PROTOCOL RV460

A Randomized, Double-Blind Phase 1 Trial to Evaluate the Safety and Immunogenicity of Priming with Env-C Plasmid DNA Vaccine Alone, with Different Adjuvants, or with an Adjuvanted HIV Env gp145 C.6980 Protein Vaccine and Boosting with the Adjuvanted HIV Env gp145 C.6980 Protein Vaccine with or without the Env-C Plasmid DNA Vaccine in Healthy HIV Uninfected Adults in Kenya

Short Title of Study:
Comparative Adjuvant Study for Env-C DNA and Protein Vaccines in Kenya

Study Conducted By:
United States Army Medical Research Directorate-Africa (USAMRD-A), Clinical Research Centre, Kericho, Kenya

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LIST OF ABBREVIATIONS

AABB	American Association of Blood Banks
Ab	Antibodies
ACTG	Aids Clinical Trials Group
ADC	Antibody-Dependent Complement
ADCC	Antibody-Dependent Cell-Mediated Cytotoxicity
ADCP	Antibody-Dependent Cell-Mediated Phagocytosis
ADCVI	Assay and Antibody-Dependent Cell Mediated Viral Inhibition
AE	Adverse Event
AESI	Adverse Events of Special Interest
AFRIMS	Armed Forces Research Institute of Medical Sciences
AIDS	Acquired Immune Deficiency Syndrome
AIDSVAX	HIV gp120 in Aluminum Hydroxide (Rehydragel®)
ALF	Army Liposome Formulation
ALF43	ALF43 Army Liposome Formulation with Monophosphoryl Lipid A (MPLA) and 43% Cholesterol
ALF43A	ALF43 Combined with Aluminum Salt-Based Adjuvant Such as Rehydragel® or Alhydrogel®
AlOH	Aluminum Hydroxide
ALT	Alanine Aminotransferase
Alum	Aluminum Salt
ALVAC	HIV Antigen-Expressing Viral Vector - Canarypox Vector
APGAR	American Pediatric Gross Assessment Record
AST	Aspartate Aminotransferase
AVEG	AIDS Vaccine Evaluation Group
BP	Blood Pressure
BB-IND	Biologics Investigational New Drug Application
BB-MF	Biologics Master File
BMI	Body Mass Index
bNAbs	Broadly Neutralizing Antibodies

CA	Cooperative Agreement
CAB	Community Advisory Board
CAP	College of American Pathologists
CBC	Complete Blood Count
CCR7	C-C Chemokine Receptor Type 7
CD4	Cluster of Differentiation
CDMRP	Congressionally Directed Medical Research Programs
CfaE	ETEC Adhesion Antigen
CFR	Code of Federal Regulation
CK	Creatine Kinase
COO	Clinical Operations Office, MHRP
CRC	Clinical Research Center, KEMRI, USAMRD-A
CRFs	Case Report Forms
CRFs	HIV Circulating Recombinant Forms
CROMC	Clinical Research Products Management Center, NIAID
CXCR5	C-X-C Chemokine Receptor Type 5
DAERS	DAIDS Adverse Experience Reporting System
DAIDS	Division of AIDS
DCAC	Data Coordinating and Analysis Center, MHRP
dmLT	Double Mutant Heat-Labile Enterotoxin
DMPC	Dimyristoylphosphatidylcholine
DMPG	Dimyristoylphosphatidylglycerol
DNA	Deoxyribonucleic Acid
DoD	Department of Defense
dscCfaE	Donor Strand Complemented Cfae (Recombinant *E. coli* Protein)
DTH	Delayed-Type Hypersensitivity
EAE	Expedited Adverse Events
EC	Ethics Committee
ECCT	Kenya Expert Committee on Clinical Trials, PPB

ELISPOT	Enzyme-Linked Immunospot
EMA	European Medicines Agency
ENV-C Plasmid DNA	HIV-1 gp120 (93MW965.26) DNA
Env	Envelope
ETEC	Enterotoxigenic *E. coli*
FDA	Food and Drug Administration
FDR	False Discovery Rate
FSH	Follicle-stimulating Hormone
GCLP	Good Clinical Laboratory Practices
GCP	Good Clinical Practices
GI	Gastrointestinal
HIV Env gp145 C.6980	gp145 C.6980 Clade C HIV-1 Env Protein
GSK	GlaxoSmithKline
GU	Genitourinary
H_20	Water
HBsAg	Hepatitis B Surface Antigen
HIV-1	Human Immunodeficiency Virus 1
HJF	Henry M. Jackson Foundation for the Advancement of Military Medicine
HLA	Human Leukocyte Antigen
HRPO	Human Research Protections Office
HSP	Henoch-Schönlein Purpura
HSPB	Human Subject Protection Branch
HVTN	HIV Vaccine Trials Network
IB	Investigator's Brochure
IBC	Institutional Biosafety Committee
ICF	Informed Consent Form
ICH	International Conference on Harmonization
ICOS	Inducible T Cell Co-Stimulator
ICS	Intra-Cellular Cytokine Staining
ID	Intradermal

IFN	Interferon
IFN-γ	Interferon-Gamma
Ig	Immunoglobulin
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IL	Interleukin
IL-21	Interleukin-21
IM	Intramuscular
IMPAACT	International Maternal Pediatric Adolescent AIDS Clinical Trials
IND	Investigational New Drug Application
INR	International Normalized Ratio
IRB	Institutional Review Board
IT	Information Technology
IUD	Intrauterine Device
IV	Intravenous
KCRH	Kericho County Referral Hospital
KEMRI	Kenya Medical Research Institute
KSH	Kenyan Shillings
LH	Luteinizing Hormone
L(MPLA)	Liposome Containing Monophosphoryl Lipid A
LN	Lymph Nodes
LPA	Lymphoproliferation Assays
LT	Heat-Labile Enterotoxin (*E. coli*)
mAb	Monoclonal Antibody
MD	Maryland
MF	Master File
MHRP	US Military HIV Research Program
mL	Milliliter
mLT	Mutant LT

MO	Medical Officer, DAIDS
MoH	Kenya Ministry of Health
MPLA	Monophosphoryl Lipid A
MPL®	Monophosphoryl Lipid A (Owned by GSK)
USAMRD-A	United States Army Medical Research Directorate-Africa
MS	Multiple Sclerosis
MVA	Modified Vaccinia Ankara
NaCl	Sodium Chloride
NCT	National Clinical Trial
NHP	Nonhuman Primate
NIAID	The National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NK	Natural Killer Cell
NSAIDS	Non-Steroidal Anti-Inflammatory Drugs
OBA	Office of Biotechnology Activities, NIH
ORP	Office of Research Protection
ORTA	Office of Research Technology and Applications
PBMC	Peripheral Blood Mononuclear Cell
PD-1	Programmed Cell Death Protein-1
PEP	Post-Exposure Prophylaxis
PI	Principal Investigator
PID	Participant Identification
PIR	Post-Injection Reactogenicity
PPB	Pharmacy and Poisons Board
PPE	Personal Protective Equipment
PSRT	Protocol Safety Review Team
QS-21	Quillaja Saponaria 21 (Fraction 21 of Purified Extract)
R4P	HIV Research for Prevention
RAC	Recombinant DNA Advisory Committee

RR	Respiratory Rate
RSC	Regulatory Support Center, DAIDS
RT	Room Temperature
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SERU	Scientific and Ethics Review Unit, KEMRI
SIV	Simian Immunodeficiency Virus
SMC	Safety Monitoring Committee, MHRP
SOE	Schedule of Evaluations
SOP	Standard Operating Procedure
STI	Sexually Transmitted Infections
SUSAR	Serious, Unexpected, Suspected Adverse Drug Reaction
TB	Tuberculosis
TCI	Transcutaneous Immunization
TEMP	Temperature
Tfh	T Follicular Helper Cell
Tfr	T Follicular Regulatory
TNF-α	Tumor Necrosis Factor
TOU	Test of Understanding
UNAIDS	Joint United Nations Programme on HIV/AIDS
UPIRTSO	Unanticipated Problems Involving Risk to Participants or Others
USAMMDA	United States Army Medical Material Development Activity
USAMRAA	United States Army Medical Research Acquisition Activity
USAMRDC	U.S. Army Medical Research and Development Command
USD	U.S. Dollars
VISP	Vaccine-Induced Seropositivity
VISR	Vaccine-Induced Seroreactivity
WB	Whole Blood
WHO	World Health Organization

WRAIR Walter Reed Army Institute of Research

PROTOCOL SUMMARY

Title: A Randomized, Double-Blind Phase 1 Trial to Evaluate the Safety and Immunogenicity of Priming with Env-C Plasmid DNA Vaccine Alone, with Different Adjuvants, or with an Adjuvanted HIV Env gp145 C.6980 Protein Vaccine and Boosting with the Adjuvanted HIV Env gp145 C.6980 Protein Vaccine with or without the Env-C Plasmid DNA Vaccine in Healthy HIV Uninfected Adults in Kenya

Design: RV460 is an exploratory study that will assess the safety, tolerability, and immunogenicity of a vaccine regimen consisting of priming with an Env-C Plasmid DNA vaccine (with or without novel adjuvants) when given with or without adjuvanted HIV Env gp145 C.6980 protein vaccine and boosting with adjuvanted gp145 C.6980 protein with or without the gp120 DNA vaccine. The study will be carried out at the KEMRI/US Army Medical Research Directorate-Africa (USAMRD-A), Clinical Research Centre, Kericho; and 126 healthy adults will be enrolled. Participants enroll randomly into one of seven groups which have 15/3 vaccine/placebo recipients per group. Each participant will receive six injections (prime at week 0, 4, 12; boost at week 20, 32 and 56) followed up for a total of 108 weeks.

Study objectives will be met by the testing of blood samples from all participants. Willing and eligible participants have the option to provide lymph node and mucosal secretion samples (semen, cervicovaginal, rectal), which will provide the data for objectives 2 and the sub-objectives.

Phase: 1

Population: 126 Healthy, HIV-uninfected male and female participants aged 18 to 40 years in Kericho, Kenya

Number of Sites: 1

Products:

- Env-C Plasmid DNA (HIV-1gp120 (93MW965.26) DNA)
- HIV Env gp145 C.6980 protein
- Double Mutant of Heat Labile E. coli enterotoxin (dmLT)
- ALF43: Army Liposome Formulation—43% Cholesterol*
- Aluminum Hydroxide Suspension—Fluid Gel (Rehydragel®) *

*From this point forward, Army Liposome Formulation—43% Cholesterol will be referred to as ALF43 and Aluminum Hydroxide Suspension—Fluid Gel will be referred to as Rehydragel®

Objectives:

Primary

Exploratory study to assess the safety and tolerability (including reactogenicity) of Env-C Plasmid DNA given IM alone, admixed with ALF43 adjuvant, topically applied with dmLT adjuvant, or given with an adjuvanted HIV Env gp145 C.6980 protein and followed by adjuvanted HIV Env gp145 C.6980 boost IM vaccinations, with or without Env-C Plasmid DNA.

Secondary

Assess the immunogenicity of the various study vaccine combinations:

1. Determine whether the adjuvants under study improve the immunogenicity of the Env-C Plasmid DNA priming.
2. Determine whether the addition of ALF43 to the Rehydragel® –HIV Env gp145 C.6980 protein boost further improves the immune response to HIV Env gp145 C.6980 protein.
3. Determine whether adjuvants improve humoral responses across vaccination regimens.
4. To evaluate the influence of various adjuvants on cellular immune responses.
5. Describe mucosal humoral responses across vaccination regimens in cervicovaginal and rectal secretions and semen.

Exploratory

1. Characterize B-cell functional specificities for each vaccination regimen.
2. Characterization of innate immunity.
3. Assess the innate/gene expression induced across vaccination regimens (DNA microarray, RNA sequencing) to compare relative influence of various

adjuvants in the gene expression profiles sorted T cell, B cell, and innate cell subsets.

4. Perform systems serology analyses.

Sub-objective

In participants who elect to undergo lymph node biopsy, characterize HIV-specific responses, T cell activation, T follicular helper (Tfh) cell and B cell distribution in intact lymph nodes (excision of intact peripheral inguinal lymph node) at weeks 14 and 58.

Duration of Individual Subject Participation	Each participant will be enrolled for 108 weeks (105 weeks of clinic visits and then contact by phone once weekly for an additional three weeks to inquire about medically attended adverse events).
Estimated Time to Last Subject/Last Visit	Approximately 137 weeks (about 2 years, 8 months)

LAY SUMMARY

The Kenya Medical Research Institute (KEMRI) in collaboration with the U.S. Military HIV Research Program (MHRP) is conducting a study on HIV vaccines. A vaccine is a medical product given to prevent certain diseases, most often infectious diseases. The vaccine may educate the human body to form a defensive response to try to prevent the disease from the beginning or taking hold of the body. This defensive response called the immune response, and it is the body's way to fight infections.

The experimental HIV vaccines tested in this study are Env-C Plasmid DNA and HIV Env gp145 C.6980 protein given with different adjuvants to increase the immune response. An adjuvant is a substance added to vaccines that can help to make the vaccine more effective by improving the immune response or causing the immune response to last longer than without the adjuvant. These adjuvants are mixed with the vaccines and injected into the muscle or placed on top of the skin. The HIV vaccines contain a piece of genetic material or a protein copied from the HIV virus cover (Env), but they do not contain the virus itself. The vaccines cannot cause HIV infection or Acquired Immune Deficiency Syndrome (AIDS).

The purpose of this study is to find out if the study vaccines with adjuvants cause side effects and are tolerable, whether humans respond, (develop immune responses) to the vaccines, and how long the effects of study vaccines last. This study will also compare the effects (both good and bad) of the study vaccines with adjuvants and adjuvant patch to those of placebo injections and placebo patch. The placebo will consist of saline (sterile saltwater), look just like the study vaccines with adjuvants and will be given the same way, but will have no active vaccine or adjuvant in it.

Study objectives will be met by the testing of blood samples from all participants. Willing and eligible participants have the option to provide lymph node and mucosal secretion samples (semen, cervicovaginal, rectal) will provide the data for objectives 2 and the sub-objections.

The sponsor of this study is National Institute of Allergy and Infectious Diseases (NIAID) Division of AIDS (DAIDS), and the Military HIV Research Program (MHRP), a part of the Walter Reed Army Institute of Research (WRAIR), funds it. A total of 126 participants will take part in this study.

ABSTRACT

A safe and effective HIV vaccine would be the cornerstone for cost-effective, user-independent prevention methods against HIV infection. Most of the successfully licensed vaccines for prevention of infections have historically relied upon aluminum salt (alum) formulations as adjuvants because they generally improve protective antibody magnitudes over the vaccine antigen alone and are reliably safe.

Antibodies are the most commonly recognized correlate of vaccine protection from infection; however, the possible protective HIV antibody levels, which were induced by the modestly protective RV144 vaccine regimen with alum, rapidly decayed. Potentially, a novel, highly selective adjuvant combined with the correctly sequenced HIV antigen could slow antibody decay, increase antibody magnitude, and induce antibodies of appropriate functional response.

RV460 is an exploratory study that will assess the safety, tolerability, and immunogenicity of a vaccine regimen consisting of priming with an Env-C Plasmid DNA vaccine (with or without novel adjuvants) when given with or without adjuvanted HIV Env gp145 C.6980 protein vaccine and boosting with adjuvanted gp145 C.6980 protein with or without the gp120 DNA vaccine. The study will be carried out at the KEMRI/US Army Medical Research Directorate-Africa (USAMRD-A), Clinical Research Centre, Kericho; and 126 healthy adults will be enrolled. Participants enroll randomly into one of seven groups which have 15/3 vaccine/placebo recipients per group. Each participant will receive six injections (prime at week 0, 4, 12; boost at week 20, 32 and 56) followed up for a total of 108 weeks.

Study objectives will be met by the testing of blood samples from all participants. Willing and eligible participants have the option to provide lymph node and mucosal secretion samples (semen, cervicovaginal, rectal), which will provide the data for objectives 2 and the sub-objections. Exploratory objectives may be met by genetic analysis including but not limited to Human Leukocyte Antigen (HLA) subtyping.

PRECIS

Title

A Randomized, Double-Blind Phase 1 Trial to Evaluate the Safety and Immunogenicity of Priming with Env-C Plasmid DNA Vaccine Alone, with Different Adjuvants, or with an Adjuvanted HIV Env gp145 C.6980 Protein Vaccine and Boosting with the Adjuvanted HIV Env gp145 C.6980 Protein Vaccine with or without the Env-C Plasmid DNA Vaccine in Healthy HIV Uninfected Adults in Kenya

Clinical Phase

Phase 1

Study Duration

Participants are seen in the clinic for 105 weeks following enrollment and randomization. Participants will be contacted by phone once weekly for an additional three weeks to inquire about medically attended adverse events. The total study-related activities may last up to 6 years (2-3 years screening and clinical activities; 2-3 years of laboratory assays and data analyses).

Participants

Healthy, HIV-uninfected male and female volunteers aged 18 to 40 years.

There will be 18 participants per group (15 vaccine recipients and 3 placebo recipients). Investigators will be blinded to vaccine versus placebo within each group but not across groups. Participants will be block randomized. Group 1 (with a prime of Env-C Plasmid DNA IM alone at weeks 0, 4, 12) will be fully enrolled first. After the 18 participants in Group 1 are enrolled, the remaining groups, 2-7, will begin enrolling participants. Once 24 participants in Groups 2-7 have been enrolled (four from each group) and received their first vaccinations, enrollment and vaccinations in Groups 2-7 will be paused for the time it takes the PSRT to review (typically 3 to 14 days) blinded safety data from these 24 participants covering the period immediately after their first vaccination through Day 7. 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.

When safety has been confirmed with PSRT review, the remaining 14 participants in each of Groups 2-7 will be enrolled, and vaccinations will continue until the first 24 participants in Groups 2-7 (four per group) have received their first boost dose (at 20 weeks—to create a time gap between boost vaccinations of the first cohort and the remaining study participants).

A second scheduled pause (this pauses boost vaccinations only; prime vaccinations can continue) will occur for Groups 2-7 after the same set of participants reviewed during the first scheduled pause (the first 24 participants (four per group) from Groups 2-7) has received its first boost (Group 1 can continue receiving vaccinations). This pause will review blinded safety data from these 24 participants, covering the period from the first boost through Day 7 after that boost. At both scheduled pauses, the PSRT will consult the SMC regarding the pause if either of the following conditions are met: 1) one (or more) participants experience an SAE that is 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. Boost vaccinations for Groups 2-7 will resume once safety data from the booster sentinel cohort have been reviewed by the PSRT and they have concluded it is safe to proceed.

Specific details of the randomization will be described in a separate randomization plan.

1 OBJECTIVES AND ENDPOINTS

1.1 OBJECTIVES

1.1.1 PRIMARY

Exploratory study to assess the safety and tolerability (including reactogenicity) of HIV Env-C Plasmid DNA given IM alone, admixed with ALF43 adjuvant, topically applied with dmLT adjuvant, or given with an adjuvanted HIV Env gp145 C.6980 protein and followed by adjuvanted HIV Env gp145 C.6980 protein boost IM vaccinations, with or without Env-C Plasmid DNA.

1.1.2 SECONDARY

1. Assess the immunogenicity of the various study vaccine combinations: Determine whether the adjuvants under study improve the immunogenicity of the HIV Env-C Plasmid DNA priming.
2. Determine whether the addition of ALF43 to the Rehydragel® /HIV Env gp145 C.6980 protein boost further improves the immune response to HIV Env gp145 C.6980 protein.
3. Determine whether adjuvants improve humoral responses across vaccination regimens.
4. To evaluate the influence of various adjuvants on cellular immune responses.
5. Describe mucosal humoral responses across vaccination regimens in cervicovaginal and rectal secretions and semen.

1.1.3 EXPLORATORY

1. Characterize B-cell functional specificities for each vaccination regimen.
2. Characterization of innate immunity.
3. Assess the innate/gene expression induced across vaccination regimens (DNA microarray, RNA sequencing) to compare relative influence of various adjuvants in the gene expression profiles sorted T cell, B cell and innate cell subsets.
4. Perform systems serology analyses.

Exploratory objectives may be met by genetic analysis including but not limited to Human Leukocyte Antigen (HLA) subtyping.

1.1.3.1 Sub-objective

In participants who elect to undergo lymph node biopsy, characterize HIV-specific responses, T cell activation, T follicular helper (Tfh) cell and B cell distribution in intact lymph nodes (excision of intact peripheral inguinal lymph node) at weeks 14 and 58.

1.2 ENDPOINTS

1.2.1 PRIMARY

Safety endpoints will be the solicited AE (local and systemic reactogenicity), and related AEs and SAEs.

1.2.2 SECONDARY

1. Comparison of plasma IgG binding antibodies to gp120 and V1V2 in terms of magnitude, durability, and area under the curve both after initial Env-C Plasmid DNA priming series among groups with differing Env-C Plasmid DNA priming regimens.

2. Comparison of plasma IgG binding antibodies to gp120 and V1V2 in terms of magnitude, durability, and area under the curve between groups with and without Rehydragel® after HIV Env gp145 C.6980 protein boosting.

3. Characterize plasma IgG and IgA binding antibodies to HIV gp120, V1V2, neutralizing antibodies, and non-neutralizing effector functions such as ADCC and ADCP with emphasis on RV144 immune correlates of risk of HIV acquisition.

4. Characterize and assess the magnitude of cell-mediated immune responses elicited across vaccination regimens including but not limited to antigen - specific CD4 and CD8 T cell responses and polyfunctionality, induced cytokines, chemokines and other inflammatory markers.

5. Characterize mucosal humoral responses in cervicovaginal and rectal secretions and semen including, but not limited to, plasma IgG and IgA binding antibodies to HIV Env proteins, IgG and IgA subclass, and functional assays.

1.2.3 EXPLORATORY

1. 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.
2. 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.
3. Compare relative influence of various adjuvants in the gene expression profiles sorted T cell, B cell and innate cell subsets using RNAseq and related methods to quantify and compare gene expression from various sorted cell subsets among study groups.
4. Utilizing systems serology analyses, link various immunological measurements, including antibody-binding data to antibody effector functional

assays to evaluate immunologic response patterns or signatures to various adjuvants.

1.2.4 SUB-SET OF PARTICIPANTS THAT ELECT TO UNDERGO LYMPH NODE BIOPSY

Between each adjuvant group, compare the magnitude and frequency of HIV-specific TfH cells in total lymph nodes and lymph node follicles, TfH and B cell distribution between intra and extrafollicular LN zones, and levels of cellular activation using ICS, immunohistochemistry, transcriptional analyses, and related techniques at weeks 14 and 58.

2 BACKGROUND AND SCIENTIFIC RATIONALE

2.1 BACKGROUND

2.1.1 PURPOSE

In the RV144 trial, a canarypox viral vector without an adjuvant and gp120 in combination with the conventional adjuvant, aluminum hydroxide was tested. The proposed clinical trial, RV460, will explore the immune responses of adjuvanted Env-C Plasmid DNA in the context of the prime/boost vaccine platform. RV460 represents an innovative strategy, guided by the results of RV144, to ascertain the ability of Env-C Plasmid DNA and HIV Env gp145 C.6980 protein antigens tested in combination with various adjuvants in prime and boost combinations given Env-C Plasmid DNA in conjunction with adjuvants to induce potent and durable adaptive immunity against HIV-1 with emphasis on antibodies to the V1V2 protein loop.

DNA is easily manufactured at low cost and has an increased stability profile compared to recombinant proteins [1], [2], [3, 4]. Thus, it is the ideal candidate for use as a vaccine. In general, DNA vaccines are highly effective in small animal models, including mice and rabbits, but are poorly immunogenic in humans [3], [5]. Therefore, different immunization and formulation approaches have been explored to increase the immunogenicity of the DNA. One strategy has been to immunize with DNA as an immune system priming immunization followed by a boost immunization with protein. This method has been effective, but is cumbersome, requiring multiple immunizations and vaccine components. In addition, a safe, effective adjuvant may also achieve a "dose-sparing" effect, i.e., the adjuvant may result in a reduction in the vaccine component dosage needed to elicit the desired immune response. Following immunization, the DNA is transcribed and translated into the protein antigen for uptake by antigen presenting cells for induction of antigen-specific immune responses. In the presence of adjuvants, this process may become more efficient, since one of the roles of the adjuvant is to activate and recruit antigen presenting cells into the injection site. These antigen-presenting cells take up antigen and migrate to the lymph nodes. There they will present the antigen to T follicular helper (Tfh) cells that provide help to both B and T cells to induce high antibody titers and potent cellular immunity.

Most of the animal and human studies with DNA vaccines have centered on the use of molecular adjuvants, such as CpG or cytokines, delivered as DNA together with the DNA of the antigen [6]. However, none of these molecular adjuvants has been successful in inducing high titer durable antibody or cellular immune responses in humans. Several studies have used additional non-traditional adjuvants to improve DNA vaccines. LT and cholera toxin B (CTB) patches have been used at the site of DNA immunization in mice demonstrating increased immune responses [7], [8], [9]. CTB has also been given as a mucosal adjuvant with an HIV DNA vaccine and was shown to enhance cellular systemic and mucosal genital tract immunity delivery [9]. An aluminum adjuvanted DNA vaccine for *Toxoplasma gondii* had a modest improvement in immunogenicity in mice [10]. MPLA has also been used in emulsions with DNA vaccines demonstrating enhance immune responses compared to DNA alone [11], [12]. Numerous studies have used various liposome or lipid-based nanoparticle formulations for DNA vaccines [3]. Gram-negative bacterial ghosts, which contain lipid A, have been used as liposome formulations to deliver DNA vaccines, have been shown to induce potent humoral and cellular immune responses in animals [13], [14], [15].

Although aluminum salts are widely used and have a proven safety record, the magnitude and durability of antibodies to HIV-1 antigens or polyfunctional T cells is low. In addition, combinations of adjuvants with different mechanisms of action may have additive or synergistic effects and may affect the magnitude and the quality of the antibody responses more than individual adjuvants. For example, ALF43+Rehydragel® (ALF43A) is an alternative to the highly successful, but proprietary, adjuvants such as AS04 (monophosphoryl lipid A (MPLA) adsorbed to aluminum salt) used in the GlaxoSmithKline (GSK) FDA-licensed vaccines, Hepatitis B (ENGERIX-B) and HPV (Cervarix™). The RV460 trial builds on the use of non-traditional adjuvants to improve the immune response of DNA-based vaccines. Evaluation of Env-C Plasmid DNA plasmid alone, with ALF43, or transcutaneous immunization with dmLT at the site of Env-C Plasmid DNA immunization will enable a mechanistic description of the adjuvants' effects on immune responses induced by DNA containing HIV Env in humans. The comparison of Rehydragel® and ALF43+Rehydragel® as adjuvants for HIV Env gp145 C.6980 protein will further enable mechanistic comparisons. A Phase 1 study IND to evaluate the safety, tolerability, and immunogenicity of an HIV vaccine that tests HIV Env gp145 C.6980 either at a 300 µg HIV Env gp145 C.6980 (high dose) or 100 µg (low dose) admixed with aluminum hydroxide adjuvant in healthy, HIV-1-uninfected adults in the United States (HVTN 122/Clinicaltrials.gov identifier NCT03382418), was submitted to the United States Food and Drug Administration (FDA) by DAIDS/NIAID with their network, the HIV Vaccine Trials Network (HVTN). The trial is underway and will provide additional immunogenicity and safety information for comparison of the adjuvant effects at the completion of both trials. This study of Env-C Plasmid DNA and HIV Env gp145 C.6980 protein that is combined with ALF43, tested in combination with various adjuvants in the same immunization, will provide exploratory data critical to describing potential mechanistic and phenotypic differences with each adjuvant.

In study arm 7, co-immunization of Env-C Plasmid DNA and HIV Env gp145 C.6980 protein-based HIV-1 vaccines are administered in the same injection site. This co-immunization strategy using DNA and protein vaccines can improve both humoral and cellular immune antigen-specific immune responses over either vaccination alone or both in series [16], [17], [18] and it can even improve control of viremia after simian immunodeficiency virus (SIV) challenge in a macaque model [18]. Preclinical studies of DNA and protein suggest that colocalizing simultaneous administration of the DNA and protein vaccines may optimize the immune response. In a study in nonhuman primates by Jalah et al., [16] the group that received co-administered DNA and protein had significantly higher levels of Env-binding antibodies (Abs) and higher avidity that persisted compared to the other groups that received either DNA alone or DNA prime with a protein boost. Also, Env-binding Abs and avidity correlated with slower SIV acquisition and in addition, cytotoxic CD4+ effector memory inversely correlated with peak viral load [18]. In another study, the Haigwood group demonstrated in rabbits that DNA + protein co-administration was superior to protein administration alone regarding antibody kinetics, magnitude, avidity, and neutralization potency [19]. A recent study with nonhuman primates reported the co-immunization of DNA vaccines with recombinant Env proteins mixed together with liposomes containing MPLA and imiquimod (TLR4 and TLR7 agonists, respectively) or liposomes containing MPLA and QS21 resulted in robust and durable cellular and humoral immune responses that efficiently disseminated to mucosal sites and protected against simian immunodeficiency virus (SIV) infection [18] Similarly, in another study of co-immunization using DNA and gp120 formulated in an oil-in-water emulsion containing a TLR4 agonist also resulted in the highest systemic binding and neutralizing antibodies to homologous or heterologous HIV Env as well as the highest Env-

specific IgG in saliva compared to DNA alone or DNA prime followed by protein boost [16]. In addition, the inclusion of DNA in the vaccine significantly increased the longevity of systemic humoral immune responses. Previously, the HVTN 096 (NCT01799954) and HVTN 105 (NCT02207920) studies reported that NYVAC vector or DNA co-immunization with AIDSVAX® B/E did not result in increased immune responses. These studies only used Rehydragel® as an adjuvant. The NHP studies discussed above demonstrate that inclusion of a TLR4 agonist in DNA protein co-immunization can induce increased and potent immune responses of longer duration. Group 7 was included in this study to investigate the immune responses induced using a TLR4 agonist in liposomes (ALF43) in humans.

However, the benefits of co-immunization with proteins and vectors have not been widely studied in humans. A recent study demonstrated that co-immunization of a modified vaccinia Ankara (MVA) influenza vaccine with a protein influenza vaccine in humans improved both memory T-cell responses and humoral responses to specific influenza antigens compared to either modality alone [20] presumably by enhancing responses to the protein via the intrinsic adjuvant activity of the viral vector to induce greater T-cell responses, or by triggering T helper cells in lymph nodes. RV460 proposes to characterize antigen-specific responses by transcriptional and antibody profiling and Tfh, memory and B-cell functional specificities across peripheral blood, peripheral lymph nodes (LN) compartments, gastrointestinal/genitourinary (GI/GU) mucosal immune cells. MHRP has performed mucosal and lymph node biopsies in multiple studies. These include RV398 and RV419 (mucosal and lymph node in Kenya, Uganda, Tanzania), and RV306 (NCT01931358). RV254/SEARCH010 (NCT03032575), RV397, RV398, RV405, RV411, RV412 in Thailand [21], [22], [23], [24]. Evaluations of LN architecture and cells before and after vaccination have used state-of-the-art assays to probe the relationship among changes in frequency and expansion of antigen-specific CD4+ and CD8+ T cells before and after vaccination [25]. Studies of LN architecture require intact lymph nodes obtained by excisional LN biopsies. Additional immune assessments will include the relative and absolute frequencies of Tfh cells and B cells and the quantification of the expression of inflammatory markers on these cell surfaces. Malaria groups at NIAID and WRAIR, are examining if and how different adjuvants promote CD4+ T cells to adopt a suppressive T follicular regulatory (Tfr) phenotype and if that differentiation occurs in LN of non-human primates (NHP). We propose to quantify the kinetics and location of Tfh differentiation following vaccination.

While there is a basic understanding of the induction of long-lived, highly matured antibody responses and the varied roles of Tfh cells, dendritic cells, and B cells in this process, it is unknown how each cell type determines the quality and function of vaccine-induced immune responses. Evaluation of intact LN structure will enable a better understanding of Tfh cell specificity and functions, as well as the coordinating mechanisms that lead to long-lived protective B-cell responses. Evaluation of signals involved in B-cell memory formation and phenotyping and functional characterization of different types of natural killer (NK) cells will be performed in relation to lymph node histochemistry observations.

2.2 SCIENTIFIC RATIONALE

2.2.1 STUDY PRODUCTS AND DOSE JUSTIFICATION

2.2.1.1 Env-C Plasmid DNA (HIV-1 gp120 (93MW965.26) DNA)

The dose of Env-C Plasmid DNA to be used for immunization is 2 mg. This was based on the dose of DNA plasmids (obtained from Dr. Shan Lu, University of Massachusetts Medical School), that are being used in PDPHV-201401, the NIAID Sponsored Network study HVTN124 (NCT03409276). The 2 mg dose was further selected based on cGMP manufacturing constraints. The maximum DNA concentration that could be obtained with precipitation was 4 mg/ml. The 2 mg dose is approximately twice that of the low dose (1.2 mg) used in the DP6-001 clinical trial [26], [27], which induced significant immune responses to HIV Env. Numerous clinical trials have used DNA vaccines in the 1-8 mg range in HIV vaccine clinical trials [28], [29] [30], [31], [32] [33], [34], [35] and other diseases [36]; for reviews see [37], [38], [39].

2.2.1.2 HIV Env gp145 C.6980 Protein

The dose of HIV Env gp145 C.6980 protein to be used for immunization is 100 µg. The dose of Env protein used in clinical trials has ranged from 5-600 µg [40], [41]. In AVEG 015 (NCT00001042), 50 µg of gp120 was used [42], [43], [44, 45], whereas in RV144 (NCT00223080) 300 µg each of gp120 MN and gp120 A244 were used [46], [47]. The dose of 100 µg matches the lower dose of HIV Env gp145 C.6980 used in the HVTN 122 trial (NCT03382418). Using ALF43 and Rehydragel® as an adjuvant, immune responses similar to those observed in AVEG015 are expected [45]. Based on the concentration of vialed HIV Env gp145 C.6980 (600 µg/ml), 100 µg was selected to allow for the mixing of the vaccine components.

2.2.1.3 ALF43: Army Liposome Formulation—43% Cholesterol

The dose of MPLA in ALF43 to be used for immunization is 200 µg. This was based on previous clinical trials with the ALF43 predecessor L(MPLA), in which approximately 200 µg was used in a Phase I malaria clinical trial [48] and 11 Phase I prostate [49], [50], [51] or colorectal cancer clinical trials [52] and two Phase II prostate clinical trials. The vaccine induced potent immune responses.

2.2.1.4 Recombinant Double Mutant Escherichia coli Heat Labile Toxin (dmLT)

The dose of dmLT to be used in transcutaneous immunization is 50 µg. This was based on the previous clinical trials with transcutaneous immunization using 50 µg of native LT [53], [54], [55], [56], [57]. In addition, the use of dmLT in doses up to 50 µg have been used as an antigen and/or adjuvant in clinical studies [58], [59], [60], [61]; that was recently reviewed [62].

2.2.1.5 Rehydragel® (Aluminum Hydroxide Fluid Gel)

The dose of aluminum in Rehydragel® to be used is 500 µg. This dose was selected to match the 500 µg dose of aluminum that was used in AVEG015 (NCT00001042) in which ALFA was used as an adjuvant [42], [43], [44], [45].

2.2.2 OVERVIEW OF PRE-CLINICAL STUDY RESULTS

RV460 evaluates Env-C Plasmid DNA with the HIV Env gp145 C.6980 protein vaccine combined with different adjuvants. The adjuvants are dmLT, ALF43, and Rehydragel®. Supporting

information detailing the evaluation of the adjuvants, the Env-C Plasmid DNA and HIV Env gp145 C.6980 protein vaccines is provided in the Investigators Brochure (IB), Nonclinical Section.

Each component of the vaccine regimen has both preclinical and clinical data or is part of a larger class of products, which, collectively, have been extensively tested. Although ALF43 has not been used in humans, it is a revised formulation of L(MPLA). ALF43 has reduced levels of phospholipids and cholesterol but maintains the same amount of MPLA. Reports relevant to assessing the potential toxicity and the immunogenicity of the combined Env-C Plasmid DNA prime-protein boost are described in detail in the IB. WRAIR conducted a preclinical rabbit study to evaluate the proposed RV460 vaccine regimen. The purpose of this study was to evaluate the magnitude, frequency and durability of immune responses induced by Env-C Plasmid DNA and HIV Env gp145 C.6980 protein in combination with the adjuvants ALF43, Alhydrogel, ® and dmLT. The rabbit immunogenicity for RV460 was evaluated with ELISA. IgG antibodies were measured against HIV Env gp145 C.6980, gp120 (C.ZA.1197MB) proteins as well as against gp70-V1V2 scaffold antigen. All groups of rabbits induced high titers of HIV Env gp145 C.6980, gp120, and gp70V1V2-specific binding IgG, which were maintained at elevated levels 6 months post-immunization. Co-administration of Env-C Plasmid DNA and HIV Env gp145 C.6980 protein resulted in titers of 100,000 after just one immunization. IM Env-C Plasmid DNA immunization with dmLT via TCI caused a delay in the induction of Abs until week 16. Additionally, neutralizing antibodies against tier 1 viruses were also evaluated. Sera from all the groups had cross-clade Tier 1 neutralizing activities (work in progress). Neutralization was observed only after the HIV Env gp145 C.6980 protein boost and declined from week 24 to week 32. However, the Env-C Plasmid DNA/HIV Env gp145 C.6980 protein co-immunization group had high neutralization titers starting at week 12, which appeared to be more durable. Overall, Env-C Plasmid DNA prime/boost vaccine regimen in combination with the ALF43, and dmLT TCI adjuvants led to the generation of high magnitude durable binding Abs and induced potent neutralizing Abs.

Additionally, toxicity and temperature of all rabbits from RV460 pre-clinical study were evaluated. The temperature of rabbits was taken before each immunization, and 6 and 24 hours after immunization. Clinically relevant elevations of temperature were not observed in any of the rabbits immunized with the different vaccine regimens. Blood samples were drawn at Weeks 0, 4, 8, 12, 16, and 20 from all immunized rabbits for blood chemistry and complete blood cell counts. Most of the animals showed blood chemistry levels within normal physiologic range except for a few fluctuations in some animals, which was considered normal by the pathologist. Samples taken after Week 8 indicate that there were no abnormal blood chemistry results in any group after the last Env-C Plasmid DNA prime was received. Additionally, blood cell counts were measured in pre-immunized animals and at 3 days and again at 1 week after each immunization. The complete blood counts of most rabbits were within the normal physiologic range. The overall conclusion from this rabbit study was that the vaccine formulations were immunogenic, and no safety concerns were identified.

Overall, the vaccine formulations were immunogenic and based on the pyrogenicity and toxicity tests conducted side by side with the immunogenicity testing, no abnormal findings or results were observed. Note that ALF43A in combination with a malaria antigen was tested in a GLP rabbit pharmacology-toxicology study without adverse findings.

See IB for details of these pre-clinical results.

2.2.3 OVERVIEW OF RELEVANT CLINICAL STUDIES

Of the five components proposed in the RV460 vaccine regimen, both the Env-C Plasmid DNA (IND 17813; HVTN124) and the HIV Env gp145 C.6980 protein (IND 17766; HVTN122) vaccines are in clinical Phase 1 trials. Clinical data on the RV460-specific formulations of Rehydragel® or dmLT are published. The most significant data sets on HIV Env proteins (gp120) with Rehydragel® (AIDSVAX® vaccine), [46], [43], [63], [64], come from efficacy trials where the product was tested alone or in a prime-boost combination with a canarypox DNA vector (RV144) and compared to placebo. dmLT has been tested as both an antigen and adjuvant in an oral vaccine for enterotoxigenic *E. coli* (ETEC) diarrhea [65], [66], [58].

2.2.3.1 Safety and Immunogenicity of the Env-C Plasmid DNA Alone or with an HIV Env Protein Boost Vaccine

DNA plasmid vaccines expressing HIV-1 Env antigens have been tested in multiple clinical studies up to 8.0 mg/dose and have demonstrated an acceptable safety profile when administered alone or with adjuvanted HIV-1 Env proteins [28], [29], [30], [31], [32], [33], [34], [35] and other diseases [36]; for reviews see [37], [38], [39] HIV-1 DNA plasmid vaccines expressing HIV-1 Env antigens are safe and relatively well tolerated in humans. For some HIV-1 DNA Env preparations, pruritus or localized delayed-type hypersensitivity (DTH) reactions have occurred as non-serious, low-grade adverse events (AEs) attributed to the HIV-1 DNA vaccine; but they were not frequent or severe enough to halt their respective studies.

Although the single plasmid of Env-C Plasmid DNA vaccine has not been previously tested in humans, a first generation of the Env-C Plasmid DNA vaccine was tested as a multigene, polyvalent DNA prime-protein boost HIV-1 vaccine, DP6-001, with the same DNA plasmid backbone [26].

In this study, each participant received two vaccine components; three sequential inoculations using a DNA plasmid vaccine administered either intradermally or intramuscularly followed by two sequential inoculations using a protein vaccine. This study involved 2 dose levels of DNA vaccines (1.2 mg [Groups A and B]; 7.2 mg [Group C]) expressing five gp120 Env antigens (HIV-1 clades A, B, C and E) and one Gag antigen (HIV-1 clade C), and a single dose level of the same five recombinant expressed gp120 protein vaccines, produced and purified from CHO cells, and mixed with QS21 adjuvant at the time of injection. Additionally, Group C in the previous trial received more than 3 times the dose of DNA compared to the 2 mg of Env-C Plasmid DNA proposed in RV460.

After each DNA immunization, Grade 1 or 2 fever was common, occurring in up to 42% of DNA vaccine recipients of which Group A (ID) recipients had significantly more frequent injection-site erythema after DNA vaccination than Groups B or C (IM). Local injection site reactions were most common (65% of participants) and included Type IV delayed-type hypersensitivity (DTH) reactions at prior DNA inoculation sites in 12 of 28 (43%) participants after protein vaccination [26]. The erythematous “recall” reactions at prior DNA vaccination sites typically appeared 36 to 72 hours after injection of the DNA (8 milder reactions) and the protein boost (12 mild to severe reactions).

Two vasculitis adverse events after the HIV-1 Env protein/QS-21 boost also occurred, which halted the study. One case was leukocytoclastic vasculitis occurring after the protein boost in the higher-dose (7.2 mg) DNA prime group. A second case occurred in a participant whose history of Henoch-Schönlein purpura (HSP) as a young adult was not reported before he entered the study. This participant experienced Grade 2 fever after DNA vaccination and experienced hepatic and renal abnormalities after protein boost that were later recognized as due to recurrence of HSP. There have been subsequent HIV-1 DNA prime HIV-1 Env boost vaccination studies (none with the adjuvant QS-21) where vasculitis has not been observed again. Grade 1, 2 or 3 local toxicity and occasional Grade 1 or 2 flu-like symptoms has been well documented after vaccinations testing QS-21 as an adjuvant. There were mild to moderate dermatological reactions following DNA immunization, however, severe reactions occurred after the protein QS21 boost as shown in Study DP6-001 [26] suggesting that the severe dermatologic reactions observed after the boost may have been due to QS-21 adjuvant.

In DP6-001, robust cross-subtype HIV-1-specific T-cell response was detected in interferon gamma enzyme-linked immunospot assays. Furthermore, high-titer serum antibody responses were detected that recognized a wide range of primary HIV-1 Env antigens and also neutralized pseudotyped viruses that express the primary Env antigens from multiple HIV-1 subtypes [67], [27]. These findings demonstrated that the DNA prime-protein boost approach is an effective immunization method to elicit both humoral and cell-mediated immune responses in humans.

2.2.3.2 Safety and Immunogenicity of the HIV Env gp145 C.6980 protein (or other HIV-1 Env Protein Vaccines) Alone or with a Plasmid Vector expressing HIV-1 Env Antigens as a Prime Vaccination

The HIV Env gp145 C.6980 protein vaccine has not previously been tested in humans, but the protein adjuvanted with Alhydrogel® is undergoing evaluation in an ongoing phase 1 clinical trial sponsored by DAIDS under investigational new drug application (IND) 17766 (HVTN122). Additionally, HIV-1 Env proteins corresponding to either a full length or a truncated version of the HIV-1 Env have been extensively tested in clinical studies and have been safe and well tolerated [68], [47], [69], [70]. Specifically, monomeric Env proteins (HIV-1 Env gp120, gp140, gp160) have been administered in many clinical trials with aluminum salt adjuvants, either alone or as a part of a prime-boost vaccine regimen.

In a series of studies of HIV-1 gp120 protein vaccines reviewed by Huang et al., the proportion of participants who experienced an unsolicited AE after any vaccination was not significantly different in vaccine and placebo recipients [71]. Most of all events (95%) in both placebo and vaccine recipients were assessed as not related to vaccination. Pain and tenderness were the most frequent local reactions observed. Vaccine-related local reactions were generally mild and resolved within 3 days. Systemic reactogenicity was observed less frequently and most often as transient fatigue and low-grade fever. 2912 SAEs were reported by 2394 study participants (14.6%) were observed but there was no statistically significant difference between vaccine (14.3%) and placebo recipients (14.9%) [71]. Most SAEs occurred outside the vaccination months with less than a 2% SAE rate during the 30 days after treatment. No deaths have been reported as related to HIV-1 Env protein vaccines even when combined with known, relatively more reactogenic, or toxic adjuvants such as Incomplete Freund's adjuvant or the saponin, QS-21 when not compounded with other molecules.

2.2.3.3 Safety and Immunogenicity Overview of Army Liposome Adjuvants

WRAIR has developed various liposomal-based adjuvants for various vaccine antigens including *Plasmodium falciparum* [48], [72], HIV-1 gp120 [43], [44], [45], prostate cancer [73], [50, 51], colorectal cancer [52], and *Neisseria meningitides* [74]. 244 participants (72 healthy and 172 with cancer) have received the ALF adjuvant, the liposome-containing monophosphoryl lipid A L(MPLA) with or without AlOH at different doses ranging from 22 µg to 2,200 µg in different formulations with various antigens and administered by different routes. Reactogenicity events were mostly mild and moderate, transient and resolving without sequelae including with the highest dose administered. The most frequent local reactogenicity events recorded include pain and tenderness, erythema, and induration. The most frequent systemic reactogenicity events consisted of malaise, myalgia, headache, fever, and nausea. There were no clinically significant laboratory abnormalities.

See IB WRAIR L(MPLA) safety summary table for details.
The newer generation of L(MPLA), ALF43, has not been directly tested in humans; however, multiple previous clinical trials using its predecessor, L(MPLA) adjuvant formulation with various antigens have been conducted and is anticipated to have similar activity and safety attributes. Both the cholesterol and MPLA are now synthetic which will minimize the possibility of the introduction of adventitious agents. ALF43 contains an 11-fold reduction in MPLA relative to the L(MPLA) adjuvant evaluated in AIDS Vaccine Evaluation Group (AVEG)015 (NCT00001042; biologics investigational new drug application [BB-IND]-5013, biologics master file [BB-MF]-4292), an adjuvant comparison study. In that study, 50 µg of a clade B HIV-1 Env gp120 SF2 vaccine was administered with seven different adjuvants and compared to AlOH given intramuscularly without vaccine [43], [44], [45]. The other adjuvants included MPL® (Ribi ImmunoChem Research, Inc.), L(MPLA) + AlOH (WRAIR), MF59 (Biocine), MF59 plus MTP-PE (Biocine), SAF/2 (Biocine), and SAF/2 plus MDP (Syntex Corp., Palo Alto, CA). 110 healthy men and women were randomized to the seven different study arms. The prime vaccination was followed by boosts at two and six months; 62 participants received an 18-month boost. Across the seven groups, the most frequent local reactogenicity events were pain and tenderness, and the most frequent systemic reactogenicity events were malaise, myalgia, headache, fever, and nausea.

Three concerning SAEs occurred after completion of the study, two individuals, whom both received 2,200 µg L(MPLA) + AlOH, developed multiple sclerosis (MS). The first is a 49-year-old female diagnosed with MS almost five years after her last AVEG015 study injection. The participant received the last vaccination on 6 January 1994 and was diagnosed with MS on 7 December 1998 which is 4 years and 11 months after the last vaccination.

The second is a 34-year-old male diagnosed with MS 12 years after the last AVEG015 vaccination. This case is complicated by the participant's subsequent enrollments in four other clinical trials where he received three unrelated experimental vaccine regimens (RSV, influenza, HCV). He received a diagnosis of MS at the end of the fourth experimental vaccine trial, 12 years after the last AVEG015 vaccination.

A third SAE occurred in a different study evaluating L(MPLA) with a prostate antigen for prostate cancer treatment (BB-MF-6205). After the study, the participant who received the experimental prostate cancer vaccine received a mouse monoclonal antibody and then experienced immediate anaphylactic shock. Although the event was assessed as related to the vaccine, the investigators

later determined the study participant had developed anti-idiotype antibodies to the mouse monoclonal antibody used in the purification of prostate-specific antigen for the study vaccine, which then reacted to the monoclonal antibody infusion in the study. In all other studies, no other related SAEs have been reported.

Immunogenicity of the encapsulated clade B HIV-1 Env gp120 SF2 and L(MPLA) + AlOH in AVEG 015 was, in some cases, better than the other regimens [43]. Additionally, preclinical studies of ALF using a mouse model demonstrated its superiority over aluminum salt adjuvants [75]. Therefore, there is a need to obtain robust safety and immunologic data on ALF adjuvants for HIV Env vaccines in humans. In AVEG015, there were significantly higher IgG antibody responses to clade B HIV-1 Env gp120 IIIB and to clade B HIV-1 Env gp120 A244 (Subtype A/E) in the liposomal arm than in the AlOH arm (see IB) [45]. Likewise, the vaccines in the liposomal arm induced significantly higher and more durable IgG antibody responses to the gp70V1V2 CaseA2 and to gp70V1V2AE Env proteins observed for the AlOH arm. These HIV-1 Env antigens correlated with a decrease in risk of HIV-1 acquisition in RV144. In addition, the AlOH-adsorbed liposomes containing MPL® (ALF43A) enhanced the magnitude and durability of V1V2-antibody responses beyond that observed in any of the previous HIV vaccine efficacy trials.

2.2.3.4 Safety and Immunogenicity Overview of Rehydragel®

AlOH has one of the largest safety databases worldwide, and it is widely accepted as safe. Aluminum adjuvants have been in use for over 90 years to increase the efficacy of licensed vaccine formulations. Many Phase 1, 2, 3 and 4 clinical trials involving AlOH demonstrate the excellent safety profile of aluminum adjuvants in humans. There are at least 10 vaccine products approved in the United States and approximately 146 worldwide that contain aluminum salts as a vaccine additive.

See IB for a list of 10 US FDA approved vaccines that contain AlOH.

Aluminum hydroxide (AlOH) and aluminum phosphate have been extensively used as adjuvants for HIV-1 vaccines since the 1990s [42], [76], [77], [78]. A review of clinicaltrials.gov has 38 trials listed in which aluminum is or was being used as an adjuvant for HIV-1 Env antigens [79]. The most well know trials were the phase 3 trials with AIDSVAX®. VAX003 [47], VAX004 [68] and RV144 [46]. Both VAX003 and VAX004 were conducted in high-risk populations, IV injection drug users and men having sex with men, respectively. VAX003 assessed the efficacy of AIDSVAX® B/E and VAX004 assessed the efficacy of AIDSVAX® B/B, immunization alone and failed to demonstrate vaccine efficacy against the acquisition of HIV-1. Both had high titer, durable antibody responses. In contrast, the RV144 trial was conducted in a low-risk cohort that received both ALVAC and AIDSVAX® B/E. A modest 31.2% efficacy was observed in RV144. Antibodies to gp70V12 were identified as the inverse correlate for the acquisition of HIV-1 [80]. The antibodies were of modest titer and waned rapidly. Taken together these data demonstrate that aluminum hydroxide can induce high titer antibodies of long duration, but the priming vaccine may be critical for antibody titer, duration, and specificity.

In adults, the most common local adverse events associated with AlOH are pain, redness, and swelling at the injection site. Rarer local reactions are erythema, subcutaneous nodules, contact hypersensitivity, and granulomatous inflammation [81], [82]. Administration of a vaccine that contains an adjuvant-like AlOH, which forms a depot after injection, results in an inflammatory

focus. The inflammatory focus attracts immune cells and results in a granuloma that contains antibody-producing plasma cells [4]. A very rare and unusual neuromuscular disorder called macrophagic myofasciitis, which is attributed to the persistence of aluminum salts in muscle, has also been reported [83]. The most common systemic AEs are fatigue, headache, myalgia, gastrointestinal symptoms, and arthralgia. Overall, the use of aluminum adjuvants in humans poses a minimal risk of AEs.

See IB for specific study summaries.

2.2.3.5 Safety and Immunogenicity Overview of Recombinant Double Mutant *Escherichia coli* Heat Labile Toxin (dmLT; R192G/L211A)

There is extensive human experience testing heat-labile enterotoxin (*E. coli*) (LT) - TCI to support the assertion that TCI with dmLT at the site of an Env-C Plasmid DNA injection is expected to be a potent and safe adjuvant strategy in humans. In addition, RV460 will test a mutated, inactivated LT (dmLT) in which the ability to activate cellular adenylate cyclase has been abolished to decrease toxicity. The proposed RV460 trial will be the first to evaluate the adjuvant dmLT delivered by transcutaneous route. dmLT has previously been used as an adjuvant in several clinical trials and as an antigen for exotoxigenic *E. coli* diarrhea in which it was administered via sublingual and intradermal (ID) routes (clinical trial number NCT03548064). However, the non-mutated form of LT has been used as an adjuvant with skin patch vaccines (TCI) in multiple clinical trials (all these trials are included in tabular form in Investigator Brochure). TCI is a unique needle-free skin patch procedure shown to be safe and well tolerated in humans [53], [54]. A variety of adjuvants has shown effectiveness in stimulating immunity by TCI such as cholera toxin or LT from *E. coli*. [84]. Transcutaneous application of LT adjuvant was first tested in several human clinical trials for ETEC diarrhea or for influenza. Doses of LT ranged from 25 µg to 500 µg placed as a gauze patch on normal skin [53]. When TCI with LT was tested as an immune-stimulant patch at the site of influenza immunization in elderly and healthy adults, an increase in seroconversion and significant increases in antibody titer to influenza vaccines were noted in both younger and elderly adults [85], [56].

The LT adjuvant given transcutaneously was also tested in an efficacy field trial for travelers to Mexico or Guatemala, but did not meet the efficacy goals [86]; study participants did develop a 4-fold higher LT antibody titer and the frequency and weight of stool samples were significantly less [57]. Of 25 SAEs, 14 occurred in the LT-patch group and 11 in the placebo group, and these events in the context of all study AEs did not suggest a safety signal related to the skin application of LT. Two deaths in the LT-patch group were a consequence of road traffic accidents. TCI with LT has been safe with usually only mild to moderate side effects that were attributable to the vaccine and its application as follows: pruritus, mild redness or rash, and hyperpigmentation at the site of patch application in varying incidence and duration depending upon the site preparation method and patch used for the trial.

More recently, transcutaneously administered mutant LT (mLT) (LTR192G) with the ETEC adhesion antigen, CfaE, given intradermal was evaluated (BB-INDs 14711 and 15163). The majority of TCI-vaccinated participants exhibited a local vaccine site reaction that was generally of mild-to-moderate severity although 68.3% reactions were characterized as erythematous and papular and another 13.9% were either erythematous or papular (8.9%) only. Erythema and papules were present after receipt of the first vaccine dose (43.5%), and their frequency appeared

to increase after receipt of the second (erythema: 76.7%; papules: 74.4%) and third (erythema: 75.6%; papules: 63.4%) vaccinations (not statistically significant). Pigmentary changes at the vaccine site were also observed and the majority (47/53; 88.7%) occurred in individuals with a preceding papular rash. Hyperpigmentation occurred in 56.5% of all participants receiving at least one vaccine dose and was less common in participants not receiving LTR192G (10.0%) compared to 69.4% in those who did ($p < 0.001$). Hypopigmentation was only observed in the group receiving 10 µg of donor strand complemented CfaE (dscCfaE) co-administered with LTR192G (62.5%). The quality and severity of the skin reactions with transcutaneous may possibly be antigen-dependent.

dmLT has also been evaluated as an oral antigen/adjuvant vaccine in a phase 1 trial for ETEC [58]. It was safe with no reported side effects and induced both immunoglobulin G (IgG) and immunoglobulin A (IgA) antibodies. In addition, the dmLT still has adjuvant activity by TCI [87].

In summary, AEs associated with the use of LT or mLT applied by TCI have generally been mild-to-moderate and have included transient local erythema and less frequently hypo/hyperpigmentation, rash, and pruritus [55]. The mild-to-moderate hyperpigmentation persisted for at least 180 days after vaccination in 150 (18%) of 849 participants who received two vaccinations and returned for final assessment in the LT-patch group, compared with none of 842 participants in the placebo group. No significant differences in either occurrence or severity of systemic AEs (e.g., fever, malaise, headache, and diarrhea) have been observed between the LT-patch and placebo groups. To date, there have been no deaths related to the use of LT or dmLT as an adjuvant. Furthermore, dmLT maintains adjuvant activity inducing both humoral and cellular immune responses.

See IB for additional details.

2.3 MILITARY RELEVANCE

The US MHRP mission is to protect the U.S. Military from HIV and improve global health by its HIV vaccine research and development, reduce new infections and find a cure. Presently, HIV is a leading cause of death in the global community. According to reports by UNAIDS, about 36.7 million people worldwide are infected with HIV-1 as of 2017, with about two million new cases in 2017 alone [88][88], [90], [81]. Sub-Saharan Africa carries the greatest burden of HIV, accounting for 71% of all HIV infections. The epidemic, however, is not just isolated to the developing world. As of 2012, there were 50,000 new HIV infections and 1.3 million people living with HIV in North America, [89] which is an increase from 1.14 million in 2009 [90].

Additionally, prevalence continues to increase across Eastern Europe, Central and East Asia and the Middle East and North Africa [91]. Unfortunately, a safe and effective HIV vaccine is presently an elusive cornerstone of HIV prevention. The impacts of HIV are widespread and include lowered life expectancy, reduced economic growth, and increased health costs. These outcomes ultimately damage social and political cohesion and impede the advancement of global health objectives, which poses a risk to national security and the stability of many nation-states.

In addition to geopolitical concerns, exposure of US Military Forces to high infection rates in both developed and developing countries pose a persistent risk to Military personnel. As reported in

2012, there were 366 incident infections in U.S military personnel. Among Active Duty Forces alone, new HIV cases have increased by 22% since 2008, and exposure to the virus, particularly among deployed Forces, continues to threaten Force readiness, Force Health Protection, and threaten the blood supply. Furthermore, improved treatment options and the younger age at diagnosis, drive higher life expectancy that has substantially increased the cost of ongoing/chronic care for Service Members and Beneficiaries. With an estimated lifetime treatment cost of $380,000 per person, the cost to the Department of Defense to provide lifelong therapy for HIV infected Service Members (based on 366 cases), estimated to be $140M. Accordingly, the tremendous and destabilizing impacts of HIV across global populations and within the U.S. Military demand the implementation of sophisticated scientific, clinical, political, and social solutions.

Through collaboration between USAMRD-A, MHRP, WRAIR and the DAIDS, NIAID, National Institutes of Health (NIH), this study aims to add to the growing knowledge and development of an HIV vaccine by building upon previous research to assess a potential vaccine regimen thus furthering MHRP's mission.

2.4 DESCRIPTION OF KERICHO SITE

USAMRD-A is a Special Foreign Activity of WRAIR, Silver Spring, Maryland (MD). USAMRD-A includes the Kericho, Kenya unit affiliated through a Cooperative Agreement with KEMRI. The unit was activated on a temporary basis in 1969 at the invitation of the Government of Kenya to study trypanosomiasis. The success of that initial venture led to the establishment of a permanent activity in 1973. Over the past 45 years, research has been conducted on malaria, trypanosomiasis, leishmaniasis, entomology, HIV/AIDS, Polio, Ebola, Tuberculosis (TB), arboviruses, and other infectious diseases, with more than 250 manuscripts published.

The USAMRD-A HIV Program is a component of MHRP. In Kenya, the HIV Program field site is located at KEMRI/USAMRD-A/Clinical Research Center (CRC) about 260 kilometers northwest of Nairobi along the Nairobi-Kisumu highway in the rural Kenyan town of Kericho. Kericho's county population is approximately 752,396 (2009 census), although the larger catchment area for both HIV research and treatment is approximately 3 million covering the southern portion of the Rift Valley Province. Kericho is located among the African Highlands in the Rift Valley and well known for the growing of tea that forms the rolling "seas of green" within the tea plantations. The primary industry in this region is tea farming, with two large international tea companies namely Finlay's Kenya Ltd and Unilever Tea Kenya Ltd, centered in Kericho County Referral Hospital (KCRH).

Kericho was ranked as the twentieth richest county in Kenya in the 2005/06 National Integrated Household Budget Survey. Income in Kericho comes mainly from agricultural activities, with about 40-50% of the population living below the poverty line. The annual national gross domestic product per capita in 2013 averaged 1,245.51 U.S. dollars (USD). In Kenya, the age of majority (i.e. legal age of recognized adulthood or independence) is 18 years. The Kenyan national literacy level according to the Kenya Adult Literacy Survey report published in March 2007 by the Kenya National Bureau of Statistics was 64%. In Kericho, the literacy level is 74.3% in men and 68.9% in women. The government through a network of hierarchical public hospitals, health centers, primarily provides the healthcare system in the larger Kericho area (southern Rift Valley) and

dispensaries ran by the Kenya Ministry of Health (MoH). The government health facilities do not charge for treatment but levy a small fee for facility maintenance, which can be waived for those not able to pay. In addition to government health care facilities, there are a few faith-based and private health care facilities providing services at a small fee. Access to most health care facilities is good given that there exists a reliable public transport system in the region.

The KEMRI/USAMRD-A CRC is the primary HIV research site in USAMRD-A. Situated on the grounds of the KCRH/MoH, the 55,050 square feet CRC supports both HIV research and care and treatment in the southern Rift Valley Province. The CRC is composed of a clinical area including a central pharmacy that can support both vaccine and therapeutic studies as well as provide additional non-study medicines for HIV and other non-study related care. The CRC is a designated satellite HIV and TB clinic under the KCRH.

The CRC laboratory is Kenya's first and the only College of American Pathologist (CAP) accredited laboratory and boasts of a state-of-the-art Information Technology (IT) center that supports both HIV research and treatment programs. Both the CRC pharmacy and laboratory are approved by NIAID/DAIDS to participate in DAIDS sponsored vaccine (Site #31352) and therapeutics AIDS Clinical Trials Group (ACTG Site #12501); International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT site #5121) studies, the only research site with such approval in Kenya.

2.5 STUDY DESIGN

The RV460 trial is an exploratory study that will assess the safety, tolerability, and immunogenicity of different adjuvant formulations in combination with an Env-C Plasmid DNA and HIV Env gp145 C.6980 protein in a prime-boost combination. Enrolled participants will receive 3 doses of Env-C Plasmid DNA as a prime (with or without adjuvants) or admixed with HIV Env gp145 C.6980 protein. The Env-C Plasmid DNA is given alone, formulated with the adjuvant ALF43 (Army Liposome Formulation with Monophosphoryl Lipid A (MPLA) and 43% Cholesterol) adjuvant, or co-administered with the transcutaneous adjuvant dmLT (*E. coli* heat labile enterotoxin) applied at the injection site. The HIV Env gp145 C.6980 protein boost will be mixed with ALF43, Rehydragel® or ALF43 plus Rehydragel®.

Study groups will be assessed for HIV-1 Env-specific antibodies responses elicited by boosting with HIV Env gp145 C.6980 protein adjuvanted with Rehydragel® with or without ALF43. Group 7 will assess whether or not the concept of co-delivery of Env-C Plasmid DNA and HIV Env gp145 C.6980 protein simultaneously will increase the frequency and magnitude of immune responses than Env-C Plasmid DNA given IM alone (after first three vaccinations – Study groups 1 and 2 or as an Env-C Plasmid DNA IM prime, protein IM boost combination as was observed in mice and non-human primates [18], [19, 92, 93].

Table 1: Study Design

Group	n =		Prime at weeks 0, 4, 12	Boost at weeks 20, 32, 56
	V	p		
1	15	3	Env-C Plasmid DNA (2 mg, IM)	HIV Env gp145 C.6980 protein (100 µg, IM)/Rehydragel® (500 µg, IM)

Group	n =		Prime at weeks 0, 4, 12	Boost at weeks 20, 32, 56
	V	p		
2	15	3	Env-C Plasmid DNA (2 mg, IM)	HIV Env gp145 C.6980 protein (100 µg, IM)/Rehydragel® (500 µg, IM)/ALF43 (200 µg, IM)
3	15	3	Env-C Plasmid DNA (2 mg, IM) + dmLT (50 µg, TCI)	HIV Env gp145 C.6980 protein (100 µg, IM)/Rehydragel® (500 µg, IM)
4	15	3	Env-C Plasmid DNA (2 mg, IM) + dmLT (50 µg, TCI)	HIV Env gp145 C.6980 protein (100 µg, IM)/Rehydragel® (500 µg, IM)/ALF43 (200 µg, IM)
5	15	3	Env-C Plasmid DNA (2 mg, IM)/ ALF43 (200 µg, IM)	HIV Env gp145 C.6980 protein (100 µg, IM)/Rehydragel® (500 µg, IM)
6	15	3	Env-C Plasmid DNA (2 mg, IM)/ALF43 (200 µg, IM)	HIV Env gp145 C.6980 protein (100 µg, IM)/Rehydragel® (500 µg, IM)/ALF43 (200 µg, IM)
7	15	3	Env-C Plasmid DNA (2 mg, IM)/HIV Env gp145 C.6980 protein (100 µg, IM)/ALF43 (200 µg, IM)	Env-C Plasmid DNA (2 mg, IM)/HIV Env gp145 C.6980 protein (100 µg, IM)/ALF43 (200 µg, IM)/Rehydragel® (500 µg, IM)

IM= intramuscular, TCI = transcutaneous immunization; ALF43 (Army Liposome Formulation with Monophosphoryl Lipid A (MPLA) and 43% Cholesterol); v= Vaccine Recipient; p= Placebo

2.6 OBJECTIVES

2.6.1 PRIMARY

Assess the safety and tolerability (including reactogenicity) of Env-C Plasmid DNA given IM, alone, admixed with ALF43 adjuvant, topically applied with dmLT adjuvant, or given with an adjuvanted HIV Env gp145 C.6980 protein and followed by adjuvanted HIV Env gp145 C.6980 protein boost IM immunizations, with or without Env-C Plasmid DNA.

2.6.2 SECONDARY

1. Determine whether the adjuvants under study improve the immunogenicity of the Env-C Plasmid DNA priming.
2. Determine whether the addition of ALF43 to the Rehydragel® –HIV Env gp145 C.6980 protein boost further improves the immune response to HIV Env gp145 C.6980 protein.
3. Determine whether adjuvants improve humoral responses across vaccination regimens.
4. Evaluate the influence of various adjuvants on cellular immune responses.
5. Describe mucosal humoral responses across vaccination regimens in cervicovaginal and rectal secretions and semen.

2.6.3 EXPLORATORY

1. Characterize B-cell functional specificities for each vaccination regimen.
2. Characterization of innate immunity.
3. Assess the innate/gene expression induced across vaccination regimens (DNA microarray, RNA sequencing) to compare relative influence of various adjuvants in the gene expression profiles of sorted T cell, B cell and innate cell subsets.
4. Perform systems serology analyses.

2.6.4 SUB-OBJECTIVE

In participants who elect to undergo lymph node biopsy, characterize HIV-specific responses, T cell activation, T follicular helper (Tfh) cell and B cell distribution in intact lymph nodes (excision of intact peripheral inguinal lymph node) at weeks 14 and 58.

2.7 ENDPOINTS

2.7.1 PRIMARY

Safety endpoints will be the solicited AE (local and systemic reactogenicity), and related AEs and SAEs.

2.7.2 SECONDARY

1. Compare plasma IgG binding antibodies to gp120 and V1V2 in terms of magnitude, durability, and area under the curve both after initial DNA priming series among groups with differing DNA priming regimens.
2. Compare plasma IgG binding antibodies to gp120 and V1V2 in terms of magnitude, durability, and area under the curve between groups with and without Rehydragel® after HIV Env gp145 C.6980 protein boosting.
3. Characterize plasma IgG and IgA binding antibodies to Env-C Plasmid DNA, V1V2, neutralizing antibodies, and non-neutralizing effector functions such as ADCC and ADCP with emphasis on RV144 immune correlates of risk of HIV acquisition.
4. Characterize and assess the magnitude of cell-mediated immune responses elicited across vaccination regimens including but not limited to antigen-specific CD4 and CD8 T cell responses and polyfunctionality, induced cytokines, chemokines and other inflammatory markers.
5. Characterize mucosal humoral responses in cervicovaginal and rectal secretions and semen including, but not limited to, plasma IgG and IgA binding antibodies to HIV-1 Env proteins, IgG and IgA subclass, and functional assays.

2.7.3 EXPLORATORY

1. Characterize B-cell functional specificities for each vaccination regimen by quantifying antigen-specific responses through B-cell ELISPOT and phenotyping the magnitude and activation status of B cell subsets via flow cytometry as well as the isolation of monoclonal antibodies from selected vaccine recipients.
2. 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.
3. Compare relative influence of various adjuvants in the gene expression profiles sorted T cell, B cell and innate cell subsets using RNAseq and related methods to quantify and compare gene expression from various sorted cell subsets among study groups.
4. Utilizing systems serology analyses, link various immunological measurements, including antibody-binding data to antibody effector functional assays to evaluate immunologic response patterns or signatures to various adjuvants.

2.7.4 SUB-SET OF PARTICIPANTS THAT ELECT TO UNDERGO LYMPH NODE BIOPSY

Between each adjuvant group, compare the magnitude and frequency of HIV-1-specific TfH cells in total lymph nodes and lymph node follicles, TfH and B cell distribution between intra and extrafollicular LN zones, and levels of cellular activation using ICS, immunohistochemistry, transcriptional analyses, and related techniques at weeks 14 and 58.

3 INVESTIGATIONAL PRODUCTS

3.1 DESCRIPTION OF STUDY AGENTS

3.1.1 ENV-C PLASMID DNA VACCINE

The Env-C Plasmid DNA vaccine is supplied as single-dose vials containing 0.9 mL of solution at a concentration of 4 mg/mL. Each 1 mL of solution contains 4 mg of Env DNA vaccine and 9 mg of sodium chloride. The Env DNA vaccine will be stored at ≤-60°C and shipped on dry ice to the clinical research site. When thawed, the study product appears as a clear, colorless liquid.

3.1.2 HIV-1 ENV GP145 C.6980

The HIV Env gp145 C.6980 protein vaccine is supplied as single-dose vials containing 0.7 mL of solution at a concentration of 600 µg/mL. The buffer is PBS, pH 7.4. The HIV Env gp145 C.6980 protein vaccine will be stored at ≤-65°C and shipped on dry ice to the clinical research site. When thawed, the study product appears as a clear, colorless liquid.

3.1.3 RECOMBINANT DOUBLE MUTANT ESCHERICHIA COLI HEAT-LABILE TOXIN LT (R192G/L211A) (DMLT)

The dmLT adjuvant will be supplied as a multiple-dose vial (up to two doses can be withdrawn from a vial) containing 0.5 mg of lyophilized product. The lyophilized product was formulated at 1 mg/mL in phosphate-buffered saline containing lactose, pH 7.4 and appears as a solid cake, consistent, and white to off-white. Each vial of dmLT will be reconstituted with 0.5 mL of sterile water for injection, resulting in a concentration of 1 mg/mL. The reconstituted product appears as a clear, colorless solution. Filled vials were labeled for storage at ≤ –10°C and are stored at –20°C ± 10°C prior to reconstitution. The dmLT product will be shipped to the clinical site on dry ice. After reconstitution, the dmLT product should be stored at 2-8°C for up to 12 hours. A maximum of two doses may be removed from each reconstituted vial for administration to study participants.

3.1.4 ALF43: ARMY LIPOSOME FORMULATION—43% CHOLESTEROL

The ALF43 adjuvant will be supplied as single-dose vials containing 240 µg of 3D-PHAD® per vial. ALF43 has a phospholipid to 3D-PHAD® molar ratio of 8.8 to 1. The ALF43 product appears as a white to off-white lyophilized powder, which may be in a cake at the bottom of the vial or dispersed throughout the vial. ALF43 is stored at -20°C ± 10°C and shipped to the clinical research site on dry ice.

3.1.5 ALUMINUM HYDROXIDE SUSPENSION—FLUID GEL (REHYDRAGEL®)

The Rehydragel® adjuvant will be supplied as 3 mL single-dose vials containing 0.7 ± 0.10 mL of aluminum suspension at a concentration of 5 ± 1 mg/mL. The vials are labeled as Aluminum Hydroxide suspension fluid gel (adjuvant). Rehydragel® appears as a white gelatinous precipitate in aqueous suspension; opaque. Rehydragel® will be stored at 2°C to 8°C and will be shipped to the clinical research site at the same temperature.

3.1.6 0.9% SODIUM CHLORIDE FOR INJECTION (STERILE SALINE)

0.9% Sodium Chloride for Injection (sterile saline) will be purchased from a commercial vendor based on availability at the time required. The sterile saline will serve as both the diluent and placebo for the trial. Sterile saline will be stored as per the manufacturer's recommendation.

Table 2: Study Agents

Short name	DP Composition	Description	Developer	Manufacturer
Env-C Plasmid DNA	DNA plasmid 3.6 mg/vial in 0.9 mL normal saline.	The Env-C Plasmid DNA drug product is a DNA plasmid vial in single-dose vials at a concentration of 4.0 mg/mL (3.6 mg/vial, 0.9 mL solution) in normal saline.	University of Massachusetts Medical School, MA	Waisman Institute, Wisconsin
HIV Env gp145 C.6980	HIV Env gp145 C.6980 0.7 mL/vial at 0.6 mg/mL in 2.7 mM potassium chloride, 136.9 mM sodium chloride, 1.5 mM potassium phosphate, and 8.9 mM sodium phosphate, pH 7.4.	The HIV Env gp145 C.6980 protein (CO6980v0c22) is vialed in PBS, pH 7.4 in single-dose vials at a concentration of 600 µg/mL with 0.7 mL/vial.	MHRP/ WRAIR	ABL through Avid, Tustin, California
dmLT	500 µg dmLT in lyophilized form in sodium phosphate buffer with 5% lactose	The double mutant heat-labile enterotoxin B of *E. coli*, dmLT (R192G/L211A), pH 7.4 is lyophilized at 500 µg/vial (0.5 mg/vial) to be reconstituted with 0.5 mL of water for injection. After reconstitution, the buffer will be 42.7 mM sodium phosphate, 10.7 mM potassium phosphate, 82 mM sodium chloride and 5% lactose.	Tulane University	GmbH Am Pharmapark 06861 Dessau-Rosslau Germany

ALF43	DMPC: 1.24 µmol/vial DMPG: 0.14 µmol/vial Cholesterol: 1.03 µmol/vial	Army Liposome Formulation consists of MPLA, dimyristoylphosphatidylcholine (DMPC), cholesterol (43%), and dimyristoylphosphatidylglycerol (DMPG) All of the lipids are synthetic. The MPLA congener that will be used is 3D-PHAD®. The final phospholipid concentration is 1.15 mM after reconstitution. 3D-PHAD®, is at concentration of 200 µg/mL of the 1.15 mM phospholipid liposomes after hydration	WRAIR	Avanti Polar Lipids/ WRAIR
Rehydragel® (Aluminum hydroxide suspension--fluid gel)	5 mg/mL aluminum in H_20	Rehydragel® was vialed at 0.7 mL ± 0.10 mL volume; aluminum concentration of 5 ± 1mg/mL	ChemTrade New Jersey	VRC Pilot Plant, NIAID, NIH

See IB for details for individual vaccine component details.

3.2 ACQUISITION AND DISTRIBUTION

Distributed through NIAID Clinical Research Products Management Center (CRPMC)

- Env-C Plasmid DNA (provided by HJF/MHRP)
- HIV Env gp145 C.6980 protein (provided by DAIDS/NIAID/NIH)
- ALF43 (provided by WRAIR)
- Rehydragel® (provided by the Vaccine Research Center (VRC)/NIAID/NIH)
- dmLT (provided by PATH, Division of Vaccines)
- 0.9% sodium chloride for injection will be available through the NIAID CRPMC.
- Sterile water for injection will be available through the NIAID CRPMC.

Locally sourced

- Empty sterile vials will be purchased from a commercial vendor.
- ECG skin preparation paper (Skin preparation paper) will be purchased from a commercial vendor.
- Sterile 2 x 2-inch, 12 ply cotton gauze pads, individually wrapped will be purchased from a commercial vendor.
- Nexcare™ Tegaderm™ Waterproof Transparent Dressings (3M), 4x4 ¾ inches, individually wrapped, will be purchased from a commercial vendor.

Upon successful completion of protocol registration procedures, the site pharmacist may obtain the study products made available to the study site through the NIAID CRPMC by following instructions provided in the Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks.

3.3 DOSAGE PREPARATION, DISPENSING AND ADMINISTRATION OF STUDY PRODUCT

The vaccines will be given by intramuscular (IM) injection into the deltoid muscle, excluding the dmLT adjuvant which is given by TCI. All products will be combined into one syringe (excluding dmLT) for IM injection, i.e., Env-C Plasmid DNA and HIV Env gp145 C.6980/ALF43A for study Group 7. Once combined, the vaccine regimen must be administered as soon as possible, although based on the stability data performed in our laboratory (data not shown but a comprehensive report was provided to DAIDS), all the vaccine formulations were stable for 24 hours. This is significantly more than required time to vaccinate a participant in the clinic. Alternating deltoids will be used for subsequent immunizations [63]. Vaccination in contralateral limbs has been successfully utilized to achieve prime boost in multiple vaccine trials [63]. Alternating sites allowed for better attribution of local reactogenicity reactions.

The site for injection cleaned with an alcohol wipe before the injection. Since the interventions are vaccines, the concept of a washout period does not apply. The duration of the interventions is unknown, but it is hoped that it would be ten years or longer.

See IB and Package Inserts for further information on each study product.

126 healthy men and women between the ages of 18 and 40 years will be randomized to one of seven groups:

Table 3: Study Groups

Group	N V/P	Prime at weeks 0, 4, 12	Boost at weeks 20, 32, 56
1	15/3	Env-C Plasmid DNA (2 mg, IM)	HIV Env gp145 C.6980 (100 µg, IM)/Rehydragel® (500 µg, IM)
2	15/3	Env-C Plasmid DNA (2 mg, IM)	HIV Env gp145 C.6980 (100 µg, IM)/Rehydragel® (500 µg, IM) / ALF43 (200 µg, IM)
3	15/3	Env-C Plasmid DNA (2 mg, IM) + dmLT (50 µg, TCI)	HIV Env gp145 C.6980 (100 µg, IM)/Rehydragel® (500 µg, IM)
4	15/3	Env-C Plasmid DNA (2 mg, IM) + dmLT (50 µg, TCI)	HIV Env gp145 C.6980 (100 µg, IM)/Rehydragel® (500 µg, IM) / ALF43 (200 µg, IM)
5	15/3	Env-C Plasmid DNA (2 mg, IM) / ALF43 (200 µg, IM)	HIV Env gp145 C.6980 (100 µg, IM)/Rehydragel® (500 µg, IM)
6	15/3	Env-C Plasmid DNA (2 mg, IM) / ALF43 (200 µg, IM)	HIV Env gp145 C.6980 (100 µg, IM)/Rehydragel® (500 µg, IM) / ALF43 (200 µg, IM)
7	15/3	Env-C Plasmid DNA (2 mg, IM) /HIV Env gp145 C.6980 (100 µg, IM)/ALF43 (200 µg, IM)	Env-C Plasmid DNA (2 mg, IM) /HIV Env gp145 C.6980 (100 µg, IM)/ALF43 (200 µg, IM)/Rehydragel® 500 µg, IM)

IM= intramuscular, TCI = transcutaneous immunization; ALF43 (ALF43 Army Liposome Formulation with Monophosphoryl Lipid A (MPLA) and 43% Cholesterol the 3D-PHAD® dose is 200 µg); V= Vaccine Recipient; P= Placebo; Rehydragel® dose is 500 µg aluminum.

3.3.1 PREPARATION OF INVESTIGATIONAL PRODUCTS

The clinical research pharmacist will prepare all doses for administration in the pharmacy and dispense to the clinic for administration. The pharmacist must be proficient in the preparation of products requiring aseptic technique under a pharmacy biological safety cabinet/isolator. Local regulations and site institutional policies and procedures for use of protective equipment, such as gloves, gowns, face masks and safety glasses, must be followed.

To preserve blinding, the pharmacist will prepare placebo syringes in the same manner as active vaccine syringes. Upon completion of placebo syringe preparation, the pharmacist will hold the prepared syringes in the pharmacy for the same length of time as it would take to prepare and dispense active vaccine syringes. Before releasing prepared study product to the clinic, the pharmacist will place an overlay on all syringes to maintain blinding (refer to protocol sections 3.3.1 and 3.3.3)

3.3.1.1 Env-C Plasmid DNA

One vial of Env-C Plasmid DNA and 0.9% sodium chloride for injection will be needed to prepare the dose. One vial of Env-C Plasmid DNA will be removed from the -70°C or colder freezer and allowed to thaw completely at room temperature (15-30°C, approximately 15 minutes). One vial of 0.9% sodium chloride for injection will be removed from storage.

When thawed, using aseptic technique, the pharmacist will add 0.9 mL of 0.9% sodium chloride for injection to the Env-C Plasmid DNA vial using a 22-25-gauge needle and a 1-mL syringe. The diluted Env-C Plasmid DNA vial will be swirled by hand for 1-2 minutes. Using a 3-mL syringe and a 22-25 gauge needle, 1 mL of the diluted product will be withdrawn from the vial for study participant administration. The pharmacist will apply an overlay to the syringe to maintain blinding.

The syringe should be labeled as "Env-C Plasmid DNA 2 mg or Placebo 1 mL", as well as "Administer IM in deltoid". The study product preparation and expiration times will also be listed on the prepared participant-specific study product label. The syringe containing the study product should be bagged for transport to the clinic where it will be administered. The study product should be administered within 4 hours of preparation.

Any unused portion of vials or expired prefilled syringes should be disposed of in accordance with institutional or pharmacy policy.

3.3.1.2 Placebo for Env-C Plasmid DNA

Using aseptic technique, the pharmacist will withdraw 1 mL of 0.9% sodium chloride for injection into a 3-mL syringe. The pharmacist will apply an overlay to the syringe to maintain blinding.

The syringe should be labeled as "Env-C Plasmid DNA 2 mg or Placebo 1 mL", as well as "Administer IM in deltoid". The study product preparation and expiration times will also be listed on the prepared study product label. The syringe containing the study product should be bagged for transport to the clinic where it will be administered. The study product should be administered within 4 hours of preparation.

Any unused portion of entered vials or expired prefilled syringes should be disposed of in accordance with institutional or pharmacy policy.

3.3.1.3 dmLT

One vial of dmLT, cold sterile water for injection, 0.9% sodium chloride for injection, and one empty sterile vial will be needed to prepare the dose. The dmLT vial will be removed from the -20℃ freezer. The sterile water for injection, the 0.9% sodium chloride for injection, and the empty sterile vial will be removed from storage and then, using aseptic technique and a 22-25-gauge needle and a 1 mL syringe, the dmLT vial will be reconstituted with 0.5 mL of cold sterile water for injection. The resulting concentration will be 1 mg/mL. The dmLT vial will be gently swirled and inverted by hand for approximately 10 times to fully suspend the contents. Following resuspension, the vial will be held at room temperature for 10-15 minutes to ensure full dissolution. The vial will be visually inspected to ensure the absence of clumps of undissolved powder. After reconstitution, a maximum of 2 doses may be removed from each reconstituted vial of dmLT for

administration to study participants. The reconstituted dmLT vial should be stored at 2-8°C for a maximum of 12 hours. Discard the reconstituted vial after this time.

Using a 22-25-gauge needle and a 3 mL syringe, 1.9 mL of cold 0.9% sodium chloride will be added to an empty sterile vial. Reconstituted dmLT will be swirled again by hand for 1-2 minutes. Then using a 0.3 mL ultra-fine insulin syringe with a 30-gauge ½-inch needle or a 0.5 mL syringe, 0.1 mL of the reconstituted dmLT solution will be withdrawn from the vial and added to the vial containing 1.9 mL of cold 0.9% sodium chloride to bring the concentration to 50 µg/mL. The vial will be swirled by hand for 1 to 2 minutes. Using a 22-25-gauge needle and a 3 mL syringe, 1 mL of the solution will be withdrawn from the vial for study participant administration.

The syringe should be labeled as "dmLT 50 µg or Placebo 1 mL", as well as "Administer by TCI". The study product preparation and expiration times will also be listed on the prepared study product label. The syringe containing the study product should be bagged for transport to the clinic where it will be administered. The study product must be placed in the refrigerator and removed for administration when clinic confirms participant is ready. The study product should be administered within 15 minutes of preparation. Any unused portion of vials or expired prefilled syringes should be disposed of in accordance with institutional or pharmacy policy.

3.3.1.4 Placebo for dmLT

Using aseptic technique, the pharmacist will withdraw 1 mL of 0.9% cold sodium chloride for injection, previously stored at 2 – 8 ºC, into a 3 mL syringe. The syringe should be labeled as "dmLT 50 µg or Placebo 1 mL", as well as "Administer by TCI". The study product preparation and expiration times will also be listed on the prepared study product label. The syringe containing the study product should be bagged for transport to the clinic where it will be administered. The study product must be placed in the refrigerator and removed for administration when clinic confirms participant is ready. The study product should be administered within 15 minutes of preparation.

Any unused portion of vials or expired prefilled syringes should be disposed of in accordance with institutional or pharmacy policy.

3.3.1.5 Env-C Plasmid DNA + ALF43

One vial of Env-C Plasmid DNA, one vial of ALF43, and 0.9% sodium chloride for injection will be needed to prepare the dose. One vial of Env-C Plasmid DNA will be removed from the -70°C or colder freezer and allowed to thaw completely at room temperature (15-30°C, approximately 10-15 minutes). One vial of ALF43 will be removed from the -20°C freezer and allowed to equilibrate to room temperature (15-30°C approximately 10-15 minutes).

Once equilibrated to room temperature, using aseptic technique with a 22-25-gauge needle and a 1-mL syringe, 0.6 mL of 0.9% sodium chloride for injection will be added the ALF43 vial. The ALF43 vial will be mixed using a Vortex mixer set at 2500 RPM for 5 minutes. The vial will be incubated at room temperature for 5 minutes.

When the Env-C Plasmid DNA vial is thawed, the pharmacist will swirl by hand for 1-2 minutes. Then, using aseptic technique, the pharmacist will add 0.6 mL of Env-C Plasmid DNA to the vial containing hydrated ALF43 using a 22-25-gauge needle and a 1-mL syringe. The Env-C Plasmid

DNA + ALF43 vial will be gently swirled by hand for 1-2 minutes. Then, using a 3-ml syringe and a 22-25-gauge needle, 1 mL of the solution will be withdrawn from the vial for study participant administration.

The syringe should be labeled as "Env-C Plasmid DNA 2 mg+ALF43 200 µg 3D-PHAD or Placebo 1 mL", as well as "Administer IM in deltoid". The study product preparation and expiration times will also be listed on the prepared study product label. The syringe containing study product should be bagged for transport to the clinic where it will be administered. The study product should be administered within 4 hours of preparation.

Any unused portion of vials or expired prefilled syringes should be disposed of in accordance with institutional or pharmacy policy.

3.3.1.6 Placebo for Env-C Plasmid DNA + ALF43

Using aseptic technique, the pharmacist will withdraw 1 mL of 0.9% sodium chloride for injection into a 3-mL syringe.

The syringe should be labeled as "Env-C Plasmid DNA 2 mg+ALF43 200 µg 3D-PHAD or Placebo 1 mL", as well as "Administer IM in deltoid." The study product preparation and expiration times will also be listed on the prepared study product label. The syringe containing the study product should be bagged for transport to the clinic where it will be administered. The study product should be administered within 4 hours of preparation.

Any unused portion of vials or expired prefilled syringes should be disposed of in accordance with institutional or pharmacy policy.

3.3.1.7 Env-C Plasmid DNA + HIV Env gp145 C.6980 + ALF43

One vial of Env-C Plasmid DNA, one vial of ALF43, one vial of HIV Env gp145 C.6980 and 0.9% sodium chloride for injection will be needed to prepare the dose. One vial of Env-C Plasmid DNA and one vial of HIV Env gp145 C.6980 will be removed from the -70°C or colder freezer and allowed to thaw completely at room temperature (15-30°C, approximately 10-15 minutes). One vial of ALF43 will be removed from the -20℃ freezer and allowed to equilibrate to room temperature (15-30°C, approximately 10-15 minutes). 0.9% sodium chloride for injection will be removed from storage. Once equilibrated to room temperature, using aseptic technique and a 22-25-gauge needle and a 1 mL syringe, 0.4 mL of 0.9% sodium chloride for injection will be added to the ALF43 vial. The hydrated ALF43 vial will be mixed using a Vortex mixer set at 2500 RPM for 5 minutes.

When the HIV Env gp145 C.6980 vial is thawed, the pharmacist will swirl by hand for 1-2 minutes. Then, using aseptic technique, the pharmacist will add 0.2 mL of the HIV Env gp145 C.6980 to the vial containing the hydrated ALF43 using a 22-25-gauge needle and a 1 mL syringe. The HIV Env gp145 C.6980 + ALF43 vial will be swirled gently with a vortex mixer set at 250 RPM for 2 minutes.

When the Env-C Plasmid DNA vial is thawed, the pharmacist will swirl by hand for 1–2 minutes. Then, using aseptic technique, the pharmacist will add 0.6 mL of Env-C Plasmid DNA to the vial containing the HIV Env gp145 C.6980 + ALF43 mixture using a 22-25-gauge needle and a 1 mL

syringe. The vial containing Env-C Plasmid DNA + HIV Env gp145 C.6980 + ALF43 will be swirled gently with a vortex mixer set at 250 RPM for 5 minutes. Using a 3-mL syringe and a 22-25-gauge needle, 1mL of this solution will be withdrawn from the vial for study participant administration.

The syringe should be labeled as "Env-C Plasmid DNA 2mg+HIV Env gp145 C.6980 100 µg +ALF3 200 µg 3D-PHAD or Placebo 1ml", as well as "Administer IM in deltoid". The study product preparation and expiration times will also be listed on the prepared study product label. The syringe containing the study product should be bagged for transport to the clinic where it will be administered. The study product should be administered within 4 hours of preparation.

Any unused portion of vials or expired prefilled syringes should be disposed of in accordance with institutional or pharmacy policy.

3.3.1.8 Placebo for Env-C Plasmid DNA + HIV Env gp145 C.6980 Protein + ALF43

Using aseptic technique, the pharmacist will withdraw 1 mL of 0.9% sodium chloride for injection into a 3-mL syringe.

The syringe should be labeled as "Env-C Plasmid DNA 2 mg + HIV Env gp145 C.6980 100 µg + ALF43 200 µg 3D-PHAD or Placebo 1 mL", as well as "Administer IM in deltoid". The study product preparation and expiration times will also be listed on the prepared study product label. The syringe containing the study product should be bagged for transport to the clinic where it will be administered. The study product should be administered within 4 hours of preparation.

Any unused portion of vials or expired prefilled syringes should be disposed of in accordance with institutional or pharmacy policy.

3.3.1.9 HIV Env gp145 C.6980 Protein + Rehydragel®

One vial of HIV Env gp145 C.6980 protein, one vial of Rehydragel®, and 0.9% sodium chloride for injection will be needed to prepare the dose. One vial of HIV Env gp145 C.6980 protein will be removed from the -70℃ or colder freezer and allowed to thaw completely at room temperature (15-30°C, approximately 15 minutes). One vial of Rehydragel® will be removed from the refrigerator and allowed to equilibrate to room temperature (15-30°C approximately 10-15 minutes) and then vortexed at 3200 RPM for 30 seconds. 0.9% sodium chloride for injection will be removed from storage.

Once the Rehydragel® vial (5 mg/mL) is equilibrated to room temperature, using aseptic technique and a 22-25-gauge needle and a 1-mL syringe, the pharmacist will add 0.85 mL of 0.9% sodium chloride for injection to the vial. The resulting dilute Rehydragel® suspension will have a concentration of 2.26 mg/mL. Swirl by hand for 1 to 2 minutes, then using a new 22-25-gauge needle and a 1-mL syringe, 0.4 mL of dilute Rehydragel® (2.26 mg/mL) will be added to an empty sterile vial.

When the HIV Env gp145 C.6980 vial is thawed, swirl by hand for 1–2 minutes. Then, using aseptic technique with a 22-25-gauge needle and a 1-mL syringe, the pharmacist will withdraw 0.3 mL of the HIV Env gp145 C.6980 solution and add it to the sterile vial containing 0.4 mL of diluted Rehydragel. With a 22-25-gauge needle and a 3-mL syringe, the pharmacist will add 1.1

mL of 0.9% sodium chloride for injection to the diluted Rehydragel + HIV Env gp145 C.6980 vial to bring the volume to 1.8 mL. The vial will be swirled by hand for 1–2 minutes and then will be incubated at 15-30°C for 10 minutes. After incubation, the vial will be swirled by hand for 1–2 minutes. Using a 3-mL syringe, 1 mL of the formulation will be withdrawn from the vial for study participant administration.

The syringe should be labeled as "HIV Env gp145 C.6980 100 µg + Rehydragel® 500 µg or Placebo 1 mL", as well as "Administer IM in deltoid." The study product preparation and expiration times will also be listed on the prepared study product label. The syringe containing the study product should be bagged for transport to the clinic where it will be administered. The study product should be administered within 4 hours of preparation.

Any unused portion of vials or expired prefilled syringes should be disposed of in accordance with institutional or pharmacy policy.

3.3.1.10 Placebo for HIV Env gp145 C.6980 Protein + Rehydragel®

Using aseptic technique, the pharmacist will withdraw 1 mL of 0.9% sodium chloride for injection into a 3-mL syringe.

The syringe should be labeled as "HIV Env gp145 C.6980 100 µg + Rehydragel® 500 µg or Placebo 1 mL", as well as "Administer IM in deltoid". The study product preparation and expiration times will also be listed on the prepared study product label. The syringe containing the study product should be bagged for transport to the clinic where it will be administered. The study product should be administered within 4 hours of preparation.

Any unused portion of vials or expired prefilled syringes should be disposed of in accordance with institutional or pharmacy policy.

3.3.1.11 HIV Env gp145 C.6980 Protein + ALF43 + Rehydragel®

One vial of HIV Env gp145 C.6980, one vial of ALF43, one vial of Rehydragel® and 0.9% sodium chloride for injection will be needed to prepare the dose. One vial of HIV Env gp145 C.6980 will be removed from the -70°C or colder freezer and allowed to thaw completely at room temperature (15-30°C, approximately 10-15 minutes). One vial of ALF43 will be removed from the -20°C freezer and allowed to equilibrate to room temperature (15-30°C, approximately 10-15 minutes). One vial of Rehydragel® will be removed from the refrigerator and allowed to equilibrate to room temperature (15-30°C, approximately 10-15 minutes) and then shaken to resuspend the aluminum hydroxide. 0.9% sodium chloride for injection will be removed from storage. Once the Rehydragel® vial (5 mg/mL) is equilibrated to room temperature, it should be vortexed at 3200 RPM for 30 seconds, then using aseptic technique and a 22-25-gauge needle and a 1 mL syringe, the pharmacist will withdraw 0.3mL of the suspension and add it to an empty sterile vial.

When the HIV Env gp145 C.6980 vial is thawed, the pharmacist will swirl by hand for 1–2 minutes. Then, using a 22-25-gauge needle and a 1-mL syringe, the pharmacist will withdraw 0.5 mL of the solution and add it to the vial containing 0.3 mL of undiluted Rehydragel®. Using a 22-25-gauge needle and a 1-mL syringe, the pharmacist will add 0.2 mL of 0.9% sodium chloride for injection to the vial containing HIV Env gp145 C.6980 + Rehydragel®, to bring the volume to 1 mL. The vial will be swirled by hand for 1 to 2 minutes and then will be incubated at 15-30°C

for 10 minutes. After incubation, the vial will be swirled by hand for 1–2 minutes. Using aseptic technique, the pharmacist will withdraw 0.4 mL of the HIV Env gp145 C.6980 + Rehydragel® using a 22-25-gauge needle and a 1-mL syringe and will add it to the vial containing ALF43. Using a 22-25-gauge needle and a 1-mL syringe, the pharmacist will add 0.8 mL of 0.9% sodium chloride for injection to the vial containing HIV Env gp145 C.6980 + Rehydragel® + ALF43, to bring the volume to 1.2 mL. The vial will be mixed using a Vortex mixer set at 250 RPM for 2 minutes. Using a 3-mL syringe and a 22-25-gauge needle, 1 mL of the formulation will be withdrawn from the vial for study participant administration.

The syringe should be labeled as "HIV Env gp145 C.6980 100 µg+ALF43 200 µg 3D-PHAD + Rehydragel® 500 µg or Placebo 1 mL", as well as "Administer IM in deltoid". The study product preparation and expiration times will also be listed on the prepared study product label. The syringe containing the study product should be bagged for transport to the clinic where it will be administered. The study product should be administered within 4 hours of preparation.

Any unused portion of vials or expired prefilled syringes should be disposed of in accordance with institutional or pharmacy policy.

3.3.1.12 Placebo for HIV Env gp145 C.6980 Protein + ALF43 + Rehydragel®

Using aseptic technique, the pharmacist will withdraw 1 mL of 0.9% sodium chloride for injection into a 3-mL syringe.

The syringe should be labeled as "HIV Env gp145 C.6980 100 µg+ALF43 200 µg 3D-PHAD+ Rehydragel® 500 µg or Placebo 1 mL", as well as "Administer IM in deltoid". The study product preparation and expiration times will also be listed on the prepared study product label. The syringe containing study product should be bagged for transport to the clinic where it will be administered. The study product should be administered within 4 hours of preparation.

Any unused portion of vials or expired prefilled syringes should be disposed of in accordance with institutional or pharmacy policy.

3.3.1.13 Env-C Plasmid DNA + HIV Env gp145 C.6980 Protein + ALF43 + Rehydragel®

One vial of Env-C Plasmid DNA, one vial of HIV Env gp145 C.6980, one vial of ALF43, one vial of Rehydragel® and 0.9% sodium chloride for injection will be needed to prepare the dose. One vial of Env-C Plasmid DNA and one vial of HIV Env gp145 C.6980 will be removed from the -70°C or colder freezer and allowed to thaw completely at room temperature (15-30°C, approximately 10-15 minutes). One vial of ALF43 will be removed from the -20°C freezer and allowed to equilibrate to room temperature (15-30°C, approximately 10-15 minutes). One vial of Rehydragel® will be removed from the refrigerator and allowed to equilibrate to room temperature (15-30°C, approximately 10-15 minutes) and then vortexed at 3200 RPM for 30 seconds. 0.9% sodium chloride for injection will be removed from storage.

The pharmacist will swirl the Rehydragel® vial by hand for 1 to 2 minutes, then using aseptic technique and a 22-25-gauge needle and a 1 mL syringe, the pharmacist will withdraw 0.3 mL of undiluted Rehydragel® (5 mg/mL) and add it to an empty sterile vial.

When the HIV Env gp145 C.6980 vial is thawed, the pharmacist will swirl by hand for 1–2 minutes. Then, using aseptic technique with a 22-25-gauge needle and a 1-mL syringe, the pharmacist will withdraw 0.5 mL of the HIV Env gp145 C.6980 solution and add it to the vial containing 0.3 mL of undiluted Rehydragel®. Using a 22-25-gauge needle and a 1-mL syringe, the pharmacist will add 0.2 mL of 0.9% sodium chloride for injection to the vial containing Rehydragel® + HIV Env gp145 C.6980 to bring the volume to 1 mL. The vial will be swirled by hand for 1–2 minutes and then will be incubated at room temperature (15-30°C) for 10 minutes. After incubation, the vial will be swirled by hand for 1–2 minutes.

Using aseptic technique, the pharmacist will withdraw 0.4 mL of the HIV Env gp145 C.6980 + Rehydragel® using a 22-25-gauge needle and a 1-mL syringe and will add it to the vial containing ALF43. The vial will be mixed using a Vortex mixer set at 2500 RPM for 5 minutes.

The pharmacist will swirl by hand for 1-2 minutes the thawed Env-C Plasmid DNA vial. Then, using aseptic technique with a 22-25-gauge needle and a 1 mL syringe, the pharmacist will withdraw 0.6 mL of Env-C Plasmid DNA from the thawed Env-C Plasmid DNA vial and will add it to the vial containing HIV Env gp145 C.6980 + Rehydragel® + ALF43. Using a 22-25-gauge needle and a 1 mL syringe, the pharmacist will add 0.2 mL of 0.9% sodium chloride for injection to the HIV Env gp145 C.6980 C.6980 + Rehydragel® + ALF43 + Env-C Plasmid DNA vial, to bring the volume to 1.2 mL. The vial will be mixed using a Vortex mixer set at 250 RPM for 2 minutes.

Using a 3 mL syringe and a 22-25 gauge needle, 1 mL of the formulation will be withdrawn from the vial for study participant administration.

The syringe should be labeled as “Env-C Plasmid DNA 2 mg+ HIV Env gp145 C.6980 100 µg+ ALF43 200 µg 3D-PHAD + Rehydragel® 500 µg or Placebo 1 mL”, as well as “Administer IM in deltoid”. The study product preparation and expiration times will also be listed on the prepared study product label. The syringe containing the study product should be bagged for transport to the clinic where it will be administered. The study product should be administered within 4 hours of preparation.

Any unused portion of vials or expired prefilled syringes should be disposed of in accordance with institutional or pharmacy policy.

3.3.1.14 Placebo for Env-C Plasmid DNA + HIV Env gp145 C.6980 Protein + ALF43 + Rehydragel®

Using aseptic technique, the pharmacist will withdraw 1 mL of 0.9% sodium chloride for injection into a 3-mL syringe.

The syringe should be labeled as “Env-C Plasmid DNA 2 mg+ HIV Env gp145 C.6980 100 µg+ ALF43 200 µg 3D-PHAD + Rehydragel® 500 µg or Placebo 1 mL”, as well as “Administer IM in deltoid”. The study product preparation and expiration times will also be listed on the prepared study product label. The syringe containing the study product should be bagged for transport to the clinic where it will be administered. The study product should be administered within 4 hours of preparation.

Any unused portion of vials or expired prefilled syringes should be disposed of in accordance with institutional or pharmacy policy.

3.3.2 PRODUCTS USED FOR DOSE PREPARATION AND ADMINISTRATION

3.3.2.1 Sterile Water for Injection

Sterile water for injection will be purchased from a commercial vendor based on availability at the time required. Sterile water for injection will be used for hydration of the dmLT. Sterile water for injection will be stored as per the manufacturer's recommendation.

3.3.2.2 0.9% Sodium Chloride for Injection

0.9% sodium chloride for injection (sterile saline) will be purchased from a commercial vendor. The sterile saline will serve as both the diluent and placebo for the trial. Sterile saline will be stored as per the manufacturer's recommendation.

3.3.2.3 Empty Sterile Vials

Empty, sterile, depyrogenated, clears glass vials will be used to mix study products. The vials will be purchased from a commercial vendor based on availability at the time required. The vials will be made of Type I borosilicate glass, with butyl stoppers and aluminum seals. The vials will be stored as per the manufacturer's recommendation.

3.3.2.4 ECG Skin Preparation Paper

ECG skin preparation paper (skin preparation paper) will be purchased from a commercial vendor and stored as per the manufacturer's recommendation. It will be used to prepare the skin prior to patch application.

3.3.2.5 Sterile 2 x 2-inch, 12 Ply Gauze Pad

Sterile 2 x 2-inch, 12-ply cotton gauze pads, individually wrapped will be purchased from a commercial vendor and stored as per the manufacturer's recommendation.

3.3.2.6 Nexcare™ Tegaderm™ Waterproof Transparent Dressing

Nexcare™ Tegaderm™ waterproof transparent dressing (3M), 4x4 ¾ inches, individually wrapped, will be purchased from a commercial vendor and stored as per the manufacturer's recommendation.

3.3.3 LABELLING TO PRESERVE BLINDING

The participants, the clinical staff and the Principal Investigator will be blinded to treatment allocation. The site pharmacist will maintain the randomization code and complete assignments of participants according to the randomization allocation.

The study statistician or DCAC will prepare a randomization list for distribution via password-protected email to the pharmacist. This will include de-identified participant study numbers, treatment assignments, and randomization codes.

Site pharmacists will prepare all doses in the pharmacy and then deliver to the clinic for administration. The pharmacist will apply an overlay to the dosing syringes before applying the

syringe label to prevent possible unblinding if the appearance of the vaccines differs. The study assignment will not appear on any label or document leaving the pharmacy. The syringes will be labeled as follows:

- Participant identifier
- Study product name/placebo
- Dose and volume of all study products/placebo in the syringe
- Route (IM or TCI)
- Preparation date and time
- Expiration date and time
- Any additional information required by the jurisdiction

3.3.4 EXPERIMENTAL VACCINATION ADMINISTRATION

The investigator will determine which arm the injection or TCI will be administered for the first dose and will use the contralateral arm alternating with each subsequent dose. The investigator will assess the planned vaccination site and determine if it is appropriate and allows for adequate post- vaccination assessment. If the participant has scarring, tattoos, or marks that would obscure assessment of the site, the investigator may determine to adjust the planned vaccination area; this could mean that the ipsilateral site is ideal. This data is captured in the case report forms as well as in the participant's study chart source document.

The injection site on the arm to be used for immunization will be gently rubbed five times with an alcohol pad. The injection site will be gently abraded with approximately 10 swipes of an ECG Skin Preparation Paper, M4606A (Philips). The injection site will then be wiped again with an alcohol pad [57]. (The purpose of these procedures is to increase the permeability of the skin. The alcohol wipe extracts lipids from the dead skin cell on the surface of the skin and loosens the dead cells. The skin preparation paper removes dead skin cells from the surface.) Next the Env-C Plasmid DNA will be injected IM at this site. In parallel, a sterile 2 x 2-inch, 12 ply gauze pad will be placed on the adhesive side of a TegadermTM waterproof transparent dressing (4 x 4 ¾ inches). Using a 22-25-gauge needle and a 3-mL syringe, 1 mL of the diluted dmLT will be added to the gauze pad. This gauze pad-TegadermTM dressing will be placed at the site of Env-C Plasmid DNA injection of the volunteer [56], [85]. Similarly, for the placebo recipients, saline will be applied to the pad instead of diluted dmLT. Site investigators will be trained on the site preparation and application prior to the start of the trial. Participants will wear the patch for 6 hours ±2 hours (range 4-8 hours) after application. The participants will remove the patch and record the time on the diary card.

3.4 STUDY PRODUCT ACCOUNTABILITY

Upon receipt at the site pharmacy, the products will be stored in and dispensed by the Investigational Pharmacy. The Food and Drug Administration (FDA) requires accounting for the disposition of all investigational products. The Investigator is responsible for ensuring that a current record of product disposition is maintained, and the product is dispensed only at the site by authorized personnel as required by applicable regulations and guidelines. Records of product

disposition, as required by federal law, consist of the date received, date administered, quantity administered, and the participant number to whom the drug was administered.

The Investigational Pharmacist will be responsible for maintaining accurate records of the shipment and dispensing of the investigational product. The pharmacy records must be available for inspection by the KEMRI SERU, Kenya PPB, the Kenya Ministry of Health, USAMRDC ORP, HRPO, the NIAID, OHRP, the US FDA, WRAIR, and other local or international regulatory entities, and is subject to inspection by a regulatory agency (e.g., FDA) at any time. An assigned Study Monitor will review the pharmacy records.

3.4.1 FINAL DISPOSITION OF STUDY PRODUCTS

All remaining unused study products at non-U.S. clinical research sites, which were supplied by the NIAID CRPMC, must be destroyed on site (as directed by the Sponsor) after the study is completed or terminated. The procedures to be followed and relevant forms are in the Pharmacy Guidelines and Instructions Manual.

4 PARTICIPANT ELIGIBILITY AND MANAGEMENT

4.1 STUDY POPULATION

126 Healthy adults aged between 18 and 40 years will be recruited from Kericho, Kenya. The site's participant screening to enrollment ratio is anticipated at four (4) screened one (1) enrolled. Approximately 504 volunteers will be screened to enroll 126 study participants.

4.2 ELIGIBILITY CRITERIA

4.2.1 INCLUSION CRITERIA

1. Healthy, male and female participant aged 18 to 40 years and available for 26 months
2. Must be at low risk for HIV infection per investigator assessment and using the study risk assessment tool.
3. Must be able to understand and complete the informed consent process.
4. Must be capable of reading English or Kiswahili.
5. Must agree to a home visit.
6. Willing to have photo or fingerprint taken for identification purposes.
7. Must complete a Test of Understanding (TOU) before enrollment. Must answer 9 out of 10 questions correctly, with a maximum of three attempts.
8. Must be in good general health without a clinically significant medical history.
9. HIV-uninfected per diagnostic algorithm within 45 days of enrollment.
10. Laboratory values:

- **Hemoglobin:**

  12.5-18.1 g/dL men

  11.0-16.1 g/dL women

- **White Cell Count:**

  2.7-7.7 x 10^3 cells/μL men

  3.0-9.1 x 10^3 cells/μL women

- **Platelets:**

  125-370 10^3 cells/μL men

  125-444 10^3 cells/μL women

- **ALT and AST:** ≤1.25 institutional upper limit of the reference range

  Creatinine: ≤1.25 institutional upper limit of the reference range

- **Urinalysis:** (dipstick) for blood and protein less than 1+ and negative glucose

Female-Specific Criteria:

11. Negative urine pregnancy test for women at screening, the day of each vaccination, and before any invasive procedure.
12. Already using and commits to continued use of an adequate birth control method for 45 days before to the first vaccine/placebo vaccination and for at least 90 days after the final vaccine/placebo vaccination. Adequate birth control is defined as follows: Contraceptive medications delivered orally, intramuscularly, vaginally, or implanted, underneath the skin, surgical methods (hysterectomy or bilateral tubal ligation), condoms with spermicide, diaphragms, intrauterine device (IUD), vasectomy in a monogamous partner, or abstinence.

4.2.2 EXCLUSION CRITERIA

A history of:

1. Three or more sexual partners in the previous 24 weeks
2. Commercial sex work
3. Non-adherence to condom use in the absence of a long-term monogamous relationship
4. Intravenous drug use in the previous year.
5. A sexually transmitted infection in the previous 24 weeks
6. Is not a sexually active MSM or a transgender person in the past 6 months.
7. Asplenia: any condition resulting in the absence of a functional spleen
8. Bleeding disorder diagnosed by a medical doctor (e.g., factor deficiency, coagulopathy, or platelet disorder requiring special precautions)
9. Breastfeeding or pregnant (positive pregnancy test) women or planning to become pregnant during the window between study enrollment and three months after the last vaccination visit.
10. Any past, ongoing, or in remission history of treated or untreated autoimmune disease.
11. Has known active Hepatitis B virus infection (or positive HBsAg).
12. Has known active Hepatitis C infection
13. History of anaphylaxis or other serious adverse reaction to vaccines or allergies or reactions likely to be exacerbated by any component of the vaccine and placebo, including antibiotics or excipients
14. Absolute Neutrophil Count (ANC):

    $<1.0 \times 10^3$ cells/μL
15. Participant has received any of the following substances:

- Chronic use of therapies that may modify immune response, such as intravenous (IV) immune globulin and systemic corticosteroids (in doses of >20 mg/day prednisone equivalent for periods exceeding 10 days)
- The following exceptions are permitted and will not exclude study participation:
    - use of corticosteroid nasal spray for rhinitis,
    - topical corticosteroids for an acute uncomplicated dermatitis; or
    - A short course (10 days or less, or a single injection) of corticosteroid for a non-chronic condition (based on investigator clinical judgment) at least 2 weeks before enrollment.

16. Blood products within 120 days before HIV screening
17. Immunoglobulins within 30 days before HIV screening
18. Any experimental vaccine containing an adjuvant other than aluminum or an adjuvant not approved by the FDA or European Medicines Agency (EMA) as part of a licensed vaccine.
19. CERVARIX vaccine against HPV (containing AS04)
20. Receipt of any investigational HIV vaccine, investigational research agents or vaccine within 30 days before enrollment.
21. Anti-tuberculosis prophylaxis or therapy during the past 90 days before enrollment
22. Any psychiatric, social condition, occupational reason, or other responsibility that, in the judgment of the investigator, is a contraindication to protocol compliance or impairs a participant's ability to give informed consent
23. Major psychiatric illness and or substance abuse problems during the past 12 months that, in the opinion of the investigator, would preclude participation
24. History of atopy or significant skin conditions
25. Study site employees who are involved in the protocol and or may have direct access to the study-related area

Final evaluation of eligibility is based on the medical judgment of the investigator. The PSRT will also remain available to PI for consultation if desired.

4.3 LYMPH NODE BIOPSY ELIGIBILITY CRITERIA

4.3.1.1 Lymph Node Biopsy Inclusion Criteria

1. Body mass index (BMI) <35
2. Platelets > 150,000
3. International normalized ratio (INR) < 1.2

4. Verbal report of no NSAIDS/aspirin for 7 days prior

5. Negative Pregnancy test for participants born female

4.3.1.2 Lymph Node Biopsy Exclusion Criteria

1. History of keloid formation

2. History of an inguinal hernia, inguinal canal cryptorchidism, varicocele, hydrocele

3. Local infections (or lymphadenitis) or rash in the groin, even if limited to the contralateral groin

4. History of inguinal excisional lymph node biopsy

4.4 PARTICIPANT MANAGEMENT

4.4.1 PARTICIPANT RESPONSIBILITIES

In addition to meeting the eligibility criteria listed above, participants will also be asked to agree to not participate in any other research study or donate blood during their participation in the RV 460 study.

4.4.2 WITHDRAWAL FROM THE STUDY, DISCONTINUATION OF STUDY PRODUCT, OR STUDY TERMINATION

4.4.2.1 Withdrawal

- 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 site investigator
- Participant requests withdrawal

Each participant has the right to withdraw from the study at any time for any reason without affecting the right to treatment. The study staff should attempt to contact participants who did not return for scheduled visits or follow-up. Although the participant is not obliged to give a reason for withdrawing prematurely, the investigator should make a reasonable effort to ascertain the reason(s) while fully respecting the participant's rights. Participants who elect to not receive additional vaccinations may be asked to continue follow up assessment for safety until resolution in the case of an on-going AE.

The study team will use several approaches that include at least three phone call attempts each to participant and to a confidant through contacts the participant would have provided and documented in the participant contact information form. If the participant is not located by phone call attempts, a home visit will be done to locate the participant. If after the home visit the participant cannot be contacted, they will be declared lost to follow-up.

For those participants who are unable to continue participation in the study, but who do not withdraw consent, an exit visit will be conducted. Any participant who withdraws consent will not have any further data collected after consent has been withdrawn.

Participants may elect to withdraw consent for future use of their biological specimens or data at any time. This will be recorded in the EDC and source and the data samples will be marked to not be used in the future. The data and specimens will be destroyed within one year of receiving notification from the protocol chair that all protocol testing is complete. Samples remaining in the KEMRI/USAMDR-A CRC repository will be destroyed by laboratory staff following approved laboratory procedures.

See Appendix A: Schedule of Evaluations

4.4.2.2 Participant Replacement

Participants who withdraw, are withdrawn from this study, or are lost to follow-up after signing the informed consent form (ICF) and administration of the study product will only 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.

4.4.2.3 Early Discontinuation of Vaccinations or Withdrawal of a Study Participant

A participant may have vaccinations discontinued or will be taken out of the study entirely under the following circumstances:

4.4.2.3.1 Immediate Early Discontinuation

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 adverse event assessed as related to the study agent, and
3. Infection with HIV-1.

4.4.3 Management of Medical Conditions

Participants will be offered pre-test counseling before HIV testing is done. When giving HIV results to participants, post-test counseling will be done, and those who test positive will be referred to KCRH, or any other health facility of their choice, for HIV care and treatment. The HIV pre and post-testing counselling will be provided by a psychological counsellor at the site, as well as by study clinicians/nurses trained in HIV counselling and testing.

All individuals with clinical sexually transmitted infections (STIs) will be treated according to the Kenya Ministry of Health local standards of care.

For all other medical illnesses and abnormal laboratory values, participants will consult study staff for treatment and or referral to the appropriate health facility during the study period.

4.4.4 MANAGEMENT OF PARTICIPANTS WHO BECOME PREGNANT

Pregnant women are excluded from enrollment, however, if a participant becomes pregnant during the study, further vaccinations will be stopped, and she will be referred for maternal and child health services. Participants will continue to be followed for the remainder of the study period for safety, and the study team will follow the course of the pregnancy until the outcome is documented. During subsequent study visits, blood draws will be conducted for safety evaluations only. No optional collections or procedures will be performed.

The Kericho site PI or designated associate investigator will be responsible for reporting any pregnancy to the PSRT and the study database. The PSRT will review this information. The site PI will forward notification as necessary to the WRAIR IRB and KEMRI SERU (Scientific and Ethics Review Unit).

Pregnancy outcomes will be recorded via a standardized case report form. Information documented on this form will include the date of last menstrual period, the date that the pregnancy was confirmed, any history of complications during prior pregnancies (such as congenital abnormalities or spontaneous abortions), and the outcome of the pregnancy including date of termination or delivery, any complications of pregnancy, and the status of the child. A separate case report form will be completed for the delivered child to document date of delivery, gender, weight, the presence of any congenital abnormalities, American Pediatric Gross Assessment Record (APGAR) score, HIV status, and any other complication of delivery.

4.4.5 MANAGEMENT OF PARTICIPANTS WHO BECOME INCARCERATED

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.

4.4.6 MANAGEMENT OF PARTICIPANTS WITH VACCINE-INDUCED SEROPOSITIVITY (VISP) OR SEROREACTIVITY (VISR)

Participants who receive study vaccine (not placebo) may subsequently receive a false positive on an HIV antibody test. Participants are asked to contact the PI or study staff if at any time during or after study participation if an HIV test is requested outside of the study. 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, and the study will provide re-testing.

4.4.7 TRANSPORTATION

The study team anticipates that nearly all participants or their confidants will have a phone. For those participants who are unable to be contacted by phone, community mobilizers will contact them with a home visit. In order to adequately locate participant's home, all participants will be dropped home after the first vaccination visit and the exact location of their homes documented in

the locator form. We will use alternative contacts to trace them if they are not home. Study participants will use public transport to and from the study site. Public transportation is good and reliable at recruitment catchment area. Transportation will not be provided by the study team except in the event of extenuating circumstances when a participant does not have adequate means of transportation for clinic visits. If the participant did not use the site's transport, they would also receive reimbursements for transportation, taking into consideration the average cost of travel to the clinic.

4.4.8 UNBLINDING PROCEDURES

Once all study visits have completed, the Kericho site investigators will contact study participants by phone or through a home visit to invite them to come back to the clinic to find out whether they received the study product or placebo. When the participant returns to the clinic for unblinding, they will be given a letter that indicates their study arm.

5 STUDY PROCEDURES

5.1 RECRUITMENT

Potential subjects will be made aware of the need for study participants through planned community sessions, church gatherings, public meetings, government administrative meetings (chief's *baraza's*) or because of previous trial participation. Participants will be invited to attend a general information session at the KEMRI/USAMRD-A Clinical Research Center. After the information session, individuals that indicate interest in the study will be invited and scheduled to attend a briefing session (using the WARIR IRB approved slides) at their convenience. The Principal Investigator (PI) or designee will conduct briefing sessions at regular intervals throughout the recruitment phase. After the briefing, the potential participants with have the opportunity for questions. All participants interested in this study will subsequently be administered informed consent individually.

5.2 CONSENT PROCEDURES AND SCREENING

All participants that present for screening will be documented in a screening log.

Potential subjects must be literate to participate in the study and the consent forms will be offered in English and Kiswahili. The PI or their designee will review the consent form in detail with potential participants and answer any questions. Written informed consent will be obtained from each participant before any study procedures are performed. All informed consent forms will be administered individually, in a private setting, with respect for confidentiality. Participants will be given ample time and opportunity to inquire about the details of the study, discuss the study with family members or friends, and ask any questions before signing the informed consent forms. A copy of the signed informed consent form will be given to the participant along with a copy of the participant event schedule. The consenting process will be documented in the case report forms (CRFs).After providing signed informed consent, the participant will complete a TOU. Participants can take the TOU three times but must have a passing score of 90% or greater by the third attempt to participate in the study. If after three attempts to pass the TOU, and the participant is unable to do so, the participant will become ineligible for study participation.

Participants who have passed the TOU and have given written informed consent will then undergo a review of complete medical history including medications, physical examination, and screening laboratory assessments to determine eligibility for trial participation.

Counseling on the potential risks of becoming pregnant during this trial will be provided. Pre-HIV test counseling and post-HIV test counseling as well as HIV prevention guidance will be provided during the screening process.

Study participants might be scheduled for a second screening visit to evaluate abnormal, not medically significant, findings (including laboratory tests). If the participant presents with an abnormality or illness that is determined to likely be related to a transient illness, they could also be invited back for reassessment.

Upon screening or rescreening, the investigator may discover an illness or condition that requires treatment. The site will provide primary care and treatment as per Kenyan National guidelines and refer these participants to an appropriate healthcare facility for further evaluation and treatment.

Participants that elect to have cervicovaginal secretion collection will undergo a routine PAP smear for assessment of inflammation or other abnormality including cellular changes. Abnormal results will be discussed with the participant and treatment or referral for care will be provided. Abnormal PAP results are exclusionary for the optional cervicovaginal secretion collection only.

5.3 ENROLLMENT AND RANDOMIZATION

There will be 18 participants per group (15 vaccine recipients and 3 placebo recipients). Investigators will be blinded to vaccine versus placebo within each group but not across groups. Participants will be block randomized. Group 1 (with a prime of Env-C Plasmid DNA IM alone at weeks 0, 4, 12) will be fully enrolled first. After the 18 participants in Group 1 are enrolled, the remaining groups, 2-7, will begin enrolling participants. Once 24 participants in Groups 2-7 have been enrolled (four from each group) and received their first vaccinations, enrollment and vaccinations in Groups 2-7 will be paused for the time it takes the PSRT to review (typically 3 to 14 days) blinded safety data from these 24 participants covering the period immediately after their first vaccination through Day 7. 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.

When safety has been confirmed with PSRT review, the remaining 14 participants in each of Groups 2-7 will be enrolled, and vaccinations will continue until the first 24 participants in Groups 2-7 (four per group) have received their first boost dose (at 20 weeks—to create a time gap between boost vaccinations of the first cohort and the remaining study participants).

A second scheduled pause (this pauses boost vaccinations only; prime vaccinations can continue) will occur for Groups 2-7 after the same set of participants reviewed during the first scheduled pause (the first 24 participants (four per group) from Groups 2-7) has received its first boost (Group 1 can continue receiving vaccinations). This pause will review blinded safety data from these 24 participants, covering the period from the first boost through Day 7 after that boost. At both scheduled pauses, the PSRT will consult the SMC regarding the pause if either of the following conditions are met: 1) one (or more) participants experience an SAE that is 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. Boost vaccinations for Groups 2-7 will resume once safety data from the booster sentinel cohort have been reviewed by the PSRT and they have concluded it is safe to proceed.

5.4 VACCINATION

The site for injection cleaned with an alcohol wipe before the injection. The vaccines will be given by intramuscular (IM) injection into the deltoid muscle, excluding the dmLT adjuvant which is given by TCI. Alternating deltoids will be used for subsequent immunizations. A study vaccination outside of a participant's vaccination window may occur at the discretion of the PSRT.

5.5 STUDY VISITS

Participants will have up to 26 clinic visits. The active study phase will last a total of 105 weeks, which will include the time of the first screening visit through the final clinic study visit. In addition, participants will be contacted once per week for 3 weeks (3 phone contacts) following the 105 week active study phase. At these contacts, staff will collect the information listed below.

- Confirmation of vital status; if deceased, attempt to learn cause and date of death;
- If participant is alive, record the participant's responses to questions regarding any occurrence of the following events since the last study contact:
- Life-threatening adverse experiences;
- Persistent or significant disability/incapacity;
- Hospitalizations and reasons;
- New diagnosis of HIV infection; and
- Pregnancies and outcomes, including congenital anomalies/birth defects

All such events will be recorded, and adverse events other than HIV infection will be assessed for relationship to study product(s). Clinic visits will only be required if HIV confirmatory testing is necessary; however, a clinic visit may be arranged for other reasons.

Evaluation of the safety of the study vaccines is outlined in the schedule of evaluations (SOE) and will include laboratory studies, medical history, physical assessment by clinicians, and participant self-assessment recorded on a 7-day diary card. Additional study visits may be required if, in the investigator's opinion, any lab value warrants repeating. The first vaccination visit could take up to 6 hours, but the other vaccination visits take about 4 hours each. Most other follow-up clinic visits will usually take about 2 hours.

Total blood volume drawn from each participant will not exceed the US Department of Defense (DoD), American Association of Blood Banks (AABB) and FDA guidelines of 450 mL in an eight-week period. If a participant is anemic, each visit's blood volume will be reviewed, and the blood drawn for research may be reduced or not drawn at all, depending on the severity of anemia.

Table 4: Visit Procedures

Clinic visit activities
Screening Visit • Study briefing and collection of contact information • Informed consents • Test of understanding • Review medical history including medications • Vital signs (blood pressure, pulse, respiratory rate, and oral temperature) • Complete physical exam including height and weight • CBC, creatinine, ALT, and AST • HIV risk counseling • HIV test with counseling before and after • Hepatitis B and C • Syphilis test • Pregnancy counseling • Urine pregnancy test for women • Urinalysis (dipstick) for blood, protein, and glucose **Optional** • Pap smear for women who agree to cervicovaginal secretion collection
Vaccination Visits • Targeted physical exam and review of current medication • Vital signs (blood pressure, pulse, respiratory rate, and oral temperature) • Review eligibility • HIV risk counseling • Blood draw for safety*, research and HIV tests • Pregnancy counseling • Urine pregnancy test for women • Enrollment and randomize* • Administer vaccination or placebo • Post-injection observation for 30-60 minutes • Adverse event documentation • Provide a diary card and instruction **Optional** • Mucosal secretion collection*

Phone/Clinic Follow Up Visits after Each Vaccination and as Needed

- Assessment of wellbeing
- Measure vital signs (blood pressure, pulse, respiratory rate, and oral temperature) *if the participant comes to the clinic*
- Review of symptoms
- Adverse event documentation

Telephone follow-up will be the default method for this visit. Home visits will be done for those participants who will not be reachable by phone.

Follow Up Visits to Include Final Visit

- Targeted physical exam and review of concomitant medication
- Measure vital signs (blood pressure, pulse, respiratory rate, and oral temperature)
- Body weight, if clinically indicated
- HIV risk counseling
- Pregnancy counseling
- Urine pregnancy test for women if mucosal secretion collection or lymph node biopsy*
- Review of diary card*
- Adverse event documentation
- Blood draw for safety, research, and HIV tests*

Optional

- Mucosal secretion collection*
- Lymph node biopsy and biopsy site assessment*

** See Appendix A: Schedule of Evaluations*

5.6 OPTIONAL PROCEDURES

5.6.1 MUCOSAL COLLECTIONS

Participants will have the option of participating in separately consented mucosal collections. These collections will be conducted at the Kericho site at baseline and subsequent intervals per the SOE. Every effort will be made to have the procedures begin early, and in the event that this is not achieved, the procedures will be split and done over two consecutive days, within the study visit window.

5.6.1.1 Female Participants

Female participants may choose to undergo in cervicovaginal secretion and or rectal sponge secretion collections. Rectal secretions will be collected by rectal sponges by use of a rectal speculum and 2-4 sponges are inserted into the rectum mucosa for at least 4 minutes. The collected sponges will be placed in a sterile conical tube and shipped to the lab for processing. Consenting women will be instructed on how to use a Softcup device to collect cervicovaginal secretions. The cup will remain in place for 4-12 hours. Women that consent for cervicovaginal secretion collection will undergo a PAP smear test at screening. A urine pregnancy test will be done prior to each collection. Pregnant women and those with a history of toxic shock syndrome will be excluded. Date of last menstrual period will be recorded at each collection visit. The collection will not take place if the woman is menstruating or has symptoms of active inflammation or

infection of the vagina or cervix. Rectal sponge secretions will also be collected however will be deferred if there are signs or symptoms of perianal inflammation. Participants will be asked to refrain from penetrative sex up to 72 hours prior to Softcup or sponge placement.

5.6.1.2 Male Participants

Male participants may participate in semen collection and or rectal sponge secretion collections. Consenting men will be asked to ejaculate into a sterile container for semen collection. Rectal secretions will be collected by rectal sponges by use of a rectal speculum and 2-4 sponges are inserted into the rectum mucosa for at least 4 minutes. The collected sponges will be placed in a sterile conical tube and shipped to the lab for processing. Rectal sponge secretions will also be collected at this visit if the participant agrees. Semen and rectal sponge collections will be deferred from men if there are signs or symptoms of urethral or perianal inflammation, respectively. Participants may participate in semen collection and or rectal sponge secretion collections.

5.6.2 LYMPH NODE BIOPSIES

Excisional lymph node biopsies have been performed (for reasons other than sentinel node staging for cancer) by research sites around the world including MHRP sites in Kenya, Uganda, Tanzania, Thailand in HIV-infected and uninfected study participants, without any post-operative severe sequelae. Moreover, a recent article describing a meeting of experts discussed the importance of evaluating germinal center responses following immunization to predict broadly neutralizing antibody potency and breadth. A review of existing data showed that risks associated with excisional biopsies are low and with few complications [94].

Willing study participants will have the option of participating in a separately consented procedure of excision of intact inguinal lymph nodes. Inguinal lymph node excision will be offered at week 14 and week 58 and will be performed by a qualified doctor trained to perform superficial lymph node excision. The procedure will be performed under local anesthesia and last approximately 30-40 minutes. Anesthetics, antiseptics, and antibiotics are per surgeon's SOP and will be modified if the participant reports an allergy or sensitivity. Participants will then be asked to rest under observation for another 2-3 hours. Post-procedure, participants will return to the clinic in about 1 week for assessment of the biopsy site.

Coagulation testing will be done approximately 1 week before each biopsy and urine pregnancy testing will be done just prior to each biopsy.

Participants will be asked for their consent for photography if the clinician wishes to document an unusual or unexpected finding. Pictures may also be taken of the biopsy site immediately after the procedure and again 6 months later in order to provide an objective reference for biopsy site healing (i.e. for participant safety) and for possible use as participant educational material (for other participants who undergo the same procedure).

Assessment of the biopsy site includes:

- Evaluation for infection
- Seroma
- Cellulitis

- Lymphedema
- Pain
- Bleeding
- Hematoma
- Erythema
- Scarring
- Adhesive tape reaction
- Numbness or altered sensation of the area.

5.7 INFORMATION SHEET ON COVID-19

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 an information sheet 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 and SERU will be notified when such a determination is made.

6 LABORATORY ASSESSMENTS

6.1 BLOOD SAMPLES

After collection, all specimens will be transported to the site laboratory according to a standard operating procedure (SOP). Samples will be packaged in appropriate isothermal containers for transportation. On arrival at the laboratory, all specimens will be logged into a sample tracking database before distribution to the different assay benches for processing.

Blood processing is performed using Good Clinical Laboratory Practices (GCLP), with personal protective equipment, including lab coats, gloves, and laminar flow hoods. All specimens will be labeled with barcode indicating participant identification numbers (PID), sample type, date and study visit number and will be indexed and cross-referenced in a specimen-tracking database. The processing lab staff will only know the PID to ensure confidentiality.

Blood collected for plasma or serum specimens will be centrifuged, aliquoted, and stored at –70ºC or colder. PBMCs will be isolated, cryopreserved and stored in the vapor phase of a liquid nitrogen freezer. Mucosal Specimens will be processed according to standard laboratory operating procedures and stored -70℃ or colder freezer. All specimens will be labeled with barcode indicating: PID, sample type, date and study visit number and will be indexed and cross-referenced in a specimen-tracking database. These procedures are detailed in the laboratory MOP.

6.1.1.1 Laboratory tests to assess safety

Clinical laboratory testing will take place in the CAP-certified laboratory and includes pregnancy testing, complete blood count, chemistry (Creatinine, ALT, AST) and HIV testing. HIV testing will be performed according to the approved laboratory algorithm including 4th generation immunoassay and nucleic acid testing when indicated. Safety labs may be repeated if clinically indicated.

6.1.2 IMMUNOGENICITY TESTS

Cryopreserved samples will be shipped to laboratories at MHRP/WRAIR, Armed Forces Research Institute of Medical Sciences (AFRIMS) or other laboratories listed to perform the assays listed below. Additional assays/analytes may be added that fall into the categories described in the schedule of events.

6.1.2.1 HIV-1-specific binding antibody assays

Binding antibody assays will be performed to detect serum, plasma, or mucosal secretion binding antibodies to a broad array of HIV-1 antigens. Serum and plasma will be also tested for their ability to block HIV-1 binding to the α4β7 receptor and other possible HIV-1 co-receptors. In addition, antibody isotype, subclass, FC gamma, complement usage, and other antibody profiling tools may be utilized in parallel with antigen specificity. Monoclonal antibodies may also be isolated from selected vaccine recipients.

6.1.2.2 Functional antibody assays

Antibody-dependent Cell-Mediated Cytotoxicity (ADCC) Assay and Antibody-dependent Cell-Mediated Viral Inhibition (ADCVI) Assays: ADCC assays will be performed at visits specified in the SOE using rapid fluorometric ADCC assay. Other functional assays may include but are not limited to Antibody-dependent Cell-Mediated Phagocytosis (ADCP) and Antibody-dependent Complement (ADC) activation assays.

6.1.2.3 Neutralizing antibody assays

Neutralization assays will be performed similarly to other HIV vaccine trials using cell line-based and PBMC assays with a panel of viruses from different HIV subtypes, including A, B, C, D, and other circulating recombinant forms (CRFs) such as AE and AG.

6.1.2.4 Cellular immune assays

Cryopreserved PBMC and lymph nodes will be stimulated with HIV-1-specific antigens and tested using standard (but not limited to) cellular immune assays which may include but are not limited to Intracellular Cytokine Staining, ELISPOT, Lymphoproliferation assays, B-cell analysis, T-follicular helper cell, and innate immune cell analysis

6.1.3 OTHER ASSAYS

- Gene expression/transcriptomic analysis using current technologies.
- Genetic analysis including but not limited to Human Leukocyte Antigen (HLA) subtyping.
- Soluble factor/cytokine analysis: Levels of soluble inflammatory markers will be evaluated representing different biological pathways such as general inflammation, tumor necrosis factor-α (TNF-α) signaling, IFN-γ signaling, and lymphocyte activation. Additional markers may be included.
- Sequencing of HIV-1 RNA to characterize viral features and assess the effect of the vaccine if a volunteer becomes infected with HIV-1 during the study.

6.2 FUTURE USE AND STORAGE OF BIOLOGICAL SAMPLES

Each study participant will be asked to voluntarily consent to their 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 biological samples stored for future use, they can consent to participate in this study only, without having their blood samples stored for future testing. In this case, their blood samples will be destroyed within one year of receiving notification from the protocol chair that all protocol testing is complete. Samples remaining in the KEMRI/USAMDR-A CRC repository will be destroyed by laboratory staff following approved laboratory procedures.

All samples for which consent has been obtained and for which additional material is available after study specified testing is complete will be stored for future testing at the KEMRI/USAMRD-A CRC Laboratory in Kericho. However, KEMRI SERU and WRAIR IRB approval will be sought before any such samples are used for analysis not specified in the protocol or a protocol amendment approved by the IRB. All samples belong to the site from which they were obtained and MHRP.

During the study, blood samples will be tested soon after collection. The remaining samples will then be stored at the KEMRI/USAMRD-A CRC lab. For specialized tests that are not available at this site some of the blood samples will be shipped to the United States for HIV immune response testing as well as genetic testing. Shipment of these samples will be done only after the approval of the KEMRI SERU.

7 DATA MANAGEMENT AND ANALYSIS

7.1 STUDY DOCUMENTATION, STORAGE, AND DATA COLLECTION

Signed Informed Consent Forms (ICFs) and study documents are kept in a room that is double locked with limited access. The investigators and staff are responsible for ensuring maintenance of a comprehensive centralized filing system of all study-related (essential) documentation, suitable for inspection at any time by the following organizations will be granted direct access to the participant's original medical records: KEMRI SERU, Kenya PPB, the Kenya Ministry of Health, NIAID, US FDA, WRAIR, USAMRDC, ORP, HRPO, and the people who work for these organizations, and other local, US or international regulatory agencies. Participants information may also be reviewed or inspected for verification of clinical trial procedures and or data. Inspections will be conducted without violating the confidentiality of the participant, to the extent permitted by the applicable laws and regulations and that, by signing a written informed consent form, the participant is authorizing such access.

Data will be collected using source documents and or Case Report Forms (CRFs). Direct entry using tablets or computers is possible depending on the current site capability. Data will be uploaded daily to the central DCAC database. Access to the data collection software is limited to PI approved staff and requires unique usernames and passwords. Data are housed in a secure database maintained by the DCAC, MHRP in accordance with Good Clinical Practices (GCP). Study data are referenced only by the study-specific identification code. The study records will be stored at the KEMRI/USAMRD-A triple-locked filing room for the period when the study is active, and thereafter transferred to the site's archive for long term storage. All participants consented will be assigned a 7-digit Participant Identification (PID). Participant IDs will be assigned sequentially, for example 460-0001.

7.2 ANALYSIS

7.2.1 POWER AND SAMPLE SIZE

The safety, reactogenicity, and tolerability of the vaccine regimens are the primary endpoints for this study. Below in Table 6, the estimated probability of a participant having at least one of these events (in percent), along with the associated exact Clopper-Pearson 95% confidence interval, is given for various numbers of observed events that correspond to approximate event rates within study groups of possible interest. Note that some event rates cannot be exactly observed within a given group, depending on the sample size (e.g. 10% of 15 participants is 1.5 participants, which is rounded to 2 in the table below).

Table 5: Estimated probability (in percent) of a participant having at least one AE, along with the associated exact Clopper-Pearson 95% CI, given varying sample sizes and numbers of participants with at least one observed AE

Group	n	Approximate AE Rate[1]							
		0%		10%		20%		50%	
		Corresponding # AEs[2]	Estimated Probability (95% CI)	Corresponding # AEs[2]	Estimated Probability (95% CI)	Corresponding # AEs[2]	Estimated Probability (95% CI)	Corresponding # AEs[2]	Estimated Probability (95% CI)
Each Individual Group	15	0	0 (0, 21.8)	2	13.3 (1.7, 40.5)	3	20 (4.3, 48.1)	8	53.3 (26.6, 78.7)
Placebo	21	0	0 (0, 16.1)	2	9.5 (1.2, 30.4)	4	19 (5.4, 41.9)	11	52.4 (29.8, 74.3)
Any DNA Prime with HIV Env gp145 C.6980 /Rehydragel Boosts	105	0	0 (0, 3.5)	11	10.5 (5.3, 18.0)	21	20 (12.8, 28.9)	53	50.5 (40.5, 60.4)
DNA alone (1+2), DNA+TCI (3+4), DNA/ALF43 (5+6)	30	0	0 (0, 11.6)	3	10 (2.1, 26.5)	6	20 (7.7, 38.6)	15	50 (31.3, 68.7)
HIV Env gp145 C.6980 /Rehydragel boost (1+3+5), HIV Env gp145 C.6980 /Rehydragel/ALF43 boost (2+4+6)	45	0	0 (0, 7.9)	5	11.1 (3.7, 24.1)	9	20 (9.6, 34.6)	23	51.1 (35.8, 66.3)
Any ALF43	75	0	0 (0, 4.8)	8	10.7 (4.7, 20.0)	15	20 (11.6, 30.8)	38	50.7 (38.9, 62.4)

[1] The approximate proportion of participants in a group reporting at least one event of interest

[2] The number of participants with observed event(s) needed to most closely correspond to the given approximate event rate, with the given sample size

For illustration, if 2 individuals among the 15 participants in an individual vaccine group experienced at least one event, the estimated event probability would be 13.3%, with an associated exact 95% CI of (1.7%, 40.5%). Eleven individuals reporting at least one event among all active participants (n=105) would lead to a similar estimated event probability of 10.5%, but with a narrower exact 95% CI (5.3%, 18%).

The table below gives the probability of observing at least one or more than one event in a group of interest, given underlying per-subject probabilities of at least one event.

Table 6: Probability of observing at least one or at least two safety events in a given group, given varying underlying per-participant probabilities of experiencing at least one event

		Underlying Probability of At Least One Event					
		1%		10%		20%	
Group	**n**	>0	>1	>0	>1	>0	>1
Each Individual Vaccine Group	15	14.0%	1.0%	79.4%	45.1%	96.5%	83.3%
Placebo	21	19.0%	1.9%	89.1%	63.5%	>99%	94.2%
Any Env-C Plasmid DNA Prime with HIV Env gp145 C.6980 /Rehydragel Boosts	105	65.2%	28.3%	>99%	>99%	>99%	>99%
Env-C Plasmid DNA alone (1+2), Env-C Plasmid DNA+TCI (3+4), Env-C Plasmid DNA/ALF43 (5+6)	30	26.0%	3.6%	95.8%	81.6%	>99%	>99%
HIV Env gp145 C.6980 /Rehydragel boost (1+3+5), HIV Env gp145 C.6980 /Rehydragel/ALF43 boost (2+4+6)	45	36.4%	7.5%	>99%	94.8%	>99%	>99%
Any ALF43	75	52.9%	17.3%	>99%	>99%	>99%	>99%

7.2.2 PRIMARY ANALYSIS

The primary endpoints of this Phase 1 study are related to the safety, reactogenicity and tolerability of the Env-C Plasmid DNA vaccine regimens. These will be monitored by PSRT and SMC as appropriate, and SAE), AEs and PIMMC will be recorded until the end of the trial.

SAEs, AEs, AESIs, reactogenicity, and tolerability will be tabulated by study group and combinations of interest. Exact 95% Clopper-Pearson confidence intervals will be calculated for each set of events, and the maximum severity and relatedness will be reported for each participant. While the study was not designed with the purpose of detecting safety differences, comparisons between regimens or between groups of interest and the placebo controls may be made. These comparisons would be made at a two-sided α=0.05 level, using the normal approximation to the binomial distribution (z-test) as mentioned in the previous section, or an alternate testing procedure as appropriate (e.g. Fisher's Exact test for smaller samples). No adjustments for multiple comparisons will be made.

7.2.3 SECONDARY ANALYSIS

The secondary endpoints of this study involve the immune response to the vaccination regimens in either the systemic and mucosal compartments. The laboratory assays used to measure these various immune response endpoints typically result in continuous measurements for each participant.

For each continuous immune response variable of interest, means, medians, ranges and 95% Wald confidence intervals will be computed. Proportions and exact 95% Clopper-Pearson confidence intervals will be computed for any categorical variables.

Comparisons of these endpoints between study groups may be made using non-parametric Mann-Whitney U tests, and once again, no adjustments for multiple comparisons will be made due to the hypothesis- generating nature of the study. The groups will be the same as used for the safety comparison but also may include other groups. For any categorical secondary endpoints, Fisher's Exact tests or asymptotic tests assuming normality will be used as appropriate.

7.2.4 EXPLORATORY AND SUBGROUP ANALYSES

Exploratory and subgroup analyses involve additional immune response endpoints as well as DNA microarrays. These will be analyzed similarly to the secondary endpoints. For any genetic analyses, control of the false discovery rate (FDR) at the 0.1 level will be made via p-value adjustment.

After the assays for the secondary and exploratory endpoints are finalized and before the final analyses have begun, a statistical analysis plan (SAP) will be created to elucidate upon these details and make any changes as necessary.

8 SAFETY AND REPORTING

8.1 ASSESSING AND RECORDING SAFETY PARAMETERS

8.1.1 REACTOGENICITY

8.1.1.1 Diary Card (7-Day Solicited Reactogenicity)

Reactogenicity events are AEs that are common and known to occur following administration of this type of study vaccine. A diary card will be used to aid in the collection of potential reactions.

Temperature and solicited local and systemic symptoms will be recorded in the clinic before vaccination and 30-60 minutes post-injection, at home about 6 hours after vaccination, and then daily by the participant for 7 days. Solicited symptoms that persist after the end of day 7 will be considered an AE or SAE, will be documented, and followed through resolution.

At each vaccination visit, participants will be given a new diary card, a thermometer and a ruler. The diary card will be used as a memory aid, on which the participant will record oral temperature, local and systemic symptoms, and concomitant medications daily for 7 days, beginning with the day of vaccination. Participants will be trained to complete the diary card, how to use the thermometer, and how to measure injection site swelling and redness using the ruler after each vaccination. Completion of the diary card training will be noted in source documents. The diary card is not a source document; the information gained from the review of the diary card and the interview with participants will be documented using a combination of progress notes and CRFs maintained in the participants research chart.

The solicited signs and symptoms on the diary card are:

Local

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

Systemic

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

- Dizziness

Where multiple assessments are taken on the same day, participants will be requested to record the day's highest measured temperature and measurement of largest dimension for redness and swelling.

Follow-up on participant well-being will be performed by telephone (preferred method) or clinic visit on the day following vaccination (and up to 48 hrs. later). Diary cards will be reviewed with the clinician on day 7 ± 2 following each vaccination. If upon reviewing the diary card, the clinician finds that local or systemic information (except swelling, redness, temperature measurements) is missing from the diary card, or the diary card is lost, the participant will be asked to provide the missing information to the best of their recollection. The missing data, such as temperature measurements will be indicated as missing in the database and a lost diary card will not result in a protocol deviation.

8.1.2 MANAGEMENT OF VACCINATION REACTIONS

Participants will be observed in the KEMRI/USAMRD-A CRC by study staff for 30 to 60 minutes after vaccination. On vaccination days, there will be at a minimum one physician investigator available for assessment of any reaction.

In the event of a severe allergic reaction, the CRC is staffed with trained medical personnel and stocked with appropriate medical emergency equipment to provide acute care for conditions such as anaphylaxis. If needed, participants will be transferred to the Unilever Hospital for further assessment and care.

8.1.3 ADVERSE EVENTS (AES)

ICH E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation participant administered a pharmaceutical product regardless of its causal relationship to the study treatment. FDA defines an AE as any untoward medical occurrence associated with the use of a drug in humans, whether considered drug related.

An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study recipient presenting for medical care, or upon review by a study monitor.

All AEs, including solicited local and systemic reactions, will be documented and included as source for the eCRF and maintained in the participant's research chart. Information to be collected for AEs includes:

- Event description
- Date of onset
- Assessment of severity
- Relationship to study product and

- Alternate etiology (assessed only by those with the training and authority to make a diagnosis and listed on the form FDA 1572 as an investigator)
- Date of resolution
- Seriousness and
- Outcome

AEs occurring during the trial collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution. Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the severity of any pre-existing medical condition increases, it should be recorded as an AE.

An SAE: An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse (21 CFR 312.32).

“Life-threatening” refers to an adverse event that at occurrence represents an immediate risk of death to the participant. An event that may cause death if it occurs in a more severe form is not considered life-threatening. Similarly, hospital admission for an elective procedure is not considered an SAE. The clinician will ask each participant about other interim adverse experiences and recorded this information in the source documents. Study staff will enter AE or SAE information into the study database within three business days of the participant visit. After study day 28 after the last vaccination, only SAEs and new chronic medical conditions that require ongoing medical management will be entered into the database, through the last study visit. All other adverse events will be recorded on source documents only. The PI or designee will assess the relationship of the study product to the events.

If an event meets both the criteria of a study endpoint and an adverse event, the event will be reported either as a study endpoint or as an adverse event (not both).

8.1.4 SERIOUS ADVERSE EVENTS (SAEs)

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

- Results in death,
- Is life-threatening,
- Requires inpatient hospitalization or prolongation of existing hospitalization,

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

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

Life-threatening adverse event: An AE is considered "life-threatening" if, in the view of either the site principal investigator or sponsor, its occurrence places the participant at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.

SAEs will be assessed for severity and relationship to study product and alternate etiology (if not related to study product) by the PI or AI listed on the Form FDA 1572 and followed through resolution.

8.1.5 POTENTIALLY IMMUNE-MEDIATED MEDICAL CONDITIONS (PIMMCS)

PIMMCs 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.

8.1.6 MEDICALLY ATTENDED ADVERSE EVENTS

The FDA requests that clinical trials of preventive vaccines with adjuvants such as ALF43 and dmLT provide for collection and analysis of data relating to medically attended adverse events (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.

8.1.7 ADVERSE EVENT 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.

8.1.8 ADVERSE EVENT DOCUMENTATION

The clinician will ask each participant about other interim adverse experiences and record this information in the source documents. Study staff will enter data into the study database within three business days of participant visits. 28 days after the last vaccination, only SAEs and new chronic medical conditions that require ongoing medical management will be entered into the database, through the last study visit. All other adverse events will be recorded on source documents only. The PI or designee will assess the relationship of the study product to the events. Participants will be asked for their consent for photography if the clinician wishes to document an unusual or unexpected finding.

8.1.8.1 Severity vs. Seriousness of Adverse Events

The term "severity" describes the intensity of a specific event. The severity of a specific event is graded, i.e., mild (Grade 1), moderate (Grade 2), severe (Grade 3), potentially life-threatening (Grade 4), or death (Grade 5) using the DAIDS AE Grading Table Version 2.1. The severity of an AE does not determine whether an event meets the definition of seriousness, which is based on participant/event outcome or action criteria associated with events that pose a threat to a participant's life or functioning (ICH E2A).

8.1.8.2 Relationship to Study Product

The site investigator is responsible for assessing the relationship between the AE and the study agents. Site investigators must determine whether there is a reasonable possibility that the study agents caused or contributed to a and AE or SAE. The relationship assessment, based on clinical judgment, often relies on the following:

- A temporal relationship between the event and administration of the study agents,
- A plausible biological mechanism for the agent to cause the AE,
- Another possible etiology for the AE,
- Previous reports of similar AEs associated with the study agent or other agents in the same class, and
- Recurrence of the AE after re-challenge or resolution after de-challenge, if applicable.

The terms used to assess the relationship of an event to study agent are:

Related – There is a reasonable possibility that the AE may be related to the study agents.

Not Related – There is not a reasonable possibility that the AE is related to the study agents.

When a SAE is assessed as "not related" to study agents, an alternative etiology, diagnosis, or explanation for the SAE should be provided. If new information becomes available, the relationship assessment of any AE should be reviewed again and updated, as required.

When the study agent is a fixed dose combination agent, an assessment of attribution will be made for each component and the combination agent.

8.1.8.3 Expectedness

Expected AEs are AEs that have been previously observed with use of the study agent(s) and are listed in the package insert or Investigator's Brochure. Expectedness is not based on what might be anticipated from the pharmacological properties of the study agent.

Unexpected AEs are AEs for which the nature or severity (intensity) is not consistent with the applicable agent information (Investigator's Brochure, package insert, or summary of agent characteristics).

8.1.8.4 Type and Duration of Follow-up of Participants after Adverse Events

AEs will be assessed and followed from initial recognition of the AE through end of the protocol defined follow-up period. SAEs will be followed up through resolution even if duration of follow-up goes beyond the protocol-defined the follow-up period. Resolution of an AE is defined as the return to pre-treatment status or stabilization of the condition with the expectation that it will remain chronic.

8.2 EXPEDITED ADVERSE EVENT (EAE) REPORTING

8.2.1 EAE REPORTING

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

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

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

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

All serious adverse events (SAEs) that are considered related or possibly related, and all deaths, will be promptly (within 48 hours) reported to the WRAIR IRB. The Principal Investigator will then submit written reports within 10 working days to the WRAIR IRB. Follow up reports will be submitted as additional information becomes available. A summary of the non-serious adverse events and SAEs (both related and unrelated) that occurred during the reporting period will be

included in the continuing review report to the WRAIR IRB. The WRAIR HSPB will report SAEs to the USAMRDC ORP HRPO as per UWZ-C-636."

8.2.1.1 EAE Reporting Requirements for the Study

The SAE or SUSAR Reporting Category, as defined in Version 2.0 of the DAIDS EAE Manual, will be used for this study.

The study products for which expedited reporting are required are:

- HIV-1gp120 (93MW965.26) DNA (Env-C Plasmid DNA)
- HIV Env gp145 C.6980 protein
- dmLT
- ALF43
- Rehydragel®

In addition to the SAE or SUSAR Reporting Category identified above, other adverse events that must be reported in an expedited manner are:

Potentially Immune-Mediated Medical Conditions (PIMMCs)

8.2.1.2 Grading Severity of EAEs

DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1 (DAIDS AE Grading Table) is used that is available on the DAIDS RSC website at https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables.

8.2.1.3 EAE Reporting Period

The EAE reporting period for this study is 108 weeks after study agent administration until study completion or discontinuation of the participant from study activity for any reason as per the DAIDS EAE Manual.

After the protocol-defined EAE reporting period, unless otherwise noted, only SUSARs as defined in Version 2.0 of the DAIDS EAE Manual will be reported to DAIDS if the study staff become aware of the events on a passive basis (from publicly available information).

All SAEs and SUSARS will be reported by the site PI or designee to the: DAIDS RSC Safety Office (DAIDSRSCSafetyOffice@tech-res.com) and MHRP COO (reportable_events@hivresearch.org).

The DAIDS Medical Officer, who determines if an IND Safety Report will be sent to the FDA, performs the final attribution of the SAE. If the SAE is assessed as related, the RSC must submit an IND Safety Reports to the FDA no later than seven (death or life-threatening) or 15 calendar days following receipt of the information from the site.

8.2.1.4 Submission of Updated Information on Expedited Adverse Events

Sites must follow each AE until the AE is resolved or stable. For each AE reported to DAIDS, sites are required to submit an updated report to DAIDS as soon as significant additional information becomes available. The following are examples that must be submitted:

- An updated report documenting the stable or resolved outcome of the AE, unless the initial report included a final outcome,
- Any change in the assessment of the severity grade of the AE or the relationship between the AE and the study agent, or
- Additional significant information on a previously reported AE (e.g., cause of death, results of re-challenge with the study agents).

8.2.1.5 Reporting Recurrent Adverse Events on an Expedited Basis

For an event previously reported to the DAIDS Safety Office, if the AE fully resolved but then reoccurs with an outcome meeting expedited reporting criteria, the AE must be reported as a new initial report to the DAIDS Safety Office.

8.3 HALTING OR PAUSING RULES

8.3.1 NOTIFICATION OF AES MEETING STUDY PAUSE CRITERIA

The PI notifies the PSRT, which includes the DAIDS MO, study chair, site PI, DoD research monitor or their designees within a few hours after the site is aware of the AE. The PSRT will convene within one business day to review these adverse events and determine disposition (including whether the SMC needs to review the event).

If a decision to resume study enrollment and study treatment administration is made, the PSRT and or SMC will record its judgment in a memorandum to the study file and notify DAIDS. The MHRP Clinical Operations Office (COO) will then forward the memorandum to the principal investigators and notify WRAIR IRB. As needed, the appropriate regulatory authorities will be informed in writing of the decision to resume or discontinue study activities. Kericho site is responsible for notifying KEMRI SERU and WRAIR IRB according to local standards and regulations. The sponsor (DAIDS) is responsible for notifying the FDA.

For SAEs, sites must also follow the DAIDS EAE Manual and submit the SAE to DAIDS Regulatory Support Center (RSC) Safety Office as soon as possible, but no later than 3 reporting days (as defined in the DAIDS EAE Manual v2.0), after the site's recognition that the event fulfills the criteria for SAE reporting.

For IND Safety Reports submitted to the FDA and received from DAIDS, the MHRP COO will complete the necessary reporting to WRAIR IRB, including telephone contact and a written report generated within 24 hours of the initial safety report. WRAIR HSPB will forward to the USAMRDC, ORP, HRPO.

If the trial is placed on an unscheduled safety pause, all enrollment and study agent administrations will be suspended until further notice. For the two scheduled safety pauses,

enrollments and vaccinations for Group 1 do not need to be paused; only Groups 2-7 would be affected. For any study pause or re-initiation after a pause, the study staff will contact the participants to inform them. If the study is on pause for vaccinations, safety blood work is continued per protocol. Vaccinations are resumed post-pause but those who have missed vaccine windows would resume based on consultation with the PSRT on a case-by-case basis based on the number of vaccinations missed, length of pause, etc.

The PSRT will closely monitor and analyze study data as they become available and will make determinations regarding the presence, severity, and seriousness of adverse events. The administration of study injections and new enrollments will be paused, and DAIDS (the IND Sponsor) will be promptly notified according to the following criteria:

- One (or more) participant experiences a SAE that is assessed as related to study agent, or
- Two (or more) participants experience the same Grade 3 or 4 AE assessed as related to a study agent.

8.3.2 Plan for Review of Pauses and Resuming Rules

The study injections and enrollments would resume only if the review of the adverse events that caused the pause resulted in a recommendation to permit further study injections and study enrollments. The reviews to make this decision will occur as follows:

Pauses for SAEs: The PSRT may consult with the SMC to conduct the review and make the decision to resume, amend, or close the study for any SAEs that meet the criteria for pausing the study. The FDA will be notified of any SAE pause review.

Pauses for two or more Grade 3 or 4 AEs (e.g., two grade 4 ALT): The PSRT will conduct the review and make the decision to consult the SMC, resume, amend or close the study for the Grade 3 or 4 events that meet the criteria for pausing the study. As part of the SMC review, the reviewers will also advise on whether the study needs to be paused again for any subsequent Grade 3 or 4 event of the same type.

When indicated, safety data reports and changes in study status will be submitted to the IRB.

8.4 Safety Oversight

8.4.1 Safety Monitoring Committee (SMC)

Oversight for several MHRP research protocols will be provided by an SMC, which consists of independent clinicians, scientists, statisticians and/or ethicists who collectively have experience in vaccines or therapeutics as well as the conduct of clinical trials.

The MHRP SMC will be consulted by the PSRT to review unblinded data to provide a recommendation on continuing enrollment if there are any questions regarding safety. The SMC may again be consulted to review data from three weeks after the 21st participant has received their first “boost” vaccination at week 20.

Subsequently, the SMC will convene every six months to review the completeness of the study data collected, the adherence to the protocol, and the PI/core team summary.

The SMC will also meet as needed to deliberate upon the disposition of study pauses, and or to provide other recommendations regarding the safe conduct of the study as requested by the PSRT, and or DAIDS. The SMC Executive Secretary will provide the Protocol Chair and DAIDS Medical Officer with SMC recommendations. MHRP COO to WRAIR HSPB will submit SMC Reports, and the RV460 PI will submit the reports to KEMRI SERU and the Kenya Expert Committee on Clinical Trials (ECCT), of the Pharmacy and Poisons Board (PPB).

8.4.2 DEPARTMENT OF DEFENSE (DOD) RESEARCH MONITOR

The DoD Research Monitor may perform oversight functions (e.g. observe recruitment, enrollment procedures, and the consent process for participants; oversee study interventions and interactions; review monitoring plans and UPIRTSO reports; oversee data collection, and analysis) and report their observations and findings to the IRB. The DoD research monitor may discuss the research protocol with the investigators, interview human participants, and consult with others outside of the study about the research. The research monitor shall have authority to stop a research protocol in progress, remove individual participants from a research protocol, and take whatever steps are necessary to protect the safety and well-being of participants until the IRB can assess the monitor's report. Research Monitors have the responsibility to report within 48 hours their observations and findings to the IRB. The DoD Research Monitor is required to review all unanticipated problems involving risks to participants or others, SAE reports, and all participant deaths. The DoD Research Monitor at a minimum must comment on the outcomes of the event or problem and in case of a serious adverse event or death, comment on the relationship to participation in the study. They must also indicate whether he/she concurs with the details of the report provided by the PI.

The DoD Research Monitor should review all initial reports for SAEs, unanticipated problems involving risks to participants or others, and all participant deaths promptly and provide their independent report. The Research Monitor will provide an unbiased written report of all unanticipated problems involving risks to participants or others, and related SAEs and deaths, within 48 hours to the WRAIR IRB by phone 301-319-9940, or by email (usarmy.detrick.medcom-wrair.mbx.hspb@mail.mil). All Research Monitor reports for unrelated SAEs and deaths should be kept with the corresponding SAE reports at the study site. A written Research Monitor report will be submitted within 10 working days to the WRAIR IRB.

The WRAIR HSPB will submit copies of these reports to the USAMRDC, ORP, HRPO as per SOP UWZ-C-636.

9 HUMAN SUBJECTS PROTECTION

9.1 INFORMED CONSENT

The legal age at which individuals can provide their own consent to participate in research is 18 years. Consent forms will be available in English and Kiswahili.

There are four consent forms to be used for this study:

1. Main Informed Consent Form
2. Optional Procedures Consent Form
3. Withdrawal of Consent Form (as needed)
4. Photography Consent Form (as needed)

Information will be given in both oral and written form whenever possible and deemed appropriate by the IRB. A clinical investigator or designee will describe the study to potential participants. The investigator or designee (e.g., study coordinator) shall give the participant ample opportunity to inquire about details of the study, discuss with other people and ask any questions before dating and signing the consent forms. Participant information and consent form language will be at a reading level to be fully comprehensible to the prospective participants.

The participant and the study staff conducting the informed consent explanation will sign and date the consent form. The participant's signature confirms that she or he has understood the information. Each participant's consent form will be kept in his or her study file and a copy will be given to the participant to take home on the same day. Any amendments to the protocol resulting in a change in study procedures will necessitate that the study participants be informed, and additional informed consent obtained if necessary.

In accordance with 32 CFR 219, 45 CFR 46 and 21 CFR 50 and Good Clinical Practice, the participant may terminate participation in the study at any time for any reason without penalty. Additionally, if the participant is unable or unwilling to adhere to the protocol design, the investigator may terminate their participation. Under such circumstances, data and sample already collected may be stored and available for analysis. In addition, if the administration of research product has occurred, following for safety will be attempted.

Informed consent is a process that is initiated prior to an individual agreeing to participate in a trial and continues throughout the individual's trial participation. Before any study procedures are performed, informed consent will be obtained and documented. An investigator or designee will give a concise and focused face-to-face presentation describing key protocol information to potential participants. The information conveyed will include the explanation that the trial involves research and identifying which aspects of the trial that are experimental, the probability for random assignment to treatment groups, any expected benefits, all possible risks and the expected duration of the participant's participation in the trial. The presentation will be organized

and presented in lay terminology and language that facilitates understanding why one might or might not want to participate.

Participants will receive an explanation as to whether any compensation and any medical treatments are available if injury occurs, and, if so, what they consist of, or where further information may be obtained. They will be informed of whom to contact (e.g., the investigator) for answers to any questions relating to the research project. Participants will be informed in a timely manner if information becomes available that may be relevant to their willingness to continue participation in the trial.

Information presented to potential participants will also include the foreseeable circumstances and or reasons under which the participant's participation in the trial may be terminated. The participants will be informed that participation is voluntary and that they are free to withdraw from the study for any reason at any time without penalty or loss of benefits to which the participant is otherwise entitled.

Participants will be informed that, as part of their responsibilities for ensuring the protection of research participant, the following will be granted direct access to the participant's original medical records: KEMRI SERU, Kenya PPB, USAMRDC, ORP, HRPO, the NIAID, the US FDA, the Kenya Ministry of Health, WRAIR, the people who work for these organizations, and other local, US or international regulatory agencies. Participants information may also be reviewed or inspected for verification of clinical trial procedures and or data. Inspections will be conducted without violating the confidentiality of the participant, to the extent permitted by the applicable laws and regulations and that, by signing a written informed consent form, the participant is authorizing such access.

Participants will be informed that records containing identifying information will be kept confidential, and, to the extent permitted by the applicable laws and or regulations, will not be made publicly available and, if the results of the trial are published, the participant's identity will remain confidential. Participants will be informed whether private information collected from this research and or specimens will be used for additional research, even if identifiers are removed.

Participants will be allowed enough time to read and review the ICFs to consider participation in this research trial and can discuss this trial with their family or friends or think about it prior to agreeing to participate. The informed consent forms will be administered individually with strict respect for confidentiality.

9.2 POTENTIAL RISKS AND BENEFITS

9.2.1 POTENTIAL RISKS

9.2.1.1 Risks of Study Agent Administration and Their Mitigation

Participants may exhibit general signs and symptoms associated with a vaccine or placebo injection including fever, chills, rash, aches and pains, nausea, headache, dizziness, and fatigue. These side effects will be monitored but are generally short-term and do not require treatment.

Participants may have an allergic reaction to the vaccination. An allergic reaction may manifest as a rash, hives or even difficulty breathing. Severe reactions are rare. To minimize this risk, those with a history of anaphylaxis or other serious adverse reaction to vaccines or allergies or reactions likely to be exacerbated by any component of the vaccine or placebo, including antibiotics, will not be allowed to participate in this study. Study staff will observe participants for 30 to 60 minutes after vaccination. On vaccination days, there will be at a minimum one physician investigator available for assessment of any reaction. In the event of a severe allergic reaction, the CRC is staffed with trained medical personnel and stocked with appropriate medical emergency equipment to provide acute care for conditions such as anaphylaxis. If needed, participants will be transferred to the Unilever Hospital for further management.

TCI is a needle-free skin patch procedure which itself (without antigen) is safe and well tolerated in humans [53], [95]. In general, TCI-vaccinated participants may exhibit a local vaccine site reaction that was generally of mild-to-moderate severity. The majority (68.3%) of vaccine site reactions were characterized as erythematous and papular, while a smaller proportion of vaccine site reactions were characterized as either erythematous (13.9%) or papular (8.9%) only. Erythema and papules were both present in participants after receipt of the first vaccine dose (both at 43.5%); however, their frequency appeared to increase after receipt of the second (erythema: 76.7%; papules: 74.4%) and third (erythema: 75.6%; papules: 63.4%) vaccinations (not statistically significant). Local site reactions also consisted of pigmentation changes, the majority (47/53; 88.7%) of which occurred in individuals with a preceding papular rash. Both hyper and hypopigmentation may occur at the site and persist for some time. It is thought these reactions are dependent on the antigen tested by the TCI method.

TCI with LT has been safe with usually only Grade 1 side effects: pruritus, mild redness or rash, and hyperpigmentation at the site of patch application in different incidence and duration depending upon the site preparation method and patch used for the trial. These side effects were most likely also influenced by the activation of adenylate cyclase from the A subunit of the LT, which should be absent in the double mutant LT to be evaluated in this study [94]. Overall, when dmLT has been tested with TCI, it has been safe and well tolerated in most of human clinical trials (See IB).

9.2.1.2 Vaccine-Induced Seropositivity (VISP) or Seroreactivity (VISR)

Participants who receive study vaccine (not placebo) may subsequently receive a false positive on an HIV antibody test. Participants are asked to contact the PI or study staff if at any time during or after study participation if an HIV test is requested outside of the study. 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 and the study will provide re-testing. A positive or indeterminate test may have negative employment and social impact; as a result, a letter of participation will be provided on request.

Participants will be discouraged from donating blood during study participation because of the potential false-positive HIV antibody test result. If these test results should occur, Western blot analysis will be augmented with PCR or other required testing to either exclude or confirm HIV

infection. Blood donation options for those participants who wish to resume blood donation will be explained at the final study follow-up visit.

9.2.1.3 Social Harms

There is a risk of social harms other than described above regarding VISP. The study staff encourage open communication with participants and are aware if the potential for harms including but not limited to embarrassment, privacy loss, judgement by family, friends, and employers. The researchers take these risks seriously, and, depending on the situation, the clinic staff will provide appropriate assistance if preventive measures have failed.

9.2.1.4 Unknown Risks

There may be other serious risks that are not known with the study vaccines.

Participants may believe that this vaccine provides HIV protection, and therefore practice riskier behavior. They will receive extensive counseling throughout the study to address this potential problem. It is not known if the vaccines increase or decrease the chance of becoming HIV infected when exposed or if becoming HIV infected the person's disease course progresses faster to AIDS.

9.2.1.5 Unknown Risks related to Immune-Based Diseases

Experimental vaccines may hypothetically unmask or worsen an immune-based disease or syndrome e.g., Guillain-Barre or true autoimmune arthritis (such that objective physical signs last longer than six months). A variety of HIV-1 Env antigens very similar to those in this study have been evaluated extensively in humans and have not caused autoimmune diseases. However, whenever new adjuvants are tested in humans, study participant will be closely monitored for immune-based diseases. In a prior study evaluating the earlier generation of ALF43, L(MPLA), two cases of multiple sclerosis (MS) were reported; but they were diagnosed several years after the last vaccination, and one of the cases received an additional three unrelated experimental vaccines before the diagnosis, so that attribution of the event to the first experimental vaccine would have been impossible.

See IB Clinical Section on ALF for details and Appendix O: List of PIMMCs.

9.2.1.6 Study Risks Related to Pregnancy and Their Mitigation

The effect of these study agents on a fetus or nursing baby is unknown so the following precautions will be taken:

- Women who are pregnant or nursing will be excluded from the study,
- Female participants of childbearing potential will be required to agree to use at least one method of effective birth control for sexual intercourse beginning 45 days before the first vaccination and continuing through 90 days after the last vaccine/placebo visit. Contraceptive medications delivered orally, intramuscularly, vaginally, or implanted, underneath the skin, surgical methods (hysterectomy or bilateral tubal ligation), condoms with spermicide, diaphragms, IUD, vasectomy in a monogamous partner, or abstinence, and

- Women must also have a negative pregnancy test before collection of cervicovaginal secretion samples, before lymph node biopsy and before receiving study agent administration.
- Women who become pregnant during the study will be referred for standard care treatment, and they will be followed up for safety reasons until the baby is born.

9.2.1.7 Risks of Phlebotomy

Venipuncture may cause pain, bruising, and infrequently, presyncope. Local infection at the phlebotomy site is also a risk of phlebotomy. Rarely, it may cause infection at the phlebotomy site. Minor infections will be treated in the study clinic or will be referred to Kericho County Referral Hospital for further care and management, based on severity and clinical judgement.

9.2.1.8 Risks Related to Mucosal Secretion Collection

Mucosal secretion samples collected in the clinic (rectal sponges, semen, and cervicovaginal cups) will be obtained noninvasively. Semen will be self-collected and for the collection of cervicovaginal secretions, women may elect to insert and remove cervical cups themselves, as well. Inserting an instrument or collection device into the anus or the vagina may cause discomfort and slight irritation. There is no evidence of rectal sponge or cervical cup sampling contributing to the risk of HIV or other sexually transmitted infection. For these non-invasive mucosal collections, men and women will be asked to refrain from receptive anal or vaginal, intercourse, douching, or inserting any product into the rectum or vagina for 3 days prior to the mucosal collection. Men will be asked not to masturbate nor ejaculate three days prior to semen collection.

9.2.1.9 Risks Related to Lymph Node Biopsy

Excisional inguinal lymph node biopsy will be performed under local anesthesia by a surgeon trained in superficial lymph node excision. As with all surgical procedures, there is a risk of scarring, bruising or bleeding at the surgical site; these risks are minimized by the small nature of the incision (approximately 1-2 centimeters) and by prior clinical and laboratory evaluation to determine any bleeding risks. Participants may experience some discomfort following the procedure despite the use of local anesthesia; for participants who complain of any subsequent discomfort, additional analgesia (such as paracetamol, diclofenac or ibuprofen) will be made available by the study site. There is a possibility of seroma formation at the biopsy site, which may require subsequent drainage or further management, or sensory nerve injury during the procedure, which could result in a temporary or permanent local reduction in feeling. Surgical site infection is a possible complication of any procedure involving an incision however, the risk is low [23]. Finally, there is the unlikely possibility that the procedure will be unsuccessful, and no lymph node will be recovered.

9.2.1.10 Risks from Human Leukocyte (HLA) testing

Blood samples donated for HLA testing is used only to provide study investigators information about the immune system. The results will be coded to protect participant identity. The test results will NOT be provided to or shared with participants because they have not been validated for clinical management.

9.2.1.11 Risks to Researchers

Risks to researchers and their work include direct exposure to blood borne pathogens, in particular to HIV. To mitigate these risks, staff members handling samples are trained on the safe use and handling of blood products utilizing universal precautions. The study personnel are provided with and trained on the use of personal protective equipment (PPE) and on the containment of biohazards. Trained staff will perform study procedures.

In case of an accidental needle-prick, the staff has access to Post-Exposure Prophylaxis (PEP) services, and there is an SOP on the use of PEP at the site.

9.2.2 POTENTIAL BENEFITS

Although study participants may benefit from clinical testing and physical examination, they may receive no direct benefit from participation.

9.3 COMMUNITY ADVISORY BOARD (CAB)

The Kericho CAB serves as a link between the research team and the community. The CAB will give feedback to the research team on issues about the study that might be of concern to the volunteers or the community at large. One of our CAB members is a lawyer who will be able to address and guide us on any legal issues that may arise or be of concern during the conduct of research studies at this site.

The CAB will be briefed about the study before community engagement commences. The briefing will be done by at least the PI/designated AI, study coordinator and the community liaison officer. The CAB will thereafter be involved on a need basis with a feedback meeting to be done at the end of participant follow-up.

9.4 MANAGEMENT OF VULNERABLE POPULATIONS

If the status of an enrolled participant changes during their participation in the study and or in that the participant's ability to exercise free choice could be limited in some way, the participant is recognized as a vulnerable participant. A vulnerable participant is any individual whose willingness to participate in a clinical trial may be unduly influenced by the expectation, whether justified or not, of benefits associated with participation; or of a retaliatory response from senior members of a hierarchy in case of refusal to participate.

The participant that is likely to be vulnerable to coercion or undue influence, might include individuals such as minors, pregnant women, prisoners, soldiers, handicapped, or mentally incompetent persons.

If a change in the status of a participant already enrolled in the study should occur, it is the responsibility of the investigators to assure that appropriate safeguards are in place to protect the rights, safety, and welfare of all study participants. The principal investigator should promptly notify KEMRI SERU and WRAIR IRB. The IRBs must decide what types of special protections are required and provide direction to the investigator.

9.4.1 PARTICIPATION OF CHILDREN

Children are not eligible to participate in this clinical trial, because it does not meet the guidelines for inclusion of children in research. These guidelines (45 CFR 46, Subpart D, 401-409) state the Department of Health and Human Services protections for children who participate in research. Generally, healthy children can be studied when the research is considered "not greater than minimal risk." Children can be involved in research with greater than minimal risk only when it presents the prospect of direct benefit to the individual child or is likely to yield generalizable knowledge about the child's disorder or condition.

9.4.2 PARTICIPATION OF PRISONERS

Participation of prisoners is not planned, and any volunteer will be suspended from study visits while incarcerated. The WRAIR IRB will be notified of the period of incarceration.

9.4.3 ILLITERATE PARTICIPANTS

Illiterate persons are not eligible to participate in this study.

9.5 FUTURE USE AND STORAGE OF BIOLOGICAL SAMPLES

Each participant will be asked to voluntarily consent to their 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 biological samples stored for future use, they can consent to participate in this study only, without having their blood samples stored for future testing. In this case, their blood samples will be destroyed within one year of receiving notification from the protocol chair that all protocol testing is complete. Samples remaining in the KEMRI/USAMRD-A CRC repository will be destroyed by laboratory staff following approved laboratory procedures.

All samples for which consent has been obtained and for which additional material is available after study specified testing is complete will be stored for future testing at the KEMRI/USAMRD-A CRC Laboratory in Kericho. However, KEMRI SERU and WRAIR IRB approval will be sought before any such samples are used for analysis not specified in the protocol or a protocol amendment approved by the IRB. All samples belong to the site from which they were obtained and MHRP.

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. For specialized tests that are not available at this site, some of the blood samples will be shipped to the United States or Thailand for HIV immune response testing as well as genetic testing. These shipments will occur in batch, when sufficient participants have reached appropriate timepoints in the schedule of events. The remaining samples will then be stored at the KEMRI/USAMRD-A CRC lab. Shipment of these samples will be done only after the approval of the KEMRI SERU.

9.6 RISKS FROM HUMAN LEUKOCYTE (HLA) AND OTHER GENETIC TESTING

Blood samples donated for HLA testing are used only to provide study investigators information about the immune system. The results will be coded to protect participant identity. The test results will not be provided to or shared with participants because they have not been validated for clinical management.

9.7 PARTICIPANT CONFIDENTIALITY

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor and their agents. This confidentiality includes documentation, investigation data, participant's clinical information, and all other information generated during participation in the study. No information concerning the study, or the data generated from the study will be released to any unauthorized third party without prior written approval of the DAIDS and the participant. Subject confidentiality will be maintained when study results are published or discussed in conferences. The study monitors, or other authorized representatives of the sponsor or governmental regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

All records will be kept locked and all computer entry and networking programs will be carried out with coded numbers only and with password protected systems. All non-clinical specimens, evaluation forms, reports, and other records that leave the site will be identified only by a coded number.

9.8 COSTS, REIMBURSEMENT, AND RESEARCH RELATED INJURIES

9.8.1 POLICY REGARDING RESEARCH-RELATED INJURIES

The US DoD is funding this protocol. As stated in the protocol and consent form, participants who experience illness or injury arising from participation in the study will receive medical care for such illness or injury with costs for such care provided by a limited set-aside fund and a clinical trials medical insurance policy that will be obtained by the study funder. While we anticipate the combination of the set-aside fund and the insurance policy is more than enough to pay for the research related injury medical care cost associated with this study, there is a limit to the amount of coverage available. If the limit is exceeded, the study participant may have to pay non-covered costs. Other than medical care, and other payments as stated in the consent form, there is no other compensation available from this research study.

9.8.2 REIMBURSEMENT

Participants will be reimbursed with Kenya shillings (KSH) 1000 for their time and any inconvenience during each scheduled study visit. Reimbursement amounts are as follows:

Study visits

(in person or by phone):	1000 Ksh
Unscheduled visits:	Up to 500 Ksh, at the discretion of the study investigator
Mucosal collection:	1000 Ksh per each collection
Lymph node biopsy:	5000 Ksh
Transportation:	Up to 3000 Ksh, at the discretion of the study investigator

10 INSTITUTIONAL REVIEW BOARDS AND REGULATORY REQUIREMENTS

10.1 INSTITUTIONAL REVIEW BOARDS

Kenya Medical Research Institute SERU
PO Box 54840-00200
Nairobi, Kenya
FWA#00002066

Walter Reed Army Institute of Research IRB
US Army Garrison-Forest Glen
503 Robert Grant Avenue
Silver Spring, MD 20910
FWA #00000373

The investigator will submit applicable information to the WRAIR IRB and KEMRI SERU for the review, to conduct the review in accordance with 45 CFR 46, ICH E6 GCP, and as applicable, 21 CFR 56 (Institutional Review Boards) and 21 CFR 50 (Protection of Human Participants), DoD regulation 32 CFR 219, and other federal, state, and local regulations.

The IRB must be registered with OHRP as applicable to the research. DAIDS must receive the documentation that verifies IRB-approval for this protocol, associated informed consent documents, and upon request any recruitment material and handouts or surveys intended for the participants, prior to the recruitment and enrollment of participants.

Any amendments to the protocol or consent materials will be approved by the IRB before they are implemented. IRB review and approval will occur at least annually throughout the enrollment and follow-up of participants and may cease if annual review is no longer required by applicable regulations and the IRB. The investigator will notify the IRB of deviations from the protocol and reportable SAEs, as applicable to the IRB policy.

In addition, the protocol and associated documents will undergo review and approval by both the KEMRI SERU, WRAIR IRB and the USAMRDC, ORP, HRPO.

10.1.1 REPORTING REQUIREMENTS TO THE KEMRI SERU

The Kericho site PI will be responsible for providing all Safety Reports and reporting all SAEs, and study pauses, social harms, major deviations to KEMRI SERU promptly according to the institution's guidelines. "When reporting to KEMRI SERU, the PI will follow the reporting requirements of the WRAIR IRB as noted below in Sections 10.2 – 10.6."

10.1.2 ADDITIONAL LOCAL REGULATORY AUTHORITY

Pharmacy and Poisons Board (PPB),
Expert Committee on Clinical Trials (ECCT)
P.O. Box 27663, Lenanna Road
Nairobi, Kenya 00506

10.2 REGULATORY REPORTING

10.2.1 IMMEDIATE AND CONTINUING REVIEW REPORTING TO THE DOD

The DoD research monitor should also review the related SAEs, deaths, and unanticipated problems involving risks to subjects or others and provide an independent assessment to the WRAIR IRB (as a DoD research monitor's report).

Reports will be submitted within 48 hours to the WRAIR IRB by phone at 301-319-9940, or by email (usarmy.detrick.medcom-wrair.mbx.hspb@mail.mil). The PI or designee will then submit written reports within ten working days to the WRAIR IRB at the following address: Walter Reed Army Institute of Research, ATTN: Human Subject Protection Branch (HSPB), 503 Robert Grant Ave, Silver Spring, MD 20910.

Follow up reports will be submitted as additional information becomes available. A summary of the non-serious adverse events and the SAEs (both related and unrelated) that occurred during the reporting period should also be included in the continuing review report to the WRAIR IRB. The WRAIR HSPB will report SAEs to the USAMRDC, ORP, HRPO as per UWZ-C-636.

All serious unanticipated problems involving risk to participants or others (UPIRTSOs) should promptly (within 48 hours) reported by telephone, or by email to the WRAIR IRB and the DAIDS MO. Prompt reporting should be submitted to the WRAIR IRB by phone at 301-319-9940, or by email usarmy.detrick.medcom-wrair.mbx.hspb@mail.mil. The Principal Investigator will then submit written reports within ten working days to the WRAIR IRB at the following address: Walter Reed Army Institute of Research, ATTN: Human Subject Protection Branch, 503 Robert Grant Ave, Silver Spring, MD 20910 and the KEMRI SERU. The WRAIR HSPB will report to USAMRDC, ORP, HRPO as per SOP UWZ-C-636.

Follow up reports should be submitted as soon as additional information becomes available. A summary of the serious unanticipated problems should also be included in the continuing review report submitted to the KEMRI SERU, and the WRAIR IRB.

The DoD research monitor should also review the unanticipated events and provide an independent assessment to the WRAIR HSPB (as a DoD research monitor's report). WRAIR HSPB will report these summaries to the USAMRDC, ORP, HRPO. MHRP COO will forward the report to the Division of AIDS (DAIDS).

10.2.2 REPORTING AND MANAGEMENT OF PARTICIPANTS WHO BECOME PREGNANT

Pregnant women are excluded from enrollment, however, if a participant becomes pregnant during the study, further vaccinations will be stopped, and she will be referred for maternal and child health services. Participants will continue to be followed for the remainder of the study period for safety, and the study team will follow the course of the pregnancy until the outcome is documented. During subsequent study visits, blood draws (6 mLs for basic chemistry and hematology) will be conducted for safety evaluations only. No optional collections or procedures will be performed.

The PI or designated associate investigator will be responsible for reporting any pregnancy to the PSRT and the study database. The PSRT will review this information. The site PI will forward notification as necessary to the WRAIR IRB and KEMRI SERU.

Pregnancy outcomes will be recorded via a standardized case report form. Information documented on this form will include the date of last menstrual period, the date that the pregnancy was confirmed, any history of complications during prior pregnancies (such as congenital abnormalities or spontaneous abortions), and the outcome of the pregnancy including date of termination or delivery, any complications of pregnancy, and the status of the child. A separate case report form will be completed for the delivered child to document date of delivery, gender, weight, the presence of any congenital abnormalities, American Pediatric Gross Assessment Record (APGAR) score, HIV status, and any other complication of delivery.

10.2.3 Reporting and Management of Participants Who Become Incarcerated

Participation of prisoners is not planned, and any participant will be suspended from study visits while incarcerated, except visits or telephone contact for ensuring participant 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.

10.2.4 Social Harms Reporting

Unanticipated events and social harms may occur during the study. When such events are related to study participation, the study staff, informed of these events, will inform the PI or designee. The PI or delegated associate investigator will then prepare a narrative summary of the event and report to KEMRI SERU, MHRP COO, and PSRT including DAIDS Medical Officer. WRAIR IRB will then be informed. The DoD research monitor should also review the social harms and provide an independent assessment of these to the WRAIR HSPB. WRAIR HSPB will report these summaries to the USAMRDC, ORP, HRPO.

10.3 Protocol Deviations

It is the responsibility of the site principal investigator and personnel to use continuous vigilance to identify and report deviations. A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or protocol-specific MOP requirements. The noncompliance may be either on the part of the participant, the site principal investigator, or the site personnel. Because of deviations, corrective actions are to be developed by the site and implemented promptly.

The investigator should not implement any deviation from, or changes of the protocol without agreement by the sponsor and prior review and documented approval from the IRB/IEC of an amendment, except where necessary to eliminate an immediate hazard to trial participants.

All protocol deviations (PD), as defined above, must be addressed in study participant data collection forms. The principal investigator and personnel are responsible for knowing and adhering to their IRB requirements.

The procedures for handling protocol deviations will occur as follows: the study staff will notify the PI as soon as possible if he or she is not present when the deviation is discovered. Any PD resulting in unanticipated problems that put participants or others at risk will be reported as an UPIRTSO.

The timeline for reporting protocol deviations to the WRAIR IRB is determined by the categorization of the deviation: (1) major or (2) minor.

10.3.1 MAJOR

A major deviation also known as protocol violation is non-adherence to the IRB approved protocol that has the potential to affect the rights and welfare of the research participant, to increase the risk to the research participant, to change the willingness of the volunteer to continue participation, or to compromise the integrity of the study data in such a way that the study objectives may not be achieved.

Examples of major deviation include exceptions to eligibility criteria, exceptions to the form and manner of obtaining informed consent, and exceptions to the schedule of administration of an investigational product.

Major deviations must be promptly reported (within 48 hours upon the PI becoming aware of the event) to DAIDS and the WRAIR IRB (via the COO) and the KEMRI SERU, as well as recorded in the study deviation log. Notification that a major deviation occurred should be submitted to the WRAIR HSPB by phone at 301-319-9940, or by email (usarmy.detrick.medcom-wrair.mbx.hspb@mail.mil). The PI will then submit a written report within ten working days to the WRAIR IRB (via the COO) at the following address: Walter Reed Army Institute of Research, ATTN: Human Participants Protection Branch, 503 Robert Grant Ave, Silver Spring, MD 20910 and the KEMRI SERU.

10.3.2 MINOR

Minor deviations are departures from the protocol that do not affect or potentially affect the health and welfare of study participants, and do not compromise the integrity of the results in such a way that the study objectives may not be achieved, although they may nonetheless affect the study results.

Examples of minor deviations include follow up visits occurring outside the protocol required time frame because of the participant's schedule, or blood samples being obtained at times close to but not precisely at the time points specified in the protocol.

Minor deviations will be reported to KEMRI SERU and WRAIR IRB as part of the protocol continuing review report and should be reported in the deviation log. Major deviations should also be summarized in the continuing review reports.

Knowledge of any instances of serious or continuing non-compliance with the regulations or requirements will be reported immediately to the KEMRI SERU and the WRAIR IRB. The WRAIR HSPB will report the deviations and instances of noncompliance with the USAMRDC, ORP, HRPO as per SOP UWZ-C-636.

10.4 PROTOCOL AMENDMENTS

All amendments and modifications will be submitted to the WRAIR IRB, KEMRI SERU and Kenya Pharmacy and Poisons Board (PPB) for review and approval. No changes in protocol conduct will be implemented until approval is obtained from the WRAIR IRB, KEMRI SERU, and Kenya PPB unless required to eliminate apparent immediate hazards to the study participants. Amendments and modifications cannot be implemented until the USAMRDC, ORP, HRPO, as applicable, and the WRAIR Commander's Authorization has been issued. The WRAIR HSPB will submit amendments and modifications to the USAMRDC, ORP, HRPO as per SOP UWZ-C-636.

Upon receiving final IRB/EC and any other applicable approvals for an amendment, sites should implement the amendment immediately. Sites are required to submit an amendment registration packet to the DAIDS PRO at the RSC. Site-specific ICF(s) WILL NOT be reviewed and approved by the DAIDS PRO and sites will receive an Amendment Registration Notification from the DAIDS PRO that approves the site specific ICFs and indicates successful completion of the amendment protocol registration process. A copy of the final amendment Registration Notification issued by the DAIDS PRO should be retained in the site's regulatory files.

10.5 CONTINUING REVIEWS AND CLOSEOUT REPORTS

The PI is responsible for submitting the required continuing review reports and associated documents to the WRAIR IRB, KEMRI SERU and Kenya PPB, allowing enough time for review and continuation determination before the established continuing review date. A closeout report will be submitted after ten years or upon completion of the study, whichever occurs first. The WRAIR HSPB will submit continuing review and the closeout reports to the USAMRDC, ORP, HRPO as per SOP UWZ-C-636.

10.6 REGULATORY AUDITS

The knowledge of any pending compliance inspection or visit by the US FDA, OHRP, Kenya PPB, 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 agencies including legal or medical actions and any instances of serious or continuing noncompliance with the regulations or requirements will be reported immediately to the sponsor (DAIDS) and WRAIR HSPB and KEMRI SERU. The WRAIR HSPB will report knowledge of any pending inspections/audits by regulatory agencies to the USAMRDC, ORP, HRPO.

11 ESSENTIAL AND SOURCE DOCUMENTS

11.1 SOURCE DOCUMENTS

Source documents are original documents, data, and records from which the participant's data are obtained. These include but are not limited to hospital records, clinical and office charts, laboratory and pharmacy records, diaries, radiographs, and correspondence. The diary card is not a source document but a memory aid for volunteers, as has been done with prior MHRP vaccine trials (e.g. RV306); the information gained from the review of the diary card and the interview with participants will be documented using a combination of progress notes and CRFs maintained in the participants research chart.

11.2 ESSENTIAL DOCUMENTS

The participating site will maintain appropriate medical and research records in compliance with ICH E6 and regulatory and institutional requirements for the protection of confidentiality of subjects. The site will permit authorized representatives of the DAIDS, its designees, and appropriate regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress. These representatives will be permitted access to all source data and source documents, which include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and subject files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial.

The investigator and staff are responsible for ensuring maintenance of a comprehensive and centralized filing system of all study-related (essential) documentation, suitable for inspection at any time by representatives of the following: KEMRI SERU, PPB, USAMRDC, ORP, HRPO the NIAID, the FDA, the Kenya Ministry of Health, WRAIR, the people who work for these organizations, and other local, US or international regulatory agencies. Essential documents for all study participants are to be maintained by the investigators in a secure storage facility. Elements include:

- Participant files containing completed informed consent forms, and copies of source documentation
- Study files containing the protocol will include all amendments and copies of correspondence between the study site, protocol team and the IRB
- All Essential Documents outlined in the ICH Good Clinical Practice Guideline.

In addition, all original source documentation must be maintained and be readily available for monitoring or auditing purposes.

12 ADMINISTRATIVE AND LEGAL OBLIGATIONS

12.1 CONDUCT OF THE RESEARCH STUDY

This research study will be conducted per GCP, International Conference on Harmonization (ICH) guidelines, DoD Directive 3216.2, the Declaration of Helsinki, the Belmont Report, the U.S. Code of Federal Regulations 21 CFR 312, 812, 50 and 56, 45 CFR 46 and 32 CFR 219, and all applicable Kenyan laws and regulations.

12.2 PROTOCOL REGISTRATION

12.2.1 INITIAL REGISTRATION

Prior to implementation of this protocol, and any subsequent full version amendments, the site must have the protocol and the protocol informed consent forms approved, as appropriate, by KEMRI SERU and any other applicable regulatory entity (RE). Upon receiving final approval, sites will submit all required protocol registration documents to the DAIDS Protocol Registration Office (DAIDS PRO) at the Regulatory Support Center (RSC). The DAIDS PRO will review the submitted protocol registration packet to ensure that all of the required documents have been received.

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

12.2.2 AMENDMENTS

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

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

12.3 COMPLIANCE WITH NIH GUIDELINES FOR RESEARCH INVOLVING PRODUCTS CONTAINING RECOMBINANT DNA

Because this study is evaluating products containing recombinant DNA, per NIH Guidelines for Research Involving Recombinant DNA Molecules, the study must be submitted to the site Institutional Biosafety Committee (IBC) and must be approved before participants are enrolled at

each respective institution. Investigators at each site are responsible for obtaining IBC approval and periodic review of the research per NIH guidelines section IV-B07-b-(6) and section IV-B-2-b. IBC review and approval must be documented by the investigator and submitted as part of protocol registration for this trial.

The NIH guidelines also require that the Recombinant DNA Advisory Committee (RAC) must submit human gene transfer trials conducted at or sponsored by institutions that receive NIH funds to the NIH Office of Biotechnology Activities (OBA) for review. The NIH guidelines create exceptions to RAC review for vaccines, which do not persist, and the RV460 Protocol team will submit a request for exemption with the study concept proposal for RAC review and response.

12.4 SPONSOR STUDY MONITORING

Kericho site visits by study monitors will be made per the IND sponsor's policy to monitor the following: study operations, the quality of data collected in the research records, the accuracy, and timeliness of data entered in the database, and to determine that all process and regulatory requirements are met.

Kericho site investigators will allow the study monitors, DAIDS, the KEMRI SERU, Kenya PPB, WRAIR IRB, USAMRDC, ORP, HRPO, other DoD regulatory agencies, and the FDA to inspect study documents (e.g., consent forms, drug distribution forms, case report forms), and pertinent hospital or clinic records for confirmation of the study data.

12.5 BIOHAZARD CONTAINMENT

As the transmission of HIV and other blood borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the Centers for Disease Control and the National Institutes of Health. All dangerous goods materials, including diagnostic specimens and infectious substances, must be transported using packaging mandated by CFR 42 Part 72.

The International Air Transport Association Dangerous Goods Regulations for UN 3373, Biological Substance, Category B and Packing Instruction 650 will send all infectious specimens using packaging that meets requirements specified. Refer to individual carrier guidelines (e.g., Federal Express or Airborne) for specific instructions.

12.6 EXPECTED APPLICATION OF RESULTS

On completion, the study findings will be shared with all authorities having oversight of this protocol including the KEMRI SERU, Kenya PPB, WRAIR IRB, and Kenya's Ministry of Health. Results obtained from this study may inform considerations for the development of an HIV vaccine for use in the prevention of HIV infection and to fulfill a US FDA application.

The description of this study and the summary of results will be available on http://www.ClinicalTrials.gov.

Any new information from this study or other studies that may affect the participant's health, welfare, or willingness to continue with the study will be given to the participant by the appropriate method of contact, i.e., the participant will be contacted as soon as possible if, likely by phone in the case of a significant lab abnormality. The results of the study will be shared with the participants through a WRAIR IRB approved letter, at the participant's written request.

12.7 USE OF INFORMATION AND PUBLICATION

It is understood by the investigator that the information generated in this study may be used in connection with the development of the product and therefore may be disclosed to government agencies in various countries. To allow for the use of information derived from the study, the investigator is obliged to provide the sponsor with complete test results, all study data, and access to all study records. Confidentiality of participants will be maintained by exclusion of personally identifiable information from the research database and from any publications that result.

WRAIR recognizes the importance of communicating medical study data and therefore encourages their publication in reputable scientific journals and at seminars or conferences. Any results of medical investigations and or publication/lecture/manuscripts based thereon shall be exchanged and discussed by the investigator, and the USAMRDC and sponsor prior to submission for publication or presentation. Details of timelines are provided in Clinical Trial Agreements.

Results from investigations shall not be made available to any third party by the investigating team outside the publication procedure as outlined previously. WRAIR will not quote from publications by investigators in its scientific information and or promotional material without full acknowledgment of the source (i.e., author and reference). All publications written by WRAIR investigators must be reviewed and approved by the WRAIR Office of Research Technology and Applications (ORTA).

12.8 VOLUNTEER REGISTRY DATA SHEET

The USAMRDC requires that investigators complete data sheets on all participants participating in this protocol for entry into the Command's Volunteer Registry Database. This Volunteer Registry Database Sheet (VRDS) contains items of personal information, such as participant name, address, national identification number, study identity, and dates of participation.

The intent of this database is twofold: first, to readily answer questions about an individual's participation in research sponsored by the USAMRDC; and second, to ensure that USAMRDC can exercise its obligation to ensure that all research study participants are adequately warned (duty to warn) of risks and to provide new information as it becomes available. This information will be stored at USAMRDC for a minimum of 75 years and is kept confidential. The Volunteer Registry Data Sheet is a separate form and is not linked to the study database.

13 PRINCIPAL INVESTIGATOR AGREEMENT

1. I will conduct the study entitled: "A Randomized, Double-Blind Phase 1 Trial to Evaluate the Safety and Immunogenicity of HIV-1 Clade C Env DNA Vaccine Alone, with Different Adjuvants or an Adjuvanted HIV-1 Clade C Recombinant gp145 C.6980 Protein Vaccine and then Boosted with the Adjuvanted gp145 C.6980 Vaccine Either with or without the Clade C Env DNA Vaccine in Healthy HIV Uninfected Adults in Kenya: in accordance with the provisions of this protocol and all applicable protocol-related documents. I agree to conduct this study in compliance with United States (US) Health and Human Service regulations (45 CFR 46 and 32 CFR 219); applicable U.S. Food and Drug Administration regulations; standards of the International Conference on Harmonization Guideline for Good Clinical Practice (E6); Institutional Review Board/Ethics Committee determinations; all applicable in-country, state, and local laws and regulations; and other applicable requirements (e.g., US National Institutes of Health, Division of AIDS) and institutional policies.

2. I agree to follow this protocol version as approved by the IRBs/ERCs.

3. I will conduct the study in accordance with applicable IRB/ERC requirements, Federal regulations and state and local laws to maintain the protection of the rights and welfare of study participants.

4. I certify that I, and the study staff, have received the requisite training to conduct this research protocol.

5. I will not modify the protocol without first obtaining an IRB/ERC approved amendment and new protocol version unless it is necessary to protect the health and welfare of study participants.

6. I have read and understood the information in the Investigators' 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.

7. I will ensure that the data and 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 applicable IRBs/ERCs.

8. I will promptly report changes to the research or unanticipated problems immediately to the KEMRI SERU at 254 020 2722541 and WRAIR IRB via the WRAIR Human Participants Protection Branch at 301-319-9940 (during duty hours) or to the usarmy.detrick.medcom-wrair.mbx.hspb@mail.mil and submit a written report within 10 working days of knowledge of the event.

9. I will prepare continuing review reports at an interval established by the IRB/ERC, and a study closure report when all research activities are completed.

10. I will immediately report to the KEMRI SERU and WRAIR Human Participants Protection Branch knowledge of any pending compliance inspection by any outside governmental agency.

11. I agree to maintain adequate and accurate records in accordance with IRB policies, Federal, state and local laws and regulations.

RV460 Principal Investigator's Name: Josphat Kosgei, MBChB, MSc

Signature: ……. Date: _____/_____/_____

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15 KERICHO BUDGET AND TIMEFRAME

15.1 KERICHO BUDGET

	Item	Total Cost (USD)
1	Personnel	113,645
2	Equipment	56,976
3	Supplies (test kits, clinic and lab supplies, office consumables, and other study related supplies)	431,607
4	Travel (Domestic and Foreign)	83,116
5	Travel Training (Domestic and Foreign)	14,500
6	Other Expenses	193,004
7	Overheads (HJF MRI)	68,481
8	Overheads (HJF)	9,613
9	Other overheads	78,095
	TOTAL BUDGET	1,049,037

15.1.1 KERICHO BUDGET JUSTIFICATION

15.1.1.1 Personnel

The amount allocated will provide salary support (and benefits to hired employees) to the Deputy Director of KEMRI and clinical research site leader in Kericho, and to the site PI and associate investigator, the Laboratory Director, Study Coordinator, Research Nurse, Pharmacist, Data Manager, Data QA/QC, Data entry specialist, a Regulatory staff and Finance Manager to assist with the study.

15.1.1.2 Equipment

The budget allocated for equipment is to purchase an ultra-low upright freezer and nitrogen tank to store patient samples in Year 3 of the study.

15.1.1.3 Supplies

The budget covers for laboratory, clinic and general supplies, to include test kits, reagents, quality control standards required by the protocol, specimen collection kits, tubes, pipets and pipet tips and other processing supplies. Office supplies include notebooks, pens, pencils, markers, labeling supplies and other materials for data collections and study execution including. General supplies cover operational expenses such as gasoline for travel, vehicle maintenance, fuel for backup generators, utilities and clinic security.

15.1.1.4 Travel

Travel expenses include Community Advisory Board member transportation expenses for meetings, volunteer recruitment, volunteer transportation and retention, hotel and per diem for staff responsible for transportation and tracking (in case required) and PI, co-PI and associate

investigators travel to local conferences to present study data and meeting in the US with the study PI and other MHRP collaborators.

15.1.1.5 Other expenses

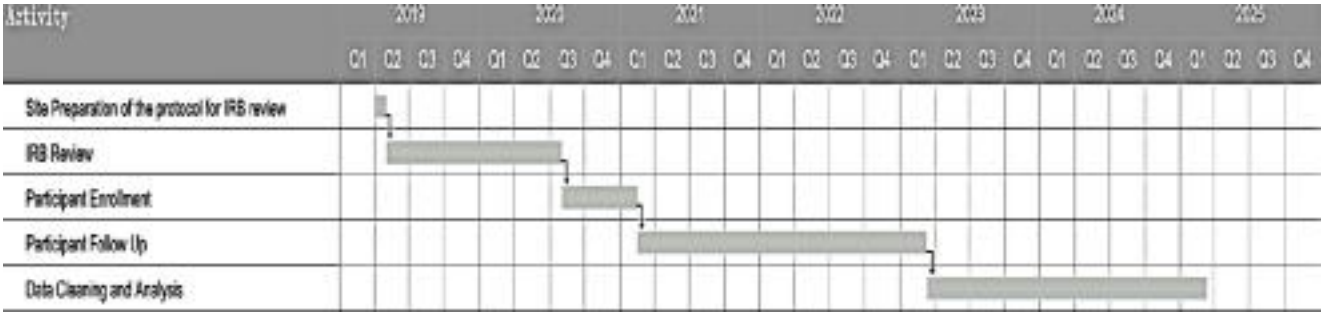
This will cover expenses related to adverse event follow- up (in case needed) including referral to medical specialist (consultation and treatment), radiographic images or medications. It also includes expenses for compensation of study participants for study visits and optional procedures.

15.1.1.6 Overheads

Overhead includes those for HJF MRI and HJF.

15.2 TIME FRAME AND DURATION AT KERICHO

Table 7: Time Frame and Duration at Kericho



16 APPENDICES

APPENDIX A. SCHEDULE OF EVALUATIONS

Visit Number	S1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26 Exit
Visit Day	-45 to -3	0	3 ± 1	7 ± 2	14 ± 3	28 ± 7	35 ± 2	42 ± 3	84 ± 7	87 ± 1	91 ± 2	98 ± 3	140 ± 7	143 ± 1	147 ± 2	154 ± 3	224 ± 7	231 ± 2	238 ± 3	392 ± 7	395 ± 1	399 ± 2	406 ± 3	476 ± 14	560 ± 14	728 ± 14	735 ± 14
Visit week		0	0.5	1	2	4	5	6	12	12.5	13	14	20	20.5	21	22	32	33	34	56	56.5	57	58	68	80	104	105
Clinical																											
Breifing & contact information	X																										
Informed consents	X																										
Test of understanding	X																										
Complete physical exam, height, weight & review medical history	X																										
Vital signs (BP, pulse, RR, oral temp)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medication	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Targeted physical exam and review of symptoms		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
HIV risk counseling; pregnancy counseling[11]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vaccination[13]		X [6]				X [6]			X [6]				X [6]				X [6]			X [6]							
Review Eligbility		X				X			X				X				X			X							
Enrollment and randomization[12]		X																									
Assess reactogenicity in clinic 30-60 min post-vaccination		X				X			X				X				X			X							
MAAE documentation [9]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Event documentation		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Diary card completion/review 10			X	X			X			X	X			X	X			X			X	X					
Clinical Laboratory Assessments [5]																											
Urinalysis dipstick for blood, protein and glucose	X																										
Urine-pregnancy test [7]	X	X				X		X	X			X[2]	X			X	X		X	X			X[2]	X	X	X	
CBC with differential	3				3			3				3				3			3				3				
Creatinine, ALT, AST	3				3			3				3				3			3				3				
Syphilis serology	6																										
HBsAg/HepC	NB																										
Pap smear for Softcup collection participants	X																										
Coagulation and safety tests prior lymph node biopsy8											9											9					
HIV testing with Pre/Post HIV test counseling	6	6										6				6			6				6		6	6	
Research Laboratory Assessments [3]																											
HIV NAb		6			6	6		6	6			6	6			6	6		6	6			6	6	6	6	6
Binding antibody/antibody profiling		6		6	6	6	6	6	6		6	6	6		6	6	6	6	6	6		6	6	6	6	6	6
Functional Antibody Assays (ADCC, ADCP, ADCVI, etc)		NB			NB	NB		NB	NB			NB	NB			NB	NB		NB	NB			NB	NB	NB	NB	NB
Cellular immune analysis		93.5	17	17	42.5		25.5	42.5		17	34	85		17	34	68		34	68		17	34	85	85	85	85	34
Cytokine/soluble factor assays		NB		NB	NB	NB	NB	NB	NB		NB	NB	NB		NB	NB	NB	NB	NB	NB		NB	NB	NB	NB	NB	NB
Gene expression/transcriptome analysis		34	17						17	17		8.5	17	17						17	17		8.5				
Mucosal secretion collection [1, 7]		X						X				X				X			X				X	X	X	X	
Lymph node biopsy [2]												X											X				
Storage & additional immunogenicity testing (mL)		25.5			17	17			8.5			17	8.5			34	17		34	17		17	51	25.5	25.5	25.5	17

Visit Number	S1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26 Exit
Visit Day	-45 to -3	0	3 ± 1	7 ± 2	14 ± 3	28 ± 7	35 ± 2	42 ± 3	84 ± 7	87 ± 1	91 ± 2	98 ± 3	140 ± 7	143 ± 1	147 ± 2	154 ± 3	224 ± 7	231 ± 2	238 ± 3	392 ± 7	395 ± 1	399 ± 2	406 ± 3	476 ± 14	560 ± 14	728 ± 14	735 ± 14
Visit week		0	0.5	1	2	4	5	6	12	12.5	13	14	20	20.5	21	22	32	33	34	56	56.5	57	58	68	80	104	105
Daily volume (mL) [4, 5]	18	171	34	23	77.5	29	31.5	60.5	37.5	34	49	134.5	37.5	34	40	126	29	40	126	46	34	66	168.5	122.5	128.5	128.5	63
Cumulative volume (mL) [4, 5]	18	189	223	246	323.5	352.5	384	444.5	482	516	565	699.5	737	771	811	937	966	1006	1132	1178	1212	1278	1446.5	1569	1697.5	1826	1889
8 week cumulative volume [4, 5]								444.5	158.5	163.5	212.5	315.5	292.5	289	295	372	29	69	195	46	80	146	314.5	122.5	128.5	128.5	191.5

NB=No extra blood required

1: Mucosal secretion collections - Rectal secretions collected by sponge.-Male participants: semen collection by masturbation. Female participants: cervicovaginal secretions collected by Softcup.

for menstruating females, mucosal collection window can be extended to -3 + 14 days.

2: Excisional inguinal lymph node biopsies will be performed in a subset of participants who agree to undergo this procedure. Urine pregnancy testing prior to procedure. Biospsy site assessment at 7 days (+2 days) after each biopsy.

3: Research labs may be performed at any visit where sufficient stored samples are available.

4: The maximum amount drawn in any 8 week interval is 444.5 mL. The maximum amount drawn in one day is 171 mL.

5: Clinical labs may be repeated if clinically significant.

6: Phone or clinic visit will be done to assess the participants well-being 24 hours (+1 day) after each vaccination.

7: Pregnancy testing must be performed prior to vaccination and mucosal secretion collection

8: 9ml should be taken if CBC/Chem results not available on day of biopsy. Otherwise, 3ml should be drawn

9: Medically Attended Adverse Events will be followed through the end of the study, a full 12 months following the last vaccination

10: Participants will take home the diary card and complete and the diary card will be reviewed at 7 days after vaccination.

11: Additional testing as clinically indicated

12: A participant is considered enrolled into the study once they are randomized into a study group

13: Study vaccination outside of a participant's vaccination window may occur at the discretion of the PSRT.

APPENDIX B. MAIN INFORMED CONSENT FORM

Kenya Medical Research Institute (KEMRI)
United States Army Medical Research Directorate-Africa (USAMRD-A)
In Collaboration with
US Military HIV Research Program and the
US Department of Defense (DoD)

WRAIR #2672/RV460 Main Informed Consent Form

Protocol Title:	A Randomized, Double-Blind Phase 1 Trial to Evaluate the Safety and Immunogenicity of Priming with Env-C Plasmid DNA Vaccine Alone, with Different Adjuvants, or with an Adjuvanted HIV Env gp145 C.6980 Protein Vaccine and Boosting with the Adjuvanted HIV Env gp145 C.6980 Protein Vaccine with or without the Env -C Plasmid DNA Vaccine in Healthy HIV Uninfected Adults in Kenya
Sponsor:	National Institute of Allergy and Infectious Diseases (NIAID), Division of AIDS (DAIDS) Bethesda, Maryland US
Funding:	United States Army Medical Research Acquisition Activity U.S. Military HIV Research Program Henry M. Jackson Foundation for the Advancement of Military Medicine
Site:	Kenya Medical Research Institute (KEMRI) US Army Medical Research Directorate-Africa (USAMRD-A), Kericho, Kenya
Principal Investigator:	Dr. Josphat Kosgei, MBCHB, MSc

Introduction

You are being asked to take part in a research study, also called a clinical trial. This study involves research and you should know that research is not the same as medical care. Research answers scientific questions and answers to these questions can help find new medicines, treatments, vaccines, and even knowledge on how the human body works.

This study is supported by the United States (US) Department of Defense and several other organizations. The box below tells you important things you should think about before deciding to join the study. We will provide more detailed information below the box. Please ask questions about any of the information before you decide whether to participate. You may also wish to talk to others (for example, your family, friends, or your doctor) about this study, before agreeing to join.

Key Information for You to Consider

- **Voluntary Consent**. You are being asked to volunteer for a research study. It is up to you whether you choose to participate or not. There are no penalties and you will not lose anything if you decide not to join or if after you join, you decide to quit.
- **Purpose**. We are doing this research to test a vaccine for HIV and vaccine additives called adjuvants that can help the vaccine be more effective.
- **Duration.** Your part of the study will last 105 weeks (26 months). You will be asked to complete at least 26 clinic visits. Additionally, you will be contacted by phone, once per week for three weeks **t**o discuss any changes you may have experienced after the first 105 weeks of being in the study.
- **Procedures and Activities.** We will ask you to receive 6 doses of the experimental vaccines and monitor your blood and symptoms after each vaccine.
- **Risks.** Most studies have some possible harms that could happen to you if you join. In this study, we expect you to have some mild or moderate symptoms like what happens after you receive some approved vaccines. Some examples of mild common and expected reactions are fever, body aches, feeling tired and pain at the injection site. Very rare but possible to have a serious and life-threatening allergic reaction right after a vaccine is given. Additionally, there is a chance that you could develop a serious illness such as autoimmune disease, but this is also very rare.
- **Benefits.** You will not directly benefit from participating in this study. The results of this study will be shared with researchers from around the world and could play a role in future HIV vaccine development which may be beneficial in the future for others. None of the experimental HIV vaccine combination groups have been shown that they work to protect against HIV infection. Also, there is a possibility that you may be assigned to receive the inactive vaccine
- **Alternatives.** Participation is voluntary, and the only alternative is to not participate.

Please contact one of the below if you have any questions concerning the study or if you have any other questions or concerns.

If you have questions about this study or if you have any symptoms that you think may be related to this study, contact:

Dr. Josphat Kosgei, MBCHB, MSc	Office: (0)5220 36302	Mobile: (0)729 110 122	Staff: (0) 5220) 36000/100
Or			
Rael Bor, BSc	Office: (0)5220 36329	Mobile: 0729110121	Staff: (0) 5220) 36000/100

1. Why are we doing this research?

Researchers are doing this study to test an experimental vaccine for HIV. A vaccine is a medical product given to prevent certain infectious diseases, most often caused by a virus or bacteria (germs). A vaccine may teach the human body to form a defensive response to try to prevent the disease from beginning or taking hold of the body. This defensive response is called the immune response, and it is the body's way to fight infections. The study also tests different adjuvants. An adjuvant is a substance added to vaccines that can help to make the vaccine more effective by improving the immune response or causing the immune response to last longer than without the adjuvant.

You are being asked to take part in this research study because you are a healthy adult and your participation will help researchers find out whether the experimental vaccines with adjuvants used in this study are safe causing mild side effects that are common, "expected" symptoms, fever, sore arm at injection site, or if the experimental vaccines cause unexpected reactions. The data we record from the symptoms you tell help tell us if it is safe enough for other people. We also want to gather information about if and how humans respond (develop immune responses) to the experimental vaccines, and how long the effects of the experimental vaccines last; we can learn that from your blood samples.

Also, this study will compare the effects (both good and bad) of the study experimental vaccines with adjuvants and adjuvant patch to those of placebo injections and adjuvant skin patch.
A placebo is a treatment for a disease or condition which is deliberately ineffective and allows researchers to compare the injection with active protection with the injection that has inactive (not protective) substance. The placebo will consist of saline (sterile saltwater), and will be given the same way, but will have no active vaccine or adjuvant in it. It will look just like the experimental vaccines so that you and the study doctors will not know if you received the placebo or active vaccine.

People who have received study experimental vaccines in other studies have still became infected with HIV when exposed to the virus. You must avoid any activities that might expose you to HIV.

2. How do I join this study?

If you agree to participate, you will have a screening visit which includes some lab tests, review of your health history and a physical exam. After your test results are available, the researchers will review the results to determine if you meet the eligibility requirements to join this study. You may take this consent form home and discuss the study with your family or friends to help you decide if this is right for you. Ask the study staff about the study schedule to plan on a time to return to enroll.

At the screening visit you will:

- Attend a briefing to learn about the study and provide your contact information

- Sign the informed consent forms
- Take a short test to check if you understand the study. *To be eligible, you must answer 9 out of 10 questions correctly but you can take the test 3 times.*
- Meet with a doctor to review your medical history
- Have a physical exam including vital signs
- Have blood drawn (18 ml which is a little more than 1 Tablespoon) to test for HIV, Hepatitis B, Hepatitis C, syphilis, and your general health
- Provide a urine sample for general health tests and pregnancy test for women

You may be allowed to participate in the study if you meet the following requirements:

- Healthy, male and female participant aged 18 to 40 years and available for 26 months
- Low risk for HIV infection
- Able to read English or Kiswahili.
- Must agree to a home visit
- Not pregnant or breastfeeding

You are not allowed to participate in this study if any of the following criteria apply to you:

- Have not been a sexually active MSM or transgender person in the last 6 months
- Have ever had a serious reaction to vaccines or other substances
- Regularly take certain medications that could affect your immune system
- Have received the CERVARIX vaccine against HPV
- Take or took anti-tuberculosis medication in the past 90 days
- Have certain skin conditions

3. What will I need to do during this study?

If you are found to be qualified to participate in the study, you will be booked to come for an appointment for enrollment and you will be selected by chance to one of the seven (7) study groups.

Additionally, as part of this study, you will have the choice to participate in the following additional optional collections:

- Cervicovaginal secretion collections (women)
- Semen collections (men)
- Rectal secretion collections
- Lymph node biopsies

These collections will be explained to you in a separate informed consent form. You will have the opportunity then to ask any questions about these collections and you will not need to say yes to them to enroll in the study and you can change your mind at any time.

It is important to remind you that to participate in this study, you have agreed to possible home visits by the study staff, and to be identified by a document with your photograph or fingerprint.

Experimental vaccine visits:
There are six (6) visits where you will receive an experimental vaccine. Vaccines will be given on study days 0, 28, 84, 140, 224 and 392. However, a study vaccination may be given outside your vaccination window if the protocol safety review team (PSRT) permits. At each of these visits, eligibility criteria will be reviewed again. You will be asked how you are feeling and if you are taking any medicines. Before the experimental vaccination, you will be asked to donate a blood sample to assess your general heath, test for HIV (at visit 1), and for research. Urine will also be collected for pregnancy testing if you are female.

If you are assigned to Groups 3 or 4, you will be receiving a patch with the experimental vaccine (or placebo) at the first three vaccination visits. You will need to wear the patch for 6 hours ±2 hours (range 4-8 hours) after application. After that time period, you will remove the patch and record the time on the diary card.

After the experimental vaccine injection, the clinic staff will observe you for 30-60 minutes and you will have your vital signs checked before leaving the clinic. You will write down your temperature and any symptoms you might have in a Diary Card while in the clinic, 30-60 minutes after the vaccination. You will be asked to complete a Diary Card at home about 6 hours after the injection and then daily for the 7 days after the experimental vaccination. The diary is for you to record your temperature and symptoms and look at the injection site each day and we will explain to you how to do this. You will be given a thermometer to take your temperature and ruler to measure any injection reactions as well as instructions. The diary card is very important for this research and needs to be returned to the study staff at the next visit. When you return to the clinic, if there is missing information on your diary or if the diary is lost, you will be asked to fill in the blanks to the best of your recollection, but you will not be able to fill in your temperature or measurements.

The clinic staff will contact you 24 to 48 hours after each vaccination to see how you feel. You will be asked to report any symptoms to the study doctor or nurse right away, and come to the study clinic for an examination, if necessary. You will also need to come to the clinic for any problem, which the nurse or doctor thinks should be checked by exam, blood, urine or other medical test. These visits may be in addition to your already planned study visits. If you are unable to be contacted by telephone, study staff will visit you at home with a home visit.

If you experience serious reactions, the study doctor may decide that you should not receive any further experimental vaccine injections. However, you may be asked to continue to be a part of the study and to return for follow-up study visits and tests. A photograph may be helpful to document an unusual or unexpected finding after the experimental vaccination is given. Also, if you undergo lymph node biopsy, the study doctor may take pictures of the biopsy site immediately after the procedure and again about 6 months later to document how well your

biopsy site healed and for possible use as educational material for other participants who undergo the same procedure.

You will be asked to sign an additional consent form for photography if pictures will be taken. No one will be able to determine your identity from these pictures.

Follow up visits:
You will be checked for any new symptoms since your last visit. You will be asked how you are feeling and if you are taking any medicines. You will be asked to give blood samples for laboratory tests to monitor your health, test for HIV (at certain visits, see your participant schedule), and for research purposes.

All clinic visits require a blood draw and the amount of blood drawn will vary from about 18.0 mL (a little more than 1 Tablespoon) to about 171 mL (11 1/2 Tablespoons) depending on the visit.

Please refer to the participant visit schedule given to you to keep track of what can be expected from you at each visit.

4. What are the experimental vaccines?

There are two experimental HIV vaccines tested in this study: the Env DNA vaccine and the gp145 protein vaccine. Each is given with different adjuvants which may make the vaccine more effective. The Env DNA vaccine has been tested in clinical trials and is relatively well tolerated by the body. The HIV gp145 protein vaccine to be tested in this trial will be the first time this vaccine is used in humans, but very similar products have also been tested in humans and are generally well tolerated.

There are three adjuvants used in this study in different combinations.

1. Rehydragel, or an aluminum hydroxide-based substance is approved for use and has been in-use in at least ten FDA licensed vaccines in the US and 146 vaccines worldwide.
2. ALF43 will be tested for the first time in humans and we are not certain if it is safe – we do know that an earlier form of ALF43 called L(MPLA) has been shown to be safe in several studies.
3. dmLT, another adjuvant, is given with a needle-free skin patch and has been used in many studies with no notable side-effects.

These adjuvants are mixed with the experimental vaccines and injected in the muscle; one of the adjuvants (dmLT) is applied to the skin where the injection is given as a skin patch and covered with a bandage. This is the first time this method will be used in humans.

It is important to remember that the HIV vaccines do NOT contain the entire HIV virus itself and cannot cause HIV infection or Acquired Immune Deficiency Syndrome (AIDS).

The following experimental vaccines will be used in this study, if you are assigned for groups 1-7, the injection you receive will contain only one experimental vaccine. If you are in group 7,

the injection will contain both. We hope to understand if different adjuvant combinations are safe, tolerable and how they stimulate your immune system.

Experimental vaccines

- Env-C Plasmid DNA (HIV-1 gp120 (93MW965.26) DNA)
- HIV Env gp145 C.6980 protein

Adjuvants

- ALF43 Army Liposome Formulation with Monophosphoryl Lipid A (MPLA) and 43% Cholesterol (referred to as ALF43)
- Rehydragel® (Aluminum Hydroxide)
- E. coli double mutant heat labile toxin (dmLT)

This research study is a double-blind study, which means that neither you nor the research team will know whether you are receiving the research study vaccines or a placebo. In the event of an emergency, there is a way to find out which one you are receiving.

Each of the 7 groups will have 15 participants that receive the experimental vaccines and 3 participants who will receive the placebo.

If you decide to participate in this study, you will be assigned randomly to one of seven (7) groups in the study and to receive either placebo (an inactive substance that does not contain the experimental vaccine—like salt-water injections) or a vaccine.

Enrollment into the groups will be done in a step-by-step way for safety reasons. Group 1 will receive a vaccine combination that has been given before and doesn't need a pause for safety reasons, while the vaccine combinations in Groups 2-7 are new and have not been tested before. Therefore, the Groups will proceed in the following manner. All of Group 1 will enroll and receive their first vaccine. Groups 2-7 will then start enrolling until 4 participants from each group (24 total) have received the first vaccine. Enrollment for Groups 2-7 will then stop for about a week to allow the researchers to look at the data and see if it is safe to give the remaining participants their vaccines.

There will also be a second pause a little later in the study once all of Group 1 and those first 24 participants in Groups 2-7 receive their 1st boost (Dose 4 at Week 20). Again, this is for safety reasons. This second pause will only stop the Week 20 boost vaccinations for the remaining participants in Groups 2-7. Earlier vaccinations (Doses 1-3) will not stop. This pause will last for about a week to allow the researchers to look at the data and see if it is safe to give the remaining participants their Week 20 boost.

None of experimental HIV vaccine combination groups have been shown that they work to protect against HIV infection. Also, there is also a possibility that you may be assigned to receive the inactive placebo.

A schedule of the assignments for each group is below:

Group	Prime at weeks 0, 4, 12	Boost at weeks 20, 32, 56
1	Env-C Plasmid DNA IM	HIV Env gp145 C.6980 protein /Rehydragel® IM
2	Env-C Plasmid DNA IM	HIV Env gp145 C.6980 protein /ALF43/Rehydragel® IM
3	Env-C Plasmid DNA IM + dmLT TCI	HIV Env gp145 C.6980 protein /Rehydragel® IM
4	Env-C Plasmid DNA IM + dmLT TCI	HIV Env gp145 C.6980 protein /ALF43/Rehydragel® IM
5	Env-C Plasmid DNA/ALF43 IM	HIV Env gp145 C.6980 protein /Rehydragel® IM
6	Env-C Plasmid DNA/ALF43 IM	HIV Env gp145 C.6980 protein /ALF43/Rehydragel® IM
7	Env-C Plasmid DNA/gp145/ALF43 IM	Env-C Plasmid DNA/HIV Env gp145 C.6980 protein /ALF43/Rehydragel® IM

IM= intramuscular (injected in the muscle), TCI = transcutaneous application (applied to the skin)

5. What are my responsibilities during this study?

If you take part in this study, you will be asked to:

- Provide complete and accurate information about your medical history, medicine use, and any symptoms or illnesses you have during the study.
- Agree to not participate in any other research study or donate blood during your participation in the study.
- Agree to have photo or fingerprint taken for identification purposes
- Follow the study staff's instructions.
- Attend all appointments and be available for telephone calls from the study staff. If you cannot keep an appointment, contact the study clinic immediately to reschedule.
- Agree to home visits. The study staff will take you home after the first vaccination in order to adequately describe your home location to allow for home visits in future should the study staff be unable to contact you by telephone.
- Female participants must use an adequate birth control method for 45 days prior to the first injection or patch and for at least 90 days after the final injection, as it is stated in the protocol.
- If female and able to become pregnant, avoid pregnancy but immediately inform the study doctor or staff if you do become pregnant during the study. If you are found to be pregnant at any study visit after the screening visit you will stop receiving study injections, but you will continue to be followed-up for the remainder of the study and

your pregnancy will be followed until your baby is born Blood will be drawn only for safety testing.

- Inform the study doctor or staff of any health problems, side effects, visits to another doctor or hospital and any changes in your plans or ability to participate in the study.

6. How long will I be in this study?

If you decide to join, you will be in this study for about 105 weeks (26 months).

You will be asked to complete at least 26 clinic visits. Please note that you may be asked to come to the clinic for an unplanned visit if you experience side effects that are of concern. Additionally, you will be contacted by phone, once per week for three weeks, to discuss any changes you may have experienced after the first 105 weeks of being in the study.

You will be vaccinated six (6) times (at weeks 0, 4, 12, 20, 32, 56) during this study. The vaccinations will be given by injection, like most approved vaccines, in the muscle of your upper arm. The first vaccination visit could take up to 6 hours, but the other vaccination visits take about 4 hours each. Most other follow-up clinic visits will usually take about 2 hours.

7. How many participants will be in this study?

126 healthy men and women, 18-40 years old will be in this study. We think that one out of every four people screened will be eligible which is about 504 potential participants.

8. What possible risks can I expect from participating in this study?

Blood draws:
In this study, study staff will take some blood from you at every visit. The total amount of blood collected over 26 months is about 128 TBSP. Blood draws can, at times, cause bruising, pain, or fainting. They rarely can cause an infection. Blood is drawn by qualified staff that have been trained in drawing blood.

Vaccines and adjuvants:
This is an early human research study to examine the safety of these vaccines and adjuvants. You are one of the first humans to receive this combination of vaccines and adjuvants. As a result, not all the side effects are known. All vaccines can cause fever, chills, rashes, pains, nausea, headache, dizziness, and sleepiness. Most people are still able to do their daily activities after getting a vaccine. Rarely do they need to go to the doctor. Frequently, vaccines cause pain and swelling where you get the injection. These local reactions are usually mild.

People who have received the DNA vaccine that will be used in this study have reported a delayed allergic reaction (appearing 24 to 48 hours after vaccination) at the site of injection. Another common reaction seen was a “recall” reaction. This reaction can range from redness to swelling, rash, and pain, which appears at the site of a previous injection, typically 36 to 72 hours after the most recent injection. Reactions that occurred after DNA vaccine was injected were described as mild. Reactions that occurred after protein was injected ranged from mild to severe.

You may take over the counter medicines to help with any discomfort you may have after the experimental vaccinations, but please report any use of these medicines to the clinic staff. Rarely, a vaccine causes an allergic reaction. This reaction can be a rash, hives, or difficulty breathing. You should tell the study staff if you have ever had a bad reaction to an injection or a vaccine. Allergic reactions can be life-threatening. If needed, participants will be transferred to the Unilever Hospital for further management.

An adjuvant is a liquid substance added to vaccines that can help to make the vaccine more effective by improving the immune response or causing the immune response to last longer than without the adjuvant. The adjuvants that will be mixed with the experimental vaccines have been used in other research studies and the most common reactions at the injection site are pain or tenderness, redness and a bump under the skin. Other common reactions are not feeling well in general, muscle aches, headache, fever, and nausea. These reactions have been reported to be mild to moderate.

The body's immune system protects the body from infections. Sometimes, a person's immune system attacks the body instead, causing an "autoimmune disease". A vaccine can cause this type of disease or make it worse, but this is very rare.

Pregnancy and breastfeeding:
Researchers do not know if the vaccines may harm unborn babies. You should not become pregnant during this study. If you are having sex that can make you pregnant, you should use birth control. Researchers will talk with you about acceptable birth control methods. You should use them from beginning 45 days prior to the first vaccination and continuing through 90 days after the last study injection. Researchers will test you for pregnancy throughout the study.

Adequate birth control is defined as follows: Oral contraceptives, injection or implant, surgery (hysterectomy or tube tie), condoms with spermicide, diaphragms, intrauterine device (IUD), vasectomy in a monogamous partner, or abstinence.

If you become pregnant during this study, you will stop getting the study injections but will remain in the study for observation which will include small blood draws but only to check your heath. Study staff will help you find out about available care for you and your baby. This study will not pay for this care. Knowing the results of your pregnancy is important, so study staff may ask you to come back for visits or may call you. Additionally, you should not breastfeed while in this study because researchers do not know if the vaccines may pass through breast-milk and may harm your baby. The study team will follow your pregnancy until your baby is born.

Transcutaneous immunization skin patch (TCI):
TCI is a needle-free skin patch applied to the skin like a bandage that, by itself, has been found to be safe and well tolerated. Common reactions to TCI are redness and bumps where the patch is applied to the skin, and changes in skin pigmentation at the site of patch application. When combined with the experimental vaccine, TCI administration side effects may include itching, mild redness or rash, and changes in pigmentation at the site of patch application. If change in pigmentation occurs, it could be permanent.

VISP:
The vaccines may cause "vaccine-induced sero-positivity" or "VISP". So, if you are in the group that receives the experimental vaccine and not the placebo, you may test HIV positive when you really do not have HIV. This clinic's HIV test can tell the difference between real HIV infection and VISP. For this reason, you should avoid all HIV testing not done at this clinic. You will be tested for HIV regularly during this study. After this, if you would like HIV testing, we encourage that you return to this clinic for testing. Researchers do not know how long you may have VISP. At your request, you will be provided with a letter explaining your participation in the study and the possibility of testing positive for HIV antibody. If you have problems because of VISP, with your written permission, the study staff will assist you with any unfair treatment you may experience by being in this study. This includes talking to insurance companies, employers, and others to verify your study participation or advocate on your behalf.

Also, if you become pregnant and have the baby while you have VISP, your baby may have VISP too. This clinic's HIV test can tell the difference between real HIV infection and VISP in your baby as well.

If you have VISP, you cannot donate blood and you must not donate blood during your participation in this research study for other reasons as well. If you wish to donate blood after you have completed participation in this study, blood donation options will be explained at the final study visit, however you may be excluded in the future from donating blood when you reveal that you participated in an HIV vaccine study. For the same reason, you may also be excluded from participation in other research studies.

Social issues:
You may face personal problems because of being in this study. Family, friends, and others may worry, get upset, or treat you unfairly. People may think that you have HIV or are likely to get it. You could lose your job because your employer thinks that you have HIV, or because you take too much time away from work to be in this study, but it is unlikely.

You may feel embarrassed when answering personal questions about sex or having a physical exam. You may feel anxious when waiting for your HIV test results. If you have these feelings, please tell the study staff so that they can find a way to help you.

Researchers and study staff try hard to protect your privacy. They also have a duty to maintain your privacy. But there is a risk that others, including your partner, may find out that you are participating in this study. So, they may treat you badly or discriminate against you. Your partner may decide to insult you, leave you, or hit you. Your partner may stop paying for things. Another social risk is that someone may use your personal information in a bad way. For example, someone finds out your test results and shares them with others. You could then have problems getting or keeping a job. You may no longer have your family's or your community's support. These situations may cause you stress and embarrassment. Researchers have ways to reduce these social risks. Some of these ways include limiting access to your study records, having your study visits in private, and using codes to identify you and your samples. If you have any of these problems, please talk to the study staff, so that they can try to help you.

Genetic testing:

The greatest risk associated with genetic testing is to your privacy. Genetic testing is described below in section 10.

9. What possible benefits can I expect from participating in this study?

You will not directly benefit from participating in this study. The results of this study will be shared with researchers from around the world and could play a role in future HIV vaccine development which may be beneficial in the future for others. These experimental HIV vaccines have not shown that they work to protect against HIV infection. Also, there is also a possibility that you may be assigned to receive the inactive placebo.

Some small benefits for study participants may include no cost detailed medical, physical and laboratory examinations and potential care for minor ailments.

10. What will happen to my samples and data?

HIV vaccine studies are done to learn how experimental vaccines affect your immune system and how your body responds. To better understand this, at each visit, the study doctors take blood samples to assess your heath and for research testing. Clinical information that may be beneficial to your health will be shared with you but your results from the research testing will not be shared with you. You may be asked to give more blood, if needed, to assess your health.

Genetic testing may be conducted on your samples during this study and in the future. For example, researchers may do "genetic variations" research. They may look at genes that affect how you fight infections. Your genes are passed to you from your birth parents. Genes are the basic "instruction book" for the cells that make up our bodies. The differences in people's genes can help explain why some people get a disease while others do not. We will not notify you of the results of any genetic test. The genetic research tests we plan to conduct are not currently used in medical practice. The results of such tests have not been approved for use in making health care decisions and the results will not be shared with you. To protect your identity, your genetic testing results will not be linked to your name and will not independently identify you as an individual. The research for this study will not include mapping of all your genes (called "whole genome sequencing").

Some of the blood samples will be shipped to the USA and or Thailand for tests are not available at this site. The blood that is left over will be stored at the KEMRI/USAMRD-A Kericho CRC lab for use in the future. We will share study information and or samples to research collaborators outside of KEMRI/USAMRD-A and to research collaborators outside of Kenya without asking for your permission and there is no time limit on how long your data and samples will be stored for future use. Documents, (including this form) notes from your visits, and photographs, if any, will be stored securely and then destroyed by authorized individuals when these records are no longer needed. You have the option to allow your specimens to be used for future research and you can also choose to allow or decline genetic research at the end of this form. If you agree now and decide later that you do not want us to use your samples for future research, please tell us. We will ask the storage facility to destroy any remaining samples that still have your study ID on them so that they cannot be used for future research. Samples that are "deidentified", meaning they do not have your study ID on them, will not be withdrawn from storage because, without your ID, there is no way for the researchers to find your samples.

The overall results and findings from this study will not be shared with you.

Please see section 12 that explains the methods we use to protect your privacy.

11. What other choices do I have?

If you choose not to participate in this study:

- You could choose to participate in other studies
- You may choose to get HIV counseling and testing outside this study

If you would like more information about the risks and benefits of each one of these choices, feel free to talk to the study staff. You can also discuss these options with your doctor. Regardless of your choice, any care that you get at this clinic outside of this study will not change.

12. How will researchers protect the privacy of my information?

You will be identified by a fingerprint identification system (with/without a picture) on a computer. We will be storing the information generated by your fingerprint along with your name and address in a secure database for the purpose of identifying participants. Only authorized study staff can access this information. Your fingerprint, photograph, name or other identifier will not be attached to any of your samples or test results or other study information, now or in the future.

Researchers have protections in place to maintain your privacy. They keep your study records in a secure place. They do not use your name in publications, meetings, or stored samples. They use a code to identify you and your samples. They do not share any information that could identify you. Complete confidentiality cannot be promised, but every effort will be made to keep the records as confidential as possible within the limits of the law. All data and medical information obtained about you as an individual will be considered important and held in confidence.

The Principal Investigator, Dr. Josphat Kosgei, is in charge of the research records of your participation in this study. All study participants will receive participant identification numbers or study ID known only to the study team at the clinic and used to ensure the confidentiality of your information. Your samples will be labeled with your participant identification number (study ID) and will not identify you by name. A key to the code connects your name to your study information and samples. The study doctor or authorized study staff will keep the key to the code in a locked cabinet in a restricted-access room here at KEMRI/USAMRD-A and will not share it with our research collaborators. No one outside of KEMRI/USAMRD-A will know which participant the samples came from. Before any data or specimens are sent to research collaborators, approvals will be obtained from the WRAIR IRB and KEMRI SERU.

The study records will be stored at the KEMRI/USAMRD-A triple-locked filing room for the period when the study is active, and thereafter transferred to the site's archive for long term storage. When your records and samples are no longer needed, they will be destroyed.

There are several organizations watching over this study. They want to make sure that researchers are protecting your rights and keeping you safe. They also want to see if researchers follow the approved study protocol. People from these organizations may review your records. These people have a duty to maintain your privacy. Some of these organizations are:

KEMRI SERU, Kenya PPB, the Kenya Ministry of Health, USAMRDC ORP, HRPO, the NIAID, the NIAID Office of Human Research Protections (OHRP), the US FDA, WRAIR, the people who work for these organizations, and other local, US, US DoD, or international regulatory agencies as part of their responsibilities for ensuring the protection of research participants. All the above representatives are bound by rules of confidentiality not to reveal your identity to others. By signing this consent form, you are authorizing this access.

Throughout the study, investigators will be collecting data related to the safety of participating in this study and receiving study products. That safety data will be stripped of any personal identifiers and will be added to a database in order to help researchers learn more about potential issues that may affect safety across multiple studies. Data collected for the safety database will include the following: study title, information on the safety event, your gender, age, and location.

Additionally, it is the policy of the USAMRDC that data sheets are to be completed on all study participants participating in research for entry into this Command's Volunteer Registry Data Base. The information to be entered into this confidential database includes your name, date of birth, address, study title and dates. The intent of the database is two-fold: first, to readily answer questions concerning an individual's participation in research sponsored by USAMRDC; and second, to ensure that the USAMRDC can exercise its obligation to ensure research study participants are adequately warned (duty to warn) of risks and to provide new information as it becomes available. The information will be stored at USAMRDC for a minimum of 75 years.

13. Will I have to pay anything to participate in this study?

You will not have to pay for any study lab tests, procedures, or exams. Your insurance provider will not have to pay either. You will not have to pay for any of the study procedures.

14. Will I receive any payment for my participation in this study?

You will receive reimbursement for your transportation costs, time, and lost wages that may result from sick days taken after the procedure. You will receive reimbursement for the main study even if you don't take part in any of the optional procedures. There is no other compensation available for your participation in this research study; however, you should also understand that signing this consent form is not a waiver or release of your legal rights.

Other than medical treatment that may be provided, there is no other reimbursement available for your taking part in this research. If you choose to end your study participation early, you will receive reimbursement only for the portion of the study that you have completed.

We may use your samples and information to develop a new product or medical test to be sold. The Sponsor and researchers may benefit if this happens. There are no plans to compensate you if your samples are used for this purpose.

Reimbursement amounts are as follows:

- Study visits: 1000 Ksh
- Unscheduled visits: Up to 500 Ksh, at the discretion of the study investigator
- Mucosal collections: 1000 Ksh per each collection
- Lymph node biopsy: 5000 Ksh
- Transportation: Up to 3000 Ksh, at the discretion of the study investigator

The clinic staff will provide compensation to participants in cash at the clinic site upon completion of the visit study procedures.

15. Can I change my mind about participating in this study?

Yes, you can change your mind at any time. Your participation in this study is completely voluntary. Tell the study staff if you are thinking about leaving or have decided to leave this study. Again, any care that you get at this clinic outside of this study will not change.

Study staff may want you to do some follow up visits and testing before you leave this study.

16. Can researchers take me off this study early?

Yes, researchers can take you off this study at any time:

- If they believe it is the best thing for you
- If you do not follow the study requirements
- If one of the groups watching over the study stops it
- If you become pregnant
- If you have a serious side effect that is related to the study vaccine.
- If you become HIV positive
- If you become incarcerated
- If you have a medical problem where continuing to be in the study would be harmful to you.

If you become pregnant or ill and can no longer participate in the study, the researchers may ask you to return for follow up visits to assess your health.

17. What happens if I become HIV infected during this study?

Researchers do not know how the experimental vaccines in this study may affect your risk of getting HIV. If you get HIV, they do not know if the vaccines would affect how bad your HIV infection will be. Also, they do not know if the vaccines would change how you may respond to other HIV vaccines in the future. Currently, there is no vaccine for HIV. If you become HIV infected during this study, study staff will tell about any available care and you will not be allowed to remain in the study. Study staff will counsel you about your HIV infection. They will also tell you how to lower the risks of giving HIV to others.

18. What are my rights and who should I contact if I have questions?

You have the right to leave this study at any time and for any reason. The study staff will continue to treat you the same no matter what you decide. You will not give up your legal rights by signing this informed consent form. You also have the right to know about any new information from this study or other studies. This information may affect your health, welfare, or decision to stay in this study.

If you have questions about your rights as a study participant, contact:

Kenya Medical Research Institute (KEMRI)	Office: 020 27541 or 0722205901 or 0733400003	Mail: Kenya Medical Research Institute (KEMRI) SERU P.O. BOX 54840-00200 Nairobi

19. Who should I contact if I think that I am hurt because of my participation in this study?

If you get sick or injured due to participation in this study, you will receive appropriate medical treatment and care as provided with costs for such treatment covered by a limited fund and a clinical trials medical insurance policy. While we anticipate the combination of the set-aside fund and the insurance policy is enough to pay for the cost of any injury associated with this study, there is a limit to the amount of coverage available. If the limit is exceeded, you may have to pay non-covered costs.

KEMRI/USAMRD-A will pay costs up to the limit from dedicated funds or through the clinical study insurance. However, you will not get any other compensation because of an injury directly related to your participation in this study. KEMRI, the US Army, or US NIH is not able to reimburse you for these treatment expenses. The United States Department of Defense will not pay for research-related injuries.

If you think you are hurt because of your participation in this study, please tell Dr. Kosgei or Rael Bor. You can do it in person or call the numbers provided at the beginning of this form.

20. How do I confirm my decision to be in this study?

My signature below confirms:

- that I voluntarily decided to participate in this study
- that I had the opportunity to read this form
- that this form was explained to me
- that I had the opportunity to ask questions
- that I had the opportunity to discuss my study participation with others

If there is any portion of this document that you do not understand, ask the study staff before signing the form. Signing this form means that you consent to participate in this research, at this time. A signed and dated copy of this document will be given to you.

21. Your choices about how your biological samples may be used:

Future Research:

Researchers may store and use my biological samples and data to find new ways to detect, treat, prevent, and cure health problems.

☐ ____ Yes, I agree. initials ☐ ____ No, I do not agree. initials

Genetic Testing:

Researchers may store and use my biological samples and data now and in future research to learn about how genes play a part in diseases.

☐ ____ Yes, I agree. initials ☐ ____ No, I do not agree. initials

Additional information about the trial is available at http://www.ClinicalTrials.gov website.

SIGNATURE OF PARTICIPANT

_____
Printed Name of Participant

_____ _____
Signature of Participant Date

SIGNATURE OF INDIVIDUAL ADMINISTERING CONSENT

(Can only be signed by an investigator or staff approved to administer consent)

_____
Printed Name of Administering Individual

_____ _____
Signature of Administering Individual Date

APPENDIX C. OPTIONAL PROCEDURES CONSENT

Kenya Medical Research Institute (KEMRI)
United States Army Medical Research Directorate-Africa (USAMRD-A)
In Collaboration with
US Military HIV Research Program and the
US Department of Defense (DoD)

RV460/WRAIR #2672 Informed Consent Form for Optional Procedures

Protocol Title: A Randomized, Double-Blind Phase 1 Trial to Evaluate the Safety and Immunogenicity of Priming with Env-C Plasmid DNA Vaccine Alone, with Different Adjuvants, or with an Adjuvanted HIV Env gp145 C.6980 Protein Vaccine and Boosting with the Adjuvanted HIV Env gp145 C.6980 Protein Vaccine with or without the Env -C Plasmid DNA Vaccine in Healthy HIV Uninfected Adults in Kenya

Sponsor: National Institute of Allergy and Infectious Diseases (NIAID), Division of AIDS (DAIDS) Bethesda, Maryland US

Funding: United States Army Medical Research Acquisition Activity
U.S. Military HIV Research Program
Henry M. Jackson Foundation for the Advancement of Military Medicine

Site: Kenya Medical Research Institute (KEMRI) US Army Medical Research Directorate-Africa (USAMRD-A), Kericho, Kenya

Introduction

This consent form is for you to learn about and to help you decide to give permission for certain optional procedures as part of this study. You do not have to agree to any of these procedures and you can still participate in the main study.

The 2 optional procedures are:

- Collection of rectal and genital secretions, also called mucosal secretions
- Lymph node biopsy

Please refer to the participant visit schedule given to you to keep track of what can be expected from you at each visit.

Key Information for You to Consider

Voluntary Consent. You are being asked to volunteer for a research study. It is up to you whether you choose to participate or not. There are no penalties and you will not lose anything if you decide not to join or if after you join, you decide to quit.

Purpose. We are doing this research to test a vaccine for HIV and vaccine additives called adjuvants that can help the vaccine be more effective. The tissue samples requested in this optional part of the study will help us learn about how your immune system responds in tissue other than blood.

Duration. Mucosal collections will occur at 9 visits (Visits 1,7, 11, 15, 18, 22, 23, 24, and 25) over the course of study. Lymph node biopsies will be performed at weeks 14 and week 58.

Procedures and Activities. Collection of mucosal secretions - cervicovaginal secretions, semen or rectal secretions at up to 9 clinic visits and up to 2 groin lymph node biopsies.

Risks. Most studies have some possible harms that could happen to you if you join.

It is possible that these optional procedures could cause an unpleasant or serious effect, for example: Collection of mucosal secretions may be uncomfortable and embarrassing. There is a rare possibility that women that use the Softcup develop a serious illness called toxic shock syndrome (TSS).

The lymph node biopsy could leave a scar, cause bleeding or an infection. You should know that there is a chance that there is a small chance of developing a large scar (keloid) or temporary or permanent loss of feeling around the biopsy site.

Benefits. You will not directly benefit from participating in this study. The results of this study will be shared with researchers from around the world and could play a role in future HIV vaccine development which may be beneficial in the future for others.

Alternatives. Participation is voluntary, and the only alternative is to not participate.

Please contact one of the below if you have any questions concerning the study or if you have any other questions or concerns.

If you have questions about this study, contact:

Dr. Josphat Kosgei, MBCHB, MSc	Office: (0)5220 36302	Mobile: (0)729 110 122	Staff: (0) 5220) 36000/100

If you have any symptoms that you think may be related to this study, contact:

Rael Bor, BSc	Office: (0)5220 36329	Mobile: (0) 729110121	Staff: (0) 5220) 36000/100

1. Why is this research being done?

The main purpose of this study is to find out if the experimental vaccines with adjuvants used in this study cause side effects, whether humans respond (develop immune responses) to the experimental vaccines, and how long the effects of the experimental vaccines last. Some results from other studies have suggested that the immune cells in your secretions might play a role in a person's chances or getting HIV. The tissue samples requested in this optional part of the study will help us learn about how your immune system responds in tissue other than blood.

2. What possible benefits can I expect from participating in this part of the study?

You will not directly benefit from participating in this study. The results of this study will be shared with researchers from around the world and could play a role in future HIV vaccine development which may be beneficial in the future for others.

3. Who can participate in this part of the study?

Optional procedures are open to all the participants that are enrolled in the main study and are eligible and willing to provide these additional samples. Not all participants will be eligible for this part of the study. There may be reasons why you would not be able to participate in the optional procedures. Your eligibility will be decided by the study doctor after reviewing your medical history and physical exam.

4. What should I know about the different optional procedures?

We would like you to know the following:

- You may not undergo these optional procedures if you become pregnant because these may cause unforeseeable risk to you or your baby.
- If you undergo any of these optional procedures and there is evidence of an abnormal finding, you will be referred for diagnosis and care. You will not be informed about any research results that come from your samples.

Collection of Genital and Rectal Secretions

1. Why is the collection of mucosal secretions done?

One of the ways that people become infected with HIV is through contact with genital secretions. HIV virus can be spread through genital secretions. Researchers do not know much about what happens in the genital tract after an HIV vaccination is given and we hope to learn about improving protection from HIV in the genital tract.

2. How is the collection of genital and rectal secretions done?

Cervicovaginal Secretion Collection (women)

You must refrain from vaginal intercourse, douching or inserting any object into your vagina for 72 hours prior to the collection. If you are menstruating (bleeding), the vaginal sampling will be done at another time. Also, the collection will be delayed if you have symptoms of active inflammation or infection in the vagina or cervix. If you become pregnant during the study, you cannot continue participation in this part of the study.

At each sample collection visit, you will be asked a series of questions to be sure that you are eligible to provide the specimens. Additionally, you cannot use the Softcup during the six-week period immediately after childbirth, miscarriage, or termination of pregnancy.

A trained nurse or doctor will show you how to insert a soft plastic cup (Softcup) into your vagina to collect the vaginal secretions. The *Instead Softcup* is approved by the United States Food and Drug Administration (FDA) as a feminine hygiene alternative to pads and tampons for use by women during menstruation. The Softcup *cannot* be used to prevent pregnancy and does not protect you from getting a sexually transmitted infection (STI).

A nurse or doctor can insert the cup for you before your appointment or you may insert it yourself. After you learn about how to use the cup, you may choose to insert the cervical cup yourself at home before you come to the clinic and remove it when you come to the clinic for your study visit. The cup should remain in the vagina for at least four hours, but not more than 12 hours to be able to obtain enough secretions. To remove the cup, you will be given a plastic container and be instructed to go alone into a private room in the clinic, remove the cup, place it in the container and close the container.

You can choose not to provide the cervicovaginal secretion sample and still participate in rectal secretion collection and lymph node biopsy described below.

Semen Collection (men)

You must refrain from masturbation or ejaculation for 72 hours prior to the collection. At each sample collection visit, researchers will provide a private room for you to provide the semen sample. In this room, you will ejaculate into a sterile container.

You can choose not to provide the semen sample and still participate in rectal secretion collection described below.

Rectal Secretion Collection (women and men)

You must refrain from any kind of sexual activity or inserting anything into your rectum for 72 hours prior to collection. You will not be allowed to participate in the rectal secretion collection part of this study if you have a gastrointestinal disorder or an illness that may result in increased

inflammation including autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, or psoriasis.

A nurse or doctor who has been trained to do an anal examination will examine you in a private room. The nurse or doctor will insert an instrument into the anus to inspect the tissue in the lower rectum and use a sponge to collect secretions in the lower rectum.
You can choose not to provide rectal secretions and still participate in the vaginal secretion (women) and semen (men) collection.

3. How often is the collection of mucosal secretions done?

You will be asked to provide a sample nine times; at visits 1,7,11, 15, 18, 22, 23, 24, and 25. You can choose which samples you wish to provide at each visit. Please see the schedule provided by the study staff.

4. What are the risks of the collection of mucosal (cervicovaginal, rectal, or semen) secretions?

Both men and women may feel shy about exposing your private areas during the examination. Nurses and doctors performing this procedure have been specially trained and will conduct the examinations in a private area.

For women, inserting the Softcup into the vagina may cause discomfort and slight irritation similar to inserting a tampon but it should not cause any pain. There is no evidence of Softcup sampling contributing to risk of HIV or other sexually transmitted infection. Squirting fluid and swabbing in the vagina may cause some irritation.

If you are a woman and you have a history of toxic shock syndrome (TSS), you cannot participate in vaginal secretion collection. TSS is a rare but serious disease that is believed to be caused by a bacterial toxin and is associated with keeping a tampon in the vagina for too long without changing it. Symptoms of TSS include a sudden high fever, vomiting, diarrhea, a rash that looks like sunburn, dizziness, fainting or near fainting, and muscle aches. TSS can quickly become a serious illness that can be fatal. If you have any of these signs and you are using the Softcup, remove it and immediately contact your physician for medical care at once. You must not use the Softcup for more than 12 hours.

Men might feel uncomfortable giving semen samples.
For both women and men, inserting an instrument into the anus may be uncomfortable but it should not cause pain. Squirting fluid and swabbing in the anus could cause some irritation.

Lymph Node Biopsy

1. What is a lymph node?

Lymph nodes are small nodules that are a part of your immune system and can be found behind the ears, in the neck, in the armpit, and in the groin. Lymph nodes are responsible for producing some white blood (immune) cells called lymphocytes as well as filtering the fluid that carries

them around in the body. They usually swell when you have an infection nearby (e.g. a wound on the leg can cause the lymph node in your groin to swell).

2. Why is lymph node biopsy done?

Two of the main types of cell that are important in the body's immune system, the T cell and the B cell, are found in large numbers within your lymph nodes. In the future, to try to develop better treatments for and vaccinations against HIV, it is important for us first to understand the interactions between these cells, their roles in immunity and how these are affected by the experimental vaccine in this study.

3. How is lymph node biopsy done?

A surgeon will perform the lymph node biopsy using local anesthetic. This means you will be awake, but the biopsy area will be numb. Please let us know if you are allergic to any medications. The surgeon will biopsy a lymph node in the groin area because this is the safest location with the smallest risk of problems. The surgeon will clean your groin with an antiseptic solution and then make a small cut in the groin (about 1 to 2 centimeters long). The surgeon will remove a lymph node, which is about the size of a peanut, through the incision. The surgeon will then close the incision with stitches and bandage the wound. The whole process will take about 30 to 40 minutes. You will remain under observation for 2-3 hours after the procedure. You will also be asked not to do any strenuous activities for the next 24 hours. You will need to return in about 1 week to have the stitches removed, depending on which type is used, and to assess the surgical site.

4. How often is the lymph node biopsy done?

You will be asked to undergo lymph node biopsy two times during this study at week 14 and week 58.

5. What are the risks of lymph node biopsy?

You may experience pain in the biopsy area once the local anesthetic has worn off. If you have pain, the study team will provide additional medications to control the pain. There is the possibility of some bleeding or bruising after the procedure. Doctors will perform blood tests before the biopsy and ask about any medications you are taking or conditions that could make you more likely to bleed. Surgery can cause a seroma to form which is a pocket of clear fluid that sometimes develops in the body after surgery. There is a small risk of infection (less than 2%). If you have any concerns that your wound might be infected, you should contact the study team immediately. Signs of infection include redness, swelling, drainage, and heat at the incision. There is the possibility of developing a scar as the wound heals. There is a possibility of nerve injury during the procedure, which could result in either temporary or permanent reduction in feeling around the biopsy site. Finally, it is possible, although unlikely, that the surgeon will be unable to find a lymph node or for some reason unable to remove it during the procedure. If you

are willing, you may return at another visit for an attempt at lymph node biopsy on the other side of your body.

Participants who develop complications after lymph node biopsy will receive immediate care at the CRC clinic, and where necessary, admitted for specialized at the Unilever Tea Kenya hospital.

6. Will I have to pay anything to participate in this study?

You will not have to pay for the procedures according to protocol.

7. Will I receive any payment for my participation in this study?

You will receive reimbursement for your transportation costs, time, and lost wages that may result from sick days taken after the procedure.You will receive reimbursement for the main study even if you don't take part in any of the optional procedures.
Reimbursement is as follows:

- Mucosal collection: 1000 Ksh per each collection
- Lymph node biopsy: 5000 Ksh

8. Am I eligible to participate in the lymph node biopsy?

You will not be able to participate in a lymph node biopsy if you have a bleeding disorder or if your platelet count is less than 150,000 count/ml or if, because of a medical condition, you are taking a blood thinner and you cannot stop it for 7 days before the procedure. You must have a negative pregnancy test if you are female. Your body mass index must be less than 35. There may be other reasons why you would not be able to participate in the optional procedures. Your eligibility for lymph node biopsy will be decided by the study doctor after reviewing your medical history and physical exam.

9. How do I prepare for the lymph node biopsy?

You should not take certain medications that may affect coagulation (clotting), for instance, aspirins or non-steroidal anti-inflammatory drugs (e.g. ibuprofen and diclofenac) for 7 days before the procedure. Those medications could increase the risk of excessive bleeding.
You should not eat for at least 8 hours (overnight) before the procedure.

10. What will happen if I am injured?

If you get sick or injured due to participation in this part of the study, you will receive appropriate medical treatment and care as provided with costs for such care covered by a limited fund and a clinical trials medical insurance policy. There is a limit to the amount of coverage available and, if the limit is exceeded, you may have to pay non-covered costs.

KEMRI/USAMRD-A will pay costs up to the limit from set aside funds or through the insurance. However, you will not get any other compensation. The U.S. National Institutes of

Health (NIH) does not have a mechanism to provide compensation for research-related injury. You are not waiving your rights by signing this consent form. You should discuss this with the study doctor before deciding to participate in this part of the study. If you have any questions about study-related sickness or injury contact the study doctor.

11. What will happen to my samples?

The samples from these optional procedures will be stored at KEMRI/USAMRD-A. They will not have your name but rather will be assigned a number and no one in the laboratory will know any personal information about you. Parts of your stored samples will be sent to study collaborators in the US and Thailand.

You can decide whether to let us use your samples for future tests that are not covered under this research study. Your decision does not affect your participation in the study or any care you receive at this clinic. Your decisions on what to do with your samples are on the "main" study consent form.

12. Can I change my mind about participating in this part of the study?

Yes. You can decide not to participate in this optional part of the study at any time, for any reason. If you decide to not participate in this part of the study, it will not affect your participation in the main study or the care you receive from this or any other health care facility.

13. How do I confirm my decision to be in this part of the study?

Consent for Lymph Node Biopsy

Please check the box of your choice and initial to the right on the blank provided.

The procedure has been explained to me and I have had a chance to have all of my questions answered. The risks of the procedure have also been explained to me and I understand that there are risks with any medical procedure.

☐ _____Yes, I agree to the lymph node biopsy procedure.
(initials)

☐ _____No, I do not agree to the lymph node biopsy procedure.
(initials)

Consent for Mucosal Secretion Collection

Please check the box(s) of your choice and initial to the right on the blank provided. You may choose one, both or none of the procedures.

The procedures have been explained to me and I have had a chance to have all of my questions answered. The risks of the procedure have also been explained to me and I understand that

there are risks with any medical procedure.

I agree to the mucosal collection procedure(s) specified below.

[redacted]_____vaginal secretion collection (***women only***)
(initials)

[redacted]_____semen collection (***men only***)
(initials)

[redacted]_____rectal secretion collection
(initials)

☐_____No, I do not agree to any secretion collection.
(initials)

Additional information about the trial is available at http://www.ClinicalTrials.gov website.

SIGNATURE OF PARTICIPANT

_____
Printed Name of Participant

_____ _____
Signature of Participant Date

SIGNATURE OF INDIVIDUAL ADMINISTERING CONSENT

(Can only be signed by an investigator or staff approved to administer consent)

_____
Printed Name of Administering Individual

_____ _____
Signature of Administering Individual Date

APPENDIX D. PHOTOGRAPHY CONSENT FORM

Kenya Medical Research Institute (KEMRI)
United States Army Medical Research Directorate-Africa (USAMRD-A)
In Collaboration with
US Military HIV Research Program and the
US Department of Defense (DoD)

RV460/WRAIR #2672 Informed Consent Form for Photography and Use of Photos

Protocol Title:	A Randomized, Double-Blind Phase 1 Trial to Evaluate the Safety and Immunogenicity of Priming with Env-C Plasmid DNA Vaccine Alone, with Different Adjuvants, or with an Adjuvanted HIV Env gp145 C.6980 Protein Vaccine and Boosting with the Adjuvanted HIV Env gp145 C.6980 Protein Vaccine with or without the Env -C Plasmid DNA Vaccine in Healthy HIV Uninfected Adults in Kenya
Sponsor:	National Institute of Allergy and Infectious Diseases (NIAID), Division of AIDS (DAIDS) Bethesda, Maryland US
Funding:	United States Army Medical Research Acquisition Activity U.S. Military HIV Research Program Henry M. Jackson Foundation for the Advancement of Military Medicine
Principal Investigator:	Dr. Josphat Kosgei, MBCHB, MSc

A photograph may be helpful to document an unusual or unexpected finding after the experimental vaccination is given. Also, if you undergo lymph node biopsy, the study doctor may take pictures of the biopsy site immediately after the procedure and again about 6 months later to document how well your biopsy site healed and for possible use as educational material for other participants who undergo the same procedure.

No one will be able to determine your identity from these pictures.

By signing below, you give study doctors permission to take and use photos taken of you during the study

Refusing to give consent for use of your photos will not affect your participation in this study. If you give consent today, you can withdraw your consent at any time without consequences.

Additional information about the trial is available at http://www.ClinicalTrials.gov website.

Please check the box of your choice and initial to the right on the blank provided.

☐ _____ Yes, I agree to allow my photos to be used.
(initials)

☐ _____ No, I do not agree to allow my photos to be used.
(initials)

SIGNATURE OF PARTICIPANT

_____
Printed Name of Participant

_____ _____
Signature of Participant Date

SIGNATURE OF INDIVIDUAL ADMINISTERING CONSENT

(Can only be signed by an investigator or staff approved to administer consent)

_____
Printed Name of Administering Individual

_____ _____
Signature of Administering Individual Date

APPENDIX E. WITHDRAWAL OF CONSENT FORM

Kenya Medical Research Institute (KEMRI)
United States Army Medical Research Directorate-Africa (USAMRD-A)
In Collaboration with
U.S. Military HIV Research Program and the
U.S. Department of Defense (DoD)

RV460/WRAIR #2672 Withdrawal of Consent of Sample Storage for Future Use

Protocol Title:	A Randomized, Double-Blind Phase 1 Trial to Evaluate the Safety and Immunogenicity of Priming with Env-C Plasmid DNA Vaccine Alone, with Different Adjuvants, or with an Adjuvanted HIV Env gp145 C.6980 Protein Vaccine and Boosting with the Adjuvanted HIV Env gp145 C.6980 Protein Vaccine with or without the Env -C Plasmid DNA Vaccine in Healthy HIV Uninfected Adults in Kenya
Sponsor:	National Institute of Allergy and Infectious Diseases (NIAID), Division of AIDS (DAIDS) Bethesda, Maryland USA
Site:	Kenya Medical Research Institute (KEMRI) United States Army
Principal Investigator:	Dr. Josphat Kosgei, MBCHB, MSc

Participant statement of withdraw of consent to have samples stored for future testing:

I wish to withdraw my consent to have my biological samples stored for future use, including genetic testing.

Please check the box next to your choice and write your initials on the line.

☐ ____ initials I wish to withdraw my consent for genetic testing only and continue to allow my samples to be stored and used for research in the future.

☐ ____ initials I wish to withdraw my consent for researchers to store and use my biological samples including genetic testing for research in the future.

We will ask the storage facility to destroy any remaining samples that still have your study ID on them so that they cannot be used for future research. Samples that are "deidentified", meaning they do not have your study ID on them, will not be withdrawn from storage because, without your ID, there is no way for the researchers to find your samples.

It has been explained to me that by signing this form, my samples will be used for all the tests

specified for this present study, but no specimens will be stored for future use.

I understand that withdrawing my consent to have my samples stored for future use will not make any difference to the care I am receiving now or in the future, or to any benefits that I am entitled to.

I have been given a chance to ask all the questions that I have about withdrawing my consent to have my samples stored. All of my questions were answered to my satisfaction.

Additional information about the trial is available at http://www.ClinicalTrials.gov website.

SIGNATURE OF PARTICIPANT

_____
Printed Name of Participant

_____ _____
Signature of Participant Date

SIGNATURE OF INDIVIDUAL ADMINISTERING CONSENT

(Can only be signed by an investigator or staff approved to administer consent)

_____
Printed Name of Administering Individual

_____ _____
Signature of Administering Individual Date

APPENDIX F. TEST OF UNDERSTANDING

RV460/WRAIR #2672

Test of Understanding

True	False	1.	**Volunteers in this vaccine study will be protected against HIV.**
True	False	2.	You will need to come to the clinic for 26 scheduled visits (including this one) over the next 2 years.
True	False	3.	The vaccines in this study will give you HIV.
True	False	4.	One purpose of this study is to determine if these vaccines are safe to administer to humans.
True	False	5.	Participants in this study will need to avoid engaging in activities that may expose them to HIV infection.
True	False	6.	You may take other experimental (test) products while you are taking part in this study.
True	False	7.	You may withdraw from the study at any time if you choose or your participation may be stopped if the study team decides it is in your best interest.
True	False	8.	Women are asked to not become pregnant while in the study.
True	False	9.	A participant in this study may experience side effects after vaccination.
True	False	10.	Because the vaccines may turn some of the standard tests for HIV infection positive, some people may incorrectly think that the study participants are infected with HIV (false-positive).

RV460/WRAIR #2672

Answer Key

False	**1.**
True	2.
False	3.
True	4.
True	5.
False	6.
True	7.
True	8.
True	9.
True	10.

APPENDIX G. PARTICIPANT VISIT SCHEDULE

RV460/WRAIR #2672

Appendix G
Participant Visit Schedule

Visit No.	Visit Day	Clinic visit activities	Optional Procedures	Approx. Time	Reimbursement
Screening	-45 to -3 days	Screening: Briefing & contact information, informed consents, test of understanding, medical history, vital signs, complete physical exam, 1 TBSP blood draw for: HIV test, hepatitis B and C syphilis, general health, urine pregnancy test for women, urine test (dipstick) for blood and protein	-Pap smear for women who agree to vaginal secretion collection	4-5 Hours	1000 Ksh
1	0	**Vaccination 1*:** Enrollment & randomize, exam & eligibility review, vaccination or placebo, examine injection site 30-60 minutes post-injection, receive diary card, urine pregnancy test for women, 12 TBSP blood draw for: research tests & HIV	-Mucosal secretion collection	5 - 6 Hours	1000 Ksh (+1000 Ksh for each mucosal secretion collection)
Phone call or Clinic Visit 24-48 hours After Vaccination					1000 Ksh
2	3	Follow Up: exam & symptom review, review diary card, 2 TBSP blood draw for: research tests. Bring diary card.		2 Hours	1000 Ksh
3	7	Follow Up: exam & symptom review, review & collect diary card, 2 TBSP blood draw for: research tests. Bring diary card.		3 Hours	1000 Ksh
4	14	Follow Up: exam & symptom review, 5 TBSP blood draw for: general health & research tests		2 Hours	1000 Ksh
5	28	**Vaccination 2*:** Exam & eligibility review, vaccination or placebo, examine injection site 30-60 minutes post-injection, receive diary card, urine pregnancy test for women, 2 TBSP blood draw for: research tests		4 Hours	1000 Ksh
Phone call or Clinic Visit 24-48 hours After Vaccination					1000 Ksh

Visit No.	Visit Day	Clinic visit activities	Optional Procedures	Approx. Time	Reimbursement
6	35	Follow Up: exam & symptom review, Review diary card, 2 TBSP blood draw for: research tests. Bring diary card.		2 Hours	1000 Ksh
7	42	Follow Up: exam & symptom review, 4 TBSP blood draw for: research tests and general health. Urine pregnancy test for women that elect for optional procedure(s). Bring diary card.	-Mucosal Secretion collection	4 - 5 hours	1000 Ksh (+1000 Ksh for each mucosal secretion collection)
8	84	**Vaccination 3*:** Exam & eligibility review, vaccination or placebo, examine injection site 30-60 minutes post-injection, receive diary card, urine pregnancy test for women, 3 TBSP blood draw for: research tests		4 Hours	1000 Ksh
Phone call or Clinic Visit 24-48 hours After Vaccination					1000 Ksh
9	87	Follow Up: exam & symptom review, review diary card, 2 TBSP blood draw for: research tests. Bring diary card.		2 Hours	1000 Ksh
10	91	Follow Up: exam & symptom review, review & collect diary card, 4 TBSP blood draw for: research tests. Bring diary card.		3 Hours	1000 Ksh
11	98	Follow Up: exam & symptom review, 9 TBSP blood draw for: general health, HIV & research tests. Urine pregnancy test for women that elect for optional procedure(s).	-Mucosal secretion collection -Lymph node biopsy	5 - 6 Hours	1000 Ksh (+1000 Ksh for each mucosal secretion collection & 5000 Ksh for lymph node biopsy)
Return in 1 week if lymph node biopsy was performed					
12	140	**Vaccination 4*:** Exam & eligibility review, vaccination or placebo, examine injection site 30-60 minutes post-injection, receive diary card, urine pregnancy test for women, 3 TBSP blood draw for: research tests		4 Hours	1000 Ksh
Phone call or Clinic Visit 24-48 hours After Vaccination					1000 Ksh
13	143	Follow Up: exam & symptom review, review diary card, 2		2 Hours	1000 Ksh

Visit No.	Visit Day	Clinic visit activities	Optional Procedures	Approx. Time	Reimbursement
		TBSP blood draw for: research tests. Bring diary card.			
14	147	Follow Up: exam & symptom review, review & collect diary card, 3 TBSP blood draw for: research tests. Bring diary card.		3 Hours	1000 Ksh
15	154	Follow Up: exam & symptom review, 9 TBSP blood draw for: general health, HIV & research tests. Urine pregnancy test for women that elect for optional procedure(s).	-Mucosal secretion collection	4 - 5 hours	1000 Ksh (+1000 Ksh for each mucosal secretion collection)
16	224	**Vaccination 5*:** Exam & eligibility review, vaccination or placebo, examine injection site 30-60 minutes post-injection, receive diary card, urine pregnancy test for women, blood draw for: 2 TBSP research tests		4 hours	1000 Ksh
Phone call or Clinic Visit 24-48 hours After Vaccination					1000 Ksh
17	231	Follow Up: exam & symptom review, review diary card, 3 TBSP blood draw for: research tests. Bring diary card.		2 Hours	1000 Ksh
18	238	Follow Up: exam & symptom review, 9 TBSP blood draw for: research tests, general health & HIV. Bring diary card. Urine pregnancy test for women that elect for optional procedure(s).	-Mucosal secretion collection	4 - 5 Hours	1000 Ksh (+1000 Ksh for each mucosal secretion collection)
19	392	**Vaccination 6*:** Exam & eligibility review, vaccination or placebo, examine injection site 30-60 minutes post-injection, receive diary card, urine pregnancy test for women, 3 TBSP blood draw for: research tests		4 Hours	1000 Ksh
Phone call or Clinic Visit 24-48 hours After Vaccination					1000 Ksh
20	395	Follow Up: exam & symptom review, review diary card, 2 TBSP blood draw for: research tests. Bring diary card.		2 Hours	1000 Ksh
21	399	Follow Up: exam & symptom review, review & collect diary card, 5 TBSP blood draw for: research tests & HIV. Bring diary card.		3 Hours	1000 Ksh

Visit No.	Visit Day	Clinic visit activities	Optional Procedures	Approx. Time	Reimbursement
22	406	Follow Up: exam & symptom review, 11 TBSP blood draw for: general health & HIV & research tests. Urine pregnancy test for women that elect for optional procedure(s).	-Mucosal secretion collection -Lymph node biopsy	5 - 6 Hours	1000 Ksh (+1000 Ksh for each mucosal secretion collection & 5000 Ksh for lymph node biopsy)
Return in 1 week if lymph node biopsy was performed					
23	476	Follow Up: exam & symptom review, 9 TBSP blood draw for: research tests. Urine pregnancy test for women that elect for optional procedure(s).	-Mucosal secretion collection	4 - 5 Hours	1000 Ksh (+1000 Ksh for each mucosal secretion collection)
24	560	Follow Up: exam & symptom review, 9 TBSP blood draw for: research tests & HIV. Urine pregnancy test for women that elect for optional procedure(s).	-Mucosal secretion collection	4 - 5 Hours	1000 Ksh (+1000 Ksh for each mucosal secretion collection)
25	728	Follow Up: exam & symptom review, 9 TBSP blood draw for: research tests & HIV. Urine pregnancy test for women that elect for optional procedure(s).	-Mucosal secretion collection	4 - 5 Hours	1000 Ksh (+1000 Ksh for each mucosal secretion collection)
26 Exit	735	Final visit: exam & symptom review, 4 TBSP blood draw for: research tests		2 Hours	1000Ksh

Reimbursement
Study visits: 1000 Ksh
Unscheduled visits: Up to 500 Ksh, at the discretion of the study doctor
Mucosal collection: 1000 Ksh per each collection
Lymph node biopsy: 5000 Ksh
Transportation: Up to 3000 Ksh, at the discretion of the study doctor

* A study vaccination may be given outside your vaccination window if the protocol safety review team (PSRT) permits.

APPENDIX H. RISK ASSESSMENT TOOL

WRAIR #2672/RV460 HIV Risk Assessment Tool

PID: __ __ __ __ __ Date: __ __/__ __ __/__ __ __ __ Visit: _____

SEXUAL BEHAVIOURS

A participant may be appropriate for inclusion if he or she meets these guidelines:

Criteria	*A participant in the **last 12 months:***	**Yes**	**No**
1.	Did not have oral, vaginal, or anal intercourse with an HIV-infected partner, or a partner who uses injection drugs.		
2.	Did not give or receive money, drugs, gifts, or services in exchange for oral, vaginal, or anal sex		
3.	Has not been diagnosed and/or been treated for a sexually transmitted infection: OR have not had any of the sexually transmitted infection symptoms as assessed by the study doctor/clinician.		
Criteria	In the ***last 6 months****:*		
1.	Has abstained from penile/anal or penile/vaginal intercourse.OR had 2 or fewer partners of the opposite birth sex for vaginal and/or anal intercourse.		
2.	Is not an MSM or a transgender person.		
3.	Uses or intends to use condoms in situations which may include penile/anal or penile/vaginal intercourse with new partners, partners of unknown HIV status, occasional partners, partners outside a primary relationship, and/or partners known to have other partners.		

NON-SEXUAL BEHAVIOURS

Criteria	*A participant* who in the ***last 12 months****:*		
1.	Did not inject drugs or other substances without a prescription.		
2.	Did not use cocaine, methamphetamine, or excessive alcohol, which in the investigator's judgment, rendered the participant at greater than low risk for acquiring HIV infection		

Comments:

Signature of Investigator or Designee Date

APPENDIX I. DIARY CARD

LABEL

Diary Card

Participant ID: |_4_|_6_|_0_|-|__|__|__|__|

Vacc. #: ____ **Vaccination Date:** ____ ____ ____
DD MM YYYY

Short title: Comparative Adjuvant Study for HIV-1 Env DNA and Protein Vaccines in Kenya 13-07-2020
RV460 / WRAIR #2672 Version 1.7

Instruction for recording No. 2-7 and 8-15
Please use the grading scale below to record the severity of your reaction to the injections in the next sections:
0 = None
1 = Mild: minimal interference with usual social and functional activities.
2 = Moderate: symptoms causing greater than minimal interference with usual social and functional activities.
3 = Severe: symptoms causing inability to perform usual social and functional activities

Time After vaccination	**30-60 min**	**6 hr**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Day/Month	__/__	__/__	__/__	__/__	__/__	__/__	__/__	__/__	__/__
Time taken *(hrs/min)*									
1. Oral temperature *(Celsius)*									
Vaccination injection site reactions	☐ **Right arm**					☐ **Left arm**			
2. Injection site pain or tenderness									
3. Swelling *(at injection site)*									
4. Itching *(at injection site)*									
5. Redness *(measure the largest dimension in cm.)*									
6. Hardness *(measure the largest dimension in cm.)*									
7. Warmth *(at injection site)*									
General Reactions *(not injection site)*									
8. Headache									
9. Chills									
10. Dizziness									
11. Tiredness / Fatigue									
12. Nausea									
13. Muscle pain									
14. Joint pain									
15. Rash *(if present, describe in "other symptoms"*									
16. Took medication for fever or for above symptoms? 0=No 1=Yes									

Other symptoms *(please describe):*

LABEL

Diary Card

Participant ID: |_4_|_6_|_0_|-|__|__|__|__|

Vacc. #: ____ **Vaccination Date:** ____ ____ ____
DD MM YYYY

If you take any medication, please record in the table below
If you take medications for fevers, please check your temperature again after 4 hours and record above in "Other symptoms"

Medication	Dose/route	Reason	Start date	Stop date

Suggestion

- Complete the diary card in as much detail as you can every day, at the same time each day
- Record any problems that occur during this period
- Bring back this diary card with you at the next appointment
- If you forget to record any data, leave it blank

If you have any problems or questions, please contact:

Staff name _____ Telephone Number _____

If you received a "patch" over the vaccination site on your upper arm, please write the time you removed the "patch" in the space below. You will need to wear the patch for 6 hours ± 2 hours (range 4-8 hours) after application.

Time patch was removed: _____ (hrs/min)

Official use only

☐ Card completed by participant and not edited by staff

☐ Card completed by participant and edited by: _____ ____ ____ ____
Staff name DD MM YYYY

APPENDIX J. EMERGENCY CONTACT CARD

Emergency Contact Card

RV460 / WRAIR #2672 **HIV Vaccine Study**

Participant ID# _____
Cardholder is a participant in an **HIV VACCINE** study conducted at **KEMRI**

While in this study, false-positive HIV serology can occur, but no infection will be present.
If this volunteer develops any unexplained illness or symptoms that could possibly be related to vaccination or other study procedure, please contact:
Rael Bor (0)5220 36329 or (0)0729110121

RV460 / WRAIR #2672 **HIV Vaccine Study**

Participant: Please keep this card with you so that you can contact the study team if you have any problems, questions, or are concerned about a symptom.

Dr. Josphat Kosgei

(0)5220 36302 or (0)729 110 122

or his research staff (0) 5220) 36000/100

APPENDIX K. VISP LETTER

KENYA MEDICAL RESEARCH INSTITUTE
US ARMY MEDICAL RESEARCH DIRECTORATE - AFRICA
Kenya Medical Research Institute (KEMRI)
P.O. Box 606, 00621 Villages Mkt.
Tel: 2729303, 2719694 Fax: 2714592
E-mail: info@usamru-k.org

WRAIR #2672/RV460 VISP Letter

Certificate of Participation

Date: _____

To Whom it May Concern,

The undersigned, Dr. Josphat Kosgei, hereby confirms that:
Mr./Mrs./Ms._____**,** date of birth / / , has been participating as a healthy, HIV-uninfected volunteer in clinical research study called: "A Randomized, Double-Blind Phase 1 Trial to Evaluate the Safety and Immunogenicity of Priming with Env-C Plasmid DNA Vaccine Alone, with Different Adjuvants, or with an Adjuvanted HIV Env gp145 C.6980 Protein Vaccine and Boosting with the Adjuvanted HIV Env gp145 C.6980 Protein Vaccine with or without the Env -C Plasmid DNA Vaccine in Healthy HIV Uninfected Adults in Kenya" at the Kenya Medical Research Institute (KEMRI) United States Army Medical Research Directorate-Africa (USAMRD-A) from_____ to _____ (date). This participant was vaccinated in a double-blinded manner with either Env-C Plasmid DNA, or HIV Env gp145 C.6980 protein, or placebo, depending on the study arm to which the volunteer was randomized.

As a consequence, if the participant received active study vaccine, HIV-specific antibodies might have developed as a result of an immune response to the candidate HIV vaccine regimen. Therefore, the results obtained in this person with commonly used HIV screening tests should be interpreted with caution as the outcome of the test could be positive without the participant truly being infected with HIV.

The research center uses special HIV testing algorithm to differentiate a positive test with no natural HIV infection from a natural HIV infection. If the HIV testing is required, the research center should be contacted for guidance or assistance in testing.

If you require any further clarification, please contact:
Dr. Josphat K. Kosgei, MBChB, MSc, DLSHTM
Kenya Medical Research Institute/Walter Reed Project HIV Program
P.O. Box 1357-20200, Hospital Road, Kericho, Kenya Tel: +254 5220 30686/ +254 5220 30388

Additional information about the trial is available at http://www.ClinicalTrials.gov website.

Yours sincerely,

_____
Dr. Josphat K. Kosgei, MBChB, MSc, DLSHTM, Principal Investigator

APPENDIX L. UNBLINDING LETTER

KENYA MEDICAL RESEARCH INSTITUTE
US ARMY MEDICAL RESEARCH DIRECTORATE - AFRICA
Kenya Medical Research Institute (KEMRI)
P.O. Box 606, 00621 Villages Mkt.
Tel: 2729303, 2719694 Fax: 2714592
Email: info@usamru-k.org

WRAIR #2672/RV460 Unblinding Letter

Date: _____

Dear Participant,

Thank you, once again, for participating in this clinical trial. You may recall that the purpose of this study was to see if the experimental HIV vaccines are safe (if it causes any side effects and how well it is tolerated). As we complete the data analysis, we can tell you whether you received the placebo or study vaccines in this trial.

Note: The study physician will edit this letter and only include the treatment that the individual received.

Number of doses received: _____

For Placebo Recipients:
During the course of the trial, you received what is called the "Placebo. This consisted of an inactive substance—like salt water injections. The liquid used as the placebo was saline (salt water). You did not receive any experimental vaccine product during your participation in the trial."

Or

For Group 1 Recipients:
During the course of the trial, you received the study experimental products called Env-C Plasmid DNA (HIV-1 gp120 (93MW965.26) DNA) and HIV Env gp145 C.6980 protein vaccines and the adjuvant, Rehydragel ®. In this group, Rehydragel was given at the last three time points of immunization. The fact that you received the experimental vaccines does not mean that you are protected from HIV infection, and you should continue to prevent HIV exposure.

Or

For Group 2 Recipients:
During the course of the trial, you received the study experimental products called Env-C Plasmid DNA (HIV-1 gp120 (93MW965.26) DNA) and HIV Env gp145 C.6980 protein vaccines and two adjuvants called ALF43 and Rehydragel ®. Both adjuvants were given at the last three time points of immunization. The fact that you received the experimental vaccines does not mean that you are protected from HIV infection, and you should continue to prevent HIV exposure.

Or

For Group 3 Recipients:
During the course of the trial, you received the study experimental products called Env-C Plasmid DNA (HIV-1 gp120 (93MW965.26) DNA) and HIV Env gp145 C.6980 protein vaccines and two adjuvants called dmLT and Rehydragel ®. The dmLT was given at the first three time points of immunization and Rehydragel ®. was given at the last three time points of the immunization. The fact that you received the experimental vaccines does not mean that you are protected from HIV infection, and you should continue to prevent HIV exposure.

Or

For Group 4 Recipients:
During the course of the trial, you received the study experimental products called Env-C Plasmid DNA (HIV-1 gp120 (93MW965.26) DNA) and HIV Env gp145 C.6980 protein vaccines and three adjuvants called dmLT, ALF43 and Rehydragel ®. The dmLT was given at the first three time points of immunization and ALF43 and Rehydragel ® were given at the last three time points of immunization. The fact that you received the experimental vaccines does not mean that you are protected from HIV infection, and you should continue to prevent HIV exposure.

Or

For Group 5 Recipients:
During the course of the trial, you received the study experimental products called Env-C Plasmid DNA (HIV-1 gp120 (93MW965.26) DNA) and HIV Env gp145 C.6980 protein vaccines and two adjuvants called Rehydragel ® and ALF43. ALF43 was given at the first three time points of immunization and Rehydragel ® was given at the last three time points of immunization. The fact that you received the experimental vaccines does not mean that you are protected from HIV infection, and you should continue to prevent HIV exposure.

Or

For Group 6 Recipients:
During the course of the trial, you received the study experimental products called Env-C Plasmid DNA (HIV-1 gp120 (93MW965.26) DNA) and HIV Env gp145 C.6980 protein vaccines and two adjuvants called Rehydragel ®.and ALF43. ALF43 was given at all six time points of immunization and Rehydragel ® was given at the last three time points. The fact that you received the experimental vaccines does not mean that you are protected from HIV infection, and you should continue to prevent HIV exposure.

Or

For Group 7 Recipients:
During the course of the trial, you received the study experimental products called Env-C Plasmid DNA (HIV-1 gp120 (93MW965.26) DNA) and HIV Env gp145 C.6980 protein vaccines, both vaccines were given at all six time points of immunization, and the two adjuvants called Rehydragel ®.and ALF43. ALF43 was given at all six time points of immunization and Rehydragel ® was given at the last three time points. The fact that you received the experimental vaccines does not mean that you are protected from HIV infection, and you should continue to prevent HIV exposure.

Please contact the clinic and/or Principal Investigator with any questions or concerns you may have.

Yours sincerely,

_____

Dr. Josphat K. Kosgei, MBChB, MSc, DLSHTM, Principal Investigator

If you require any further clarification, please contact:
Dr. Josphat K. Kosgei, MBChB, MSc, DLSHTM
Kenya Medical Research Institute/Walter Reed Project HIV Program
P.O. Box 1357-20200, Hospital Road, Kericho, Kenya Tel: +254 5220 30686/ +254 5220 30388

Additional information about the trial is available at http://www.ClinicalTrials.gov website

APPENDIX M. ROLES AND RESPONSIBILITIES

Roles and Responsibilities

Protocol Chair:
Responsible for study design and serving as a liaison between the site, the vaccine developers including the Sponsor, and contributing support to overall project management and the analysis and reporting of study data. The chair will support the site PI with clinical trial guidance, protocol preparation, protocol review and response to regulatory reviews. The Chairs do not have contact with study subjects.

Protocol Co-Chair:
Responsible for study design and serving as a liaison between the site and the vaccine developers including the Sponsor and contributing support to overall project management and the analysis and reporting of study data. The Co-Chair will provide support with clinical trial guidance, protocol preparation, protocol review and response to regulatory reviews. The Chairs do not have contact with study subjects.

Principal Investigator:
Responsible for all aspects of the study at the site including performing briefing, consenting, and all other participant interactions, while maintaining the blind.. The principal investigator is the primary person responsible for individual participant safety.

Associate Investigators at the Site:
Assist the Protocol Chairs and Principal Investigators in performing briefing, consenting, and all other participant interactions under their supervision, while maintaining the blind. Site associate investigators can act in place of the PI, if necessary.

Associate Investigators at MHRP:
Assist the Protocol Chairs and will not have contact with study subjects or identifiers.

Laboratory Investigators:
Responsible for laboratory analysis. Lab investigators will not have contact with study subjects or identifiers.

Protocol Statistician:
Responsible for statistical analysis for this study. The protocol statistician will not have contact with study subjects.

Overall Study Pharmacist:
The qualified pharmacist with responsibility for overseeing the import, distribution, inventory, and accountability of vaccine, communication with sponsor/MHRP regarding pharmacy related issues, training and consultation with the study site pharmacy personnel.

Protocol Data Management:
Responsible for data management for this study. The data management team will not have contact with study subjects.

DAIDS Medical Officer:
Responsible for protocol development and liaison for the submission to the DAIDS Clinical Sciences Review Committee review The DAIDS medical officer will not have contact with study subjects or identifiers.

DoD Research Monitor:

The DoD Research Monitor may perform oversight functions (e.g. observe recruitment, enrollment procedures, and the consent process for participants; oversee study interventions and interactions; review monitoring plans and UPIRTSO reports; oversee data collection, and analysis) and report their observations and findings to the IRB. The DoD research monitor may discuss the research protocol with the investigators, interview participants, and consult with others outside of the study about the research. The research monitor shall have authority to stop a research protocol in progress, remove individual participants from a research protocol, and take whatever steps are necessary to protect the safety and well-being of participants until the IRBs can assess the monitor's report. Research Monitors shall have the responsibility to promptly report their observations and findings to the IRB. The DoD Research Monitor is required to review all unanticipated problems involving risks to subjects or others, serious adverse event (SAE) reports, and all participant deaths. The DoD Research Monitor at a minimum must comment on the outcomes of the event or problem and in case of a serious adverse event or death, comments on the relationship to participation in the study. They must also indicate whether he/she concurs with the details of the report provided by the PI.

APPENDIX N. PARTICIPATING LABORATORIES

Participating Labs

MHRP Laboratories
508 Robert Grant Ave.
Silver Spring, MD
20910 USA

MHRP/SPL Repository
13 Taft Court
Rockville, MD.
20850 USA

AFRIMS Laboratory
Department of Retrovirology
315/6 Rajvithi Rd.
Bangkok 10400, Thailand

Local Laboratory (Kenya):
Kenya Medical Research Institute
U.S. Army Medical Research Directorate-Africa
Clinical Research Centre
PO Box 1357, Off Hospital Rd.
Kericho, Kenya 20200

Back-up/Fee for service laboratories (Kenya):
The Kenya Medical Research Institute/Walter Reed Project
Clinical Research Center Laboratory
Kombewa, Kisumu, Kenya

Walter Reed Project/Kenya Medical Research Institute
Along Kakamega Road PO Box 54 – 40100
Kisumu, Kenya

Kenya Medical Research Institute/Center for Disease Control (KEMRI/CDC)
New Nyanza Provincial General Hospital Grounds,
Off Kisumu-Kakamega Road
Kisumu, 40100 Kenya

Kenya Medical Research Institute/Walter Reed Project
Clinical Research Centre Laboratory
P.O. Box 1357 KERICHO
Kericho, Kenya

AMPATH Reference Laboratory
1st floor, West Wing
Nandi Road, Moi Teaching and Referral Hospital Grounds,
P.O Box 4606-30100, Eldoret, Kenya

Aga Khan Health Services, Kenya
Kericho Medical Centre, Ushma Plaza
On Hospital/Temple Road,
P.O. Box 530, Kisumu 40100,
Tel: 052-2020623
Email: ksm.admin@akhskenya.org

Lancet Kericho Lab
Sinendet Towers, 3rd Floor,
Tel: 0703061176/0724658439,
Email: Kericho@lancet.co.ke
Kisumu-Kericho Road

PathCare Kenya Ltd
Regal Plaza, Limuru Road,
P.O. Box 1256-00606,
Nairobi, Kenya.
Tel: 254 20 3753416-9/020-2430854/2430753
Fax: 254 20 3753420

Moi Teaching and Referral Histopathology Laboratory
Moi Teaching & Referral Hospital
P.O. Box 3-30100, Eldoret, Kenya.
Nandi Road,Uasin Gishu County

Chulalongkorn University
1873 Rama 4 Road, Pathumwan
Bangkok, 10330, Thailand

APPENDIX O. LIST OF PIMMCS

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 spondyloarthritis
- 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
- Morphoea
- 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 P. EXTERNAL COLLABORATORS

External Collaborators

Collaborator	Role	Access
Shan Lu, MD, PhD University of Massachusetts Medical School Professor of Medicine 55 Lake Ave. North, Worcester, MA 01655 Phone: 508-856-6791 Email: shan.lu@umassmed.edu	• Performs potency assay for gp120 DNA. • Provides the gp120 DNA plasmid used in this trial • Subject matter expert on gp120 DNA • May assist with writing any publication	No contact with study subjects, samples, or access to identifiable data.

APPENDIX Q. TELEPHONE VISIT SCRIPT

Telephone Visit Script

Site staff may use the telephone script as guidance when carrying out a phone call visit.

1) If you reach the participant:

   a) Introduce yourself.

   b) Ask if this is a good time for the participant to talk. If so, proceed. If not, try to ascertain a better time to call the participant back.

   c) State why you are calling with the following: "I am calling to see how you are doing since you received the vaccination"

   d) If the participant is silent or says something unrevealing such as "Everything is fine, ask the following: "Have you had any complaints?" OR "Are you able to carry out your daily activities without a problem?"

   e) If the participant identifies a symptom, try to take further history and, based on your judgement, provide remedy, or invite the participant to the clinic for further assessments. Participants with symptoms assessed to be grade 3 and above should be invited for further assessment at the clinic.

   f) If the participant identifies no symptoms; finish the call with a brief summary, such as "Thank you for your time and I would like to request that you continue filling the diary card and remember to come for your next appointment on the date that you were provided."

   g) If the participant had a sign or symptom, and you want to evaluate the participant at the clinic, remind he/she to come to the clinic at the earliest opportunity.

2) If you do not reach the participant and reach someone else or if connected to voice mail;

   a) Introduce yourself.

   b) If you reach someone else, ask if that person knows the participant and request that the participant calls back or ask the best time to call back to talk to the participant.

   c) In case of voicemail, leave a message for the participant to call back.

   d) Once the participant is reached, go through the procedure 1 and 2.

3) Document the participant's responses and your recommendations, if any, and attempts to contact the participant including time and date.

APPENDIX R. BRIEFING SLIDES

RV460 Protocol Briefing

Conducted by:

- Kenya Medical Research Institute/Walter Reed Project (KEMRI/WRP)
- U.S. Military HIV Research Program (MHRP)

Sponsored by:

- National Institutes of Health:

National Institute of Allergy and Infectious Diseases (NIAID) Division of AIDS (DAIDS)

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Study Title: A Randomized, Double-Blind Phase 1 Trial to Evaluate the Safety and Immunogenicity of Priming with Env-C Plasmid DNA Vaccine Alone, with Different Adjuvants, or with an Adjuvanted HIV Env gp145 C.6980 Protein Vaccine and Boosting with the Adjuvanted HIV Env gp145 C.6980 Protein Vaccine with or without Env-C Plasmid DNA Vaccine in Healthy HIV Uninfected Adults in Kenya

Study Location: KEMRI Clinical Research Centre

Principal Investigator: Dr. Josphat Kosgei

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Purpose of this Briefing

This Presentation will tell you details about the study so that you can decide if participation is right for you.

The next slide lists key information and the slides following will give you more details.

We invite you to ask questions at any time.

Detailed information is included in Informed Consent Forms

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Voluntary Consent. You are being asked to volunteer for a research study. It is up to you whether you choose to participate or not. There are no penalties and you will not lose anything if you decide not to join or, if after you join, you decide to quit.

Purpose. We are doing this research to test a vaccine for HIV and vaccine additives called adjuvants that can help the vaccine be more effective.

Key Information

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Duration. Your part of the study will last 105 weeks (26 months). You will be asked to complete at least 26 clinic visits**.** Additionally, you will be contacted by phone, once per week for three weeks to discuss any changes you may have experienced after the first 105 weeks of being in the study.

Procedures and Activities. We will ask you to receive 6 doses of the experimental vaccines and monitor your blood and symptoms after each vaccine.

More Key Information

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Risks. Most studies have some possible harms that could happen to you if you join. In this study, we expect you to have some mild or moderate symptoms like what happens after you receive some approved vaccines. Some examples of mild common and expected reactions are: fever, body aches, feeling tired and pain at the injection site. It is very rare, but possible, to have a serious and life-threatening allergic reaction right after a vaccine is given. Additionally, there is a chance that you could develop a serious illness such as an autoimmune disease, but this is also very rare.

More Key Information

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Benefits. You will not directly benefit from participating in this study. The results of this study will be shared with researchers from around the world and could play a role in future HIV vaccine development which may be beneficial in the future for others.

Alternatives. Participation is voluntary, and the only alternative is to not participate.

More Key Information

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Intro

Vaccines are a safe and effective way to prevent diseases, but we do not have a vaccine for every disease. HIV is one of the diseases that we still need a vaccine for.

We are doing this research to test a vaccine for HIV and vaccine additives, called adjuvants, that can help the vaccine be more effective.

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Human Immuno-deficiency Virus (HIV)

HIV is passed from person to person through:

- **Sex**
- **Blood**
- **From mother to child**

There is currently no vaccine or cure for HIV.

However, treatment options are available.

Vaccine studies are on-going worldwide.

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Why is this study being done?

Determine	Determine the safety of the vaccines and any side effects they may cause.
Learn	Learn how they act on the body, if the body develops an immune response and how long the effects of the vaccines last.
Compare	Compare effects of each combination of vaccines to each other and to placebo.

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A vaccine is a substance that teaches the body to defend itself against an infection (or germ).

This defensive response is called the immune response, and it is the body's way to fight infections.

Vaccines teach your immune system to recognize certain germs and defend you if you come into contact with that germ.

About Vaccines

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The vaccines in this study:

- **are experimental**
- **do not contain HIV**
- **cannot give you HIV**
- **may not protect you from HIV**

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What vaccines are being tested?

Env-C Plasmid DNA

HIV Env gp145 C.6980 protein

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Adjuvants

This study also tests 3 different adjuvants that are mixed with the vaccines

- An adjuvant is a substance added to vaccines that can help to make the vaccine more effective by improving the immune response or causing the immune response to last longer than without the adjuvant.

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Adjuvants can make vaccines more effective

These adjuvants will be mixed with the vaccines in different combinations:

- ALF43
- Rehydragel®
- dmLT

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Eligibility

Some of the reasons you might be eligible:

- ❑ Between 18 and 40 years old
- ❑ Healthy
- ❑ Able to provide consent
- ❑ Low risk for HIV infection
- ❑ Agree to use reliable contraception
- ❑ Not pregnant, breast-feeding or planning to become pregnant

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Eligibility

Some of the reasons why you would not be eligible:

- ❑ Unwilling or unable to use effective contraception
- ❑ History of a severe allergic reaction
- ❑ Had 3 or more sexual partners in the last 6 months
- ❑ Unable to attend all visits
- ❑ Suffer from a serious illness
- ❑ Unable to provide written consent

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Study Overview

- 126 healthy men and women, 18-40 years old
- Just over 2 years long
- 26 + clinic visits
- 6 vaccines
- Optional samples

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Study Visits

Screening Visit:	Vaccine Visits:	Follow-up Visits:
• Study Info and Eligibility Check	• 6 Vaccine Visits	• Heath and Research Assessments

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Screening Visit (4-5 hours)

- ❑ Study briefing
- ❑ Informed consents
- ❑ Test of understanding
- ❑ Review medical history
- ❑ Physical exam
- ❑ Blood draw to check your general health, HIV, hepatitis B, hepatitis C, and syphilis
- ❑ Urine pregnancy test for women
- ❑ Urine test for general health
- ❑ Pap smear for women who agree to cervicovaginal secretion collection

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Vaccine Visits (4-6 hours)

- ❑ Physical exam & eligibility review
- ❑ Observation after vaccination
- ❑ Instructions on how to monitor any symptoms
- ❑ Urine pregnancy test for women
- ❑ Blood draw for research tests and HIV
- ❑ There are six (6) visits where you will receive an experimental vaccine. After the first vaccine, vaccines will be given at 1 month, 3 months, 5 months, 8 months and 14 months.

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Follow up Visits (2-3 hours)

- ❑ Brief physical exam
- ❑ Review any symptoms you may have experienced
- ❑ Blood draw for HIV, research tests and to check your general health

We will call you after each vaccination so we need to have a number where we can reach you.

It is very important to tell us how you are doing. *If we have trouble contacting you, we may need to come to your house.*

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Optional Specimens

You can choose to participate in the following additional optional collections:

- ❑ Cervicovaginal secretion collections (women)
- ❑ Semen collections (men)
- ❑ Rectal secretion collections
- ❑ Lymph node biopsies

These collections will be explained to you in detail in a separate informed consent form. You will have the opportunity to ask any questions about these collections. You will not need to say yes to them to enroll in the study and you can change your mind at any time.

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If you are found to be qualified to participate in the study, you will be booked to come for an appointment for enrollment and you will be selected by chance to one of the seven (7) study groups.

The group that you are in is randomly selected and neither you nor your study doctor will know which group you are in.

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Study Groups

Group	Vaccines 1, 2 and 3	Vaccines 4, 5, and 6
1	Env-C Plasmid DNA IM	HIV Env gp145 C.6980/Rehydragel® IM
2	Env-C Plasmid DNA IM	HIV Env gp145 C.6980/ALF43/Rehydragel® IM
3	Env-C Plasmid DNA IM + dmLT TCI	HIV Env gp145 C.6980/Rehydragel® IM
4	Env-C Plasmid DNA IM + dmLT TCI	HIV Env gp145 C.6980/ALF43/Rehydragel® IM
5	Env-C Plasmid DNA/ALF43 IM	HIV Env gp145 C.6980/Rehydragel® IM
6	Env-C Plasmid DNA/ALF43 IM	HIV Env gp145 C.6980/ALF43/Rehydragel® IM
7	Env-C Plasmid DNA/HIV Env gp145 C.6980/ALF43 IM	Env-C Plasmid DNA/HIV Env gp145 C.6980/ALF43/Rehydragel® IM

IM= intramuscular (injected in the muscle), TCI = transcutaneous application (applied to the skin)

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Duration

You will be asked to complete at least **26 clinic visits** over **2 years and 2 months**

In addition, you will be asked to be available for **phone contact once per week for three weeks.**

Are you available?

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Risks:

Blood draws

There is a blood draw at each visit.

Blood draws can, at times, cause bruising, pain, or fainting. They rarely can cause an infection.

The blood amounts are different at each visit and range from 1 TBSP (tablespoon) to 12 TBSP.

Remember to drink plenty of water before clinic visits

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Risks:

Experimental Vaccines

You are one of the first humans to receive this combination of vaccines and adjuvants and so not all of the side effects are known.

Common expected symptoms of all vaccines are: fever, chills, rashes, aches and pains, nausea, headache, dizziness, and feeling tired.

Frequently, vaccines cause pain and swelling where you get the injection (your arm).

Most people are still able to do their daily activities after getting a vaccine.

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Risks:

More Info About Experimental Vaccines

Rarely, a vaccine causes a serious allergic reaction soon after you get the injection. This reaction can be a rash, hives, or difficulty breathing. ***Tell the study staff if you have ever had a bad reaction to an injection or a vaccine***. We will monitor you in the clinic for at least 30 minutes after each vaccine and we have emergency staff, equipment and medications here, if needed.

Some participants will get a Transcutaneous Immunization skin patch (TCI) placed over the injection site like a bandage.

The TCI bandage may cause itching, mild redness or rash, and changes in pigmentation at the site of patch application. If change in pigmentation occurs, it could be permanent.

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Risks:

Genetic Testing and Your Privacy

Researchers may use your samples for genetic testing. For example, researchers may do "genetic variations" research which looks at genes that affect how you fight infections to try to learn why some people get a disease while others do not. We will not notify you of the results of any genetic test.

Your privacy is very important. To protect your identity, all study samples and data including genetic testing results will not be linked to your name and will not be enough to independently identify you as an individual.

Researchers keep your study records in a secure place. We do not share any information that could identify you. **Complete confidentiality cannot be promised**, but every effort will be made to keep the records as confidential as possible.

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Risks:

VISP

The HIV vaccines in this study do NOT contain the entire HIV virus itself and cannot cause HIV infection or Acquired Immune Deficiency Syndrome (AIDS).

But the experimental vaccines may cause "vaccine-induced sero-positivity" or "VISP" so *you may test positive for HIV when you really do not have HIV.*

This clinic's HIV test can tell the difference between real HIV infection and VISP. For this reason, you should avoid all HIV testing not done at this clinic. You will be tested for HIV regularly during this study. After this, if you would like HIV testing, we encourage that you return to this clinic for testing.

Also, if you become pregnant and have the baby while you have VISP, your baby may have VISP too. This clinic's HIV test can tell the difference between real HIV infection and VISP in your baby as well.

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Risks:

More info about VISP

If you have VISP, you cannot donate blood. You must not donate blood during your participation in this research study for other reasons as well. You may also be excluded from participation in other research studies. At your request, you will be provided with a letter explaining your participation in the study and the possibility of testing positive for HIV antibody.

We do not know how long you will have VISP.

If you have problems because of VISP, tell the study staff and they will assist you.

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Risks:

Possible Social Issues

If you join this study:

- Your family or partner might worry or treat you badly
- You could lose your job
- You may feel embarrassed
- You may feel anxious when waiting for your HIV test results
- Your samples and information (data) may be accidentally released

Researchers and study staff try hard to protect you and have a duty to maintain your privacy.

But there is a risk that others, including your partner, may find out that you are participating in this study. They may misunderstand and think you have HIV.

If you have any of these problems, please talk to the study staff, so that they can try to help you.

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Risks:

Pregnancy and Breast-feeding

You should not become pregnant during this study. Researchers do not know if the vaccines may harm unborn babies.

Use effective contraception beginning 45 days prior to the first vaccination and continuing through 90 days after the last vaccine/placebo visit.

You must not breastfeed while in this study. Researchers do not know if the vaccines may pass through breast-milk and may harm your baby.

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HIV Infection Risk

These experimental HIV vaccines have not shown that they work to protect against HIV infection.

None of the experimental HIV vaccine combinations in this study have been shown to protect against HIV infection.

There is also a possibility that you may be assigned to receive the inactive placebo.

You MUST continue to use all precautions to protect yourself from HIV.

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Your Choices

Participation in this study is 100% voluntary

You may choose opt in or out of:

- ❑ Genetic testing
- ❑ Sample storage for future research
- ❑ Optional sample collections

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Your Rights

You can change your mind at any time. We do ask that you tell the study staff if you are thinking about leaving or have decided to leave this study. We will be very concerned if you "disappear", but we will not be angry with you.

Study staff may want you to do some follow up visits and testing before you leave this study.

Any care that you get at this clinic outside of this study will not change.

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Reimbursement

You will need to spend some time and maybe some money to participate in this study and researchers will reimburse you for some of these expenses.

Reimbursement amounts:

Study visit:	1000 Ksh
Unscheduled visit:	Up to 500 Ksh
*Secretion collection:	1000 Ksh
*Lymph node biopsy:	5000 Ksh
Transportation:	Up to 3000 Ksh

* optional

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Thank for your time, interest and attention!

Ask questions at any time, now or in private later.

Please take all the time you need to decide to participate or not.

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ATTACHMENT 1: COVID INFORMATION SHEET

Kenya Medical Research Institute (KEMRI)
United States Army Medical Research Directorate-Africa (USAMRD-A)
In Collaboration with
US Military HIV Research Program (MHRP) and the
US Department of Defense (DoD)

WRAIR #2672/RV460 Information Sheet for COVID-19

In light of the circulating severe acute respiratory syndrome Coronavirus-2 (SARS-CoV-2) and the associated coronavirus disease (COVID-19) pandemic, we want you to know that coming to the clinic and being around other people can increase your exposure to SARS-CoV-2.

In order to reduce your risk of contracting the virus, KEMRI/WRP CRC is practicing COVID-19 control measures like social distancing, handwashing, wearing masks and taking temperatures of all persons entering the institution at the entrance.

In the event that there is an increase in infections that would prompt the need to modify study visits to reduce the risk of transmission, the site may institute the following modifications.

- Visit windows may be extended in order to conduct study visits early or late within the window
- Ability to conduct study visits – in full or in part – off-site if permitted by applicable government, health authority, and institutional policies
- Certain study procedures may be prioritized during a particular visit so that the participant does not have to be present for the full duration of the scheduled study visit.

Other modifications may be undertaken that are not listed above. However, any proposed modifications put in place are to safeguard the health and well-being of participants. The modifications provide flexibility for conducting study visits and procedures when needed to monitor your 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 and the local IRBs/ECs will be notified when such a determination is made.

If you should have any questions or concerns about coming to the clinic and/or continuing with the study, please contact

Dr. Josphat Kosgei, MBCHB, MSc	Office: (0)5220 36302	Mobile: (0)729 110 122	Staff: (0) 5220) 36000/100

If you consent, please read and then sign below.

This form will be attached to the consent form you initially signed. If you choose to continue participating in the RV 460 study, please read and then sign below. If you still have questions, please ask the study doctor or one of the study staff, before signing this form. You will receive a copy of this signed Participant Information Form.

Agreement to continue taking part in the study

- I have read this information.
- It has been written in a language that I can read and understand.
- The information regarding COVID-19 risk for this study has been explained to me.
- All my questions about how the site is managing the risks associated with COVID-19 during study participation have been answered to my satisfaction.

_____
Printed Name of Participant in full

_____
Signature of Participant Date (dd-MON-yyyy)

_____
Printed Name of Person Obtaining Consent

_____ _____
Signature of Person Obtaining Consent Date (dd-MON-yyyy)