research Program Walter Reed Army Institute of Research (WRAIR) 503 Robert Grant Avenue Silver Spring, MD 20910 Walter Reed Army Institute of Research Specimen Processing Laboratory and Biorepository (SPL/BioR) Diagnostics and Countermeasures Branch (DCB) Center for Infectious Disease Research (CIDR) 13 Taft Ct., Suite 100 Rockville, MD 20850 External Research Collaborators are listed in Attachment 2.

Clinical Laboratories:	Walter Reed Army Institute of Research HIV Diagnostics and Reference Laboratory (HDRL) Diagnostics and Countermeasures Branch (DCB) Center for Infectious Disease Research (CIDR) 9100 Brookville Road, Building 508 Silver Spring, MD 20910 Quest Diagnostics Inc. 1715 Twin Springs Road Baltimore, MD 21227-2997
Institutional Review Boards:	Walter Reed Army Institute of Research IRB US Army Garrison-Forest Glen 503 Robert Grant Avenue Silver Spring, MD 20910 FWA #00000015

PROTOCOL AMENDMENT HISTORY

Version No.	Date	Description
0.5	21JUN21	Team review
1.0	04AUG21	Medical writing review, original version submitted to the Protocol Review Board
1.1	27SEP21	Included response to the FDA
1.2	14JAN22	Included DAIDS comments and response to the IRB
1.3	04APR22	WRAIR HSPB comments addressed
1.4	21JUN22	Change in PI and IRB comments addressed
1.5	26JUL22	Response to IRB stipulations
1.6	11OCT22	Response to FDA non-hold comments and includes administrative updates (office name changes and contact information) and clinical updates
1.7	26OCT22	Updated to clarify compensation language
1.8	09NOV22	Response to WRAIR review: updated blood donation inclusion criterion
1.9	14MAR23	Added Planned Interim Analyses - Section 9.6.6
2.0	15AUG24	Updated study personnel to include change in PI from MAJ Adjei to MAJ Cebula.
2.1	24SEP24	Response to WRAIR review: updated funding CA number
2.2	07JAN25	Added exploratory objective and endpoint

INVESTIGATOR'S AGREEMENT

1. I agree to follow this protocol version as approved by the IRB.
2. I will conduct the study in accordance with applicable IRB requirements, Federal regulations, and state and local laws to maintain the protection of the rights and welfare of study participants.
3. I certify that I, and the study staff, have received the requisite training to conduct this research protocol.
4. I will not modify the protocol without first obtaining an IRB approved amendment and new protocol version unless it is necessary to protect the health and welfare of study participants. PRB approval of amended study documents prior to IRB submission is required.
5. I have read and understand the information in the Investigators' Brochure (or Manufacturer's Brochure) regarding the risks and potential benefits. I agree to conduct the protocol in accordance with Good Clinical Practices (ICH-GCP), the applicable ethical principles, the Statement of Investigator (Form FDA 1572), and with local regulatory requirements. In accordance with the U.S. FDA Modernization Act and NIH policy, I will ensure the registration and reporting results of the trial on the www.clinicaltrials.gov website.
6. I will ensure that the data (and/or specimens) are maintained in accordance with the data (and/or specimen) disposition outlined in the protocol. Any modifications to this plan should first be reviewed and approved by the WRAIR IRB.
7. I will promptly report changes to the research or unanticipated problems to the WRAIR IRB immediately via the WRAIR Human Subjects Protection Branch at 301-319-9940 (during duty hours) or to usarmy.detrick.medcom-wrair.mbx.hspb@health.mil and submit a written report within 10 working days of knowledge of the event.
8. I will prepare continuing review reports at an interval established by the IRB, and a study closure report when all research activities are completed.
9. I will immediately report to the WRAIR Human Subjects Protection Branch knowledge of any pending compliance inspection by any outside governmental agency.
10. I agree to maintain adequate and accurate records in accordance with IRB policies, Federal, state, and local laws and regulations.

Brennan R. Cebula, MD, MTM&H Principal Investigator Date

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation or Specialist Term	Explanation
3D-PHAD®	Synthetic monophosphoryl lipid A
AAE	Acquired angioedema
Ab	Antibody
ADCC	Antibody-Dependent Cell-Mediated Cytotoxicity
ADCP	Antibody-Dependent Cell-Mediated Phagocytosis
AE	Adverse event
AESIs	Adverse events of special interest
AIDS	Acquired immunodeficiency disease syndrome
AHFG	Aluminum Hydroxide Fluid Gel
ALF	Army Liposome Formulation
ALFQ	ALF mixed with the saponin QS21
AlOH	Aluminum hydroxide
ALT	Alanine aminotransferase
ATO	Army Technology Objective
AUC	Area under the curve
CD4	Cluster of Differentiation
CFSE	Carboxyfluorescein succinimidyl ester, a fluorescent cell staining dye
CRFs	Case Report Forms
CRR	Continuing review report
CTC	WRAIR Clinical Trials Center
DAIDS	Division of AIDS
DODI	Department of Defense Instruction
DSMB	Data Safety Monitoring Board
eCRF	Electronic Case Report Forms
EDC	Electronic Data Capture
EDTA	Ethylenediaminetetraacetic acid
ELISPOT	Enzyme-Linked Immunospot
FDA	U.S. Food and Drug Administration
FSH	Follicle stimulating hormone
GBS	Guillain-Barré syndrome
GCLP	Good Clinical Laboratory Practices
GCP	Good Clinical Practices
HAE	Hereditary angioedema
HBsAg	Hepatitis B surface antigen
HIV-1	Human Immunodeficiency Virus 1
HRT	Hormonal replacement therapy
HSPB	Human Subjects Protection Branch, WRAIR
IBs	Investigational brochures
ICH	International Conference on Harmonization
ICS	Intra-cellular cytokine staining
ID	Infectious disease

Abbreviation or Specialist Term	Explanation
IEC	Independent Ethics Committee
IFNγ	Interferon gamma
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IL2	Interleukin 2
IM	Intramuscular
IND	Investigational New Drug
IP	Investigational product
IQR	Interquartile range
IRB	Institutional Review Board
ISM	Sponsor Independent Safety Monitor
IUD	Intrauterine device
IUS	Intrauterine hormone-releasing system
LPLV	Last Participant Last Visit
MAAE	Medically Attended Adverse Events
MHRP	U.S. Military HIV Research Program
MOP	Manual of Procedures
MPLA	Monophosphoryl lipid A
NAAT	Nucleic Acid Testing
NCO	Non-commissioned officer
NHP	Non-human primate
NK	Natural Killer Cell
NKT	Natural Killer T cell
NSAIDs	Nonsteroidal anti-inflammatory drugs
OAE	Other significant adverse events
OHARO	Office of Human and Animal Research Oversight
OHRO	Office of Human Research Oversight
ORA	Office of Regulated Activities
PBMC	Peripheral blood mononuclear cell
PI	Principal Investigator
PIMMCs	Potentially Immune-Mediated Medical Conditions
PIN	Participant identification number
PRB	Protocol Review Board
PSRT	Protocol Safety Review Team
PSSO	Product Safety Surveillance Office
PVG	Pharmacovigilence
RFADCC	Rapid and fluorometric ADCC
SAE	Serious adverse event
SD	Standard deviation
SGTP	Serum glutamic pyruvic transaminase
SOE	Schedule of Evaluations
SOP	Standard operating procedure
STI	Sexually transmitted infection

Abbreviation or Specialist Term	Explanation
TOU	Test of understanding
UPIRTSO	Unanticipated problems involving risks to subjects or others
USAMRDC	United States Army Medical Research and Development Command
WRAIR	Walter Reed Army Institute of Research

1. PROTOCOL SUMMARY

1.1. Synopsis

Name of Sponsor:

The Surgeon General, Department of the Army

Name of Investigational Product:

A244, B.63521, and ALFQ

Name of Active Ingredient:

A244 consists of the gp120 envelope glycoprotein HIV-1 subtype CRF_01E A244 derived from the CM244 CRF_01AE. The A244 gp120 envelope has an 11 amino N-terminal deletion, similar to the A244 protein used in AIDSVAX B/E.

B.63521 gp120 is a recombinant HIV-1 Env protein containing an 11 amino acid truncation at the N-terminus of gp120 that enhances antigenicity and immunogenicity.

ALFQ (Army Liposomal Formulation) is a liposomal adjuvant containing a synthetic monophosphoryl lipid A (MPLA) with the addition of QS-21.

Protocol Number(s): RV 575

Title of Study: A Phase 1, Randomized Study to Evaluate the Safety, Tolerability, and Immunogenicity of Ranging Doses of ALFQ Adjuvant in a Candidate HIV Vaccine Containing A244 and B.63521

Study Site(s): WRAIR Clinical Trials Center (CTC)

Principal Investigator:

MAJ Brennan R. Cebula, MD, MTM&H

*Protocol Team Roster included as Attachment 1.

Studied Period (years):	**Phase of Development:** Phase I
Estimated date first participant enrolled: May 2022 Estimated date last participant completed: October2023 Study duration: Specimen analysis and monitoring of medically attended adverse events (AEs) will continue 12 months beyond the final participant vaccination. Anticipated end date is October 2028.	

Objectives:

Primary:

To evaluate the safety and tolerability (including reactogenicity) of candidate vaccine A244/B.63521 (300 µg each) with ALFQ adjuvant at the 200 µg, 100 µg, and 50 µg doses

Secondary:

To evaluate the effect of candidate vaccine A244/B.63521 (300 µg each) with ALFQ adjuvant at the 200 µg, 100 µg, and 50 µg doses on cellular, and humoral immune responses

Exploratory:

To characterize B-cell functional specificities for each vaccination regimen

To characterize innate immunity

To assess the innate/gene expression induced across vaccination regimens

To perform systems serology analyses

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To characterize the impact of vaccination with the ALFQ adjuvant on the permissiveness and expression to in vitro HIV infection of CD4+ T cells and differentiated macrophages
Endpoints **Primary** Evaluate the occurrence and severity of solicited local and systemic AEs following candidate vaccine administration. Evaluate the occurrence, severity, and relationship to vaccination of unsolicited AEs after candidate vaccine administration. Evaluate the occurrence of SAEs, and new-onset medical conditions. Evaluate the occurrence of AEs of special interest (AESIs) following candidate vaccine administration. **Secondary** Compare plasma Immunoglobulin G (IgG) binding antibodies to gp120 in terms of magnitude, durability, and area under the curve among groups with differing ALFQ doses. Characterize and assess the magnitude of cell-mediated immune responses elicited across vaccination regimens including antigen -specific CD4 and CD8 T cell responses and polyfunctionality. **Exploratory** Characterize plasma IgG and Immunoglobulin A (IgA) binding antibodies to HIV gp120, neutralizing antibodies, and non-neutralizing effector functions such as Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) and Antibody-Dependent Cell-Mediated Phagocytosis (ADCP) with emphasis on RV144 immune correlates of risk of HIV acquisition. Characterize B-cell functional specificities for each vaccination regimen by quantifying antigen-specific responses through B-cell Enzyme-Linked Immunospot (ELISPOT), phenotyping the magnitude and activation status of B cell subsets via flow cytometry and isolation of monoclonal antibodies from selected vaccine recipients. Characterize innate immunity through quantification of soluble chemokines and cytokines and assessing the phenotype and function of cellular innate immune subsets such as Natural Killer Cell (NK), NKT, and dendritic cells. Characterize effects of host genetic polymorphisms on immune responses and characterize differentially expressed genes. Characterize the impact of vaccination with the ALFQ adjuvant on the permissiveness and expression to in vitro HIV infection of CD4+ T cells and differentiated macrophages through integrated HIV-DNA PCR, cell associated-DNA and RNA, flow cytometry of various activation markers and HIV Gag protein, ELISA of HIV p24 in the cell culture supernatant, and production of cytokines/chemokines.
Methodology This study is exploratory in nature. The purpose of this study is to optimize ALFQ dosing. We will also evaluate the B.63521 protein. Safety will be assessed through the frequency of the overall and specific post-vaccination reactions. Blood will be collected to assess humoral, cell-mediated, and innate immune responses. Healthy adults not living with HIV, who are available for 14 months will be enrolled. A total of 60 participants will be enrolled in one of three arms, each comprised of 20 candidate vaccine recipients. Each arm will receive identical doses of A244 and B.63521 (300 micrograms of each protein). In

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addition, Arm 1 will receive 200 micrograms of ALFQ. Arm 2 will receive 100 micrograms of ALFQ. Arm 3 will receive 50 micrograms of ALFQ. The safety, reactogenicity, and immunogenicity will then be compared among the three arms to determine the optimal dose of ALFQ.

All vaccinations will be split into 2 half doses, which will be administered intramuscularly (IM) into the same deltoid muscle. Vaccinations will occur at months 0, 1, and 2. **The second vaccination will be administered into the contralateral deltoid at study month 1 compared to the first vaccination at study month 0. The third vaccination at study month 2 will be administered into the same deltoid as the first vaccination at study month 0.** Participants will be followed for 12 months following the last study vaccination.

Number of Subjects:

Maximum number of subjects to be screened: 150

Maximum number of subjects to be enrolled: 60

Inclusion Criteria

Potential participants are required to meet all of the following criteria for enrollment into the study.

1. Healthy adults between the ages 18-55 years (inclusive)
2. Must be at low risk for HIV infection per investigator assessment and using the study risk assessment tool.
3. Able and willing to provide written, informed consent
4. Able and willing to comply with all research requirements, in the opinion of the Investigator
5. Agreement to refrain from blood donation during the course of the study and within the 2 months prior to study entry
6. Minimum body weight of 110 pounds (lbs) (50kg)
7. Laboratory Criteria within 45 days before enrollment:
    a. Hemoglobin ≥ 11.7 g/dL for women; ≥ 12.5 g/dL for men
    b. White Blood Cell count = 3,500-10,800 cells/mm^3
    c. Platelets ≥140,000/mm^3 and ≤ 450,000/mm^3
    d. Alanine aminotransferase (ALT; SGPT) <1.25 x ULN
    e. Serum creatinine ≤ 1.25 x institutional upper limit of the reference range
    f. Negative HIV testing (HIV Ab / antigen 4th generation screen with reflex confirmatory RNA testing)
    g. Negative HBsAg and hepatitis C antibody testing

Note: As above, Grade 1 lab abnormalities detected on screening may be repeated at PI discretion. Persistent Grade 1 abnormalities that are felt to represent the non-pathologic baseline for the subject will be documented before a subject is enrolled in the trial and are allowable per discretion of the PI.

8. Birth control requirements:

All participants assigned female at birth must meet one of the following two criteria:

a. No reproductive potential due to post-menopausal status (12 months of natural [spontaneous] amenorrhea) or hysterectomy, bilateral oophorectomy, or tubal ligation
b. People of childbearing potential should agree to practice highly effective contraception at least 30 days before enrollment and through 3 months post-last vaccination, using one of the following methods: condoms (male or female) with spermicide; diaphragm, or cervical cap with spermicide; intrauterine device; contraceptive pills, patch, injection,

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intravaginal ring or other FDA-approved contraceptive method; male partner has previously undergone a vasectomy; abstinence

c. All participants are encouraged to engage in safe sex practices to prevent HIV acquisition

9. For all participants assigned female at birth, except those with a history of hysterectomy or bilateral oophorectomy, a negative β-HCG pregnancy test (urine) on the day of enrollment and each vaccination day is required. Because tubal ligations have a failure rate that is not insignificant, and because 12 months of spontaneous amenorrhea can be a result of polycystic ovarian syndrome and does not completely preclude pregnancy, a negative β-HCG pregnancy test at enrollment and on each vaccination day is also required for participants assigned female at birth with a history of either of these).
10. No plans to travel outside the Washington DC metro area (DC, Maryland, and Virginia) that would prevent compliance with planned study visits
11. Test of Understanding (TOU) (minimum passing score of 80% with 2 attempts permitted)

Exclusion Criteria

Potential participants meeting any of the following criteria will be excluded from enrollment.

1. Receipt of any investigational HIV vaccine or investigational adjuvant
2. Received an investigational product in the 30 days before enrollment, or planned to receive during the study period. **This does not include products with emergency use authorization.**
3. Concurrent participation in another clinical research study
4. Any serious medical illness or condition
5. Receipt of immunoglobulins or blood products within 3 months before enrollment
6. Any history of anaphylaxis or allergy to study product
7. History of sickle cell trait or disease
8. Pregnancy, lactation, or intention to become pregnant during the study
9. History of active/recent cancer still within treatment or active surveillance follow-up (except basal cell carcinoma of the skin and cervical carcinoma in situ). Treated/resolved cancers with no likelihood of recurrence may be deemed acceptable at Principal Investigator (PI) discretion.
10. History of autoimmune disease
11. History of Potentially Immune-Mediated Medical Conditions (PIMMCs)
12. Suspected or known current alcohol or drug abuse as defined by an alcohol intake of greater than 3 drinks a day on average for a man, and greater than 2 drinks a day on average for a woman
13. Any other significant disease, disorder or finding which may significantly increase the risk to the volunteer because of participation in the study, affect the ability of the volunteer to give informed consent, participate in the study, or impair interpretation of the study data, in the opinion of the Investigator
14. History of splenectomy
15. History of confirmed or suspected immunodeficiency
16. History of hereditary angioedema (HAE) acquired angioedema (AAE), or idiopathic forms of angioedema

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17. History of asthma that is unstable or required emergent care, urgent care, hospitalization, or intubation during the past 2 years
18. History of diabetes mellitus (type I or II), with the exception of gestational diabetes
19. History of thyroid disease (except for well controlled hypothyroidism)
20. History of idiopathic urticaria within the past year
21. History of hypertension that is not well controlled by medication or that is persistently greater than 140/95 at screening
22. History of bleeding disorder diagnosed by a doctor (e.g., factor deficiency, coagulopathy, or platelet disorder requiring special precautions) or significant bruising or bleeding difficulties with IM injections or blood draws
23. History of chronic or active neurologic disease to include seizure disorder and chronic migraine headaches. Exceptions are: i) childhood febrile seizures, or ii) seizures secondary to alcohol withdrawal more than 3 years ago
24. Subjects receiving any of the following substances:
    a. Systemic immunosuppressive medications or cytotoxic medications within 12 weeks before enrollment [with the exception of a short course of corticosteroids (≤ 14 days duration or a single injection) for a self-limited condition at least 2 weeks before enrollment; inhaled, intranasal or topical steroids are not considered exclusionary
    b. Treatment with known immunomodulators including allergy immunotherapy (other than nonsteroidal anti-inflammatory drugs [NSAIDs] or stable maintenance immunotherapy (doses not in the process of being increased), at the discretion of the Protocol Safety Review Team (PSRT)) for any reason
    c. Live attenuated vaccines within 30 days before initial study vaccine administration
    d. Medically indicated subunit, mRNA, or killed vaccines, e.g., influenza, pneumococcal, vaccines with QS-21 as an adjuvant, or allergy treatment with antigen injections, planned for administration 14 days before or after study vaccine administration
25. History of arthritis diagnosis other than osteoarthritis
26. History of other diagnosed rheumatoid disorders
27. Has an acute illness or temperature ≥38.0 ºC/100.4 ºF on any study injection day or within 48 hours of planned study injection.

Note: Participants will not be excluded from further consideration for enrollment and study injections. Volunteers with fever or an acute illness on the day of study injection or in the 2 days before the study injection may be re-assessed by a study physician for resolution of the condition and enrolled and receive the study injection so long as the injection is within allowable windows. Military personnel will be excluded from participation in this study, regardless of leave status due to the potential for a false-positive HIV test result on mandatory HIV testing. This could have adverse effects on deployment status.

Final evaluation of eligibility will be based on the medical judgment of the investigator based on his/her medical and research experience.

Investigational Product, Dosage, and Mode of Administration

Arm 1: An injection containing A244/B.63521 with 200 μg of ALFQ adjuvant will be administered as an IM injection into the deltoid muscle at visits 1, 3, and 5 (corresponding to Day 1, Day 29, and Day 57).

Arm 2: An injection containing A244/B.63521 with 100 μg of ALFQ adjuvant will be administered as an IM injection into the deltoid muscle at visits 1, 3, and 5 (corresponding to Day 1, Day 29, and Day 57).

Arm 3: An injection containing A244/B.63521 with 50 μg of ALFQ adjuvant will be administered as an IM injection into the deltoid muscle at visits 1, 3, and 5 (corresponding to Day 1, Day 29, and Day 57).

Investigational Products

Products: A244, B.63521, and ALFQ

Dosage: 200 μg, 100 μg, and 50 μg of ALFQ (Dose of MPLA/3D-PHAD® component), **300** μg A244, and **300** μg of B.63521

Adjuvant: ALFQ (200 μg of MPLA/3D-PHAD® and 100 μg of QS-21 diluted in 0.5 mL Sorenson's buffer)

Total Volume: 1.8 mL

Mode and Location of Administration: IM injection into the same deltoid muscle (same side) for all injections.

Duration of Study Participation: Participants will receive IM administration of the investigational products at months 0, 1, and 2, after which they will be followed for 12 months after the 3rd injection for safety analyses. Total duration of participation will be 14 months.

Criteria for Evaluation:

Immunogenicity:

Humoral responses will be assessed by HIV-specific binding antibody assays, HIV-specific neutralizing antibody assays, and non-neutralizing antibody function assays.

HIV-specific cell-mediated responses will be assessed by intracellular cytokine staining, CD4 and CD8 T cell proliferation, and related assays. Innate immune responses and genetic responses will be quantified.

Safety:

Safety will be assessed both by direct physical examination and by diary cards, which serve as memory tools for better identification of reactions. Participants will be assessed at pre- vaccination baseline and will remain in clinic under direct observation for at least 30 minutes post vaccination. Participants will return to clinic post-vaccination to be assessed for symptoms of local and systemic reactogenicity. Results will then be compared among the three ALFQ dose arms.

Participants will be followed for a total of 12 months post final vaccination. Serious Adverse Events and SAEs will be recorded at all visits along with timing and possible attribution to Investigational Product. Because this clinical trial involves an adjuvant, Adverse Events of Special Interest AESIs and Medically Attended Adverse Events (MAAE) will also be assessed. Safety laboratory analyses of complete blood count, liver function tests, urinalysis, and pregnancy test in females will also be performed. Safety results will then be compared among dose arms.

Statistical Methods:

RV 575 is designed to optimize ALFQ dosing. The primary objective is to compare dose- dependent safety and reactogenicity of ALFQ. For safety analyses, the occurrence of local and systemic reactogenicity symptoms, AEs, and SAEs will be assessed as the proportions of participants experiencing such safety events and compared among the three study arms. The secondary objective is to assess immunogenicity.

1.2. Schema

The study design is illustrated in Figure 1.

Figure 1: Study Design

Number of participants (N) = 60		Vaccination schedule in months (days)		
	Number of participants (N)	**Month 0 (Day 1)**	**Month 1 (Day 29)**	**Month 2 (Day 57)**
Arm 1	20	300µg A244 300µg B.63521 200µg ALFQ	300µg A244 300µg B.63521 200µg ALFQ	300µg A244 300µg B.63521 200µg ALFQ
Arm 2	20	300µg A244 300µg B.63521 100µg ALFQ	300µg A244 300µg B.63521 100µg ALFQ	300µg A244 300µg B.63521 100 µg ALFQ
Arm 3	20	300µg A244 300µg B.63521 50µg ALFQ	300µg A244 300µg B.63521 50µg ALFQ	300µg A244 300µg B.63521 50µg ALFQ

1.3. Schedule of Activities

The detailed schedule of study events is provided in Table 1 and a summary of laboratory assessments is provided in Table 2 .

Table 1: Schedule of Evaluations (SOE)

Visit Number	0	1	2	3	4	5	6	7	8	9	10^2/Exit 4	Phone Follow-up #1	Phone Follow-up #2
Visit Type	Screen	V1	Safety & Immun	V2	Safety & Immun	V3	Safety & Immun	Safety & Immun	Safety & Immun	Safety& Immun	Safety & Immun	MAAE follow-up	MAAE follow-up
Visit Day	-45 to -3	1	15	29	43	57	58-59	64	71	169	337	365	393
Window			± 3d	± 7d	± 3d	±7d		± 3d	± 3d	± 7d	± 7d	± 7d	± 7d
Visit Week		0	2	4	6	8	8	9	10	24	48	52	56
Clinical													
Briefing and Contact Information	X												
Informed Consent	X												
Test of Understanding	X												
Enrollment and Randomization		X											
Vaccination		X		X		X							
Vital Signs and Physical exam[1]	X	X	X	X	X	X	X	X	X	X	X		
Medical History & Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X		

Visit Number	0	1	2	3	4	5	6	7	8	9	10[2]/Exit 4	Phone Follow-up #1	Phone Follow-up #2
Visit Type	**Screen**	**V1**	**Safety & Immun**	**V2**	**Safety & Immun**	**V3**	**Safety & Immun**	**Safety & Immun**	**Safety & Immun**	**Safety& Immun**	**Safety & Immun**	**MAAE follow-up**	**MAAE follow-up**
Visit Day	**-45 to** -3	**1**	15	**29**	**43**	**57**	**58-59**	**64**	**71**	**169**	**337**	**365**	**393**
Window			**± 3d**	**± 7d**	**± 3d**	**±7d**		**± 3d**	**± 3d**	**± 7d**	**± 7d**	**± 7d**	**± 7d**
Visit Week		**0**	**2**	**4**	**6**	**8**	**8**	**9**	**10**	**24**	**48**	**52**	**56**
Adverse Event Documentation (AE, SAE, AESI)[3]		X	X	X	X	X	X	X	X	X	X		
Medically Attended Adverse Event (MAAE) Documentation[4]	X	X	X	X	X	X	X	X	X	X	X	X	X
Diary Card		X	X	X	X	X	X	X	X				
HIV Risk Counseling	X	X	X	X	X	X			X	X	X		
Pregnancy Test[7] & Pre/Post Counseling	X	X		X		X			X	X	X		
CBC w/ diff	4	4	4	4	4	4			4		4		
Creatinine, ALT	4	4	4	4	4	4			4		4		
Hepatitis B and Hepatitis C Serology	7.5												
HIV Testing*	7.5		7.5		7.5				7.5	7.5	7.5		

Visit Number	0	1	2	3	4	5	6	7	8	9	10[2]/Exit 4	Phone Follow-up #1	Phone Follow-up #2
Visit Type	Screen	V1	Safety & Immun	V2	Safety & Immun	V3	Safety & Immun	Safety & Immun	Safety & Immun	Safety& Immun	Safety & Immun	MAAE follow-up	MAAE follow-up
Visit Day	-45 to -3	1	15	29	43	57	58-59	64	71	169	337	365	393
Window			± 3d	± 7d	± 3d	±7d		± 3d	± 3d	± 7d	± 7d	± 7d	± 7d
Visit Week		0	2	4	6	8	8	9	10	24	48	52	56
Research[5]													
HIV Binding Antibody		SP	SP	SP	SP	SP			SP	SP	SP		
HIV Neutralizing Antibody Assays		7.5	7.5	7.5	7.5	7.5			7.5	7.5	7.5		
Functional Antibody Assays		SP	SP	SP	SP	SP			SP	SP	SP		
B-Cell Analysis		17	17	17	17	17		17	17	17	17		
Multiparameter Flow Cytometry		17	17	17	17	17		17	17	17	17		
Innate Cell Analysis		17	17		17		17	17	17	17	17		
Transcriptomics		2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5				
Additional Immunogenicity Testing		42.5	8.5	8.5	8.5	8.5		17	25.5	34	34		

Visit Number	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10[2]/Exit 4**	**Phone Follow-up #1**	**Phone Follow-up #2**
Visit Type	**Screen**	**V1**	**Safety & Immun**	**V2**	**Safety & Immun**	**V3**	**Safety & Immun**	**Safety & Immun**	**Safety & Immun**	**Safety& Immun**	**Safety & Immun**	**MAAE follow-up**	**MAAE follow-up**
Visit Day	**-45 to** -3	**1**	15	**29**	**43**	**57**	**58-59**	**64**	**71**	**169**	**337**	**365**	**393**
Window			**± 3d**	**± 7d**	**± 3d**	**±7d**		**± 3d**	**± 3d**	**± 7d**	**± 7d**	**± 7d**	**± 7d**
Visit Week		**0**	**2**	**4**	**6**	**8**	**8**	**9**	**10**	**24**	**48**	**52**	**56**
Daily Volume (mL)[6]	23	111.5	85	60.5	85	60.5	19.5	70.5	102	100	108		
Cumulative Volume (mL)	23	134.5	219.5	280	365	425.5	445	515.5	617.5	717.5	825.5		
8-Week Cumulative Volume (mL)						402.5	422	381	483	100	108		

SP = Assay performed from stored plasma; no additional blood volume required

[1] Full physical examination at screening (visit S1) and targeted physical examination at all other visits; Vitals to be collected both pre- and at about 30 minutes post-vaccination at Visit Day 1, 29, and 57.

[2] Participants will be contacted for the unblinding visit after the clinical data is locked.

[3]This includes unsolicited AEs which will be collected until 28 days post each vaccination.

[4]Medically Attended AEs will be followed through the end of the study, a full 12 months following the last vaccination. After clinic visits end, follow-up for MAAEs will occur by monthly phone calls for two months.

For those participants who are unable to continue participation in the study, but who do not withdraw consent, an exit visit will be conducted.

5 Research labs may be performed at any visit where sufficient stored samples are available

6 Blood volumes for clinical lab assays may be adjusted as long as maximum daily blood volume is not exceeded.

7 Pregnancy tests can be conducted at any visit if clinically indicated.

8 Any clinically indicated safety labs can be obtained at investigator discretion

*10 mL EDTA verification blood collection for HIV incident case resolution for enrolled participants.

**Blood will be collected into vaccutainer tubes according to standard operational procedures. Substitution of tube types may be made as long as the substitution does not interfere with the study performance. A 10% variance in blood volume is acceptable, such as in the case of an error or a broken or clotted vial.

Table 2: Protocol-Required Safety Laboratory Assessments

Laboratory Assessment	**Parameters** [a]
Hematology	CBC with differential
Clinical Chemistry	Creatinine, ALT
Laboratory Assay	HIV

[a] Acronyms

2. INTRODUCTION

The investigational products to be used in this trial include A244, B.63521, and ALFQ.

A244, a Duke University product, manufactured by Vetter Development Services USA, Inc. for NIH/NIAID, consists of the gp120 envelope glycoprotein HIV-1 subtype CRF_01E A244 derived from the CM244 CRF_01E. The A244 gp120 envelope has an 11-amino N-terminal deletion, similar to the bivalent AIDSVAX® B/E protein used in RV144[1], which has been safely administered to over 9,000 participants.

B.63521Δ11mutc gp120 is a recombinant HIV-1 Env protein containing an 11 amino acid truncation at the N-terminus of gp120 that enhances antigenicity and immunogenicity. It is manufactured for NIH/NIAID by Vetter Skokie IL.

ALFQ, a US Army product, is a liposomal adjuvant containing a synthetic monophosphoryl lipid A (MPLA/3D-PHAD®) with the addition of QS-21. ALF55 is manufactured by Avanti thru DAIDS-ABL contract. QS-21 is procured from Desert King thru DAIDS-ABL contract. ALF55 and QS21 will be combined fill/finish at Walter Reed Pilot Bioproduction Facility to produce ALFQ.

This trial will be conducted in compliance with the protocol, Good Clinical Practices (GCP), and the applicable regulatory requirements(s).

2.1. Study Rationale

Given the global burden of the human immunodeficiency virus (HIV) and specific burden to the Department of Defense (DoD), as the cost of treatment for its HIV-infected service members ranges from $21 to $54 million per year, HIV vaccine development would offer an immeasurable benefit. Models suggest that even a partially effective vaccine would make a substantial contribution to HIV incidence reduction. A study conducted by Harmon et al suggested that a vaccine with 70% efficacy could reduce disease incidence by 44% in the first decade, and up to 78% in under 50 years.[2] A model presented by Stover et al posited that even a vaccine with 50% efficacy could reduce the annual transmission rate by 34% over 15 years.[3] Both studies concluded that a vaccine would be a cost- effective intervention. This prevention strategy would confer multiple advantages over other interventions in that it requires time-limited delivery, minimal maintenance, and offers long-term protection.

Efforts to produce an HIV vaccine have primarily focused on the HIV-1 gp 120 subunit vaccines which incorporate outer envelope viral proteins. Protein based vaccines are commonly paired with an immunostimulatory adjuvant to improve durability of the immune response. ALFQ is an adjuvant containing both MPLA and saponin QS21 which has elicited a potent immune response in preclinical studies. Determining the optimal dose of ALFQ adjuvant may reduce the amount of material and cost necessary for future HIV vaccine production.

Similarly, improving the rapidity of our response to new emerging infectious diseases will be facilitated by use of an adjuvant that can be broadly coupled to various candidate vaccines.

2.2. Background

Although global initiatives have made great strides in controlling the HIV pandemic, HIV and acquired immunodeficiency disease syndrome (AIDS) continues to impact the lives and livelihoods of a significant portion of the population. In 2019, 38 million individuals were living with HIV worldwide and 690,000 died of AIDS-related causes.[4] Despite extensive global efforts for disease control over the last 20 years, 1.7 million individuals contract HIV annually.[4] Given these successes and challenges, the development of a safe and efficacious preventive HIV vaccine as part of a comprehensive prevention program remains among the highest global health priorities and the best long-term tool for the control of the HIV-1.[5-7] To date, seven HIV vaccine efficacy trials have been conducted, including VAX003[8], VAX004[9] RV144[1] , STEP[10] , Phambili/HVTN503[11] , HVTN505[12] and HVTN702[13]. Of these, only the RV144 Phase 3 trial demonstrated efficacy. Healthy participants who received the 6-month vaccination regimen containing live recombinant ALVAC-HIV (vCP1521) and AIDSVAX B/E, adjuvanted with Rehydragel®, were 31% less likely to acquire HIV than participants who received placebo over a 3.5-year follow-up period.[1] In a post hoc analysis, efficacy was 60% at 12 months after initial vaccination, indicating that protective immunity may have waned rapidly[14] . A follow-on study of immunologic correlates associated with HIV acquisition found that the magnitude of plasma immunoglobulin (Ig) G antibodies to variable regions 1 and 2 (V1/V2) of HIV-1 Env was inversely correlated with probability of HIV acquisition, and plasma IgA antibody titers to HIV-1 gp120 Env were directly correlated with probability of HIV acquisition.[15] Given these findings, the B/E envelope proteins were selected for this study, based on their similarity to proteins utilized in the RV144 trial.

2.2.1. A244d11 gp120

The A244 protein is an immunogen that consists of the gp120 envelope glycoprotein HIV-1 subtype CRF_01AE. It has demonstrated its antigenicity and immunogenicity in human and non-human primate (NHP) studies, as described below.[16]

A244d11 gp 120 gD has been administered to thousands of clinical trial participants as a component of AIDSVAX® B/E. A phase 1/2 study performed in Thailand enrolled 133 participants to test the safety and immunogenicity of the combination of ALVAC-HIV and AIDSVAX B/E. No serious vaccine-related events were reported.[17] Only 4 AEs were determined to be vaccine related. These included myalgia, pruritis, and erythematous rash.[17] The RV144 trial, which enrolled 16,402 individuals, tested the efficacy of the combination of ALVAC and AIDSVAX B/E. Local reactogenicity was mild or moderate for the AIDSVAX component, and overall AEs were primarily mild or moderate in severity. As previously discussed, vaccine efficacy was 60% At 12 months, but decreased to 31% by 3.5 years. The RV306 trial assessed the immunologic effect of a late boost of either AIDSVAX B/E or the combination of AIDSVAX B/E and ALVAX-HIV at month 12, 15, or 18 after receiving the standard RV144 series at months 0, 1, 3, and 6. There were no vaccine-related serious AEs recorded. Groups which received a late boost had increased peak plasma IgG-binding antibody levels against gp70 V1V2, and those that received a late boost at 15 or 18 months, specifically, had improved gp 120 responses. Additionally, groups with late boosts had increased functionality and polyfunctionality scores compared to the RV144 regimen only group.[18]

In addition to combination regimens with ALVAC-HIV, AIDSVAX B/E has also been administered as a stand-alone investigational product. A randomized, double-blind, placebo-controlled trial assessing the efficacy of AIDSVAX B/E was performed in a population of more than 2500 injection drug users in Thailand. Although the product was well-tolerated, it did not prove efficacious. HIV acquisition rate was almost identical between the vaccine and placebo groups.[8] AIDVAX was also administered alone in the AVEG 036 study, which, similarly, did not demonstrate vaccine efficacy.

The A244-rpg 120 immunogen utilized in the AIDSVAX B/E product was created with an 11 amino acid deletion at the gp120 N terminus and a herpes simplex virus gD peptide tag.[19] The effects of these modifications were explored by comparing gp120 expression, antigenicity, and immunogenicity. The study demonstrated that the deletion of the N-terminal improves both envelope antigenicity and immunogenicity, and is sufficient to induce an enhanced immune response without the gD protein-derived tag.[16] As a result, the A244d11 gp120 investigational product was formulated without the gD tag. This investigational product has not yet been used in humans, but is planned for use as a late boost for RV306 participants in the RV546 trial.

2.2.2. B.63521

The B.63521 gp120 is an investigational recombinant HIV- 1 Clade B T/F envelope protein containing an 11 amino acid truncation at the N-terminus of gp120. It has been shown to elicit HIV binding antibodies, neutralizing antibodies with wide antigenic breadth, and antibody dependent cellular cytotoxicity in animal models.[20, 21] Although the B.63521 investigational product has not yet been used in humans, it is sufficiently similar to other HIV-1 gp120 proteins administered in multiple previous clinical trials, that it is thought to have a similar safety profile. There have been a large number of clinical trials with other HIV-1 Env gp120 proteins, and the most common adverse effects were local and resolving within 3 days.

2.2.2.1. Non-Human Primate Study

A non-human primate study was designed to augment the diversity of gp120 motifs in the immunogen utilized in the RV144 trial and to assess whether a broader antibody response could be elicited. Eighteen rhesus macaques received six doses of ALVAC–HIV delivered alone at doses 1 and 2 at weeks 0 and 4, respectively, and combined with intramuscular bivalent (B/E) or pentavalent (B/E/E/E/E) at doses 3-6 in weeks 13, 21, 47 and 88, respectively. A 100 mcg protein dose was administered for the first 3 protein immunizations at weeks 13, 21 and 47, and a 300 mcg protein dose was administered for the 4th protein immunization at week 88. All doses were combined with GLA-SE adjuvant. The B.63521 gp120 was combined with CRF01 AE.A244 fp120, and the pentavalent boost contained three additional subtype E gp120s (AE.AA058 / AE.AA104 / AE.AA107). Immunizations were well-tolerated and no AEs were observed.

Both vaccine regimens induced Env-reactive plasma antibodies, with greater breadth in the monkeys in the pentavalent cohort. Additionally, all animals had detectable mucosal IgG antibodies against at least one of twelve tested gp120 antigens, and all but one animal in the pentavalent group had mucosal antibodies specific to B.63521gp120 Env. Tier-1 neutralizing antibodies were induced in both groups, and both could mediate antibody-dependent cellular phagocytosis of vaccine Env-coated targets, with weak phagocytosis of Tier-2 SHIV Env by

3 animals in the pentavalent group. Protection from SHIV challenge was achieved in 55% of the pentavalent cohort.[22]

2.2.3. ALFQ

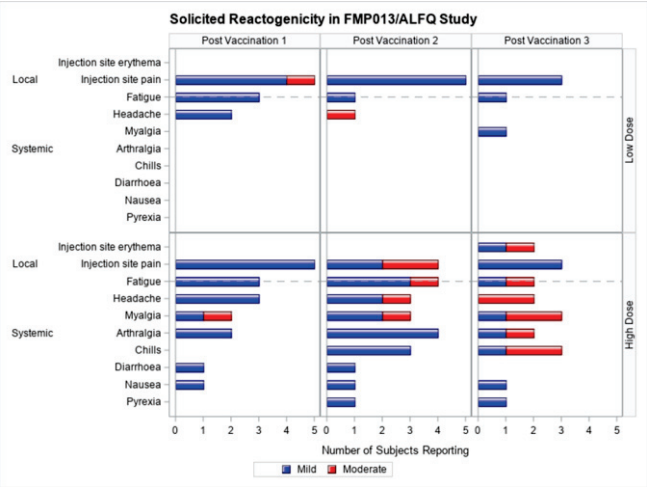
Modulation of the response to vaccines with immunostimulatory adjuvants is one very promising approach to improve durability. The Army Liposome Formulation (ALF) family of adjuvants are ALFA (ALF adsorbed to aluminum salt), ALFQ (ALF mixed with the saponin QS21; US patent pending) and ALFQA (ALFQ adsorbed to aluminum salt). Both ALFA and ALFQ are potent adjuvants, non-pyrogenic, and nontoxic in rabbit pyrogenicity and toxicology studies.[23] ALFQ is an alternative to the successful but proprietary adjuvant, AS01B (liposomes containing both MPLA and the saponin QS21). ALFQ is potent in preclinical studies and contains 4 times the amount of MPLA and twice the amount of QS21 when compared to AS01B. An ongoing NHP study comparing HIV gp120 A244 formulated with either ALFQ or with Aluminum hydroxide fluid gel (AHFG) has shown that animals that received ALFQ as the adjuvant compared to animals that received AHFG mounted significantly higher binding antibody responses to A244, gp70V1V2 A244, and V2 peptide (Haynes 2020, unpublished). The responses were significantly higher after each immunization, as well as in ADCC responses, quantified using gp120 coated target cells. Given that antibody functions such as ADCC may have contributed to protection by non-neutralizing antibodies in RV144 vaccine recipients, ALFQ may contribute to increasing protective vaccine efficacy.

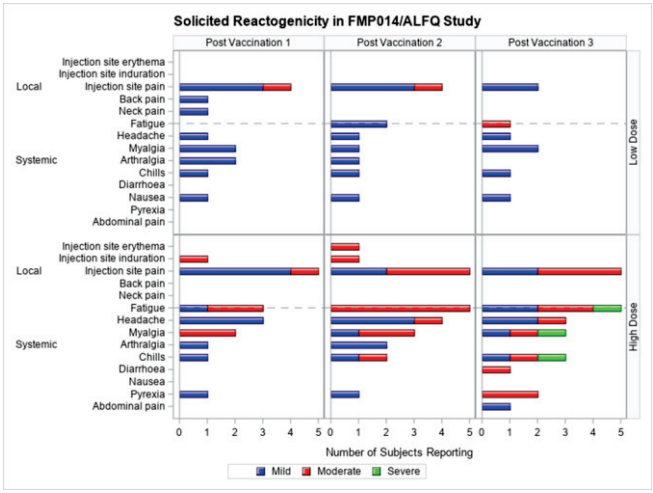
ALFQ is currently being investigated in three clinical trials. The FMP013 and FMP014 (NCT04268420 and NCT04296279) malaria investigational vaccines are adjuvanted with ALFQ at the 100mcg and 200mcg doses in the low dose and high dose arms, respectively. The FMP013 and FMP014 groups were designed to enroll 10 participants each, which underwent investigational vaccine administration at months 0, 1, and 2, with 5 participants assigned to each of the high and low dose groups.

In the FMP013 trial, 5 pariticipants received dose 1 and 2 in the low dose group and 4 individuals recived dose 3. The participant who did not receive the final dose left the study for reasons unrelated to the study design. In the high dose group, 5 participants received product adminstration 1, 4 pariticpants received product admin istration 2, and 3 participants received product adminstration 3. One participant dropped out for reasons unrelated to the trial, and another participant was removed due to a likely pre-exisiting medical condition. In the low dose group, the majority of participants reported mild local and systemic symptoms, with a minority of partiicpants reporting moderate local and systemic symptoms (Figure 2). In the high dose group, participants reported mild to moderate local and systemic symptoms. The study product was well tolerated with no SAEs (Hutter, 2020).

In the FMP014 trial, 10 out of 10 participants completed all 3 vaccinations. In the low dose group, the majority of pariticpants reported mild local and systemic symptoms, with a minority of participants reporting moderate local and systemic symptoms (Figure 2). In the high dose group, the majority of participants reported mild to moderate local and systemic symptoms (Figure 2). All severe systemic reactions were reported by a single individual who had 24 hours of flu like symptoms that resolved spontaneously. The study product was well-tolerated, with no SAEs (Hutter, 2021) (Hutter ASTMH 2020).

Figure 2: Reactogenicity Results for FMP013 and FMP014





ALFQ has also been administered in the EID030 (SpFN) (IND27301, NCT04784767) trial. The spike ferritin nanoparticle was administered with 200 mcg of ALFQ at months 0, 1, and 6. In Arm 1, 24 partiticpants received dose 1 and 2, and 9 participants received dose 3. In Arm 2, 5 participants received dose 1, 4 participants received dose 2, and 2 participants received dose 3. No SAEs were reported. A total of 8 unsolicited AEs occurring in 5 participants, including 5 determined to be at least possibly related to study vaccination were reported. All unsolicited AEs reported were of mild severity.

In Arm 1, a total of 14 participants (58.3%) reported at least one mild solicited event, and 7 (29.2%) reported at least one moderate solicited event. Five participants reported moderate systemic events and 3 reported moderate local events. Sixteen participants reported mild injection site pain and 2 reported moderate injection site pain. Eight individuals reported mild myalgia and 1 reported moderate myalgia. Five participants reported mild fatigue and 2 reported moderate fatigue. Clinical safety laboratory assessments demonstrated that 12 participants had mild decreases in hemoglobin 8 days after product administration. (Personal communication with study PI)

In Arm 2, 5 participants experienced Grade 1 solicited reactions, and one participant experienced a Grade 2 muscle pain at the injection site. Grade 1 change in hemoglobin was reported for two participants. One participant had a Grade 1 change in WBC and a Grade 3 absolute neutrophil count which was similar to baseline. (Personal communication with study PI)

ALFQ will also be administered in one additional clinical trial which may initatie prior to the start of RV575. In the upcoming RV546 trial (IND 27011, NCT04658667), participants in arms 3 and 4 will receive a single IM injection of 200 mcg of ALFQ. These ALFQ doses refer to the MPLA/3DPHAD component of ALFQ.

Manufacture of protein-based vaccine antigens can be technically complex and expensive, both of which can limit vaccine supply, particularly in times of high demand during an epidemic.

Optimization to reduce vaccine dosing can help to alleviate these issues. RV 575 will perform a dose de-escalation study of the ALFQ adjuvant to determine the optimal dose for safety and

immunogenicity. Even a similar, rather than superior, immunogenicity of decreased dosing would result in significant cost savings, thereby facilitating scale-up and delivery to populations at greatest need for a preventive HIV vaccine.

2.3. Military Relevance

Historically, infectious diseases have had a major impact on US Armed Forces. With an estimated 34 million infected individuals worldwide at the end of 2011, HIV poses a significant and persistent threat in terms of force readiness and protection and may act as a war-starter by affecting the stability and security of nation-states.

HIV's relevance the US Military has been recognized from the very beginning of the pandemic. In 1985, the US Military recognized the emerging HIV-1 epidemic as a new threat to US and allied forces worldwide. The United States Congress mandated the establishment of the US Military HIV Research Program to develop effective preventive measures to include prevention education, vaccine development and implementation of novel anti-viral therapies and clinical management tools for the US DoD and Allied Forces. In 2001, the Armed Forces Epidemiology Board identified HIV as a disease of military importance, and Army FOC 09-07, Global Casualty Prevention, requires detection, identification, and vaccination in order to protect US personnel against potential infectious disease (ID) threats. The 2001 DoD Report on Biological Warfare Defense Vaccine Research and Development identified HIV as the 4th greatest infectious disease threat to DoD forces. Department of Army Headquarters designated HIV vaccine development as an Army Technology Objective (ATO), a status reserves for the highest priority science and technology efforts. Furthermore, The National Security Strategy of the United States (2002, 2006, and 2010) clearly identifies the threat of HIV/AIDS as a destabilizing force that threatens US National Security. Finally, in January 2011, The Department of the Army approved the HIV Vaccines Capability Development Document for HIV Vaccine, which addresses the need to provide service members with vaccine protection against HIV and AIDS.

Medical care for HIV infection is extremely costly to the US Defense Health Program. The estimated lifetime cost of HIV infection is at least USD $400,000 and the estimated average yearly cost varies from $20,000 to $25,000, bringing the estimated lifetime healthcare cost of the 5,000 HIV infected servicemen/women to $8B to $10B dollars. Total annual cost of treatment for HIV-infected service members ranges from $21 to $54 million per year.

2.4. Benefit/Risk Assessment

The potential risks and benefits based on the study design and what is known about the product are summarized in this section. More detailed information about the known and expected benefits and risks and reasonably expected AEs can be found in the Investigator's Brochure (IB).

2.4.1. Local Reactions

Participants may exhibit post-vaccination reactions including local reactions at the injection site such as erythema, induration, pain/tenderness, swelling and limitation of arm movement. These reactions are generally of short duration and rarely require treatment. Should such reaction persist and require treatment, the participant will be referred to appropriate medical care services.

2.4.2. Systemic Reactions

Participants may exhibit general signs and symptoms associated with administration of a vaccine injection, including fever, tiredness, chills, rash, myalgia, arthralgia, nausea, and headache.

These side effects will be monitored but are generally of short duration and rarely require treatment. Should such reaction persist and require treatment, the participant will be referred to appropriate medical care services. As with all vaccines, an allergic reaction is possible and will be managed as per site standard operating procedure (SOP) for anaphylaxis. To mitigate this risk, participants will be observed in the clinic for at least 30 minutes post-injection.

2.4.3. Risks to Study Personnel and the Environment

The principal risk to personnel in the clinical setting is that associated with potential exposure to infectious agents (e.g., in the handling of needles that may be contaminated and the attendant risks, including exposure to hepatitis, human immunodeficiency virus, and other human pathogens). Adherence to SOPs for working with infectious agents and universal precautions will reduce the risk of exposure.

This protocol poses no known risks to the environment other than those associated with the generation of biohazardous waste attendant to study procedures. All biohazardous waste will be disposed of and specimens collected as stipulated by local, state, and Federal regulations and in accordance with study site SOPs.

2.4.4. Other Risks

Venipuncture: Blood sampling carries a minimal risk of minor discomfort, the possibility of minor bruising at the site of the needle puncture, and, rarely, the possibility of infection at the needle puncture site. To minimize risk, blood will be drawn by trained health care providers. Changes at the needle puncture site will be tracked with the diary card and will be examined at the follow up visit. Participants with evidence of severe bruising or infection will be referred for appropriate medical care.

Allergic Reaction: No matter what precautions are taken, the administration of any product always carries the risk of a serious, even life-threatening, allergic reaction. Medical emergency equipment is located at the study site. This is available to handle emergencies, such as anaphylaxis, angioedema, bronchospasm, and laryngospasm.

Vaccine Induced Seroreactivity: As with every candidate HIV vaccine trial, there is a risk that participants will mount an antibody response to the vaccine, which may cause them to test positive on a standard clinical diagnostic assay for HIV. If a participant has an issue due to a false-positive HIV test result from receiving the vaccination, the PI will provide an explanatory letter (Appendix I). The study will provide re-testing for participants should they receive a positive HIV test elsewhere and require proof of vaccine induced seroreactivity. Should a participant move out of the D.C. metro area, they will be provided with a contact number to call and ask to be connected to an MHRP study physician for further guidance on what to do if they need to receive HIV testing.

Social Harm and Discrimination: In the unlikely event of HIV infection during this study, the primary concern is related to ascertaining and providing HIV diagnostic information and, in

particular, involuntary disclosure of HIV status to others. These disclosures may result in depression and rarely suicide among individuals learning that they are infected with the HIV virus. Furthermore, involuntary disclosure to others may result in prejudice by the community, family, employers, and psychosocial factors including stigma and discrimination. This risk will be minimized as study counselors will be trained in pre- and post-test counseling for HIV and will inform participants of all activities in the study and attendant risks and benefits. The candidate vaccines may also induce false positivity to standard HIV antibody tests and may result in problems when applying for life or health insurance, international travel, employment, or hospitalization. If a participant has VISP and becomes pregnant, the baby may also have VISP. If there are any problems related to the above situations, the investigators will provide HIV testing to confirm HIV status. They will provide this information to the persons involved. Currently, people who have received experimental HIV vaccines are deferred from blood donation even though they do not have HIV infection. The participant will not be able to donate blood if they have VISP.

In addition to the risk of social harms resulting from HIV diagnosis, becoming aware of a positive test results for Hepatitis B and C or the discovery of incidental findings may also result in the emotional effects one may experience after becoming aware of a positive HIV test. The study staff will take appropriate action to assist participants with any discrimination they may experience by participating in this study. Such measures will be accompanied with appropriate counseling.

Guillain–Barré Syndrome: Guillain-Barré syndrome (GBS) is a disorder in which the body's immune system attacks part of the peripheral nervous system. It afflicts only about one person in 100,000, and in rare instances vaccinations may increase the risk of GBS. The first symptoms of this disorder include varying degrees of weakness or tingling sensations in the legs. These symptoms can increase in intensity until certain muscles cannot be used at all. Most individuals recover from even the most severe cases of GBS, although some continue to have a certain degree of weakness.[1]

2.4.5. Unknown Risks

As with all research, there is the remote possibility of risks that are unknown or that cannot be foreseen based on current information. This would include late-onset effects.

Risks to unborn babies are unknown at this time; the effect of the candidate HIV vaccines on pregnancy and fetus are unknown as they have not been specifically studied in pregnant people. Participants who are pregnant or plan to become pregnant during the first 3 months post-vaccination will be excluded from enrollment. Female participants must use an adequate form of birth control including condoms (male or female) with spermicide, diaphragm, or cervical cap with spermicide, intrauterine device, contraceptive pills, patch, injection, intravaginal ring or other FDA-approved contraceptive method, male partner has previously undergone a vasectomy, or abstinence for 30 days prior to study injection until at least 3 months after receipt of the final study injection. A pregnancy test will be performed at screening, prior to the study injection (same day), and prior to any of the invasive procedures. Should a participant become pregnant within the 3 months post-study injection, she will be followed for safety to term. Partners of male participants who become pregnant will not be followed.

All participants are encouraged to engage in safe sex practices to prevent HIV acquisition.

2.4.6. Alternative to this Product or Study

The alternative is not to participate in this protocol.

2.4.7. Benefits

Study participants will receive no direct benefit from study participation. HIV and other sexually transmitted infection (STI) testing may reveal HIV or STI transmission. In this case, the participant would be referred for counseling and care. Findings of medical concern will be referred for appropriate care and treatment. Others may benefit from knowledge gained in this study that may contribute to the development of an HIV vaccine.

3. OBJECTIVES AND ENDPOINTS

The objectives and associated endpoints are provided in Table 3.

Table 3: Objectives and Endpoints

Objective	Endpoint
Primary:	
To evaluate the safety and tolerability (including reactogenicity) of candidate vaccine A244/B.63521 with ALFQ adjuvant at the 200 µg, 100 µg, and 50 µg doses	Evaluate the occurrence and severity of solicited local and systemic AEs following candidate vaccine administration. Evaluate the occurrence, severity, and relationship to vaccination of unsolicited AEs after candidate vaccine administration. Evaluate the occurrence of SAEs, and new-onset medical conditions. Evaluate the occurrence of AEs of special interest (AESIs) following candidate vaccine administration.
Secondary:	
To evaluate the effect of candidate vaccine A244/B.63521 with ALFQ adjuvant at the 200 µg, 100 µg, and 50 µg doses on cellular, and humoral immune responses	Compare plasma IgG binding antibodies to gp120 in terms of magnitude, durability, and area under the curve among groups with differing ALFQ doses. Characterize and assess the magnitude of cell-mediated immune responses elicited across vaccination regimens including antigen-specific CD4 and CD8 T cell responses and polyfunctionality.
Exploratory:	
To characterize B-cell functional specificities for each vaccination regimen	Characterize plasma IgG and IgA binding antibodies to HIV gp120, neutralizing antibodies,

Objective	Endpoint
Primary:	
To characterize innate immunity To assess the innate/gene expression induced across vaccination regimens To perform systems serology analyses To characterize the impact of vaccination with the ALFQ adjuvant on the permissiveness and expression to in vitro HIV infection of CD4+ T cells and differentiated macrophages	and non- neutralizing effector functions such as ADCC and ADCP with emphasis on RV144 immune correlates of risk of HIV acquisition. Characterize B-cell functional specificities for each vaccination regimen by quantifying antigen-specific responses through B-cell ELISPOT, phenotyping the magnitude and activation status of B cell subsets via flow cytometry and isolation of monoclonal antibodies from selected vaccine recipients. Characterize innate immunity through quantification of soluble chemokines and cytokines and assessing the phenotype and function of cellular innate immune subsets such as NK, NKT, and dendritic cells. Characterize effects of host genetic polymorphisms on immune responses and characterize differentially expressed genes. Characterize the impact of vaccination with the ALFQ adjuvant on the permissiveness and expression to in vitro HIV infection of CD4+ T cells and differentiated macrophages through integrated HIV-DNA PCR, cell associated-DNA and RNA, flow cytometry of various activation markers and HIV Gag protein, ELISA of HIV p24 in the cell culture supernatant, and production of cytokines/chemokines.

4. STUDY DESIGN

4.1. Overall Study Design

The purpose of this Phase I randomized, double-blind clinical trial is to optimize ALFQ dosing. Safety will be assessed through the frequency of the overall and specific post-vaccination reactions. Blood will be collected to assess humoral, cell-mediated, and innate immune responses.

Healthy adults not living with HIV who are available for 14 months will be enrolled. A total of 60 participants will be enrolled to one of three arms, each comprised of 20 candidate vaccine recipients. Each arm will receive identical doses of A244 and B.63521 (300 micrograms of each). In addition, Arm 1 will receive 200 micrograms of ALFQ. Arm 2 will receive 100 micrograms of ALFQ. Arm 3 will receive 50 micrograms of ALFQ. The safety, reactogenicity, and immunogenicity will then be compared among the three arms to determine the optimal dose of ALFQ.

All vaccinations will be split into 2 half doses which will both be administered intramuscularly (IM) into the same deltoid muscle. Vaccinations will occur at months 0, 1, and 2. The second vaccination will be administered into the contralateral deltoid at study month 1 compared to the first vaccination at study month 0. The third vaccination at study month 2 will be administered into the same deltoid as the first vaccination at study month 0. Participants will be followed for 12 months following the last study vaccination. Safety and tolerability will be assessed with both clinical and laboratory monitoring. Vaccine- related reactions will be observed and solicited for 30 minutes post-vaccination and with the aid of a diary card and interview of participants during the 14 days post vaccination.

The information gained from the review of the diary card and the interview with the participants will be documented in the clinical study chart. In addition, AEs will be documented at each clinical encounter. AEs will be graded for seriousness, severity, and relationship to the investigational product.

4.2. Number of Participants

The site will screen approximately 150 participants to achieve a total of 60 enrolled.

4.3. Sentinel Group Allocation

A sentinel group including 6 participants will be enrolled. Four participants in the sentinel group will be randomized to the 200 mcg ALFQ group (Arm 1), one participant will be randomized to the 100 mcg ALFQ group (Arm 2), and one participant will be randomized to the 50 mcg ALFQ group (Arm 3). Safety data for seven days following vaccination one will be reviewed by a PSRT. If it is determined to be necessary by the PSRT, the MHRP SMC will be consulted to review unblinded data to provide a recommendation on continuing enrollment. Additionally, the PSRT will consult the SMC if either of the following conditions are met:

1) One (or more) participants experience an SAE that is assessed as related to a study agent, or

2) Two (or more) participants experience grade 3 or 4 AEs of the same type (e.g., elevated ALT) related to a study agent. This would exclude monitoring lab values if a clear alternate etiology is identified. Additionally, CBC differential counts would not contribute.

After safety has been confirmed by PSRT review, the remaining 54 participants will be enrolled.

4.4. Scientific Rational for Study Design

To date there have been no comparative studies regarding optimal dosing of adjuvant ALFQ. The ALFQ doses in Arm 1, 2, and 3 are based on the MPLA/(3D-PHAD®) component of ALFQ. ALFQ has been demonstrated to elicit potent antibody binding responses in animal models.

ALFQ adjuvant dose optimization has the potential to limit AEs and improve efficiency and cost effectiveness of vaccine administration while maximizing immunogenicity. As a result, RV575 will perform a dose de-escalation study.

4.5. Justification for Dose

The A244 protein was included in the AIDSVAX vaccine at the 300 microgram dose. This dose was found to be safe and well tolerated.[24]

B.63521has been tested in animal trails at doses up to 300 micrograms. It has not yet been utilized in human trials. B.63521 is very similar to the MN protein utilized in AIDSVAX which was administered at the 300 microgram dose in RV 144.[10]

ALFQ had already demonstrated safety and immunogenicity at the 200 microgram and 100 microgram dose.(Hutter, ASTMH, 2020)

This study will contribute information about safety and immunogenicity of ALFQ at the 50 microgram dose and perform a direct dose comparison for both safety and immunogenicity. This data is essential to optimizing efficiency of adjuvant inclusion in future vaccine trials.

4.6. Method of Treatment Assignment

Randomization will be conducted using a randomization block scheme described in a separate randomization plan held by the protocol statistician. The randomization list will be generated by the protocol statistician and provided and trained on with the site delegated un-blinded study staff/nurse. Once a randomization number has been assigned, it will not be re-assigned.

4.7. End of Study Definition

The end of the study primary completion date is defined as the last subject last visit (LSLV). Each participant will be followed for 12 months after the final vaccination. Follow up includes 10 months of clinic visits and two additional telephone follow up visits at 1-month intervals for a total of 12 months. The completion of laboratory assays and data analysis is anticipated to take up to 5 years from the primary completion date.

5. STUDY POPULATION

5.1. Participant Recruitment

The study population will consist of 60 healthy adults not living with HIV between the ages of 18 and 55 years at the time of study enrollment at the WRAIR CTC.

Healthy adult volunteers, male and female, will be recruited by noncoercive means through the WRAIR CTC according to applicable US Army regulations. Participants will be recruited from the Baltimore-Washington, D.C. metro area through the WRAIR CTC according to applicable U.S. Army regulations (i.e., 32 CFR 219 and 21 CFR 50, 54, 56, and 312). Adult men and women will be recruited by the use of WRAIR IRB approved advertisements using multiple media formats, to include, but not limited to: newspapers, flyers, emails, the WRAIR CTC website, postings on public listservs, social media (such as Facebook or other apps/websites), posters, bus ads, generic radio and television ads, word of mouth and referrals from volunteers currently in studies. Email announcements will include information on ad posters excluding any photos unless attached as complete flyer. See Appendix F below for the recruitment script. Recruitment may also include oral presentations (using standardized audio script) at events and

meetings. All advertisements, both general and specific to this study, will have been reviewed and approved by the WRAIR IRB prior to their use. All recruiting methods will direct the volunteer to contact the CTC via e-mail or phone, or to the CTC web site where the same contact information will be given.

In the case of oral presentations and other meeting-related recruitment, potential subjects may be given the opportunity to sign up directly for a briefing and screening appointment. Any subject who so desires will be asked to provide basic contact information and an appointment made.

Upon calling the recruitment phone number, a recruitment script will be used by study personnel to inform potential subjects of the details of the study. If the potential subject desires, some basic contact information will be obtained and an appointment for a formal briefing and screening will be made at that time.

Upon contacting the CTC via e-mail, potential participants will be provided an electronic version of the recruitment script to inform them of the details of the study. If the potential subject desires, some basic contact information will be obtained and an appointment for a formal briefing and screening will then be made (via further e-mail or phone conversations).

Given the value of the information in this recruitment script, a version of the script without compensation figures will be submitted as a separate "information flier" to be used on the WRAIR CTC web site, email correspondence (as noted above), and other electronic or social networking platforms.

Participants who have participated in studies at the WRAIR CTC will be given the opportunity to receive compensation for referring additional subjects. Subjects will be asked to direct interested persons to contact the CTC. Subjects will receive $50 for each referred person who then attends a screening session and meets all inclusion and none of the exclusion criteria.

Compensation will be independent of the referred person's decision to enroll. Final authority over dispensation of referral compensation will lie with the CTC Director.

Volunteers can also be recruited via WRAIR 2038, which is a generic screening protocol managed by the WRAIR CTC. Under this screening protocol, volunteers will undergo a generic screening visit with medical history, exam, vitals, lab tests, along with other potential screening tests. If the volunteer is interested in learning about this clinical trial, they will need to attend a briefing and sign this study's informed consent form. If the volunteer consents, the screening documentation from WRAIR 2038 can be shared with this study to fulfill screening requirements, and a copy of said documents can be added to the volunteer's study file for this clinical trial.

5.2. Participant Identification Numbers

All participants consented will be assigned a Participant Identification Number (PIN), which is a 7-digit number. The first three digits are the protocol number (575), followed by a 4-digit number which, will be assigned sequentially**.**

The PIN will be used to label all source documents, Case Report Forms (CRFs) and specimens. Source documents, CRFs and specimens are never labeled with participant names or national identification numbers.

5.3. Participant Eligibility

Screening for eligible participants will be performed within 45 days prior to administration of the investigational vaccine. Participants who have passed the TOU and have given written informed consent will undergo a complete medical history, physical examination, and screening laboratory assessments to determine eligibility for study participation. Screening assessments (found in the SOE) will be completed after the informed consent process has been completed.

Screening evaluations for specific eligibility criteria (see Section 5.3.1 and Section 5.3.2) must be completed within the screening visit window specified (Table 1) prior to enrollment.

Counseling for HIV risk reduction and the potential risks of becoming pregnant during participation will be provided. The PI or designee will make the final decision regarding eligibility. Clinical data and specimens collected during screening from participants subsequently found to be ineligible for participation in the study will become part of the study records and specimens will be evaluated in accordance with protocol procedures. One re-screening attempt will be allowed if the there was an elevation and laboratory values on the first screening attempt which is suspected to be temporary, and or insignificant by the study personnel. Only eligible participants will be given the investigational product at the enrollment visit. A participant is considered enrolled in the study upon receipt of the first study vaccination.

5.3.1. Inclusion Criteria

Potential participants are required to meet all of the following criteria for enrollment into the study.

1. Healthy adults between the ages 18-55 years (inclusive)
2. Must be at low risk for HIV infection per investigator assessment and using the study risk assessment tool.
3. Able and willing to provide written, informed consent
4. Able and willing to comply with all research requirements, in the opinion of the Investigator
5. Agreement to refrain from blood donation during the course of the study and within the 2 months prior to study entry
6. Minimum body weight of 110 pounds (lbs) (50kg)
7. Laboratory Criteria within 45 days before enrollment:
   a. Hemoglobin ≥ 11.7g/dL for women; ≥ 12.5 g/dL for men
   b. White Blood Cell count = 3,500-10,800 cells/mm^3
   c. Platelets ≥140,000/mm^3 and ≤ 450,000/mm^3
   d. Alanine aminotransferase (ALT; SGPT) <1.25 x ULN
   e. Serum creatinine ≤ 1.25 x institutional upper limit of the reference range

f. Negative HIV testing (HIV Ab / antigen 4th generation screen with reflex confirmatory RNA testing)

g. Negative HBsAg and hepatitis C antibody testing

Note: As above, Grade 1 lab abnormalities detected on screening may be repeated at PI discretion. Persistent Grade 1 abnormalities that are felt to represent the non-pathologic baseline for the subject will be documented before a subject is enrolled in the trial and are allowable per discretion of the PI.

8. Birth control requirements:

All participants assigned female at birth must meet one of the following 2 criteria:

a. No reproductive potential due to post-menopausal status (12 months of natural [spontaneous] amenorrhea) or hysterectomy, bilateral oophorectomy, or tubal ligation

b. People of childbearing potential should agree to practice highly effective contraception at least 30 days before enrollment and through 3 months post-last vaccination, using one of the following methods: condoms (male or female) with spermicide; diaphragm, or cervical cap with spermicide; intrauterine device; contraceptive pills, patch, injection, intravaginal ring or other FDA-approved contraceptive method; male partner has previously undergone a vasectomy; abstinence

c. All participants are encouraged to engage in safe sex practices to prevent HIV acquisition

9. For all participants assigned female at birth, except those with a history of hysterectomy or bilateral oophorectomy, a negative β-HCG pregnancy test (urine) on the day of enrollment and each vaccination day is required. Because tubal ligations have a failure rate that is not insignificant, and 12 months of spontaneous amenorrhea can be a result of polycystic ovarian syndrome which does not completely preclude pregnancy, a negative β-HCG pregnancy test at enrollment and on each vaccination day is also required for all participants assigned female at birth with a history of either of these.

10. No plans to travel outside the Washington DC metro area (DC, Maryland, and Virginia) that would prevent compliance with planned study visits

11. Test of Understanding (TOU) (minimum passing score of 80% with 2 attempts permitted)

5.3.2. Exclusion Criteria

Potential participants meeting any of the following criteria will be excluded from enrollment.

1. Receipt of any investigational HIV vaccine or investigational adjuvant
2. Received an investigational product or vaccine in the 30 days before enrollment. This does not include products with emergency use authorization.
3. Concurrent participation in another clinical research study
4. Any serious medical illness or condition

5. Receipt of immunoglobulins or blood products within 3 months before enrollment
6. Any history of anaphylaxis or allergy to study product
7. History of sickle cell trait or disease
8. Pregnancy, lactation, or intention to become pregnant during the study
9. History of active/recent cancer still within treatment or active surveillance follow-up (except basal cell carcinoma of the skin and cervical carcinoma in situ). Treated/resolved cancers with no likelihood of recurrence may be deemed acceptable at PI discretion
10. History of autoimmune disease
11. History of PIMMCs
12. Suspected or known current alcohol or drug abuse as defined by an alcohol intake of greater than 3 drinks a day on average for a man, and greater than 2 drinks a day on average for a woman
13. Any other significant disease, disorder or finding which may significantly increase the risk to the participant because of participation in the study, affect the ability of the participant to give informed consent, participate in the study, or impair interpretation of the study data, in the opinion of the Investigator
14. History of splenectomy
15. History of confirmed or suspected immunodeficiency
16. History of Hereditary angioedema (HAE) acquired angioedema (AAE), or idiopathic forms of angioedema
17. History of asthma that is unstable or required emergent care, urgent care, hospitalization, or intubation during the past 2 years
18. History of diabetes mellitus (type I or II), with the exception of gestational diabetes
19. History of thyroid disease (except for well controlled hypothyroidism)
20. History of idiopathic urticaria within the past year
21. History of hypertension that is not well controlled by medication or that is persistently greater than 140/95 at screening
22. History of bleeding disorder diagnosed by a doctor (e.g., factor deficiency, coagulopathy, or platelet disorder requiring special precautions) or significant bruising or bleeding difficulties with IM injections or blood draws
23. History of chronic or active neurologic disease to include seizure disorder and chronic migraine headaches. Exceptions are: i) childhood febrile seizures, or ii) seizures secondary to alcohol withdrawal more than 3 years ago
24. Participants receiving any of the following substances

a. Systemic immunosuppressive medications or cytotoxic medications within 12 weeks before enrollment [with the exception of a short course of corticosteroids (≤ 14 days duration or a single injection) for a self-limited condition at least 2 weeks before enrollment; inhaled, intranasal or topical steroids are not considered exclusionary
b. Treatment with known immunomodulators including allergy immunotherapy (other than NSAIDs) for any reason
c. Live attenuated vaccines within 30 days before initial study vaccine administration
d. Medically indicated subunit, mRNA, or killed vaccines, e.g., influenza, pneumococcal, vaccines with QS-21 as an adjuvant, or allergy treatment with antigen injections, planned for administration 14 days before or after study vaccine administration

25. History of arthritis diagnosis other than osteoarthritis
26. History of other diagnosed rheumatoid disorders
27. Has an acute illness or temperature ≥38.0 ºC/100.4 ºF on any study injection day or within 48 hours of planned study injection.
28. Note: Participants will not be excluded from further consideration for enrollment and study injections. Volunteers with fever or an acute illness on the day of study injection or in the 2 days before the study injection may be re-assessed by a study physician for resolution of the condition and enrolled and receive the study injection so long as the injection is within allowable windows. Military personnel will be excluded from participation in this study, regardless of leave status due to the potential for a false-positive HIV test result on mandatory HIV testing. This could have adverse effects on deployment status.

Final evaluation of eligibility will be based on the medical judgment of the investigator based on his/her medical and research experience.

5.4. Lifestyle and Reproduction Restrictions

Only restrictions to contraception and pregnancy are required.

5.4.1. Contraception and Pregnancy

Risks to unborn babies and nursing infants are unknown at this time. Therefore, participants childbearing potential must agree to follow contraceptive guidance and must agree not to breastfeed while in the study and for 2 months after the study.

5.4.1.1. Individuals of Childbearing Potential

Participants are not considered of childbearing potential if they meet any of the following criteria:

- Premenopausal with 1 of the following:
  - Documented hysterectomy

  - Documented bilateral salpingectomy
- Documented bilateral oophorectomy
- Premenarchal
- Postmenopausal
  - Defined as no menses for 12 months without an alternative medical cause (A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.)
  - People assigned female sex at birth on HRT and whose menopausal status is in doubt will be required to use one of the nonhormonal highly effective contraception methods if they wish to continue their HRT during the study, or they must discontinue HRT to allow confirmation of postmenopausal status before protocol enrollment

5.4.1.2. Participants of Childbearing Potential

Participants of reproductive potential are eligible to participate if they agree to use a highly effective method of contraception as described the section below.

5.4.1.3. Effective Methods of Contraception

Highly effective contraceptive methods that have a failure rate of <1% per year when used consistently and correctly:

- User Dependent Methods:
  - Oral, intravaginal, or transdermal combined (estrogen- and progestogen- containing) hormonal contraception associated with inhibition of ovulation
  - Oral or injectable progestogen-only hormonal contraception associated with inhibition of ovulation
- User Independent Methods:
  - Implantable progestogen-only hormonal contraception associated with inhibition of ovulation
  - Intrauterine device (IUD)
  - Intrauterine hormone-releasing system (IUS)
  - Bilateral tubal occlusion

- Vasectomized partner
- Sexual abstinence

5.4.1.4. Pregnancy Testing

Participants of childbearing potential will be included in the study only after a negative pregnancy test. Additional pregnancy testing will be performed according to Table 1 Schedule of Evaluations and whenever a menstrual cycle is missed or when pregnancy is otherwise suspected.

6. STUDY INTERVENTION

6.1. Treatments Administered

All products to be administered in this study are described in Table 4. Investigational product is defined as any investigational treatment or a product/device being used for an indication that has not been approved/cleared by the U.S. Food and Drug Administration (FDA).

Table 4: Products Administered

	Investigational Product	**Investigational Product**	**Investigational Product**
Compound	A244	B.63521	ALFQ
Dosage formulation:	0.5 mg/mL of the A244 gp120 protein, 20 mM Tris, 123 mM NaCl pH 7.4	0.60 mg/mL of the B.63521gp120 protein, 20 mM sodium phosphate, 150 mM NaCl, 0.02% polysorbate 80(PS80), pH 6.5.	The concentration of DMPC is 25.8+/-3.9 mM. The concentration of DMPG 2.9 +/- 0.4 mM. The synthetic cholesterol concentration is 34.9+/1 5.2mM
Unit dose strength(s)/dos age level(s):	300 ug	300 ug	Arm 1 will contain 200ug MPLA/3D-PHAD®, Arm 2 will contain 100 ug MPLA/3D-PHAD®, and Arm 3 will contain 50ug MPLA/3D-PHAD®. ALFQ also contains 100ug of QS-21.
Route of administration:	IM	IM	IM
Manufacturer:	Vetter Development Services USA Inc. For: NIH/NIAID	Vetter Skokie, IL for NIH/HIAID	Walter Reed Pilot Bioproduction Facility

6.2. Preparation, Handling, Storage, and Accountability

6.2.1. Acquisition Accountability

The sponsor's representative is responsible for distributing the investigational product to the study site and has ultimate responsibility for product accountability.

Responsibility for investigational product (IP) accountability at the trial site rests with the investigator/institution.

The sponsor's representative may release the product to other clinical study sites.

After the investigational product is distributed, the site pharmacy personnel are responsible for maintaining logs of investigational product receipt, storage, reconstitution, accountability by subject, and remaining before final disposition within the site. The investigational product should be stored as specified by the sponsor. The PI may delegate, in writing, this responsibility to another individual, but the PI is ultimately responsible for the investigational product and its proper storage upon receipt at the study site until it is transferred back to the sponsor's representative, or designee, or is destroyed, as directed by the sponsor's representative.

The empty vials and the unused portion of the vial should be discarded in a biohazard containment bag and incinerated after verification from the monitor. Any unopened vials (past the re-test date or otherwise) that remain will be destroyed at the discretion of the sponsor in accordance with local site SOP as directed by the Sponsor's representative and as stipulated by local, state, and Federal regulations. Partially used vials or expired prepared doses will not be administered to other participants or used for *in vitro* experimental studies. They will be disposed of in accordance with institutional pharmacy policy. Retest dates shall be updated as per analytical analyses of the IP. No IP shall be administered to a participant beyond the current retest date. The decision for final disposition of the investigational product rests with the sponsor.

Any temperature excursions during product storage must be reported to the sponsor and the affected IP must be placed in quarantine status. The sponsor will determine the disposition on the affected product.

Further guidance and information for the final disposition of unused study treatment are provided in the pharmacy section of the Manual of Operations (MOP).

6.2.2. Product Storage and Stability

All study products will be transported in accordance with the labeled storage conditions.

Once at the study site, all study products will be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the investigator and authorized site staff. Only participants enrolled in the protocol may receive study injections, and only authorized site staff may supply or administer study injections.

The investigator or designee will confirm that appropriate temperature conditions have been maintained during transit for all study products received and that any discrepancies are reported and resolved before use of the study products.

6.2.3. Product Preparation

All investigational product preparation will be performed by the unblinded study nurse on the day of vaccine administration, after confirmation by the clinical staff that the participant is present, is eligible for enrollment into the study, and has received their randomization assignment. The unblinded study nurse will perform all manipulations of investigational products in accordance with GCP guidelines. The unblinded study nurse will wear a lab coat and gloves for all manipulations. The center seals of the vials will be removed and the vial stoppers wiped with an alcohol wipe prior to insertion of a sterile needle attached to a sterile syringe. The Pharmacy section of the MOP outlines specific product preparations.

6.3. Study Intervention Compliance

All IP is administered by the study team and documented on the Study Vaccine Administration CRF. A study vaccination outside of a participant's vaccination window may occur at the discretion of the PSRT.

6.4. Measures to Minimize Bias: Randomization and Blinding

The PI, study staff, and participants will be blinded as to group allocation as it pertains to the dose of ALFQ. Since the vaccines are not identical in appearance, to preserve blinding the material inside the syringe will be masked. In addition, the delegated un-blinded study staff/nurse preparing the vaccine syringes will not be involved in the clinical assessment of participants and will be instructed not to comment on the appearance of experimental agent to study staff. For all participants, the volume of injection will be consistent.

Delegated un-blinded study staff/nurse staff will be trained in GCP and instructed not to discuss the vaccine randomization lists, codes, or participant assignments with study personnel. Unblinded study staff will be the only person(s) on site who will know the randomization assignment. The randomization assignment will not appear on any label or source document. Samples will be labeled on site using labels containing participant study numbers, protocol number and visit number. The samples will be accompanied by a specimen tracking form that records the study number, date and time of sample collection.

Any request for unblinding, with its rationale, must be forwarded through the PI. The PI will evaluate the request and will notify the Protocol Chair/Sponsor's Medical Expert for the Trial and HIV Vaccine Product Manager. They will evaluate the request and will advise the United States Army Medical Research and Development Command (USAMRDC) Office of Regulated Activities (ORA) Product Safety Surveillance Office (PSSO) regarding a course of action. A PVG physician from the USAMRDC ORA PSSO will decide whether to approve the request for unblinding.

In the case of a decision to unblind, the Sponsor will authorize the independent statistician to provide this information to the PI, who must notify the WRAIR IRB (see Table 7) and provide the study assignment to the site physician. It should be noted that there are very few circumstances in which unblinding will be essential to the medical management of a vaccine recipient. The site investigator will report episodes of accidental unblinding, with an explanation, to Protocol Chair/Sponsor's Medical Expert for the Trial, the Institution Review Boards (IRBs)

and the USAMRDC ORA PSSO. Follow-up of unblinded participants will continue through the duration of the trial.

After completion of the study and database lock, the Sponsor will authorize the independent statistician to prepare a list containing each participant's unblinded treatment assignment.

Participants will be contacted to return to clinic or will be notified via phone or letter (Appendix A) of their unblinded treatment assignment.

6.5. Concomitant Therapy

Concomitant medications will be recorded from 45 days prior to first vaccination until 10 months after the final vaccination. An initial review of past medical history will include a review of medications. Information pertaining to receipt of non-study vaccines, research agents, immunoglobulin preparations, immunosuppressive medication, antiretroviral drugs, and any blood products will be elicited at study visits and recorded in source documents.

To ensure appropriate medical follow-up for study participants, information regarding concomitant medications used in association with an AE will be collected and recorded in the source documents.

Study participants can receive medications such as acetaminophen, non-steroidal anti-inflammatory drugs (NSAIDs), or antihistamines as required, although they must be documented.

6.6. Dose Modification

Excluding the aforementioned study arm dose-escalation of ALFQ, no modification of dosage for any of the vaccine products will be allowed in this study.

6.7. Treatment After the End of the Study

There is no plan to continue with study injections after the end of the study.

7. DISCONTINUATION CRITERIA

Each participant has the right to withdraw from the study at any time for any reason without penalty. In those cases where a "withdrawal of consent" is requested by the participant, documentation of the withdrawal of consent and the reason(s) for the request will be captured in the participant's clinical research records and/or on the status change CRF. Although the participant is not obligated to give reason(s) for withdrawing, the investigator should make a reasonable effort to ascertain the reason(s) while fully respecting the participant's rights. The participant will also be asked if she/he will agree to complete the clinical assessments listed for the study termination/exit visit.

7.1. Screening Failures

Screen failures are defined as subjects who consent to participate in the clinical study but either are deemed not eligible or are not subsequently randomized. The investigator will ensure that the

following information is collected for each screen failure—demographic information, and the reason for the screen failure.

7.2. Discontinuation of Study Intervention

Under certain circumstances, a participant will be terminated from participating in further vaccinations. These specific experiences include:

- Investigator's clinical judgment is that it is in the best interest of the subject
- Confirmed diagnosis of HIV
- Pregnancy
- Clinically significant Type 1 hypersensitivity associated with vaccination
- Serious concomitant illness that is not expected to resolve prior to the next scheduled vaccination or will require contact isolation during the next scheduled vaccination.
- Treatment with chronic systemic glucocorticoids (e.g., prednisone) or other immunomodulators other than NSAIDs for greater than 10 days. Inhaled and topical steroid use is not exclusionary
- Need for concomitant vaccine that requires discontinuation
- After initial vaccination, missing any subsequent vaccination by more than 15 days

Participant's request Immediate Early Discontinuation will occur:

1. Upon learning of pregnancy;
2. Due to an SAE that is believed to be related to study vaccination, including:
   a. Any Grade 3 systemic adverse reaction that is assessed as related to the study agent,
   b. Any Grade 4 AE assessed as related to the study agent, or
3. If a participant develops infection with HIV-1.

Each case will be reviewed by the Protocol Safety Review Team (PSRT) to assess impact on study conduct overall and continuation of individual participants with referral to the U.S. FDA and WRAIR IRB, in consultation with the study sponsor.

7.2.1. PSRT

The PSRT will include the following:

- Sponsor's Medical Expert or designee
- HIV Vaccine Product Manager or designee
- ORA Pharmacovigilance (PVG) Physician
- PI or designee
- Independent Safety Monitor (ISM)

Additional PSRT participants may include, as needed, the following:

- Associate Investigators
- Clinical research nursing staff
- Laboratory directors

- Data management and regulatory staff
- DAIDS Medical Officer

PSRT conference calls or meetings will require, at minimum, the participation of the Sponsor's Medical Expert or designee, ISM, and the PI or designee. The WRAIR IRB will be notified of the decisions taken by the PSRT if they involve suspensions, clinical holds (voluntary or involuntary), or terminations of this research in accordance with Table 8. The USAMRDC ORA PSSO will be responsible for safety reporting to the U.S. FDA.

The DAIDS Medical Officer will serve in a consultant role on the PSRT but is not required for PSRT or any other protocol team decisions.

Participants who are discontinued from additional study vaccinations will be discussed with the PSRT to determine the duration, if any, of follow-up visits to further evaluate safety and monitor adverse experiences.

Wherever possible, the tests and evaluations listed for the termination visit should be carried out if the subject refuses follow-up according to the protocol visit schedule.

7.2.2. Pregnancy

Subjects who become pregnant post investigational product administration will not receive any additional investigational product. The subject will be followed for safety to term by phone interview and laboratory assessment.

7.3. Participant Discontinuation/Withdraw from the Study

A participant may withdraw from the study at any time at his/her own request, or may be withdrawn at any time at the discretion of the investigator for safety, behavioral, or administrative reasons. Reasons may include the following:

- Repeated failure to comply with protocol requirements
- Recommended withdrawal by the study investigator, e.g., because of worsening health status, intercurrent illness, or AEs interfering with study assessments as determined by the investigator
- Participant requests withdrawal.

If the participant withdraws consent for disclosure of future information, the sponsor's representative may retain and continue to use any data collected before such a withdrawal of consent. If a participant withdraws from the study, he/she may request destruction of any samples taken and not tested, and the investigator must document this in the site study records. Participants who acquire HIV will be discontinued from study vaccinations, and additional research blood draws will not be performed. However, the participant will continue with safety assessments: pregnancy test, CBC with differential, and Creatinine/ALT.

Refer to the schedule of activities in Table 1 for data to be collected at the time of study discontinuation and follow-up and for any further evaluations that need to be completed.

7.3.1. Replacement of Withdrawn Participants

Participants who withdraw or are withdrawn from study vaccinations or the study completely, or are lost to follow-up after signing the ICF and administration of the study product will be replaced if enrollment is still open. If a participant withdraws from the study or discontinues vaccination before all slots have been filled, the vacated randomization slot will be filled with the next available participant. Participants who did not receive the study injection will not count toward the total participants enrolled in the study.

7.3.2. Follow-up for Participants Who Withdraw Consent

After withdrawal of consent, the study team will engage in no further communication with the participant except as directed by an IRB and/or USAMRDC regarding information concerning participant safety.

7.4. Lost to Follow-up

A participant will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site.

The following actions must be taken if a participant fails to return to the clinic for a required study visit.

The site must attempt to contact the participant and reschedule the missed visit as soon as possible and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain whether or not the participant wishes to and/or should continue in the study.

- In cases in which the participant is deemed lost to follow up, the investigator or designee must make every effort to regain contact with the participant (where possible, three (3) telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record.
- Should the participant continue to be unreachable for 3 months, he/she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8. STUDY ASSESSMENTS AND PROCEDURES

Study procedures and their timing are summarized in Section 1.3, Schedule of Activities.

All screening evaluations will be completed and reviewed to confirm that potential subjects meet all eligibility criteria. The investigator will maintain a screening log to record details of all subjects screened and to confirm eligibility or record reasons for screening failure, as applicable.

The maximum amount of blood collected from each subject during the study will not exceed 550 mL per 8 weeks. Blood will be collected into vacutainer tubes according to standard operating procedures. Substitution of tube types may be made as long as the substitution does not interfere with the study performance. A 10% variance in blood volume is acceptable, in case of an error or a broken or clotted vial.

8.1. Safety Assessments

8.1.1. Demographic/Medical History

Age, sex at birth, gender identity, date of birth, birthplace, level of education, occupation and baseline medical history of participants will be recorded. Participant's medical conditions, previous procedures, medications, and history of abnormal laboratory values, if available, will be recorded in the participant's medical history.

8.1.2. Physical Examination

For the purpose of assessing AEs, symptoms and directed medical examination will be performed based on the medical judgment of the investigator. In addition, participants will be asked to record their temperature and complete a diary card at home in the evening after the vaccination and each evening thereafter for a total of 14 days. Study staff will provide thermometers and participants will be trained how to complete the diary card, use the thermometer, and to measure injection site induration and redness using the ruler after each vaccination.

Investigators will pay special attention to clinical signs related to previous serious illnesses.

8.1.3. Vital Signs

Body temperature, pulse, respiratory rate, and blood pressure will be measured prior to study vaccination and again at 30 minutes post-vaccination. Blood pressure and pulse measurements will be assessed in the seated position with legs uncrossed with a completely automated device. Manual techniques will be used only if an automated device is not available. Blood pressure and pulse measurements will be preceded by at least 5 minutes of rest for the subject in a quiet setting without distractions (e.g., television, cell phones).

8.1.4. Clinical Laboratory Assessments

Clinical laboratory assessments will be performed according to standard operating procedures by the laboratory listed in the General Information of this protocol. The clinical laboratory tests to be performed and the timing and frequency is provided in Section 1.3, Schedule of Activities.

Screening labs may be repeated one time.

Laboratory values will be graded and their clinical significance evaluated according to the following procedures:

- All abnormal laboratory values included in the Toxicity Grading Scale are graded by the investigator per Appendix A.
- The investigator will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant; however abnormal laboratory results will be judged by investigators to be clinically significant if they meet one or both of the following criteria:

  - The abnormality suggests a disease and/or organ toxicity that is new or has worsened from baseline.
  - The abnormality is of a degree that requires additional active management (e.g., change of dose, discontinuation of the drug, close observation, more frequent follow-up assessments, or further diagnostic investigation).

- Abnormal laboratory findings or other abnormal assessments judged by the investigator to be clinically significant will be recorded as AEs or SAEs if appropriate.
- All events (Grade 1 or higher) must be recorded, regardless of suspected causal relationship or clinical significance (e.g., including those determined by the physician investigator as non-clinically significant).
- Clinically significant abnormal laboratory findings or other abnormal assessments that are detected before the first administration of investigational product will be documented on medical history as pre-existing conditions and will be reported as AEs or SAEs only if the pre-existing conditions are worse following the vaccination.
- Clinically significant abnormal laboratory findings or other abnormal assessments that are detected after the first administration of investigational product during the study will be reported as AEs or SAEs.
- Chronic clinically significant laboratory abnormalities will be followed until they return to normal or to baseline, stabilize with the probability of becoming chronic, or a satisfactory explanation is provided by the investigator.

8.1.4.1. Hematology

Complete blood cell count (with differential) will be performed on whole blood as per the SOE (Table 1).

8.1.4.2. Blood Chemistry

Creatinine and alanine amino-transferase will be tested on plasma or serum according to the SOE.

8.1.4.3. Laboratory assays

HIV EIA and HIV Western blot or immunochromatographic test U.S. FDA-approved kits maybe used.

HIV nucleic acid tests (NAAT): serum and/or plasma collected in a suitable anti-coagulant (EDTA or ACD) will be assayed for HIV NAAT using at least one nucleic acid test platform. Supplemental nucleic acid tests may also be incorporated.

Samples that are Western Blot or immunochromatographic test indeterminate may also be subject to supplemental Nucleic Acid testing.

8.1.4.4. Pregnancy Screen

Pregnancy tests will be performed at screening, and prior to the vaccination as indicated in the SOE. No vaccine procedures will be performed if the pregnancy test is positive.

8.2. Adverse Events

The following terms, as defined by 21 CFR 312.32, apply to Investigational New Drug (IND) application safety reporting.

8.2.1. Definitions

8.2.1.1. Adverse Event and Suspected Adverse Reaction

An adverse event:

- Is any untoward medical occurrence associated with the use of a drug in humans, whether or not it is considered drug related
- Including any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal (investigational) product

A suspected adverse reaction:

- Is any AE for which there is a reasonable possibility that the investigational product caused the adverse event, with "reasonable possibility" meaning there is evidence to suggest a causal relationship between the investigational product and the AE
- Implies a lesser degree of certainty about causality than adverse reaction, which means any AE caused by an investigational product

8.2.1.2. Solicited Adverse Event

A solicited adverse event:

- Is a pre-determined event identified in the IB that may reflect safety concerns related to the investigational product
- Is specifically asked about (orally or in writing) during subject questioning

The solicited AEs for this protocol include:

<u>Local</u>

- Pain/tenderness
- Itching
- Warmth
- Redness/erythema measurement of largest dimension

- Induration measurement of largest dimension

Systemic

- Fever
- Myalgia
- Arthralgia
- Headache
- Fatigue
- Chills
- Rash
- Nausea
- Dizziness

Solicited adverse events will be recorded daily by the participant, starting the evening of each IP administration and each evening thereafter for a total of 14 additional days following each IP administration. They will use a diary card, for recording any reactions they experience, which will be provided to participants at the time of each IP administration. The diary card will act as a memory tool for the participant and may be used as a source document. Participants must complete the full diary card prior to the administration of any subsequent vaccine.

8.2.1.3. Serious Adverse Event

An AE or suspected adverse reaction is considered serious if it results in any of the following outcomes:

- Death
- Life-threatening AE
  - In the view of either the investigator or sponsor, its occurrence places the participant at immediate risk of death
  - It does not include an AE or suspected adverse reaction that, had it occurred in a more severe form, might have caused death
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Congenital anomaly/birth defect

- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment:
  - They may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above 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

Planned hospitalizations or surgical procedures for pre-existing conditions are not to be considered SAEs unless the condition worsens or requires additional interventions post investigational product administration. Pre-existing conditions, in general, are not to be considered SAEs unless the condition worsens (in frequency, severity, or intensity) or requires additional interventions post-investigational product administration.

8.2.1.4. HIV Diagnostic Algorithm

Diagnostic HIV testing will utilize a series of validated tests that will differentiate between vaccine induced seroreactivity and true HIV infection. HIV test information provided to the study staff at each vaccine trial site will not include the test results of individual tests, but rather will be provided with an aggregate interpretation as HIV "infected" or "not infected", or that repeat testing is needed (as in the case of need for a verification specimen). Report of results will be delayed to at least 10 days from blood collection so that the timing of HIV test reporting does not compromise the double-blind nature of the trial, as a result returned immediately after EIA testing could signal the clinical team that this specimen did not require the Western blot or immunochromatographic testing which would be needed if vaccine had induced an antibody response.

In the case of suspected HIV infection with a positive nucleic acid test, a verification specimen may be requested immediately. If HIV infection is confirmed, results may be returned to the participant immediately.

8.2.1.5. Medically Attended Adverse Events (MAAE)

The FDA requests that clinical trials of preventive vaccines with adjuvants such as ALFQ provide for collection and analysis of data relating to MAAEs among subjects in all treatment groups through 12 months or longer following the last study vaccination, due to the theoretical potential for induction of autoimmune or auto-inflammatory diseases.

MAAEs are defined as AEs with medically attended visits including hospital, emergency room, urgent care clinic, or other visits to or from medical personnel for any reason. Adverse events (e.g., abnormal vitals) identified at a routine study visit will not be considered MAAEs. MAAEs include, but are not limited to, PIMMCs.

8.2.1.6. Unexpected Adverse Event or Unexpected Suspected Adverse Reaction

An AE or suspected adverse reaction is considered unexpected if:

- It is not listed in the IB or is not listed at the specificity or severity that has been observed
- If an IB is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended
- It is mentioned in the IB as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug but is not specifically mentioned as occurring with the particular drug under investigation

For example:

- Hepatic necrosis would be unexpected (by virtue of greater severity) if the Investigator's Brochure referred only to elevated hepatic enzymes or hepatitis
- Cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the IB listed only cerebral vascular accidents.

8.2.2. Timeframe for Collecting Adverse Event Information

All AEs and SAEs will be collected from the start of treatment until the end of the protocol-defined follow-up period, at the time points specified in Section 1.3, Schedule of Activities. Adverse events and SAEs will be assessed at all study visits, documented in the source records, and recorded on the CRFs using accepted medical terms and/or the diagnoses that accurately characterize the event. All unsolicited AEs will also be collected from at least 28 days after each vaccination. Medical occurrences that begin before the start of study treatment but after obtaining informed consent will be recorded on the Medical History/Current Medical Conditions section of the CRF.

Unsolicited adverse events (AEs) and Medically Attended Adverse Events (MAAEs) will be collected from the day of IP administration and for the next 28 days. The investigator will also document SAEs, MAAEs and AESIs (including PIMMCs) for at least 12 months after subjects receive their last study vaccination.

8.2.3. Methods for Obtaining AE and SAE Data

Adverse events will be reported by the subject or detected by the investigator or designee during examination or assessment.

The investigator and any designees are responsible for detecting, documenting, and reporting events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study treatment or the study, or that caused the subject to discontinue the study treatment or the study (Section 7.3). Participants will be asked for their consent for photography if the clinician wishes to document an unusual or unexpected finding. Participants will not be able to be identified by the photographs, which will be kept indefinitely.

When an AE or SAE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory, and diagnostics reports) related to the event.

8.2.4. Recording Adverse Event and Serious Adverse Event Data

When a diagnosis is known, the AE term recorded on the case report form will be the diagnosis rather than a constellation of symptoms. The investigator will assess all AEs for seriousness (Section 0), severity (Section 8.2.5), relationship to investigational product (Section 8.2.6), and other possible etiologies. When an event has not resolved by study closure, it will be documented on the AE case report form as "not recovered/not resolved." The AE verbatim (in the clinical database) and the SAE verbatim (in the safety database) should be recorded consistently and that any updates to the event should be made in both places.

Serious adverse events will be recorded on the SAE report form as follows:

- Start date - date the AE met serious criteria.
- Resolution date - date the event returned to baseline status or the condition stabilized or was determined chronic
- If not resolved by study closure, the event will be documented on the SAE report form as "not recovered/not resolved."

8.2.5. Severity Assessment

All AEs (laboratory and clinical symptoms) will be graded for severity and assessed for relationship to study product using the DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), Version 2.1 dated July 2017 that is found on the website https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables. AEs characterized as intermittent require documentation of onset and duration of each episode. The start and stop date of each reported AE will be recorded on the appropriate data collection form and eCRF. Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of intensity. Adverse events not identified in the grading table, follow the guidelines provided at the beginning of the table.

All other events will be graded according to the scale in Table 5. The case report form for AEs will reflect only the highest severity for continuous days an event occurred.

8.2.6. Estimating Severity Grade for Parameters Not Identified in the Grading Table

The functional table below 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 5: DAIDS Adverse Event Grading Scale

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

8.2.7. Exceptions to the Grading Table

8.2.7.1. Creatinine

Creatinine is required to be reported as an AE only if it is gradable per the increase from local lab upper limit of normal (ULN) parameter. Do not grade elevated creatinine based on the change from the baseline parameter.

Do not grade creatinine clearance or eGFR based on the change from the baseline parameter. Do not grade on the basis of eGFR if there is clinical concern for kidney injury.

8.2.7.2. Injection Site Erythema and Induration

Injection Site Erythema and Injection Site Induration will not consider surface area and interference with usual social and functional activities such that:

- Grade 1 is: 2.5 to < 5 cm in diameter;
- Grade 2 is: ≥ 5 to < 10 cm in diameter;
- Grade 3 is: ≥ 10 cm in diameter OR ulceration OR secondary infection OR phlebitis OR sterile abscess OR drainage;
- Grade 4 is: Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue

8.2.7.3. Unintentional Weight Loss

Unintentional weight loss is required to be reported as an AE only if it is considered to be potentially deleterious to the participant's health.

8.2.8. Causality Assessment

A physician must assign a relationship of each AE to the receipt of the investigational product using the relationship categories in Table 6.

Table 6: Causality Relationships

Relationship	Description
Not related	No relationship to investigational product. Applies to those events for which evidence exists that there is an alternate etiology.
Unlikely	Likely unrelated to the investigational product. Likely to be related to factors other than the investigational product but cannot be ruled out with certainty.
Possible	An association between the event and the administration of investigational product cannot be ruled out. There is a reasonable temporal association, but there may also be an alternative etiology, such as the subject's clinical status or underlying factors (including other therapy).
Probable	There is a high degree of certainty that a relationship to the investigational product exists. There is a reasonable temporal association, and the event cannot be explained by known characteristics of the subject's clinical state or factors including other therapy.
Definite	An association exists between the receipt of investigational product and the event. An association to other factors has been ruled out.

8.2.8.1. Serious Adverse Events

The investigator will report SAEs to the ORA PSSO according to the requirements in Table 8. A summary of the required information is provided in Table 9. When reporting via email, the subject lines should be formatted as follows:

> **Initial:** SAFETY REPORT – IND # xxx_____, Sponsor Study #S-xx-xx_____,Subject# _____, Event term: _____
>
> **Follow-Up:** SAE Case Number provided by PSSO (e.g., US-000123), SAFETY REPORT – IND # xxx_____, Sponsor Study #S-xx-xx_____, Subject#_____, Event term: _____

Investigators must also follow all relevant requirements regarding the timely reporting of AEs to the WRAIR IRB and the USAMRDC Office of Human and Animal Research Oversight (OHARO).

There may be instances when copies of medical records for certain cases are requested by the sponsor's safety office. In this case, all subject identifiers, with the exception of the subject number, must be redacted on the copies of the medical records before submission to the sponsor's safety office.

8.2.9. Sponsor Independent Safety Monitor

Independent Safety Monitor (ISM): The Sponsor Independent Safety Monitor (ISM) is a physician with relevant expertise whose primary responsibility is to provide independent safety monitoring in a timely fashion. The ISM is required to review and provide an unbiased written report for all SAEs and subject deaths to the ORA PSSO (Safety Office) within 24 hours of their

awareness of the event. The report provided must include, at a minimum, a brief summary of the ISMs review of the event and event outcome, relationship of the event to the investigational product/device and alternate etiology if the event is deemed unrelated to the investigational product/device.

In addition to the responsibilities above, the ISM maybe required to attend safety meetings and providing assessments on subject withdrawals, sentinel subject data reviews, study halting criteria, etc., and must be clearly defined in the sponsor approved clinical trial protocol.

8.2.10. Reporting Serious and Unexpected Adverse Events

Contact information for reporting SAEs is provided in Table 7.

Table 7: Study Contacts for Reporting Serious and Unexpected Adverse Events

Sponsor's Safety Office	US Army Medical Research & Development Command Office of Regulated Activities Product Safety Surveillance Office ATTN: FCMR-ORA 1430 Veterans Drive Fort Detrick, MD 21702-5009 Fax: 301-619-7790 Phone Number: +1 301 619 1005 Email: usarmy.detrick.medcom-usamrmc.mbx.sae-reporting@health.mil
Institutional Review Board	Walter Reed Army Institute of Research IRB 503 Robert Grant Avenue Silver Spring, MD 20910, USA Phone Number: 301-319-9940 Email: usarmy.detrick.medcom-wrair.mbx.hspb@health.mil
USAMRDC Office of Human and Animal Research Oversight	Office of Human and Animal Research Oversight (OHARO) Office of Human Research Oversight (OHRO) US Army Medical Research and Development Command, ATTN: FCMR-RPH 504 Scott Street Fort Detrick, MD 21702-5012 Fax: +1 301 619 7803 Phone Number: 301-619-2165 Email: usarmy.detrick.medcom-usamrmc.other.hrpo@health.mil
Independent Safety Monitor	Melanie McCauley, MD Research Physician | HJF in support of the Emerging Infectious Diseases Branch (EIDB) Walter Reed Army Institute of Research (WRAIR) 6720A Rockledge Drive, Suite 400 Bethesda, MD 20817 Phone Number: 301- 500-3636

Sponsor's Safety Office	US Army Medical Research & Development Command Office of Regulated Activities Product Safety Surveillance Office ATTN: FCMR-ORA 1430 Veterans Drive Fort Detrick, MD 21702-5009 Fax: 301-619-7790 Phone Number: +1 301 619 1005 Email: usarmy.detrick.medcom-usamrmc.mbx.sae-reporting@health.mil
	Email: mmccauley@eidresearch.org
Alternate Independent Safety Monitor	Roger Ying, MD Research Physician | HJF U.S. Military HIV Research Program (MHRP) 6720A Rockledge Drive, Suite 400 Bethesda, MD 20817 Email:RYing@global-id.org

8.2.10.1. Reporting to WRAIR IRB

All SAEs related to participation in the study, and all deaths will be promptly (within 48 hours) reported to the WRAIR IRB and written reports will be submitted to the WRAIR IRB within 10 working days. The WRAIR Human Subjects Protection Branch (HSPB) will report all related SAEs and all participant deaths to USAMRDC Office of Human and Animal Research Oversight (OHARO) Office of Human Research Oversight (OHRO) as per USAMRDC Command Policy 21 Follow up reports will be submitted as additional information becomes available.

A summary of the non-serious AEs and SAEs (both related and unrelated) that occurred during the reporting period will be included in the continuing review report (CRR) to the WRAIR IRB.

Investigators are required to forward safety information provided by the sponsor's representative to the WRAIR IRB in a timely manner.

8.2.11. Additional Immediately Reportable Events

8.2.11.1. Potentially Immune-Mediated Medical Conditions (PIMMCs)

PIMMCs (Appendix L) constitute a group of AEs that includes diseases which are clearly autoimmune in etiology and other inflammatory and/or neurologic disorders which may or may not have autoimmune etiologies.

8.2.11.2. Unanticipated Problems Involving Risks to Subjects or Others

Unanticipated problems involving risks to subjects or others (UPIRTSO) are any incident, experience, or outcome that meets all of the following criteria:

- Is unexpected (in terms of nature, severity, or frequency) given
  - the procedures that are described in the protocol, IB, or informed consent document; and
  - the characteristics of the subject population
- Is related or possibly related to participation in the study
- Suggests that the protocol places participants or others at a greater risk of harm than was previously known or recognized

These events encompass a broader category of events than SAEs and may include issues such as problems with loss of control of participant data or the investigational product; adverse psychological reactions; or breach of confidentiality.

The PI will determine whether a given incident, experience or outcome constitutes a UPIRTSO and will promptly (within 48 hours) report UPIRTSOs to the WRAIR IRB and to the sponsor. The PI will then submit a written report within 10 working days to the WRAIR IRB.

Follow up reports should be submitted as soon as additional information becomes available. A summary of UPIRTSOs will also be included in the continuing review report submitted to the WRAIR IRB. The WRAIR HSPB will report UPIRTSOs to the USAMRDC OHARO OHRO as per USAMRDC Command Policy 21.

8.2.11.3. Study Pause or Termination

The PSRT will closely monitor and analyze study data as they become available and will make determinations regarding the presence, severity, and seriousness of AEs. The administration of study injections and new enrollments will be paused, and the Sponsor will be promptly notified regarding a safety issue according to the following criteria:

- One (or more) participant experiences an SAE that is assessed as related to study agent, or
- Two (or more) participants experience grade 3 or 4 AEs of the same type related to a study agent. This would exclude monitoring lab values if a clear alternate etiology is identified. Additionally, CBC differential counts would not contribute.

The WRAIR IRB will be immediately notified of any suspensions, clinical holds (voluntary or involuntary), or terminations of this research by any of the WRAIR IRB, the PSRT, the institution, the sponsor, or regulatory agencies. WRAIR HSPB will forward the notification to USAMRDC OHARO OHRO as per USAMRDC Command Policy 21.

8.2.11.4. Participant Status Change: Incarceration

Participation of prisoners is not planned, and any participant will be suspended from study visits while incarcerated, except visits or telephone contact for ensuring their safety. No study product

will be administered to a participant who is incarcerated. The IRB will be notified of the period of incarceration. Data and samples that were collected before the period of incarceration may still be stored and used for analyses. After release from incarceration, either the participant may return to participation in study visits according to the SOE or study participation may be terminated at the discretion of the PI.

Any participant who is incarcerated will be re-consented before rejoining the study.

When a previously enrolled participant becomes a prisoner and the relevant research protocol was not reviewed and approved by the IRB in accordance with the requirements of Department of Defense Instruction (DODI) 3216.02 subparagraphs 7.b (1) and (2), to include prisoners as research participants, the PI shall promptly notify the WRAIR IRB. WRAIR HSPB will forward the notification to USAMRDC OHARO OHRO as per USAMRDC Command Policy 21.

8.2.11.5. AE-Related Withdrawal of Consent

Any AE-related withdrawal of consent during the study must be reported by email or fax to USAMRDC ORA PSSO within 24 hours of event awareness. The WRAIR IRB will be notified of the withdrawal in a follow-up report as per Table 8.

8.2.12. Pending Inspections/Issuance of Reports

The knowledge of any pending compliance inspection/visit by the FDA, Office for Human Research Protections (Department of Health and Human Services), or other government agency concerning clinical investigation or research, the issuance of Inspection Reports, FDA Form 483, warning letters, or actions taken by any regulatory agency including legal or medical actions and any instances of serious or continuing noncompliance with the regulations or requirements will be reported immediately to the WRAIR IRB and the sponsor's representative. The WRAIR HSPB will report knowledge of any pending inspections/audits by regulatory agencies to the USAMRDC OHARO OHRO as per USAMRDC Command Policy 21.

8.2.13. IND Annual Report to the FDA

The sponsor's representative will notify the PI of the due date of the annual report with sufficient time for the PI to assemble and submit all of the required clinical study information to the sponsor's representative.

The Sponsor and Sponsor's representative (USAMRDC) will be responsible for the preparation of the detailed annual synopsis of clinical activity, including AEs, for submission to the US FDA. Each annual report will summarize IND activity for one year beginning approximately 3 months before the IND US FDA anniversary date.

8.2.14. Final Report

A final study report will be prepared in accordance with "Guidance for Industry: Submission of Abbreviated Reports and Synopses in Support of Marketing Applications" and ICH E3 Guideline "Structure and Content of Clinical Study Reports" and provided to the sponsor's representative for review and approval. The sponsor's representative will use this report to prepare the final clinical study report for submission to the U.S. FDA.

Table 8: Sponsor's Adverse Event Reporting Requirements

Event	Report	Notification Time	Notification Method
Serious adverse events	SAE Report	Within 24 hours of event awareness [a]	Email: usarmy.detrick.medcom-usamrmc.mbx.sae-reporting@health.mil If email is not available, the information can be faxed or called in: Fax: 301-619-0197 Phone: 301-619-1005
	Independent Safety Monitor Report	Within 24 hours of event awareness	
Pregnancy	Pregnancy Report	Within 24 hours of event awareness	
Events of Special Interest (including PIMMCs)	SAE/AESI) Report	Within 24 hours of event awareness [a]	
	Independent Safety Monitor Report	Within 24 hours of event awareness	
Adverse Event Related Withdrawals	Contact PSSO	Within 24 hours of awareness of event awareness	
Safety-related Deviations	Contact PSSO	Within 5 business days of event awareness	

[a] Submission of the reports to the PSSO must not be delayed. Do not wait to receive the independent safety monitor's report.

Table 9: Summary of Information Required When Reporting Adverse Events to the Sponsor

Information to Include on the Serious Adverse Event Report Form includes, but is not limited to:
Investigational new drug application number, sponsor study number
Name of the investigational product
Investigator name and contact number
Subject identification number
Serious adverse event term, description
Onset date
Date(s) of investigational product administration
Severity
Relationship to the investigational product
Subject's current status
Admission/Discharge Summary
Consultations
Medical record progress notes including pertinent laboratory/diagnostic test results

8.2.15. Pregnancy Reporting

Each pregnancy must be reported within 24 hours of identification by completing and submitting the Pregnancy Report Form by email or fax to the sponsor's safety office (ORA, Product Safety

Surveillance Branch (PSSO)). Pregnancies must be reported within 48 hours of becoming aware of the event to the IRB. WRAIR HSPB will forward the notification to USAMRDC OHARO OHRO as per USAMRDC Command Policy 21.

The following information will be reported on the Pregnancy Report: type and date of delivery, Apgar scores, and health status of the mother and child including the child's sex, head circumference, gestational age at delivery, length, and weight. Complications and or abnormalities should be reported including any premature terminations. A pregnancy is reported as an AE or SAE only when there is suspicion that the investigational product may have interfered with the effectiveness of contraception or there was a serious complication in the pregnancy including a spontaneous abortion or an elective termination for medical rationale. Otherwise, pregnancy will not be categorized as an AE.

8.2.16. Hepatitis B and C and HIV Reporting to the Local Health Authorities

Hepatitis B and C, and Human Immunodeficiency Virus (HIV) test results are required by law to be reported to the local health authorities. The test results reported to local health authorities will contain the participant’s name, contact information, including address and telephone numbers, and the type of testing conducted.

8.2.17. Serious Adverse Event Follow-up

After the initial /SAE report, the investigator will proactively follow each subject at subsequent visits or contacts via phone or in person. All SAEs, and events of special interest, if applicable, will be followed until resolution, even if this extends beyond the prescribed reporting period. Resolution is the return to baseline status or stabilization of the condition with the probability that it will become chronic. The SAE outcomes will be reported to the ISM and ORA PSSO using the SAE Report Form. The investigator will submit any updated SAE data to the sponsor and ISM within 24 hours of receipt of the information.

If a subject dies during participation in the study or during a recognized follow-up period, the investigator will need to provide a copy of any post-mortem findings, including histopathology, if available.

Investigators are not obligated to actively seek SAEs in former subjects; however, if a SAE that is considered to be related to the investigational product occurs in a former subject and is brought to the attention of the investigator at any time following completion of the study, the event will be reported to the sponsor’s representative (Table 8).

8.2.18. Safety Monitoring Committee

An independent safety monitoring committee (SMC) will be convened to review safety data and provide recommendations to the sponsor’s representative. The SMC for this study will be comprised of an independent group of experts who will review cumulative safety data during scheduled in-process and ad hoc reviews to evaluate safety, study conduct, and scientific validity and integrity of the trial. The SMC will meet annually. After each review of study data, the SMC will make a recommendation to the sponsor’s representative. The SMC recommendations will be documented and provided in writing (via the SMC Chair Recommendation Form) to the ORA PSSO. The sponsor’s representative will then decide whether to continue, modify, or suspend the

study. The ORA PSSO will communicate the final decision to the investigator, who in turn will notify the IRB as appropriate. The ORA regulatory affairs scientist will communicate the final decision to the U.S. FDA, as appropriate. A further description of the SMC roles and responsibilities, functions, reporting requirements, meeting frequency, and the study stopping/continuation criteria is included in the SMC charter.

8.3. Immunogenicity Assessments

8.3.1. Specification of Immunogenicity Endpoints

Table 10: Immunology Assays

Humoral Assays	**Serum or plasma**	**Function Measurement**
ADCC, ADCP, and other non-neutralizing antibody functions	Frozen Plasma/ Serum	Measures lysis of HIV expressing targets mediated by HIV specific antibodies
HIV-specific binding	Frozen Plasma/ Serum	Binding antibody to vaccine antigens
HIV-specific neutralizing antibodies	Frozen Serum	Neutralizing activity against luciferase reporter gene expression
Cellular and Innate Assays	**Serum or plasma**	**Function Measurement**
Cellular response by cytokines such as IFN-γ and IL2 after stimulation with HIV-specific antigens	Frozen PBMC	CD4+ and CD8+ antigen-specific response
Lymphocyte proliferation	Frozen PBMC	Characterize the function of proliferating cells in response to HIV antigens
B-cell ELISPOT	Frozen PBMC	Measures cytokine secretion from B cells in response to HIV antigens
Flow cytometry for innate immune cell phenotyping and a cytokine array assay	Frozen Plasma/ Serum	Phenotype NK and other innate cells and characterize the cytokines elicited by the different vaccine regimens
DNA Microarray: gene expression to vaccine antigens	Frozen PBMC	Host gene expression profile and signature to vaccine antigens
RNA sequencing: gene transcription to vaccine antigens	Frozen PBMC	Host gene transcription profile and signature to vaccine antigens

8.3.2. Immunogenicity Endpoints

Immunogenicity will be assessed using the following assays at time points defined in the SOE.

8.3.3. Humoral Immune Responses

HIV-specific Binding Antibody ELISA Assays will be performed to detect serum or plasma IgG and IgA binding antibodies to HIV-1 gp120, in particular to the V1V2 loops. Capture antigens will include V1V2 sequences from both subtype AE and Subtype B HIV-1 Env (gp70V1V2 92TH023 and gp70V1V2 Case A2), and HIV-1 Env gp120 proteins matched to sequences in AIDSVAX® B/E without the gD tag and with an 11 amino acid N-terminal deletion, represented as gp120 A244gD- D11 and gp120 MNgD- D11. Durability of IgG response will be assessed for each participant by estimating the decline in log10 IgG from peak to 6 and 12 months post final vaccination.

Neutralizing Antibody Assays will be measured as a function of reductions in luciferase (Luc) reporter gene expression after a single round of infection in TZM-bl cells using high throughput analysis. Stocks of molecularly cloned envelope-pseudotyped viruses have been prepared by transfection in 293T/17 cells (American Type Culture Collection) and titrated in TZM-bl cells. This assay has been formally optimized and validated and was performed in compliance with Good Clinical Laboratory Practices (GCLP), including participation in a formal proficiency testing program. Tier 2 neutralization was assessed using a panel of 11 pseudoviruses, and a global panel.

Antibody Profiling will be conducted to assess antibody breadth and correlation of Fc receptor usage with antibody function. The current capacity for Antibody Profiling is the assessment of over 300 samples in one experiment. The beads presenting the HIV antigens will be distributed robotically in a high-throughput manner into 384 well plates. Similarly, the samples will be diluted robotically and incubated with the beads. Detection reagents will be added to the wells after washing. These detection reagents are inclusive of subclass, isotype and Fc receptor usage. Therefore, in one well, we will be able to capture 900 to 1000 data points from a single sample, and over 250,000 data points per experiment that will allow evaluation of the humoral immune response to these vaccine strategies.

Antibody-dependent cell-mediated cytotoxicity and antibody-dependent cellular phagocytosis will evaluated using an amine reactive staining (Aqua) to identify killed target cells compare to loss of carboxyfluorescein succinimidyl ester (CFSE) in the traditional rapid and fluorometric ADCC (RFADCC). This Aqua-based assay is high throughput, has a better signal to noise ratio and a lower coefficient of variation than the conventional CFSE-based RFADCC assay. The ADCP phagocytic score will also be calculated and compared with Antibody profiling data to evaluate the impacts on IgG subclass and Fc receptor usage on non-neutralizing function between groups.

8.3.4. Cellular Immune Responses

Intra-cellular cytokine staining (ICS) assay. Cryopreserved peripheral blood mononuclear cell (PBMC) will be stimulated with HIV-specific antigens and gated on cell surface markers such as CD4 and CD8 to determine antigen-specific cellular responses by individual cytokines, such as interferon gamma (IFNγ) and interleukin 2 (IL2), as well as a composite, calculated functional and polyfunctional response using the COMPASS analysis method.

Cellular Proliferation. The proliferative responses of participant's PBMC to HIV-specific antigens and mitogens will be measured by the CFSE assay to characterize the function of proliferating cells.

B-cell Responses. A highly sensitive Env-specific B-cell ELISPOT system was developed, which allows the enumeration of antibody-secreting cells from diverse anatomical compartments directed against different structural determinants of HIV envelope. Both plasmablast and memory B cell responses will be quantified to detect gp120 A244-specific IgG producing plasmablasts and long-lived memory B cells using cryopreserved PBMC.

8.3.5. Innate Immunity

Characterization of Innate Immunity. Flow cytometric panels will be used to phenotype the different types of NK cells and functional ICS to characterize their function. Luminex® multiplex assays will be performed to compare soluble cytokines and other factors in plasma/serum across vaccine regimens, to further characterize innate biologic pathways engaged.

Innate and Early Adaptive Responses by Gene Activation Assessment. DNA microarrays will explore the innate and early adaptive immune responses to vaccination by screening genomic expression profile in PBMCs.

Transcriptomics. Transcriptome data will be generated using RNASeq to investigate gene expression that correlates with increased immunogenicity to particular vaccine combination.

8.4. Specimen Archiving and Transfer

Biological samples such as serum, plasma, PBMCs, whole blood, remaining after all assays described in this protocol have been completed will be bar coded and archived using electronic specimen storage and tracking system. PBMC will be stored at -125°C or lower and plasma/sera will be stored at -70°C or lower. Samples will be archived at the WRAIR Diagnostics and Countermeasures Branch (DCB) Specimen Processing Laboratory and Biorepository (SPL/BioR) indefinitely. If the study team determines to destroy these specimens, they will be destroyed per applicable SOPs of the archiving institutions. The PI, the Sponsor and authorized regulatory bodies may have access to the data regarding the archived specimens.

Archived samples will only be used for research and will not be sold; nor will study participants receive payment, should samples lead to the development of new products in the future. Any future study requesting these samples must first be reviewed and approved by the IRB of each applicable institute. With concurrence from these parties, archived specimens will be shipped to the U.S. Military HIV Research Program (MHRP) and collaborating laboratories for assays.

8.5. Future Use and Storage of Data and Biological Samples

Each participant will be asked to voluntarily consent to their data and blood samples to be stored for other research studies that may be done after this study is complete. Future testing may involve genetic tests. In case the participant is unwilling to have their data and/or biological samples stored for future use, they can consent to participate in this study only, without having their data and blood samples stored for future testing. In this case, their data and blood samples will be destroyed within one year of receiving notification from the PI that all protocol testing is

complete. Samples remaining in the biorepository will be destroyed by laboratory staff following approved laboratory procedures.

All data and samples for which consent has been obtained and for which additional material is available after study specified testing is complete will be stored indefinitely for future testing. However, WRAIR IRB approval will be sought before any such data and/or samples are used for analysis not specified in the protocol or a protocol amendment approved by the IRB. Data and samples may be shared with external collaborators but only after WRAIR IRB approval is received.

During the study, blood samples for safety testing will be tested as soon as possible and within the allowable sample stability timeframe for each test.

9. STATISTICS

This study intends to descriptively characterize safety and immune responses following administration of candidate vaccine A244/B.63521 with ALFQ adjuvant in healthy adults across three doses of ALFQ: 200 µg, 100 µg and 50 µg, at baseline, month 1 and month 2. Statistical analysis will be performed on the following three groups:

- **Arm 1:** A244/B.63521 + **200 µg** ALFQ, N=20
- **Arm 2:** A244/B.63521 + **100 µg** ALFQ, N=20
- **Arm 3:** A244/B.63521 + **50 µg** ALFQ, N=20

Of specific interest is determining the safety of the candidate vaccine A244/B.63521 with ALFQ regimens as well as whether immunologic differences can be detected between the 3 study arms. Response rates, and, where applicable, titer level distributions with appropriate summary statistics will be estimated for each immunologic assay performed. The study will evaluate a series of cellular, humoral, and innate immune assays.

Due to limited sample size, rapid enrollment of subjects and short period of vaccination delivery over two months, no seasonal and other temporal effects are expected.

9.1. Statistical Criteria for the Termination of the Trial

There are no statistical criteria for the purpose of study termination in this clinical trial.

9.2. Procedures for Reporting Deviations from the Statistical Plan

Any deviation(s) from the statistical plan as indicated in the protocol will be described in an amendment to the protocol or the clinical study report. The protocol amendment will be submitted to the WRAIR IRB as per Section 10.1.1.

9.3. Accounting for Missing, Unused, and Spurious Data

Non-analyzable data will be documented in the deviations.

9.4. Sample size and Power calculation

The safety, reactogenicity, and tolerability of the vaccine regimens are the primary endpoints for this study. The table below demonstrates effect size with a power of 80%, calculated at a variety of sample sizes.

In Table 11, the sample size calculation for the antibody titer (secondary outcome) will be based on the published data from Pitisuttithum P et.al[18] . For the analysis of this experiment we plan on observing a single measurement of peak antibody level per participant. Thus, the study is designed around a 1-way ANOVA model. Tukey's method will be used in the pairwise comparison test. In the analysis we looked at the size of the error bars on the peak of the log antibody titer and based upon our visual inspection we assumed that the residual standard deviation is = 0.2. A sample size of approximately 20 participants per arm was selected to provide ≥80% power to detect the effect size of ≥ 0.202 between study arms. Statistical significance will be set at p=0.05.

Table 12 depicts SAE or AESI rates and associated 95% confidence intervals in each potential grouping of interest given 0-5 participants with observed events. If we were to observe zero events within a single active study group (n=20), the upper limit of a 2-sided exact 95% CI would be 13.9% and true events above this could be ruled out at the α=0.025 level.

Table 11: Sample size per arm and minimum effect size with 80% power, assuming residual standard deviation of 0.2

	Part A: 3 Active Arms	
n per arm	**Minimum effect size**	**Power**
5	0.448	0.801
6	0.398	0.801
7	0.362	0.801
8	0.334	0.800
9	0.312	0.800
10	0.294	0.801
11	0.280	0.804
12	0.266	0.802
13	0.254	0.800
14	0.244	0.800
15	0.236	0.803
16	0.228	0.804
17	0.220	0.801
18	0.214	0.803
19	0.208	0.803

	Part A: 3 Active Arms	
20	0.202	0.802
21	0.198	0.806
22	0.192	0.801
23	0.188	0.803
24	0.184	0.804
25	0.180	0.803
26	0.176	0.802
27	0.174	0.808

Table 12: Exact 95% Clopper-Pearson CI for the SAE Rate in Each Study Group

Number of Participants with Event	All Active Groups (n=60)	Any Active Group (n=20)	Arm 1 (n=22)	Arms 2 or 3 (n=19)
0	(0%, 4.9%)	(0%, 13.9%)	(0%, 12.7%)	(0%, 14.6%)
1	(<0.1%, 8.9%)	(0.1%, 24.9%)	(0.1%, 22.8%)	(0.1%, 26.0%)
2	(0.4%, 11.5%)	(1.2%, 31.7%)	(1.1%, 29.2%)	(1.3%, 33.1%)
3	(1.0%, 13.9%)	(3.2%, 37.9%)	(2.9%, 34.9%)	(3.3%, 39.6%)
4	(1.8%, 16.2%)	(5.7%, 43.7%)	(5.2%, 40.3%)	(6.0%, 45.5%)
5	(2.8%, 18.4%)	(8.7%, 49.1%)	(7.8%, 45.4%)	(9.1%, 51.2%)

9.5. Populations for Analyses

Analysis populations are defined in Table 13.

Table 13: Analysis Populations

Population	Description
Enrolled	All participants who receive the first study vaccination
Randomized	All participants who are randomized to a study arm
Evaluable	All participants who receive the first study vaccination
Safety	All randomized participants who take at least 1 dose of study treatment. Participants will be analyzed according to the treatment they actually received
Immunogenicity	The immunogenicity population will include all participants who received at least one dose of vaccination and have at least one post-vaccination blood sample

9.6. Statistical Analyses

Immunologic measurements and AEs including solicited and unsolicited events and clinically significant abnormal laboratory values are the main database for RV575. The statistical analysis plan will be developed and finalized before database lock and will describe the selection of participants to be included in the analyses, and procedures for accounting for missing, unused, and spurious data.

Below is a summary of planned statistical analyses of the primary and secondary endpoints.

9.6.1. Demographics and Baseline Characteristics

Participant demographics and baseline characteristics will be summarized and may be presented in table form. The number of participants who enroll in the trial and the number and percentage of participants who complete each assessment will be presented. The percentage of patients who withdraw from the trial or discontinue the study drug and reasons for withdrawal will be summarized.

9.6.2. Safety Analysis

The primary endpoints of this Phase 1 study are related to the safety, reactogenicity, and tolerability of the candidate vaccine A244/B.63521 with varying doses of ALFQ adjuvant. The occurrence and severity of SAEs, AEs, AESIs, and unsolicited AEs will be tabulated by study group and compared between and among groups.

The occurrence of local and systemic reactogenicity symptoms will be assessed as the proportions of participants experiencing such safety events along with 95% confidence intervals. Pairwise, between group comparisons will be performed. These comparisons may be performed at a two-sided α=0.05 level, using the normal approximation to the binomial distribution or an alternate testing procedure as appropriate (e.g. Fisher's Exact test for smaller samples).

9.6.3. Immunogenicity Analysis

The primary immunogenicity endpoint is the magnitude of immune response to the endpoint titer of plasma IgG. Immune response over time (durability) will be assessed by the positive incremental area under the curve (AUC) based on a graph with log endpoint titer on the y-axis and visit week on the x-axis. Summary statistics at specific time points of interest will also be provided using the mean and standard deviation (or median and interquartile range (IQR) as appropriate). Primary comparisons may use a 2-sample t-test with an unadjusted alpha of 0.05. When differences are found, pairwise comparisons between vaccination groups will be made using the Holm-Bonferroni method and a 5% significance threshold for the adjusted p-values.

Further exploratory comparisons may be performed and adjusted for multiplicity as appropriate.

Secondary immunogenicity endpoints will entail primarily descriptive analyses. In general, continuous outcomes will be assessed using summary statistics such as the mean, median, standard deviation and IQR. Binary responses will be described using frequencies and percentages. Changes from baseline may be assessed within groups using paired t-tests (or Wilcoxon signed rank as appropriate) or McNemar's test for binary outcomes. Changes over

time between groups may be evaluated using longitudinal models with unstructured covariance matrices where possible or covariance strictures selected as appropriate for the observed data.

9.6.4. Missing Values and Outliers

All attempts will be made to collect all data per protocol. Frequencies of missingness for primary outcomes will be calculated overall and within each study group. Less than 10% missing data that are missing completely at random will be considered ignorable missingness and complete case analysis will be utilized. No imputation will be performed for missing values. Data visualizations such as boxplots, histograms, or scatter plots will be used to identify outliers. Single outliers may be identified if values are outside of 1.5x the interquartile range depending on normality assumptions. Outliers will not be excluded from the primary analyses. Outliers identified during the analysis will be discussed in the analysis report.

9.6.5. Clinical Laboratory Data Analyses

Clinical laboratory values, including change from baseline, will be summarized for each participant according to time and study arm. The values will be graded according to the DAIDS grading scale in Appendix A and, if clinically significant, reported as AEs.

Descriptive summary statistics (mean, standard deviation (SD), median, minimum, and maximum) for clinical laboratory data at admission and each applicable post-dosing visit, including changes from the baseline value collected at admission, will be calculated. If multiple baseline values are obtained, only the most recent value will be analyzed. For change-from-screening summaries, participants with an undefined change from screening, due to missing data, will be excluded. Clinical significance of abnormalities will be indicated in the listings.

9.6.6. Planned Interim Analyses

No formal interim safety analyses are planned but may be performed if requested by the safety monitoring committee as outlined in Section 8.2.18.

Interim immunogenicity analyses will be conducted after all enrolled participants have reached at least week 10 (20.8% of follow-up accrual) and may be conducted at additional time points if requested by the safety monitoring committee. The main goal of planned interim analyses through week 10 is to assess short-term effect of the candidate vaccine A244/B.63521 (300 µg each) with ALFQ adjuvant at the 200, 100, and 50 µg doses on cellular and humoral immune responses. This immunogenicity group analysis will not compromise the integrity of the trial in terms of the maintenance of the study blind, participant retention, or safety or immunogenicity endpoint assessments. All participant IDs will be replaced with alternate IDs by the unblinded study staff and only the minimum necessary summary level of information will be shared with blinded study staff. Results from planned interim immunogenicity analyses will be used to inform the design of a subsequent clinical trial.

To minimize the risk of type-I error, an alpha-spending function, such as O'Brien-Fleming boundary will be used to monitor primary and secondary endpoints using an overall, two-sided alpha of 0.05, with the overall goal being to maximize the amount of alpha reserved for the final study analysis. Specific details of alpha-spending methods will be included in the statistical analysis plan.

The Principal Investigator will ensure concurrence from USAMRDC ORA as outlined in Section **Error! Reference source not found.** prior to presentation or publication of data from interim analyses. Any analyses conducted prior to the end of the scheduled follow-up visits will not compromise the integrity of the trial in terms of participant retention or safety or immunogenicity endpoint assessments.

10. ETHICS

10.1. Ethics Review

The protocol is based on adequately performed laboratory and animal experimentation and will be conducted under a protocol reviewed by the IRB (see Table 7) and USAMRDC OHARO OHRO. The study is to be conducted by scientifically and medically qualified persons. The WRAIR IRB will determine whether the benefits of the protocol are in proportion to the risks. The rights and welfare of the participants will be respected, the physicians conducting the study will ensure that the hazards do not outweigh the potential benefits, the results to be reported will be accurate, participants will give their informed consent and will be competent to do so and not under duress, and all study staff will comply with the ethical principles in 21 CFR Part 50 and the Belmont Principles.

10.1.1. Protocol Modifications

All modifications to the protocol and supporting documents (informed consent, protocol-specific procedures, standard operating procedures, recruitment materials, etc.) will be reviewed and approved prior to implementation:

1. Integrated product team review and approval
2. Scientific Review Board review and approval (if applicable)
3. Protocol Review Board (sponsor's representative) review and approval
4. Institutional Review Board review and approval
5. U.S. FDA submission

Any protocol amendment will be agreed upon and approved by the sponsor's representative, through the PRB prior to submission to the IRB and approved by the IRB prior to implementation of said change or modification.

Any modification that could potentially increase risk to participants will be submitted to the US FDA prior to implementation. Substantive modifications to the protocol and any modifications that could potentially increase risk to participants must also be submitted to the USAMRDC OHARO OHRO for approval prior to issuance of the WRAIR Commander Approval Authorization. The USAMRDC OHARO OHRO defines a substantive modification as a change in PI, change or addition of an institution, elimination or alteration of the consent process, change in the IRB of Record, change to the study population that has regulatory implications (e.g., adding children, adding active duty population, etc.), significant change in study design (i.e., would prompt additional scientific review) or a change that could potentially increase risks

to participants. Any changes of the IRB used to review and approve the research will be promptly reported to the USAMRDC OHARO OHRO and ORA PRB. The WRAIR HSPB will submit protocol amendments and modifications to the USAMRDC OHARO OHRO as per USAMRDC Command Policy 21.

The Informed Consent Form must be revised to concur with any significant amendment that directly affects participants and must also be reviewed and approved with the amendment. New participants enrolled in the study will be consented with the most recent approved consent form. Any participant already enrolled in the protocol will be informed about the revision and asked to sign the revised informed consent document if the modification directly affects the individual's participation in the protocol. A copy of the revised, signed, and dated informed consent document will be given to the participant. All original versions of the informed consent document will be retained in the protocol regulatory file.

Modifications or updates to the IBs will also be submitted as protocol amendments to the WRAIR IRB for review and approval.

10.1.2. Protocol Deviations

All subject-specific deviations from the protocol (e.g., failure to return for follow-up visits or blood collection within the time indicated in the protocol) are to be documented. These are considered non-emergent or minor deviations. The PI or designee will be responsible for identifying and reporting all deviations, which are defined as isolated occurrences involving a procedure that did not follow the protocol or protocol-specific procedure. Non- emergent/minor deviations will be reported annually in the continuing review report to the IRB and, if appropriate, in the final study report. Action taken in response to the deviation and the impact of the deviation will be assessed by the PI or subinvestigator and recorded as significant or nonsignificant.

For any protocol deviation that adversely affects the safety or rights of a subject or scientific integrity of the study (emergent deviation), the deviation will be reported within 24 hours to the sponsor's representative's clinical trial monitor or clinical trial manager and the IRB. Emergent/significant deviations should be reported promptly (within 48 hours) to the IRBs and the sponsor, upon becoming aware of the event, by telephone or email. A written report is required to be submitted by the PI to the WRAIR IRB and sponsor within 10 working days of knowledge of the significant deviation. Deviations will be reported by the WRAIR HSPB to the USAMRDC OHARO OHRO as per USAMRDC Command Policy 21.

Waivers and exemptions to protocol procedures are not allowed with the exception of immediate safety concerns and these will be discussed with the sponsor immediately upon occurrence or awareness to determine whether the subject should continue or discontinue study treatment.

10.2. Ethical Conduct of the Study

This study will be conducted in accordance with all applicable Federal and DoD human research protections requirements and the Belmont Principles of respect for persons, beneficence, and justice. The procedures set out in this protocol are designed to ensure that the sponsor's representative and all study personnel abide by the principles of the ICH GCP Guideline and the CFR. The PI confirms this by signing this protocol and FDA Form 1572.

Clinical research supported (directly or indirectly) by all HHS agencies, including the NIH, must comply with 45 CFR 46 (See 45 CFR 46.116), the OHRP regulations, in addition to all other applicable requirements.

10.3. Confidentiality

HIPAA requires that researchers obtain the subject's permission (HIPAA authorization) to use and disclose health information about the subject that is either created by or used in connection with this protocol. The information includes the entire research record and supporting information from the subject's medical records, results of laboratory tests, and both clinical and research observations made during the individual's participation in the protocol. In this protocol, the subject's health information will be collected and used to conduct the study; to monitor the subject's health status; to measure effects of the investigational product; to determine research results; and possibly to develop new tests, procedures, and commercial products. Health information is used to report results of research to the sponsor's representative and Federal regulators and may be reviewed during study audits for compliance with study plans, regulations, and research policies. After the study ends, each subject has the right to see and receive a copy of his/her information.

Release of personally identifiable genetic information may pose a risk of discrimination or increased difficulty in obtaining certain types of insurance for participants and their family members. However, every measure will be taken to keep the information confidential. An added protection of privacy is the Genetic Information Nondiscrimination Act of 2008 (Pub. L. 110-233), also known as "GINA", which is a federal law that prohibits discrimination in health insurance coverage and employment based on genetic information. However, GINA does not apply to employers with fewer than 15 employees. GINA's protections in employment do not extend to the US military, nor does it apply to health insurance through the TRICARE military health system, the Indian Health Service, the Veterans Health Administration, or the Federal Employees Health Benefits Program. Lastly, the law does not cover long term care insurance, life insurance, or disability insurance.

The Surgeon General (as the IND sponsor) the sponsor's representative, the representatives of USAMRDC OHARO OHRO, WRAIR IRB, the U.S. FDA, the U.S. DoD, and other regulatory agencies, as part of their responsibilities for insuring the protection of research participants (the DoD, and the FDA), are eligible to photocopy and review records related to this protocol as a part of their responsibility to protect the subjects of this protocol. In addition, representatives from these organizations may witness study procedures to ensure the safety of subjects.

When study information is communicated or published to support this protocol, no personal identifier will be used. The subject's identification number will be used in the event it becomes necessary to identify data specific to a single subject.

10.4. Compensation for Participation

Volunteers will be compensated $200 for their time, blood draws, and any inconvenience during each scheduled vaccination visit, $130 for other follow-up visits, and $50 for each screening visit. Unscheduled visits may be compensated up to $100 each if the PI deems appropriate. Participants who are ineligible to continue with the study and are subsequently withdrawn, will

be compensated $130 at their exit visit. This totals a maximum of $1,910 if the subject returns for each visit, and meets all study requirements for Diary Card return, and end of study completion fee of $350. The two phone call follow-ups, conducted after clinical study visits have concluded, are not compensated but must be completed in order to receive the study completion fee.

By regulation, federal employees can be compensated only for visits in which blood draws occur, and then only $50 per visit, unless the visits occur during off-duty hours or when they are on leave. If the volunteer is off-duty or on leave, he or she will be paid the same as non-federal personnel.

The total amount of compensation may vary depending on the number of visits completed. Civilian (non-Federal employee) volunteers who undergo injections may receive approximately $1,910 in compensation. Subjects who are federal employees who are part of a vaccine group are expected to receive approximately $900 in compensation, unless they are on approved leave or participating outside of normal duty hours.

Compensation for visits that are not specifically planned (scheduled) in the protocol, such as may be required to repeat labs to verify/clarify results or labs drawn to better evaluate abnormal lab values or AEs may be compensated at the discretion of the PI relative to the severity of the event and scope of evaluations. Compensation for transportation to the clinic for study visits may be provided at the discretion of the PI.

Participants will receive $50 for each referred person who then attends a screening session and meets all inclusion and none of the exclusion criteria.

Other than the payments discussed in this section, there is no other compensation available for your participation in this research. For all volunteers receiving more than $600.00, an IRS Form 1099 will be issued.

10.5. Medical Care for Research-Related Injury

All nonexempt research involving human subjects shall, at a minimum, meet the requirement of 32 CFR 219.116(a)(6).

If a subject is injured because of participation in this protocol and is a DoD healthcare beneficiary (e.g., military spouse or dependent), the subject is entitled to medical care for that injury within the DoD healthcare system, as long as the subject remains a DoD healthcare beneficiary. This care includes, but is not limited to, free medical care at Army hospitals or clinics.

If a subject is injured because of participation in this protocol and is not a DoD healthcare beneficiary, the subject is entitled to medical care for that injury at an Army hospital or clinic; medical care charges for care at an Army hospital or clinic will be waived. The subject is also entitled to care for that injury at other DoD (non-Army) hospitals or clinics, but such care for that injury at non-Army hospitals or clinics may be limited by time, and the subject's insurance may be billed. It cannot be determined in advance which Army or DoD hospital or clinic will provide care. If the subject obtains care for research-related injuries outside of an Army or DoD hospital or clinic, the subject or the subject's insurance will be responsible for medical expenses.

Participants who experience illness or injury arising from participation in the study should contact the study site immediately. An emergency contact card will be provided to study participants for this purpose. The site will either treat and care for the participant directly or refer the participant to an appropriate medical facility for care and treatment. Other than medical care and other payments as stated in this protocol, there is no other compensation available to the study participant from this research study.

10.6. COVID-19 Information

In light of the circulating severe acute respiratory syndrome Coronavirus-2 (SARS-CoV-2) and the associated coronavirus disease (COVID-19) pandemic, the clinic will provide to the participant at time of consent information regarding proposed modifications put in place to safeguard the health and well-being of participants. The modifications provide flexibility for conducting study visits and procedures when needed to monitor participant safety. These modifications are expected to be time-limited in relation to the COVID-19 pandemic. In consultation with the Sponsor and MHRP, the study team will determine when, in the future, the guidance is no longer applicable. WRAIR will be notified when such a determination is made.

Study personnel will follow the CTC SOP for conducting clinical trials in the setting of COVID-19 for the duration of the pandemic in order to ensure that proper precautions are in place for the minimization of COVID-19 exposure.

10.7. Source Documents

The primary source document for this study will be the participant's study folder. If separate research records are maintained by the investigator(s), the medical record and the research records will be considered the source documents for the purposes of auditing the study. The source documents will be retained at each site. During the study, all hard copy data collection forms will be locked in cabinets accessible only by appropriate study staff when not in use.

For this study, an electronic data capture (EDC) database system will be used for the collection of the study data in an electronic format. The EDC database system will be designed based on the protocol requirements, the approved eCRF layouts and specifications, and in accordance with 21 CFR Part 11. The eCRF layouts and specifications define and identify the applicable source data that will be collected and captured into the EDC database system. The applicable source data will be electronically transcribed by the site designee onto the electronic Case Report Forms (eCRF) (data entry screens) in the EDC database system. No source data will be recorded directly in the eCRF without a prior written record of the data. The investigator is ultimately responsible for the accuracy of the data transcribed on the eCRF. Data monitoring and management will be performed in the EDC database system by the study clinical monitor and DCAC. After database lock, data will be transferred from DCAC to the PI and Sponsor in a secure manner.

A detailed data management plan will be written and approved by the study team and the PI prior to study start, with approval by the sponsor's data manager in the USAMRDC ORA. All updates to the data management plan must be approved before study close-out and database lock.

10.8. Study and Site Closure

The PI, the sponsor's representative, the IRB, the USAMRDC Office of Research Protections, or the U.S. FDA, may stop or suspend the use of this product at any time. Otherwise, the study will be closed after all study procedures are completed.

At study closure, all electronic source documents will be filed in the sponsor's electronic Trial Master File (eTMF) and all hard copy documents will be delivered to USAMRDC ORA for archiving. Hard copy documents should be shipped to:

USAMRDC ORA
ATTN: Document Control Group 1430 Veterans Drive
Fort Detrick, MD 21702-5009

10.9. Written Informed Consent

The PI (or designated staff) will obtain PRB and IRB approval of the informed consent document prior to initiation of the study. The informed consent will be administered individually with strict respect for confidentiality. The investigators or their designees will present the protocol in lay terms to individual participants.

The PI or designee will solicit questions from participants on the purpose of the protocol, protocol procedures, and risks and allow the participant time to review the document. The person conducting consent will ensure that the participant understands the following principles of consent

- Participation is entirely voluntary
- The participant may withdraw from participation at any time
- Refusal to participate involves no penalty
- The individual is free to ask any questions that will allow him/her to understand the nature of the protocol

Participants will be asked to indicate on the consent how their samples and/or data may be used in future studies.

The designated individuals will obtain a copy of the consent form signed by each participant before any study-related procedures are initiated for that participant. The participant will also be given a copy of the participant event schedule. This signed and dated consent document will be retained as part of the study records. Each participant will receive a copy of the signed informed consent document.

Should the protocol be modified, the informed consent document must be reviewed and revised to reflect the changes to the protocol, if applicable. If a previously enrolled participant is directly affected by the change, the approved revision must be read, signed, and dated by the participant. A copy of the signed and dated consent must be provided to the participant.

The participant will be informed that a description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by US law.

11. ADMINISTRATIVE

11.1. Study Monitoring

Study monitoring will be the responsibility of the USAMRDC ORA. Upon successful approval of the protocol and establishment of the regulatory file, the clinical monitor will establish a clinical monitoring plan. To ensure that the investigator and the study staff understand and accept their defined responsibilities, the clinical monitor will maintain regular correspondence with the site and may be present during the course of the study to verify the acceptability of the facilities, compliance with the investigational plan and relevant regulations, and the maintenance of complete records. As needed, the clinical monitor may witness the informed consent process or other applicable study procedures to assure the safety of participants and the investigators' compliance with the protocol and GCPs.

Monitoring visits conducted by a sponsor's representative-designated clinical monitor will be scheduled to take place at the initiation of the study, during the study at appropriate intervals, and after the last participant has completed the study. A report of monitoring observations will be provided to the PI (for corrective actions), USAMRDC ORA, and the product manager. A copy of the study monitoring report will be provided to the WRAIR IRB, upon request.

11.2. Data Quality Assurance

All data management activities will be conducted according to the approved study-specific data management plan.

All participant data relating to the study will be recorded on printed or electronic case report forms unless transmitted to the sponsor or designee electronically (e.g., laboratory data).

The investigator is responsible for:

- Verifying that data entries are accurate and correct by physically or electronically signing the case report forms
- Maintaining accurate documentation (source data) that supports the information entered in the case report forms.
- Permitting study-related monitoring, audits, IRB review, and regulatory agency inspections and providing direct access to source data documents.

The sponsor or designee is responsible for the data management of this study, including quality checking of the data.

Study monitors will:

- Perform ongoing source data verification based on the study-specific monitoring plan to confirm that data entered into the case report forms by authorized site personnel are accurate, complete, and verifiable from source documents
- Verify that the safety and rights of participants are being protected

- Verify that the study is being conducted in accordance with the currently approved protocol and any other study agreements, International Conference on Harmonisation Good Clinical Practice, and all applicable regulatory requirements

11.3. Record Retention

Records and documents, including signed informed consent forms, pertaining to the conduct of this study will be retained by the investigator for 5 years after study completion

No records may be destroyed during the retention period without the written approval of the sponsor.

No records may be transferred to another location or party without written notification to the sponsor.

11.4. Pending Inspections/Issuance of Reports

The knowledge of any of the following will be reported immediately to the [IRB and/or the USAMRDC Office of Research Protections] and the sponsor's representative through USAMRDC Office of Regulated Activities Quality and Compliance Office:

- Pending compliance inspection/visit by the U.S. FDA, Office for Human Research Protections (Department of Health and Human Services), or other government agency concerning clinical investigation or research
- The issuance of Inspection Reports, FDA Form 483, warning letters, or actions taken by any regulatory agency, including legal or medical actions
- Any instances of serious or continuing noncompliance with the regulations or requirements

11.5. Reports and Publications

11.5.1. Annual Report to the FDA

The PI will be responsible for the preparation of a detailed annual synopsis of clinical activity, including AEs, for submission to USAMRDC ORA. Each annual report will summarize IND activity for 1 year beginning approximately 3 months before the IND FDA anniversary date. USAMRDC ORA will notify the PI of the due date with sufficient time for the PI to assemble the required information.

11.5.2. Final Clinical Study Report

A final clinical study report will be prepared in accordance with the following guidelines will be provided to the USAMRDC ORA for review and approval:

- Guidance for Industry: Submission of Abbreviated Reports and Synopses in Support of Marketing Applications
- ICH E3 Guideline "Structure and Content of Clinical Study Reports"
- The Comprehensive Table of Contents Headings and Hierarchy

- All applicable electronic Common Technical Document standards

The USAMRDC ORA will use this report to prepare/review the final clinical study report for submission to the FDA.

11.5.3. Continuing Review and Closeout Reports

The PI is responsible for submitting the required CRRs and associated documents to the WRAIR IRB for review and approval, allowing sufficient time for review and continuation determination prior to the established continuing review date. Summaries of enrollment and safety reports will be provided in the CRR, as they are made available by the sponsor.

After all study related activities, including data analysis are completed, a closeout report will be submitted as required to the same bodies. A closeout report will be submitted after 5 years or upon completion of the study, whichever occurs first. The WRAIR HSPB will forward CRRs and closeout report to the USAMRDC OHARO OHRO as per USAMRDC Command Policy 21.

11.5.4. Publication Policy

All data collected during this study will be used to support this investigational new drug application. Data may be published in the open medical or military literature with the identity of the participants protected. Anyone desiring to publish or present data obtained during the conduct of the study will forward the publication to the USAMRDC Office of Regulated Activities prior to submission.

11.6. Roles and Responsibilities

A list of study personnel will be maintained by the PI, or designee. The list must be available for review by clinical study monitors and representatives of the sponsor (ORA). Study roles and responsibilities are described in Appendix B.

12. LIST OF REFERENCES

1. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. N Engl J Med. Dec 3 2009;361(23):2209-20. doi:10.1056/NEJMoa0908492

2. Harmon TM, Fisher KA, McGlynn MG, et al. Exploring the Potential Health Impact and Cost-Effectiveness of AIDS Vaccine within a Comprehensive HIV/AIDS Response in Low- and Middle-Income Countries. PLOS ONE. 2016;11(1):e0146387. doi:10.1371/journal.pone.0146387

3. Stover J, Bollinger L, Hecht R, Williams C, Roca E. The impact of an AIDS vaccine in developing countries: a new model and initial results. Health Aff (Millwood). Jul-Aug 2007;26(4):1147-58. doi:10.1377/hlthaff.26.4.1147

4. UNAIDS 2020 Data (Joint United Nations Programme on HIV/AIDS) (2020).

5. Barouch DH. Challenges in the development of an HIV-1 vaccine. Nature. Oct 2008;455(7213):613-9. doi:10.1038/nature07352

6. Johnston MI, Fauci AS. An HIV vaccine--challenges and prospects. N Engl J Med. Aug 2008;359(9):888-90. doi:10.1056/NEJMp0806162

7. Koff WC, Russell ND, Walport M, et al. Accelerating the development of a safe and effective HIV vaccine: HIV vaccine case study for the Decade of Vaccines. Vaccine. Apr 2013;31 Suppl 2:B204-8. doi:10.1016/j.vaccine.2012.10.115

8. Pitisuttithum P, Gilbert P, Gurwith M, et al. Randomized, double-blind, placebo-controlled efficacy trial of a bivalent recombinant glycoprotein 120 HIV-1 vaccine among injection drug users in Bangkok, Thailand. J Infect Dis. Dec 2006;194(12):1661-71. doi:10.1086/508748

9. Flynn NM, Forthal DN, Harro CD, et al. Placebo-controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. J Infect Dis. Mar 2005;191(5):654-65. doi:10.1086/428404

10. Buchbinder SP, Mehrotra DV, Duerr A, et al. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. Lancet. Nov 2008;372(9653):1881-1893. doi:10.1016/S0140-6736(08)61591-3

11. Gray GE, Allen M, Moodie Z, et al. Safety and efficacy of the HVTN 503/Phambili study of a clade-B-based HIV-1 vaccine in South Africa: a double-blind, randomised, placebo-controlled test-of-concept phase 2b study. Lancet Infect Dis. Jul 2011;11(7):507-15. doi:10.1016/S1473-3099(11)70098-6

12. Hammer SM, Sobieszczyk ME, Janes H, et al. Efficacy trial of a DNA/rAd5 HIV-1 preventive vaccine. N Engl J Med. Nov 2013;369(22):2083-92. doi:10.1056/NEJMoa1310566

13. Gray GE, Bekker LG, Laher F, et al. Vaccine Efficacy of ALVAC-HIV and Bivalent Subtype C gp120-MF59 in Adults. N Engl J Med. 03 2021;384(12):1089-1100. doi:10.1056/NEJMoa2031499

14. Robb ML, Rerks-Ngarm S, Nitayaphan S, et al. Risk behaviour and time as covariates for efficacy of the HIV vaccine regimen ALVAC-HIV (vCP1521) and AIDSVAX B/E: a post-hoc analysis of the Thai phase 3 efficacy trial RV 144. Lancet Infect Dis. Jul 2012;12(7):531-7. doi:10.1016/S1473-3099(12)70088-9

15. Haynes BF, Gilbert PB, McElrath MJ, et al. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. N Engl J Med. Apr 2012;366(14):1275-86. doi:10.1056/NEJMoa1113425

16. Alam SM, Liao HX, Tomaras GD, et al. Antigenicity and immunogenicity of RV144 vaccine AIDSVAX clade E envelope immunogen is enhanced by a gp120 N-terminal deletion. J Virol. Feb 2013;87(3):1554-68. doi:10.1128/JVI.00718-12

17. Nitayaphan S, Pitisuttithum P, Karnasuta C, et al. Safety and immunogenicity of an HIV subtype B and E prime-boost vaccine combination in HIV-negative Thai adults. J Infect Dis. Aug 2004;190(4):702-6. doi:10.1086/422258

18. Pitisuttithum P, Nitayaphan S, Chariyalertsak S, et al. Late boosting of the RV144 regimen with AIDSVAX B/E and ALVAC-HIV in HIV-uninfected Thai volunteers: a double-blind, randomised controlled trial. Lancet HIV. 04 2020;7(4):e238-e248. doi:10.1016/S2352-3018(19)30406-0

19. Berman PW. Development of bivalent rgp120 vaccines to prevent HIV type 1 infection. AIDS Res Hum Retroviruses. Oct 1998;14 Suppl 3:S277-89.

20. Moody MA, Santra S, Vandergrift NA, et al. Toll-like receptor 7/8 (TLR7/8) and TLR9 agonists cooperate to enhance HIV-1 envelope antibody responses in rhesus macaques. J Virol. Mar 2014;88(6):3329-39. doi:10.1128/JVI.03309-13

21. Liao HX, Tsao CY, Alam SM, et al. Antigenicity and immunogenicity of transmitted/founder, consensus, and chronic envelope glycoproteins of human immunodeficiency virus type 1. J Virol. Apr 2013;87(8):4185-201. doi:10.1128/JVI.02297-12

22. Bradley T, Pollara J, Santra S, et al. Pentavalent HIV-1 vaccine protects against simian-human immunodeficiency virus challenge. Nat Commun. 06 2017;8:15711. doi:10.1038/ncomms15711

23. Cawlfield A, Genito CJ, Beck Z, et al. Safety, toxicity and immunogenicity of a malaria vaccine based on the circumsporozoite protein (FMP013) with the adjuvant army liposome formulation containing QS21 (ALFQ). Vaccine. 06 2019;37(29):3793-3803. doi:10.1016/j.vaccine.2019.05.059

13. APPENDICES

APPENDIX A. DAIDS TABLE FOR GRADING THE SEVERITY OF ADVERSE EVENTS

APPENDIX B. ROLES AND RESPONSIBILITIES

- Principal Investigator
  - Supervises sub investigators and protocol nurse coordinator
  - Is responsible for completing informed consent process
  - Trains study staff
  - Screens individuals and reviews all of the screening tests to determine eligibility of an individual to participate in the study
  - Makes the final participant eligibility decisions or appoints a qualified designee
  - Permits auditing by the site quality unit, the US Army MRDC Quality Unit, and the ORA Quality and Compliance Office
  - Assures adequate resources, time, and participant population to meet study requirements
  - Provides medical care for AEs
  - Documents protocol deviations
  - Reports all SAEs to (WRAIR IRB, MRDC) and sponsor's safety office (US Army Medical Research and Development Command) Product Safety Surveillance Office (PSSO), WRAIR IRB
  - Delegates investigational product accountability and administration
  - May reconstitute vaccine
  - May administer investigational product to study participants
  - Evaluates AEs for diagnosis, relationship, and severity
  - Ensures documentation and safety reporting
- Sub-investigator
  - Assists the PI in conduct of the study and may include (as indicated on the Delegation of Responsibilities Log) the following duties:
  - Is responsible for completing informed consent process
  - Assists in supervising study staff
  - Reviews all of the screening tests to determine eligibility of an individual to participate in the study

  - Screens individuals for protocol participation (physical examination and medical history)
  - Can make final eligibility decisions
  - May reconstitute vaccine as designated by the PI
  - May administer investigational product to study participants
  - Evaluates AEs
  - If designated by the PI, to assist the PI in protocol adherence and execution, management and reporting, and any other duties described under PI responsibilities

- Protocol Nurse/Study Coordinator
  - May be responsible for investigational vaccine accountability
  - May be responsible for completing informed consent process
  - Reviews all of the screening tests to determine eligibility of an individual to participate in the study
  - May reconstitute vaccine as designated by the PI
  - May administer investigational product to study participants
  - Schedules follow-up appointments
  - Follows up on all moderate and SAEs
  - Coordinates all study activity to include all participant visits and any non-compliant participants
  - Completes case report forms
  - Trains staff on protocols/protocol changes
  - Documents protocol deviations
- Protocol Support Staff
  - Screens study participants
  - Collects pre- and post-vaccination vital signs
  - Contacts study participants to collect AE data
  - Ensures efficient clinic flow
- Clinical Monitor

  - Conducts qualification, initiation, interim, and closeout study site visits
  - Monitors protocol adherence – reviews informed consent, standard operating procedures, regulations, and Good Clinical Practices
  - Monitors study and regulatory documents
  - Performs source data verification of case report form data
  - Checks investigational product accountability
- Statistical Support Team
  - Assist in the review of statistical analysis
  - Assist with the review of/support the writing of the final Clinical Study Report
- Data Manager
  - Provides study database end-user training to users requiring access to the study database
  - Oversees the data entry and data discrepancy management activities
  - Ensures data consistency, quality, and integrity
  - Performs Medical Dictionary for Regulatory Affairs (MedDRA) and World Health Organization (WHO) coding
  - Manages and maintains the study database
- Statistician
  - Analyzes the verified data according to the protocol, statistical analysis plan, and any amendments in place
  - Provides documentation of statistical findings to the PI for incorporation into required reports
- Sponsor's Safety Office
  - Receives safety events, reviews, follows until resolution and performs SAE case processing
  - Performs medical evaluation of SAEs and provides the final determination on relatedness and expectedness and determines expedited reporting
  - Queries site for additional information as appropriate
  - Reports expedited SAEs to regulatory authorities

- DAIDS Medical Officer
  - Responsible for protocol development and liaison for the submission to the DAIDS Protocol Sciences Review Committee (PSRC) for review. The DAIDS Medical Officer (MO) will be consulted on safety issues, if necessary, but will not have contact with study participants or identifiers.

APPENDIX C. PARTICIPANT VISIT SCHEDULE

APPENDIX D. INFORMED CONSENT FORM

APPENDIX E. TEST OF UNDERSTANDING

APPENDIX F. RECRUITMENT SCRIPT

APPENDIX G. INFORMATION SHEET REGARDING COMPENSATION TO FEDERAL PERSONNEL WHEN THEY PARTICIPATE IN RESEARCH

APPENDIX H. VISP LETTER

APPENDIX I. UNBLINDING LETTER

APPENDIX J. EMERGENCY CONTACT CARD

APPENDIX K. BRIEFING SLIDES

APPENDIX L. LIST OF POTENTIALLY IMMUNE-MEDIATED MEDICAL CONDITIONS

Gastrointestinal

- Celiac disease
- Crohn’s disease
- Ulcerative colitis
- Ulcerative proctitis

Liver

- Autoimmune cholangitis
- Autoimmune hepatitis
- Primary biliary cirrhosis
- Primary sclerosing cholangitis
- Metabolic diseases
- Addison’s disease
- Autoimmune thyroiditis (including Hashimoto thyroiditis)
- Diabetes mellitus type I
- Grave's or Basedow’s disease

Musculoskeletal

- Dermatomyositis, Polymyositis and Antisynthetase Syndrome
- Juvenile chronic arthritis (including Still’s disease)
- Mixed connective tissue disorder
- Polymyalgia rheumatic
- Psoriatic arthropathy
- Relapsing polychondritis
- Rheumatoid arthritis
- Scleroderma, including diffuse systemic form and CREST syndrome
- Spondyloarthritis, including ankylosing spondylitis, reactive arthritis (Reiter's Syndrome) and undifferentiated spondylarthritis
- Systemic lupus erythematosus

	• Systemic sclerosis
Neuroinflammatory:	• Acute disseminated encephalomyelitis, including site specific variants (e.g., non-infectious encephalitis, encephalomyelitis, myelitis, radiculomyelitis) • Cranial nerve disorders, including paralyses/paresis (e.g., Bell's palsy) • Guillain-Barré syndrome, including Miller Fisher syndrome and other variants • Immune-mediated peripheral neuropathies and plexopathies, including chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy and polyneuropathies associated with monoclonal gammopathy • Multiple sclerosis • Narcolepsy • Optic neuritis • Transverse myelitis • Myasthenia gravis, including Eaton-Lambert syndrome
Skin:	• Alopecia areata • Autoimmune bullous skin diseases, including pemphigus, pemphigoid and dermatitis herpetiformis • Cutaneous lupus erythematosus • Erythema nodosum • Morphea • Lichen planus • Psoriasis • Rosacea • Sweet's syndrome • Vitiligo
Vasculitides:	• Large vessels vasculitis including: giant cell arteritis such as Takayasu's arteritis and temporal arteritis

- Medium sized and/or small vessels vasculitis including: polyarteritis nodosa, Kawasaki's disease, microscopic polyangiitis, Wegener's granulomatosis, Churg–Strauss syndrome (allergic granulomatous angiitis), Buerger's disease thromboangiitis obliterans, necrotizing vasculitis and anti-neutrophil cytoplasmic antibody (ANCA) positive vasculitis (type unspecified), Henoch-Schonlein purpura, Behcet's syndrome, leukocytoclastic vasculitis

Other:

- Antiphospholipid syndrome
- Asthma and other immune-based lung diseases such as idiopathic pulmonary fibrosis, BOOP
- Autoimmune hemolytic anemia
- Autoimmune glomerulonephritis (including IgA nephropathy, glomerulonephritis rapidly progressive, membranous glomerulonephritis, membranoproliferative glomerulonephritis, and mesangioproliferative glomerulonephritis)
- Autoimmune myocarditis/cardiomyopathy
- Autoimmune thrombocytopenia
- Goodpasture syndrome
- Pernicious anemia
- Raynaud's phenomenon* note: Up to 10% of healthy female population has this without ever developing an autoimmune disease
- Sarcoidosis
- Sjögren's syndrome
- Stevens-Johnson syndrome
- Uveitis

APPENDIX M. RISK ASSESSMENT TOOL

ATTACHMENT 1: PROTOCOL TEAM ROSTER

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ATTACHMENT 2: TABLE OF EXTERNAL COLLABORATORS

(When external collaborators are identified, they will be added here.)