Randomized, Double Blind Evaluation of Different One-Year Boosts after Sanofi Pasteur Live Recombinant ALVAC-HIV (vCP1521) and Global Solutions for Infectious Diseases (GSID) gp120 B/E (AIDSVAX® B/E) Prime-Boost Regimen in HIV-uninfected Thai Adults

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Department of the Army

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Investigator's Agreement

Randomized, Double Blind Evaluation of Different One-Year Boosts after Sanofi Pasteur Live Recombinant ALVAC-HIV (vCP1521) and Global Solutions for Infectious Diseases (GSID) gp120 B/E (AIDSVAX® B/E) Prime-Boost Regimen in HIV-uninfected Thai Adults

"I have read this protocol and agree to conduct the study as outlined herein in accordance with International Conference on Harmonization Good Clinical Practice Guideline and FDA, DoD, and United States Army Regulations."

_____ _____

Punnee Pitisuttithum, MD, DTM&H
Principal Investigator
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Date

Procedures in Case of Emergency

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2. Synopsis

Name of Sponsor:
Office of the Surgeon General, Department of the Army

Name of Investigational Products:
ALVAC-HIV (vCP1521) and AIDSVAX® B/E

Name of Active Ingredients:
Recombinant canarypox vector vaccine expressing gag, protease HIV-1 subtype B (LAI strain) and gp120 subtype E with transmembrane anchoring portion of gp41 (LAI strain)

Bivalent HIV gp120 envelope glycoprotein vaccine containing a subtype B envelope from the HIV-1 strain MN and a subtype E envelope from the HIV-1 strain A244 with a 27aa gD (HSV-1) tag on the N-terminal part of this protein.

Title of Study: Randomized, Double Blind Evaluation of Different One-Year Boosts after Sanofi Pasteur Live Recombinant ALVAC-HIV (vCP1521) and Global Solutions for Infectious Diseases (GSID) gp120 B/E (AIDSVAX® B/E) Prime-Boost Regimen in HIV-uninfected Thai Adults.

Study Center(s): Clinical Sites: Vaccine Trial Centre, Faculty of Tropical Medicine, Mahidol University Bangkok, Royal Thai Army Clinical Research Center AFRIMS, Bangkok, RIHES Chiang Mai.

Sites for optional invasive procedures:

King Chulalongkorn Memorial Hospital: leukapheresis, sigmoid biopsy and bone marrow aspiration

Thai Red Cross AIDS Research Centre: cervical biopsy

Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University: bone marrow aspiration

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Senior Site Investigators: LTG. Sorachai Nitayaphan, MD, PhD; Suwat Chariyalertsak, MD, Dr.PH; Nittaya Phanuphak, MD, PhD

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Investigator for Data Management: Jaranit Kaewkangwal, PhD

Research Laboratory Investigators: Nicos Karasavva, PhD, Alexandra Schuetz, PhD, Silvia Ratto-Kim, PhD

Study Period (years):	**Phase of development:** 2
Estimated date first subject enrolled: October 2013 Estimated date last subject completed: April 2017 Estimated duration of the trial: 10 years (2-3 years screening and clinical activities: 3-10 years laboratory assays and data analysis)	

Objectives:

Primary:

- Characterize and compare cellular and humoral immune responses in the systemic compartment and humoral responses in the mucosal compartments in accordance with the RV144 primary vaccination series and following booster vaccination with either the ALVAC-HIV/AIDSVAX®B/E combination or ALVAC-HIV alone or AIDSVAX®B/E alone or placebo.
- Assess the safety and tolerability of the vaccination regimens.

Secondary:

- Evaluate and compare the lymphoproliferation responses of participant PBMCs to HIV antigens and mitogens between vaccination regimens
- Characterize and compare innate immune responses (characterization of NK cells and APOBEC anti-retroviral factor) between the vaccination regimens

Exploratory:

- Characterize and compare cellular immune responses in the mucosal compartment in accordance with the RV144 primary vaccination series and following booster vaccination with either the ALVAC-HIV/AIDSVAX®B/E combination or ALVAC-HIV alone or AIDSVAX®B/E alone or placebo.
- Characterize B cell functional specificities in the systemic and bone marrow compartments for each vaccination regimen
- Assess the local B-cell responses in ex-vivo cervical mucosal explants for each vaccination regimen
- Assess the HIV mobility in semen and cervico-vaginal secretions for each vaccination regimen
- Assess the innate and early adaptive responses to vaccination by gene activation for Groups I, II, and III
- Evaluate systemic and mucosal activation of T cell targets for HIV infection for each vaccination regimen

Methodology:

This study is exploratory in nature.

The primary purpose of the study is to better define the relative contributions of AIDSVAX®B/E alone or ALVAC-HIV/AIDSVAX®B/E combination to the observed immune profile in the weeks and months after receiving the original prime and boost vaccine regimen in RV144, and their booster effects in both the systemic and mucosal compartments. In addition, this study will provide more intensive and comprehensive characterization of the innate, cell-mediated and humoral immune responses than possible within RV144. Specifically, in addition to large volume blood collections provided by leukapheresis, mucosal samples will be collected to assess both humoral and cell-mediated immune responses and activation of potential T-cell targets for HIV infection at the mucosal surface. In addition, B cell

functional specificities will be characterized in peripheral blood, bone marrow (provided by bone marrow aspiration) and sigmoid compartments.

Healthy, HIV-uninfected volunteers, at low risk for HIV infection, between 20 and 40 years of age, weighing over 45 kilograms and available for follow-up in the next 24 months will be enrolled. A total of 360 volunteers will be enrolled: 27 vaccines recipients and 3 placebo recipients in Group I, 100 vaccine recipients and 10 placebo participants each from Groups II, III, IV. Group IV will be divided into Groups IVa and IVb, with 50 vaccine recipients and 5 placebo participants in each group. ALVAC-HIV or placebo will be administered to all groups at weeks 0 and 4 followed by ALVAC-HIV + AIDSVAX®B/E or placebos at weeks 12 and 24 with an additional boost at 48 weeks for the two Groups II and III regimens. ALVAC-HIV + AIDSVAX®B/E or placebos will be administered at week 60 in Group IVa and at week 72 in Group IVb. Volunteers will be followed up until week 96 after enrollment. Contemporary assays will be performed on samples obtained from study participants at weeks 4, 12, 14, 24, 26, 36, 48, 50, 60, 62, 72, 74 and 96. Measurement of CD8+ T-cell effector function will be evaluated by using surrogate markers of CD8+ effector function including IFN-γ ELISPOT and intra-cellular cytokine staining (ICS). Innate immunity will be assessed by measuring antibody-dependent cellular toxicity (ADCC) and antibody-dependent cellular viral inhibition (ADCVI) assays, flow cytometric panels to phenotype NK cells and a cytokine array to characterize the type of cytokines elicited by the vaccine regimens, assessment of APOBEC expression, and gene activation by DNA microarray studied at Day 3 and 2 weeks post fourth and boost vaccinations and 6 months post boost (will not be performed in Group IV). All assays will be conducted on batched cryopreserved samples. The CD4+ function will be assessed by the lymphoproliferation responses as measured by the 3H-thymidine incorporation assay and/or the functional CFSE assay. HIV-specific humoral responses will be assessed by HIV-specific binding antibody assays and neutralizing antibody assays. Leukapheresis will be performed at weeks 26 (for Group I), or 50 (for Groups II, III) in a subset of willing volunteers for in-depth investigations of the T-cell responses and establishment of T-helper cell lines. Humoral mucosal immune responses in the rectal, semen and cervico-vaginal compartments will be assessed using non-invasive sampling methods (sponge or lavage, cup, and masturbation) at baseline and weeks 14, 26, 48 (except Groups IVa and IVb), 50, 60 (Group IVa only), 62 (Group IVa only), 72, 74 (Group IVb only), and 96. Invasive sigmoid and cervical biopsies will be performed in a subset of willing volunteers to assess cellular mucosal immune responses at weeks 26 (Group I), week 50 (Groups II and III), week 62 (Group IVa), and week 74 (Group IVb). Bone marrow aspirations will be performed at week 50 on a subset of willing volunteers from Group II only (the group of participants from whom the most robust immunologic response is expected). The mucosal samples and bone marrow aspirations will be subject to the volunteer's acceptance and tolerability and laboratory constraints.

Biological specimens will be archived for potential immunological assessments as techniques develop.

Estimated Number of Subjects Screened: Approximately 1080 (Screening-to enrollment ratio is 3:1)

Estimated Number of Subjects Enrolled: 360 (60 enrolled in Chiang Mai and 300 enrolled in Bangkok sites)

Criteria for Inclusion/Exclusion:

Inclusion Criteria

- Healthy, HIV-uninfected male and female volunteers between age 20 and 40, weighing over 45 kilograms, and available for a period of 24 months and having a Thai identity card.
- Must be at low risk for HIV infection per investigator assessment
- Must be able to understand and complete the informed consent process.
- Must be capable of reading Thai
- Must successfully complete a Test of Understanding prior to enrollment as described in section 8.2
- Must be in good general health without clinically significant medical history.
- HIV-uninfected per diagnostic algorithm within 45 days of enrollment.
- Laboratory screening analysis:
    - Hemoglobin: Women ≥12.0 g/dL, Men ≥12.5 g/dL
    - White cell count: 4,000 to 11,000 cells/mm3
    - Platelets: 150,000 to 450,000/mm3
    - ALT and AST ≤1.25 institutional upper limit of reference range
    - Creatinine: ≤1.25 institutional upper limit of reference range
- Urinalysis (dipstick) for blood and protein no greater than 1+ and negative glucose
- Female-Specific Criteria:
    - Negative pregnancy test for women at screening and prior to each vaccination (same day) and prior to any of the invasive procedures.
    - Be using an adequate birth control method for 45 days prior to the first vaccine/placebo vaccination and for at least 3 months 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, diaphragms, intrauterine device (IUD), or abstinence.

Exclusion Criteria

- Asplenia: any condition resulting in the absence of a functional spleen
- Bleeding disorder diagnosed by a medical doctor (e.g., factor deficiency, coagulopathy, or platelet disorder requiring special precautions)
- Therapeutic anticoagulation resulting in an abnormal prothrombin (PT) / international normalized ration (INR) of partial thromboplastin time (PTT)
- Women breast-feeding or pregnant (positive pregnancy test) or planning to become pregnant during the window between study enrollment and 3 months after the last vaccination visit.
- 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 eggs, egg products, streptomycin, or neomycin.
- Subject has received any of the following substances:
    - Chronic use of therapies that may modify immune response, such as IV immune globulin and systemic corticosteroids (in doses of $\geq$ 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 (duration of 10 days or less, or a single injection) of

corticosteroid for a non-chronic condition (based on investigator clinical judgment) at least 2 weeks prior to enrollment in this study.
  - Blood products within 120 days prior to HIV screening.
  - Immunoglobulins within 30 days prior to HIV screening.
  - Any licensed vaccine within 14 days prior to initial study vaccine administration in the present study.
  - Receipt of any investigational HIV vaccine.
  - Investigational research agents or vaccine within 30 days prior to enrollment in the present study.
  - Anti-tuberculosis prophylaxis or therapy during the past 90 days prior to enrollment.
- Active sexually transmitted infection confirmed by clinical exam and diagnostic test.
- Any medical, psychiatric, social condition, occupational reason, or other responsibility that, in the judgment of the investigator, is a contradiction to protocol compliance or impairs a subject's ability to give informed consent.
- Psychiatric condition that precludes compliance with the protocol; past or present psychoses; past or present bipolar disorder; disorder requiring lithium; or within 5 years prior to enrollment, a history of suicide ideation or attempt.
- Study site employees who are involved in the protocol and/or may have direct access to study related area.

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

Investigational Product, Dosage and Mode of Administration:

ALVAC-HIV (vCP1521), a Sanofi Pasteur product (manufactured by IDT Biologika, Germany), $>10^6$ CCID50 per vial, is lyophilized and reconstituted in 0.4% NaCl, given as a 1 mL intramuscular injection into the left deltoid muscle.

ALVAC Placebo, a Sanofi Pasteur product (manufactured by IDT Biologika, Germany), is supplied as a sterile, lyophilized product that consists of a mixture of virus stabilizer, and freeze-drying medium. The diluent supplied for reconstitution of ALVAC-Placebo consists of sterile 0.4% NaCl. ALVAC Placebo is given as a 1 mL intramuscular injection into the left deltoid muscle.

AIDSVAX® B/E manufactured by Genentech Inc. for GSID, in the United States, is a bivalent HIV gp120 glycoprotein vaccine with subtype B (MN) and subtype E (A244) co-formulated and administered in aluminum hydroxide gel at a combined concentration of 600 µg/mL (300 µg of each antigen), given as a 1 mL intramuscular injection into the right deltoid muscle.

AIDSVAX® B/E Placebo manufactured by Hollister-Stier Laboratories LLC for GSID, in the United States, is a sterile suspension of 600 µg of aluminum hydroxide adjuvant, given as a 1 mL intramuscular injection into the right deltoid muscle.

Duration of Treatment: 24 , 48 , 60 and 72 weeks. Four administrations at 0, 4, 12 and 24 weeks (Group I), five administrations that include a booster vaccination at week 48 (Groups II, III), at week 60 (Group IVa) and at week 72 (Group IVb).

Reference Therapy, Dosage, Schedule, and Mode of Administration:

Vaccination Schedule

GROUP	NUMBER OF SUBJECTS VACCINE / PLACEBO	Week of Study				
		0	**4**	**12**	**24**	**48**
		ALVAC-HIV or placebo, 1mL, IM, left deltoid muscle **AIDSVAX® B/E or placebo**, 1 mL, IM, right deltoid muscle				
I	**27**	**ALVAC-HIV**	**ALVAC-HIV**	**ALVAC-HIV + AIDSVAX® B/E**	**ALVAC-HIV + AIDSVAX® B/E**	
	3	**ALVAC-HIV placebo**	**ALVAC-HIV placebo**	**ALVAC-HIV placebo + AIDSVAX® B/E placebo**	**ALVAC-HIV placebo + AIDSVAX® B/E placebo**	
II	**100**	**ALVAC-HIV**	**ALVAC-HIV**	**ALVAC-HIV + AIDSVAX® B/E**	**ALVAC-HIV + AIDSVAX® B/E**	**ALVAC-HIV + AIDSVAX® B/E**
	10	**ALVAC-HIV placebo**	**ALVAC-HIV placebo**	**ALVAC-HIV placebo +**	**ALVAC-HIV placebo +**	**ALVAC-HIV placebo +**

				AIDSVAX® B/E placebo	**AIDSVAX® B/E placebo**	**AIDSVAX® B/E placebo**
III	**100**	**ALVAC-HIV**	**ALVAC-HIV**	**ALVAC-HIV + AIDSVAX® B/E**	**ALVAC-HIV + AIDSVAX® B/E**	**AIDSVAX® B/E**
	10	**ALVAC-HIV placebo**	**ALVAC-HIV placebo**	**ALVAC-HIV placebo + AIDSVAX® B/E placebo**	**ALVAC-HIV placebo + AIDSVAX® B/E placebo**	**AIDSVAX® B/E placebo**
IVa		**0**	**4**	**12**	**24**	**60**
	50	**ALVAC-HIV**	**ALVAC-HIV**	**ALVAC-HIV + AIDSVAX® B/E**	**ALVAC-HIV + AIDSVAX® B/E**	**ALVAC-HI.V + AIDSVAX® B/E**
	5	**ALVAC-HIV placebo**	**ALVAC-HIV placebo**	**ALVAC-HIV placebo + AIDSVAX® B/E placebo**	**ALVAC-HIV placebo + AIDSVAX® B/E placebo**	**ALVAC-HIV placebo + AIDSVAX® B/E placebo**
IVb		**0**	**4**	**12**	**24**	**72**
	50	**ALVAC-HIV**	**ALVAC-HIV**	**ALVAC-HIV + AIDSVAX® B/E**	**ALVAC-HIV + AIDSVAX® B/E**	**ALVAC-HIV + AIDSVAX® B/E**
	5	**ALVAC-HIV placebo**	**ALVAC-HIV placebo**	**ALVAC-HIV placebo + AIDSVAX® B/E placebo**	**ALVAC-HIV placebo + AIDSVAX® B/E placebo**	ALVAC-HIV placebo + **AIDSVAX® B/E placebo**

Targeted Number of Volunteer for Invasive Procedures

Invasive procedures*	Targeted Number		Gender	Weeks
	Vaccination	Placebo		
Sigmoid biopsy	35	6	Male and female	26, 50, 62, 74 Group I: week 26 Groups II, III: week 50 Group IVa: week 62 Group IVb: week 74
Cervical biopsy	35	6	Female	26, 50, 62, 74 Group I: week 26 Groups II, III: week 50 Group IVa: week 62 Group IVb: week 74
Leukapheresis	25	4	Male and female	26, 50 Group I: week 26 Groups II, III: week 50
Bone marrow aspiration	5	5	Male and female	50 Group II only

*Each volunteer can participate in one procedure only

Criteria for evaluation:

Immunogenicity:

Vaccine-induced immune responses will be assessed in study participants at weeks 4, 12, 14, 24, 26, 36, 48, 50, 60, 62, 72, 74, and 96. Measurement of CD8+ T-cell effector function will be evaluated by using surrogate markers of CD8+ effector function including IFN-γ ELISPOT and intra-cellular cytokine staining (ICS). The CD4+ function will be assessed by the lymphoproliferation responses as measured by the 3H-thymidine incorporation assay and/or the functional CFSE assay. Leukapheresis will be performed at weeks 26 (Group I) or 50 (Groups II, III) in subset of willing volunteers for in-depth investigations of the T-cell responses and establishment of T-helper cell lines. Innate immunity will be assessed by measuring antibody-dependent cellular toxicity (ADCC) and antibody-dependent cellular viral inhibition (ADCVI) assays, and by flow cytometric panels to phenotype NK cells and a cytokine array assay to characterize the type of cytokines elicited by the vaccine regimen. Gene expression to vaccine antigens will be studied by DNA microarray technique at Day 3 and 2 weeks post fourth and boost vaccinations and 6 months post boost for Groups I, II, and III only. HIV-specific humoral responses will be assessed

by HIV-specific binding antibody assays and neutralizing antibody assays and B cell ELIspot. Humoral mucosal immune responses at the rectal, cervico-vaginal and semen compartments will be assessed using non-invasive sampling methods (sponge or lavage, cup and masturbation) at weeks 0, and weeks 14, 26, 48 (except Groups IVa and IVb), 50, 60 (Group IVa only), 62 (Group IVa only), 72, 74 (Group IVb only), and 96. Invasive sigmoid and cervical biopsies will be performed at weeks 26 (Group I), week 50 (Groups II and III), week 62 (Group IVa) and week 74 (Group IVb) in a subset of willing volunteers for cell-mediated immunological assessments (2 weeks after final vaccination). Bone marrow aspirations will be performed at week 50 on a subset of willing volunteers from Group II (the group of participants from whom the most robust immunologic response is expected). The mucosal samples and bone marrow aspirations will be subject to the volunteer's acceptance and tolerability and laboratory constraints.

Safety:
Post-vaccination reactions will be assessed including local (at the injection site) reactions such as erythema, induration, pain/tenderness, swelling and limitation of arm movement, and systemic reactions such as fever, tiredness, chills, myalgia, arthralgia, headache, nausea, dizziness, and rash. They will be recorded on diary card during the 3 days following each vaccination.

Serious Adverse Events will be recorded until the end of the trial. All adverse events occurring through visit 11 (Groups I, II and III), visit 12 (Group IVa) and visit 14 (Group IVb) will be elicited and recorded. After these visits, only AE's that are "medically significant" events, defined as requiring multiple visits (two or more) to a physician for the same condition, or that result in hospitalization or an emergency room visit, will be captured.

Statistical consideration: A total of 360 volunteers will be enrolled and randomized in four groups. (27 vaccines recipients and 3 placebo recipients in Group I and 100 vaccines recipients and 10 placebos each from Groups II, III and IV (divided in 50 vaccine and 5 placebo recipients for Groups IVa and IVb, respectively), for a total 327 vaccine recipients and 33 placebo recipients). The study sample size will permit detection of large differences in boost vaccination arm response rates (>20 percentage points) with adequate power (80%) across the expected response range for selected assays (e.g. ICS with p1=0.30, p2=0.60 power=99%, for 0.4 vs. 0.6 power=83%). Losses to follow-up or non-adherence are not expected to occur in more than 5% of the cases resulting in a small, expected power loss (e.g. ICS with p1=0.30, p2=0.60, power=98%, for 0.4 vs. 0.6 power=79%). The study is also designed to detect differences in response rates of greater than 30% (power=81%) between boost arms (n=100) and the non-boost arms (n=27). Sample size was derived using a pooled Z- test and, given the explorative nature of the study, a 2-tailed 5% level test for the pairwise comparisons. In addition differences in mean assay levels of up to .40 standard deviations for the pairwise comparison of the active boost regimens will be detectable (with power=80%). For directional contrasts with the combined placebo recipients, large differences in response rate for several antibody measures are anticipated. If true rates after placebo injection are 5%, then increases to >20% can be detected with 80% power.
Groups IVa and IVb will still be compared as described above at week 50. In addition, the pairwise comparisons of Group IVa and IVb (50 subjects per group) will have at least 80% power for a two-tailed 5% level test to detect an approximate 30% difference in response rates between the arms at week 62, week 74, and week 96 as well approximately 0.6 standard deviation differences between the two groups. Given the exploratory nature of the comparison, no adjustments for loss to follow-up or multiple comparisons are incorporated.

3. Table of Contents, List of Tables, and List of Figures

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4. List of Abbreviations and Definitions of Terms

The following abbreviations and definitions are used in this study protocol.

Table 2: Abbreviations and Definitions

Abbreviation / Acronym	Explanation
ACD	Acid citrate dextrose
ADCC	Antibody-dependent cellular cytotoxicity
ADCVI	Antibody-dependent cellular viral inhibition
AE	Adverse event, adverse experience
AIDS	Acquired Immunodeficiency Syndrome
AFRIMS	Armed Forces Research Institute of Medical Sciences
ALT	Alanine transferase
APOBEC	Apolipoprotein B mRNA editing enzyme
ARCL	AFRIMS Retrovirology Clinical Laboratory
AST	Aspartate transferase
BM	Bone marrow
C	Celsius
CBC	Complete blood count
$CCID_{50}$	Cell culture infectious dose (50%)
CD4+, CD8+	Helper (CD4+) and cytotoxic (CD8+) T cells
CFR	Code of Federal Regulation
CFSE	Carboxyfluoresceindiacetatesuccinimidyl ester lymphoproliferationassay
CHO	Chinese hamster ovary
CI	Confidence Interval
CID	Chimpanzee infectious dose (50%)
CIOMS	Council for International Organizations of Medical Sciences
Cr	Chromium
CRA	Clinical Research Associate
CRC	Clinical Research Coordinator
CRF01_AE	Circulating Recombinant Form of HIV-1 subtypes A and E
CSSD	Clinical Services Support Division
CTL	Cytotoxic T lymphocyte
CVM	Cervico-vaginal mucus
DA	Department of the Army
DAIDS	Division of AIDS, NIAID, NIH
DDC	Department of Disease Control (Thai MOPH)
DNA	Deoxyribonucleic Acid

Abbreviation / Acronym	Explanation
DoD	Department of Defense
DRAC	Division of Regulated Activities and Compliance
eCRF	Electronic Case Report Form
EDTA	Ethylene diamine tetra-acetic acid
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
ELISpot	Enzyme-linked immunospot assay
ERC	Ethical Reviewing Committee
FDA	Food and Drug Administration
FSH	Follicle stimulation hormone
FWA	Federal Wide Assurance
GBS	Guillain-Barré Syndrome
GCP	Good Clinical Practice
GGT	Gamma glutamyl transferase
GMT	Geometric mean titer
GSID	Global Solutions for Infectious Diseases
HCG	Human choriogonadotropin
HEC	Human Experimentation Committee
HIV and HIV-1	Human Immunodeficiency virus, type 1
HIPAA	Health Insurance Portability Accountability Act
HLA	Human leukocyte antigen
HMJF	Henry M. Jackson Foundation for the Advancement of Military Science
HPV	Human Papilloma Virus
HSPB	WRAIR Human Subjects Protection Branch
HSV	Herpes Simplex Virus
HVRC	HIV Vaccine Research Center of Excellence
ICH	International Conference on Harmonization
ICS	Intracellular cytokine staining measurement
IDT	IDT Biologika, Dessau-Rosslau, Germany – Contract Manufacturer for ALVAC-HIV and ALVAC Placebo
IFN-γ	Interferon gamma
IND	Investigational New Drug
IRB	Institutional Review Board
ITT	Intention to treat
IUD	Intrauterine device
IV	Intravenous
IVS	In vitro stimulation

Abbreviation / Acronym	Explanation
LH	Luteinizing hormone
LMRM	Local (Independent) Medical Research Monitor
LPA	Lymphocyte proliferation assay
LSI	Lymphocyte stimulation index
MedDRA	Medical dictionary for regulatory activities
Mg	Milligram
MHC	Major histocompatibility complex
MHRP	U.S. Military HIV Research Program
ml	Milliliter
Mm	Millimeter
mM	Millimole
MOP	Manual of procedure
MOPH	Ministry of Public Health, Royal Thai Government
MRKAd5 HIV-1	Merck recombinant Ad5 vector HIV vaccine
NIAID	National Institute of Allergy and Infectious Disease
NIH	National Institutes of Health
NK	Natural killer cells
OHRP	Office for Human Research Protections, Department of Health and Human Services
ORP, HRPO	Office of Research Protections, Human Research Protection Office
OTSG	Office of The Surgeon General
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PI	Principal investigator
PPA	Per-protocol analysis
PTT	Partial thromboplastin time
RBC	Red blood cell
RIHES	Research Institute for Health Sciences, Chiang Mai University
RNA	Ribonucleic acid
ROC	Regulatory Operations Center, MHRP
RPR	Rapid Plasma Reagin
RSS	Research Support System
RTA	Royal Thai Army
SAE	Serious adverse event
SMM	Study Medical Monitor
SOP	Standard operating procedure
STI	Sexually transmitted infection

Abbreviation / Acronym	Explanation
TAVEG	Thai AIDS Vaccine Evaluation Group
$TCID_{50}$	Tissue culture infectious dose (50%)
TCLA	Tissue culture laboratory adapted
TOU	Test of understanding
UA	Urinalysis
µg	Microgram
UNAIDS	The Joint United Nations Programme on HIV/AIDS
USAMC	US Army Medical Component
USAMMDA	United States Army Medical Materiel Development Activity
USAMRMC	United States Army Medical Research and Materiel Command
VE	Vaccine efficacy
VTC	Vaccine Trials Centre, Mahidol University
WB	Western Blot
WHO	World Health Organization
WRAIR	Walter Reed Army Institute of Research

5. Introduction

Rationale for Study

At the end of 2012, an estimated 35.3 million people [32.2–38.8] were living with HIV worldwide. This reflects the continued large number of new HIV infections and a significant expansion of access to antiretroviral therapy, which has helped reduce AIDS-related deaths, especially in more recent years [1]. Despite promising but still fragile successes in prevention and care and treatment, the development of a safe and efficacious preventive HIV vaccine as part of a comprehensive prevention program remains among the highest global health priorities and the best long-term tool for the control of the HIV-1 [2-4].

In response to the epidemic, the Royal Thai Government developed and implemented a comprehensive plan for prevention and control of HIV; preventive HIV vaccines are an integral component of this plan [5]. Consequently, the National AIDS Commission of Thailand established a Subcommittee for HIV Vaccine Development and Trials with responsibility for coordinating and overseeing efforts in this area. In 1991, the World Health Organization (WHO) selected Thailand as a site for evaluation of candidate HIV vaccines [6]. Since then, WHO/UNAIDS has provided consultation to the Subcommittee for HIV Vaccine Development and Trials of the National Commission for the Prevention and Control of AIDS. Through multiple partnerships and collaborations, Thailand has actively carried out its National Plan and implemented several vaccine clinical trials including the first Phase III trial in the developing world [7]. The combination of planning, collaboration and commitment to HIV vaccine development has put Thailand in a position of international leadership concerning HIV vaccine development. These efforts led to the implementation of a second efficacy trial in Thailand.

A community-based, randomized, multicenter, double blind, placebo-controlled phase III efficacy trial of the prime–boost combination of vaccines containing ALVAC-HIV (vCP1521) and AIDSVAX®B/E was recently conducted [8]. The study was designed to evaluate two co-primary end points: the prevention of HIV-1 infection and the effect of vaccination on the early viral load after infection. The trial was conducted

through facilities of the Thai Ministry of Public Health in Rayong and Chon Buri provinces. From September 2003 through December 2005, a total of 16,402 volunteers were enrolled. Thai men and women who were between the ages of 18 and 30 years and who were not infected with HIV were recruited from the community without regard to HIV risk. Written informed consent was obtained from all volunteers, who were required to pass a written test of understanding. Women were counseled to practice effective contraception until 3 months after the last vaccination; pregnant and breast-feeding women were excluded.

The study vaccines were administered at baseline (day 0), 4 weeks, 12 weeks (range, 10 to 15), and 24 weeks. The ALVAC-HIV (vCP1521) vaccine was administered at each of the four visits. Boosting with AIDSVAX®B/E occurred at weeks 12 and 24. For 3 days after each dose of vaccine, subjects reported local and systemic vaccine reactions on a diary card. All other adverse and serious adverse events were documented at each visit. All subjects who underwent randomization were included in the safety analysis. All volunteers were followed with the use of HIV testing at day 0, at 24 and 26 weeks, and every 6 months during the 3-year follow-up phase. Peripheral-blood mononuclear cells were isolated and archived at 0, 6, 12, and 42 months. Assessment of behavior associated with an increased risk of HIV infection occurred at baseline, at week 26, and at each 6-month follow-up visit. HIV-prevention counselling was provided during each vaccination and post-test counseling visit.

At various time points after vaccination, plasma and cells from volunteers who did not have HIV infection were analyzed to evaluate immunogenicity. After removal of a small subgroup of samples for future matched case–control studies, random samples were identified and provided in a blinded fashion to the Armed Forces Research Institute of Medical Sciences laboratory at a ratio of samples from the vaccine group to samples from the placebo group of approximately 4:1. The immunogenicity of the vaccine regimen was measured with the use of the following validated assays: IFN-γ ELISPOT and CD4+ and CD8+ intracellular cytokine staining (ICS) for interferon-γ and interleukin-2 to Gag and Env; binding antibody to gp120 in the MN strain, gp120 in the A244 strain (CM244), and p24 Gag; and lymphoproliferation to gp120 MN, gp120 A244, and p24.

Both intention-to-treat (ITT) and per-protocol (PP) analyses were conducted. The ITT analysis included all subjects who underwent randomization. Seven persons who were enrolled and vaccinated were found to be positive for HIV-1 RNA at baseline. The PP analysis included a subgroup of subjects in the ITT analysis who received the entire series of vaccinations within the defined time period, who remained eligible to participate in the study, and who did not have HIV infection at the time of the fourth vaccination. A separate subgroup analysis called the modified ITT (mITT) analysis and used for the interim and final analyses, excluded the seven volunteers who were found to have HIV infection at baseline.

A total of 26,676 volunteers were screened and 16,402 were enrolled (ITT group). The 12,542 subjects who completed all vaccination visits on schedule and were not found to have HIV-1 infection after receiving the full vaccination regimen were included in the PP analysis. Seven volunteers who were found to be seropositive for HIV-1 on the first test after vaccination were determined by RNA testing to have been infected at enrollment and were not included in the mITT analysis, leaving 16,395 volunteers: 8197 in the vaccine group and 8198 in the placebo group. This group consisted of 10,064 men (61.4% of the subjects) and 6331 women (38.6%). No data were collected on the status of male circumcision or on serologic analyses for adenovirus type 5 or herpes simplex virus type 2. There were 52,985 person-years of follow-up (15% more than planned). At 42 months, 14,672 of the volunteers (89.5%) had completed the trial and were HIV-seronegative.

Most local and systemic reactions to the vaccine were mild to moderate and reflected the findings of studies on the safety of these products that have been reported previously [9-13]. In AVEG 022A, 5 to 6

administrations over a 12-month period of the combination regimen of ALVAC-HIV at $10^{6.7}$ TCID50 and gp120 in MF59 adjuvant (Chiron) in healthy volunteers were well tolerated [13]. In RV144, most reactions resolved within 3 days after vaccination. At least one adverse event was reported in 69.4% of subjects in the two study groups. The number of deaths and the frequency and severity of adverse events and serious adverse events were similar in the two groups.

HIV-1 infection was diagnosed in 132 subjects (56 in the vaccine group and 76 in the placebo group) during 52,985 person-years of follow-up in the ITT analysis, in 86 subjects (36 in the vaccine group and 50 in the placebo group) during 36,720 person-years of follow-up in the PP analysis, and in 125 subjects (51 in the vaccine group and 74 in the placebo group) during 52,985 person-years of follow-up in the mITT analysis.

With the use of the Cox proportional-hazards method, the observed vaccine efficacy was 26.4% (95% confidence interval [CI], −4.0 to 47.9; P = 0.08) in the ITT analysis; 26.2% (95% CI, −13.3 to 51.9; P = 0.16) in the PP analysis; and 31.2% (95% CI, 1.1 to 52.1; P = 0.04 by the O'Brien–Fleming method) in the mITT analysis. Because HIV testing was done at week 24, it is not possible to discern which dose of vaccine might have been associated with an early effect. The overall observed effect in the mITT analysis was evaluated with the use of several different analyses: event rates by Barnard's test (P = 0.04), the log-rank test (P = 0.04), the Wilcoxon test (P = 0.03), modification of the time-to-seroconversion end point (P = 0.04), exclusion of the in-hospital diagnosed case (P = 0.05), and analysis of interval-censored data (P = 0.04). Covariates were analyzed for the populations with similar results. Simultaneous adjustment for sex, age, living with a partner, and baseline risk factors did not affect estimates of vaccine efficacy, even though between-group differences in age, living with a partner, and baseline risk factors were significant.

There was no significant difference in the mean viral load among subjects who were found to have HIV infection in the vaccine group, as compared with those in the placebo group. The mean viral-load values were 4.36 log10 copies/mL in the vaccine group and 4.21 log10 copies/mL in the placebo group in the ITT analysis. The viral-load values were 4.24 log10 copies/mL in the vaccine group and 4.19 log10 copies/mL in the placebo group in the PP analysis and 4.30 log10 copies/mL and 4.20 log10 copies/mL, respectively, in the mITT analysis. In all three analyses, there were there were no significant between-group differences in post-infection CD4+ T-cell counts. The mean early post-infection CD4+ T-cell count was 541 cells/μL in the vaccine group and 568 cells/ μL in the placebo group in the ITT analysis (P = 0.47 by the Wilcoxon test), 572 cells/μL in the vaccine group and 532 cells/μL in the placebo group in the PP analysis, and 555 cells/μL in the vaccine group and 568 cells/μL in the placebo group in the mITT analysis.

Vaccination induced an HIV-specific response, as measured by IFN-γ ELISPOT assay to either Env or Gag antigen, in 19.7% of volunteers 6 months after the final dose of vaccine was administered. This result was similar to the rate of 17% in the phase II trial [10]. Response rates for CD4+ Env-specific ICS were higher in the vaccine group than in the placebo group (32% vs. 2%). Rates of positivity in the gp120 and p24 binding-antibody assays and the lymphoproliferation assay were similar to those in the phase II study. Binding antibody for Env was nearly uniformly present, with GMT of 31,207 for the MN strain and 14,558 for the A244 strain, whereas p24 responses were less frequent (138). The median lymphocyte stimulation index (LSI) was 2 for all subjects at baseline and subsequently in placebo recipients. The LSI was significantly higher in vaccine recipients (median LSI, 24 for gp120 MN, 32 for A244, and 4 for p24).

Taken together, these data are consistent with a modest protective effect of vaccine in this study. However, there was no significant difference in the HIV-1 viral load or the post-infection CD4+ count between the two study groups. A simple, combined analysis of phase I and II ALVAC-HIV and gp120 prime–boost

studies showed a rate of HIV-1 infection of 0.59 per 100 person-years in the vaccine group and 1.2 per 100 person-years in the placebo group, for a vaccine efficacy of 50% (95% CI, −39 to 80), a difference that was not significant; the results also showed no effect on viral load [14].

The relative contribution of either component of the vaccine strategy to the RV144 outcome remain unclear. The RV144 trial provided a unique opportunity to perform a case control study of correlates of risk. Plasma IgG binding antibody to scaffolded gp70 V1V2 envelope proteins correlated inversely with risk, while Env plasma IgA correlated directly with risk, raising the hypotheses that IgA responses against Env and IgG responses directed against V1V2 may be mechanistically associated with protection. Neither low levels of V1V2 antibodies nor high levels of Env-specific IgA antibodies were associated with higher rates of infection than in the placebo group. In vaccinees with low levels of Env-specific IgA antibodies, IgG avidity, ADCC, neutralizing antibodies, and Env-specific CD4+ T cells, were inversely correlated with risk of infection [15-17]. Two weeks post last vaccination 97% of RV144 studied plasma samples from vaccine recipients contained antibodies to V2 region synthetic peptides, falling to 19% at 48 weeks, suggesting that waning vaccine efficacy may be correlated to waning V2 antibody response. Interestingly, gp70 V1V2 antibodies were lower in HVTN 505 compared to RV144 [18]. The response to V3 CRF01_AE also inversely correlated with the risk of HIV infection in vaccine recipients with lower levels of Env-specific plasma IgA and neutralizing antibodies. In Vax003 and Vax004 (no protection), serum IgG responses targeted the same epitopes as in RV144 with the exception of an additional C1 reactivity in Vax003 and infrequent V2 reactivity in Vax004. These results along with a recent sieve analysis [19] generate the hypothesis that IgG to linear epitopes in the V2 and V3 regions of gp120 are part of a complex interplay of immune responses that contributed to protection in RV144 [20].

Approximately 90% of incident infections in RV144 were CRF01_AE, the predominant circulating strain in much of South East Asia. A sieve analysis identified two vaccine-associated genetic signatures in V2 corresponding to sites 169 and 181, further supporting the hypothesis that vaccination-induced immune responses directed against the V2 loop were associated with protection [21]. Monoclonal antibodies from RV144 vaccine recipients contact the V2 K169 residue, providing further evidence that vaccine-induced antibodies correspond to the observed sieve effect. These V2-specific antibodies can mediate ADCC, neutralization and low-level virus capture [22,23]. These findings generate the hypothesis that V2 IgG may play a role in protection against HIV-1 acquisition but do not provide evidence of a mechanistic or non-mechanistic mechanism of protection [24].

Sequences in gp70 V1V2 antigens other than V2, such as C1 and V1, may significantly contribute to the binding responses. Some light has recently been shed on the role of plasma IgA in RV144. In the presence of low anti-Env IgA, both ADCC and NAb responses correlated with decreased risk of infection. ADCC responses were predominantly directed to the C1 conformational region of gp120 [25,26]. IgA antibodies elicited by RV144 block C1 region-specific IgG-mediated ADCC [27]. Whether V2 antibodies might block the gp120-$\alpha 4\beta 7$ interaction and contribute at least partially to the protective effect against HIV-1 sexual transmission remains to be demonstrated [28-30]. Assessing IgG and IgA to V1V2 binding antibody immune responses in the mucosal compartments will be key.

In previous clinical studies, monomeric gp120 induced high levels of Env-specific IgG4 antibodies [31] while ALVAC (vCP1452) prime and gp120 MN in alum boost elicited lower IgG4 relative to IgG1 and IgG3 antibodies [32]. Antigen-specific IgG3 antibodies are associated with long-term control of *Plasmodium falciparum* [33] and monocyte-mediated cellular inhibition of parasite growth in vitro [34]. Similarly, early appearance of chikungunya virus-specific IgG3 neutralizing antibodies is associated with clearance of the virus and long-term clinical protection [35]. Conversely, IgG4 have been associated with progression to AIDS [36]. IgG3 can fix complement and have a high affinity for FcγR. In RV144, Env IgG3 was correlated with decreased risk of HIV infection, response that declined rapidly compared to overall IgG responses [37]. A recent comparison of RV144 and Vax003 showed that Env-specific IgG3 and V1/V2 IgG3 response rates were higher in recipients of the RV144 vaccine compared to those of Vax003 vaccinees and conversely that IgG4 were considerably lower in RV144. V1/V2 IgG3 responses and IgG3 responses specific for V1/V2 169K correlated with decreased risk of HIV-1 infection after IgA adjustment [38]. It is speculated that ALVAC priming due to its unique proinflammatory cytokine and chemokine response following vaccination in rhesus monkeys and infection in human PBMC [39] may shape the IgG subclass response to IgG3 in response to envelope protein boost in humans compared to envelope vaccination alone. Do V1V2-specific IgG subclasses modulate ADCC and antibody-mediated cellular phagocytic activity [40]? The contribution of Fc–FcγR interaction-mediated antibody function through mechanisms including ADCC, antibody-dependent cell mediated viral inhibition (ADCVI), and antibody-dependent cellular phagocytosis (ADCP) remains to be explored [41,42]. A recent post hoc analysis of RV144 showed an association between the FcγRIIC polymorphism and vaccine efficacy and correlates of risk, emphasizing the potential role of FcR genetics in predicting vaccine efficacy [43].

HIV-infected participants from RV144 were evaluated in RV152. There was no effect on early post-infection HIV-1 RNA VL or CD4+ T-cell count. Vaccination did not affect the clinical course of HIV-1 disease after infection, though there was evidence of reduction in seminal fluid viral load [44].

Although the study provided preliminary evidence that an HIV vaccine regimen has the potential to prevent infection, it did not have the power to address two intriguing considerations: vaccine efficacy may have decreased over the first year after vaccination, and vaccine efficacy may have been greater in persons at lower risk for infection. The data also do not answer the related question of whether it was a single vaccine or the combination of vaccines that induced a potentially protective immune response (AIDSVAX® B/E did not demonstrate any protective efficacy in a previous Phase III trial). Finally, the study supports the possibility that immunologic mechanisms mediating protection against HIV may be different from those mediating early post-infection control of viral replication [45,46]. These considerations underscore the need to conduct further exploring studies using the prime-boost regimen tested in the phase III trial.

The first HIV vaccine approaches investigated different forms of the HIV envelope protein. Unfortunately, these products failed to protect volunteers in phase III efficacy trials [47,48]. Current AIDS vaccine candidates are unable to induce neutralizing antibodies against primary HIV isolates or only to a very limited and narrow extent [49]. Natural history studies of HIV infection provided growing evidence of the role of T cells in the control of disease progression [50]. The immune response elicited by a successful vaccine likely will require both antibodies and T cells that recognize, neutralize and/or inactivate diverse strains of HIV and that reach the site of infection before infection becomes irreversibly established [51].

The control of viral replication could conceivably slow the rate of disease progression and/or reduce transmission of HIV from the infected vaccine recipient to his/her partner [52]. Indeed, several non-human primate (NHP) challenge studies demonstrated that vaccine candidates that elicited T-cell responses enabled animals to better control viral replication after challenge with a pathogenic virus [53-55].

The Step and RV144 efficacy trials illustrate the limits and need to revisit the concepts for immune protection and the laboratory assays available for assessment of vaccine immunogenicity [56]. While the MRKAd5 (Merck recombinant Ad5 vector) HIV-1 vaccine induced IFN-γ ELISPOT responses in a majority of vaccine recipients, it did not confer protection from HIV acquisition or reduce in post-infection setpoint viral load. In contrast the RV144 prime-boost regimen conferred a modest efficacy for HIV acquisition without affecting HIV viral load while IFN-γ ELISPOT responses were elicited in less than 20% of vaccine recipients. ELISPOT assays and intracellular cytokine analysis should no longer be the only tools used assess vaccine potency. Novel technologies aiming at exploring humoral, innate and adaptive cell-mediated immune responses have been developed to better assess systemic and mucosal immune responses to various vaccination regimens [57]. The development and validation of additional assays measuring lymphoproliferation, mucosal responses [58], cytotoxic capacity, *in-vitro* viral inhibition [59], or other immune functions such non-neutralizing antibody avidity [60] and antibody-dependent cytotoxicity and antibody-dependent cell-mediated viral inhibition [61,62] may provide a more robust indication of functional antiviral activity.

The immunogenicity profile of the ALVAC-HIV and Envelope gp120 subunit prime-boost regimen has been evaluated in numerous phase I/II clinical trials and shown to elicit both cellular and humoral immune responses [10-13, 63-70]. Interestingly in small animals (mice and guinea pigs) ALVAC, either infectious or heat-inactivated, induced in both animal species a nearly inflammatory response, as evidenced by a rapid migration of neutrophils to the site of inoculation. In parallel, ALVAC was shown to strongly adjuvant the co-administered immunogen, resulting in a marked increase in Env-specific IgG, IgG1 and particularly IgG2(a) serum titers. Of further interest, the heat-inactivated preparation of ALVAC retained this immunostimulatory activity. Whether or not a link between the inflammatory and immunomodulatory properties of ALVAC exists remains to be established [71]. The adjuvant effect of ALVAC on the response to an envelope subunit administration has however not been demonstrated in humans since the two products are administered separately in different arms. In AVEG 022A, neutralizing antibodies to HIV-1 MN were higher when the gp120 subunit was administered sequentially compared to co-administration with ALVAC, although a strict comparison between groups with sequential and co-administration was not clearly performed [13]. The prime-boost regimen also induced antibody-dependent cell-mediated cytotoxicity (ADCC) activity [12]. In one of these ALVAC studies using the identical RV144 vaccination regimen detected ADCC activity in 93% and 78% of vaccines to HIV subtype B and E gp120, respectively [72]. Interestingly, ADCC was not observed in participants receiving ALVAC-HIV only, suggesting that the combination of ALVAC-HIV and AIDSVAX® BE is necessary for ADCC induction. The nature of the protective immunity and specifically, the relative contribution of either component of the vaccine strategy to the RV144 outcome remain however unknown. ADCC was detected in all controllers and significantly higher than in viremic individuals while the magnitude and breadth of HIV-specific neutralizing antibodies were heterogeneous and lower than in viremic individuals [73].

The efficacy and practical application of HIV-1 vaccines may depend in part on the longevity of the immune responses generated, particularly those in the memory compartment. Candidate vaccines based on the HIV-1 envelope glycoproteins generate binding and neutralizing antibodies in humans but there have been no prior studies on the long-term persistence and recall of those responses. Evans *et al*. evaluated six healthy, HIV non-infected adults who had received a combination of recombinant canarypox HIV-1 vaccines

boosted by gp120 and who had achieved a high serum titer of neutralizing antibody to HIV-1 MN. These individuals were administered a gp160 boost 4-5 years after their last vaccination. Four volunteers had detectable binding and neutralizing antibodies at the time of boosting and all six volunteers exhibited recall binding and neutralizing antibody responses. The antibodies neutralized multiple T cell line-adapted strains of virus, including the vaccine strain, but not primary isolates. These results demonstrate that memory B-cell responses can last for many years following HIV-1 envelope glycoprotein immunization. However, the study did not evaluate recall responses after recombinant vector and combination revaccination [74]. The persistence and boosting of HIV vaccine-induced effector and central memory T cell differentiation as well as of humoral immune responses in the mucosal compartments after a long interval post primary vaccination has not been studied systematically. Long-term maintenance of the memory T-cell response is the hallmark of immune protection and, hence, constitutes one of the most important objectives of vaccine-development strategies [75]. Recently, multi-parameter flow cytometry has allowed a simultaneous assessment of the phenotype and multiple effector functions of single T cells; the delineation of T cells into distinct functional populations defines the quality of the response. New evidence suggests that the quality of T-cell responses is crucial for determining the disease outcome to various infections [18,76]. Breadth and depth of the CD8+ T cell vaccine-induced responses have been shown to be critical for the control of SIV replication in non-human primates [77].

The mucosal immune system acts as a first line of defense against infection caused by luminal pathogens. HIV infection results in depletion of gut-associated lymphoid tissue. New vaccine strategies are required that elicit both potent high avidity CD8+ T-cell effector/memory and central memory responses that can clear the nidus of initial virus infected cells at mucosal surfaces in order to prevent mucosal transmission or significantly curtail development of disease. The objective of an HIV-1 T-cell vaccine is to generate functional CD8+ effector memory cells at mucosal portals of virus entry to prevent viral transmission. The transmission event and systemic dissemination occur however with such rapidity in HIV (3-5 days) that there is might be not enough time for a secondary antibody response or activation of memory T cells to impact transmission [78]. Although we cannot exclude that vaccine-induced memory T-cell responses may contribute to decreasing the viral load in blood and mucosal tissues and impact on transmission, prevention of HIV transmission probably must likely rely on the presence of the formed elements of the acquired immune response (antibodies and/or activated effector T cells) being present at the site of transmission at the time of transmission. Vaccines that induce high levels of effective mucosal immunity should impact viral replication rate and prevent dissemination of virus from the mucosa into the systemic circulation. The ideal mucosal vaccine would induce HIV-specific secretory IgA and mucosal CD8+ cytotoxic T cells as a first line of defense at a very early stage of HIV infection, before the virus can seed into the secondary lymphoid organs in mucosal and systemic tissues [79,80].

The RV306 study will provide more intensive and comprehensive characterization of the innate, cell-mediated and humoral immune responses than possible in RV144. Results obtained from this trial will directly benefit Thailand and other countries in South East Asia where CRF01_AE infections are prevalent and indirectly provide the world with crucial information on the immunogenicity of this prime-boost strategy of HIV vaccines. It may inform the design of future studies with regard to the importance of boost regimens [81]. The results generated by this study may allow us to determine how the ALVAC + AIDSVAX combination regimen was able to modestly protect against HIV infection in the RV144 efficacy trial, understand which component of the combination contributed to the efficacy, and perhaps augment or sustain the vaccine efficacy observed. The results of this study may inform future vaccine design, including possible refinement or improvement of this combination regimen and future product development plans and timelines. This study is exploratory in nature and hypothesis-driven. The hypotheses can be formulated as follows:

- In RV144, although not significant, a higher efficacy rate of 60% was observed one year post vaccination, of 44% at 18 months, and waning over time down to 31% at 3.5 years, suggesting a humoral immune component perhaps in association with a cell-mediated immune component as possible immune mechanisms for protection. Residual immune responses may be enhanced by administration of additional booster doses of vaccine one year after the last primary series vaccination. Therefore, a booster vaccination at month 15 or at month 18 might significantly boost the antibody response and maintain a higher efficacy rate. Humoral and cell-mediated immune responses will be assessed post-boost in both the systemic and the mucosal compartments.

- Recently, the RV305 trial enrolled RV144 vaccine recipients who had received the complete RV144 vaccination regimen. These volunteers received a late boost 6-8 years later with either ALVAC-HIV/AIDSVAX®B/E combination, AIDSVAX®B/E alone or ALVAC-HIV alone, or placebo. The ALVAC-HIV/AIDSVAX®B/E combination induced antibody titers higher than 2 weeks post fourth RV144 vaccination, suggesting an anamnestic response and long term memory after a long interval between vaccinations. Conversely, ALVAC-HIV alone poorly boosted both peripheral and mucosal Env antibody and cell-mediated responses [82-84]. Since the respective contribution of the RV144 vaccine components in the protective immune responses is unknown, booster doses will compare AIDSVAX®B/E alone with the ALVAC-HIV/AIDSVAX®B/E combination at different intervals and with no boost. The hypothesis tested in this study is that simultaneous boost with ALVAC-HIV/AIDSVAX®B/E provides superior immune responses to AIDSVAX®B/E alone and that a longer interval between last primary series vaccination and ALVAC-HIV/AIDSVAX®B/E booster vaccination will provide higher antibody titers in comparison to a shorter interval.

- Vaccination may activate T-cell targets for HIV infection in the systemic and mucosal compartments.

Military Relevance

Historically, infectious diseases have had a major impact on U.S. Armed Forces. With an estimated 33.3 million infected individuals worldwide at the end of 2009, HIV poses a significant and persistent threat in terms of readiness and force protection, and may act as a war-starter by affecting the stability and security of nation-states.

HIV military relevance has been recognized from the very beginning of the pandemic. In 1985, the U.S. military recognized the emerging HIV-1 epidemic as a new threat to U.S. and allied forces worldwide. The United States Congress mandated the establishment of the U.S. Military HIV Research Program (MHRP) to develop effective preventive measures to include prevention education, vaccine development and implementation of novel anti-viral therapies and clinical management tools for the U.S. Department of Defense (DoD) and Allied Forces. In 2001, the Armed Forces Epidemiology Board identified HIV as a disease of military importance, and Army FOC 09-07, Global Casualty Prevention, requires detection, identification, and vaccination in order to protect US personnel against potential infectious disease (ID) threats. The 2001 DOD Report on Biological Warfare Defense Vaccine Research and Development identified HIV as the 4th greatest infectious disease threat to DOD forces. Department of Army Headquarters designated HIV vaccine development as an Army Technology Objective (ATO), a status

reserved for the highest priority science and technology efforts. Furthermore, The National Security Strategy of the United States (2002, 2006, and 2010) clearly identifies the threat of HIV/AIDS as a destabilizing force that threatens US National Security. Finally, in January 2011, The Department of the Army approved the HIV Vaccines Capability Development Document, which addresses the need to provide service members with vaccine protection against HIV and AIDS.

Medical care for HIV infection is extremely costly to the U.S. Defense Health Program. The estimated lifetime cost of HIV infection is at least USD $400,000 and the estimated average yearly cost varies from $20,000 to $25,000. The estimated lifetime healthcare cost of the 5,000 HIV infected servicemen/women is therefore $8B to $10B dollars. Total annual cost from the approximately 1,500 infected members who are still on active duty is estimated at $30M per year.

Infection rates could rise precipitously if forces are deployed to areas of high HIV prevalence. We developed an estimate based on the US Army Surgeon General's estimate of gonorrhea cases/year during the Vietnam war and determined the estimated number of visits to gonorrhea infected commercial sex workers necessary to generate that number. This equates to roughly one visit per service member per year during the height of the Vietnam War (this is widely viewed as an underestimate, since most cases of STI were treated without being reported). Given the known infection rate of 1/300 (no inflammation or ulceration) and estimating commercial sex worker HIV prevalence at 30% (lower than currently estimated in Thailand, Vietnam or many African nations) this equates to 1,200 to 1,800 new HIV infections per year for a deployed force of 400,000.

5.1. Name and Description of the Investigational Product

Refer to section 7.4 for additional information.

a. ALVAC-HIV (vCP1521), a Sanofi Pasteur product, manufactured by IDT Biologika, Germany, is formulated as a lyophilized vaccine for injection and is reconstituted with 1 mL of sterile sodium chloride (0.4% NaCl) for a single dose.

b. ALVAC Placebo, a Sanofi Pasteur product, manufactured by IDT Biologika, Germany, is supplied as a sterile, lyophilized product that consists of a mixture of virus stabilizer, and freeze drying medium and is reconstituted in 1 mL of sterile sodium chloride (0.4% NaCl).

c. AIDSVAX® B/E, manufactured by Genentech Inc. for GSID, the United States. Each 1 mL dose contains 600 μg of bivalent HIV gp120 glycoprotein vaccine (300 ug of subtype B (MN) and 300 μg subtype E (A244) proteins) absorbed onto a total of 600 ug of aluminum hydroxide gel adjuvant.

d. AIDSVAX® B/E placebo, manufactured by Hollister-Stier Laboratories LLC for GSID, the United States, is a sterile suspension of aluminum hydroxide adjuvant (concentration of 600 ug/mL) in each single use vial.

5.2. Summary of Nonclinical and Clinical Trials

5.2.1 Nonclinical Studies

As both products have a long-standing preclinical and clinical development, please refer to the ALVAC-HIV and AIDSVAX® B/E specific investigator's brochures.

5.2.2 Clinical Studies

For detailed description of RV144 design and results and conclusions, please refer to section 5 – Rationale for Study.

5.3. Known and Potential Risks and Benefits to Human Subjects

5.3.1 Risks/Discomfort to Subjects and Precautions to Minimize Risk

Outlined below are anticipated and unexpected adverse reactions, and a brief description of procedures to ameliorate risks and symptoms. All known risks and precautions described here are explained in detail in the informed consent.

5.3.1.1 Social Harm and Discrimination

Participants eligible for this study are at low risk for HIV infection. In the unlikely event of HIV infection during this study, the primary concern is related to ascertaining and providing HIV diagnostic information and, in particular, involuntary disclosure of HIV status to others. These disclosures may result in depression and rarely suicide among individuals learning that they are infected with the HIV virus. Furthermore, involuntary disclosure to others may result in prejudice by the community, family, employers, and psychosocial factors including stigma and discrimination. This risk will be minimized by the fact that all counselors will be trained in pre- and post-test counselling for HIV and will aim at fully informing volunteers of all activities in the study and attendant risks and benefits. The candidate vaccines may also induce false positivity to standard HIV antibody tests and may result in problems when applying for life or health insurance, international travel, employment or hospitalization. If there are any problems related to the above situations, the investigators will provide HIV testing to confirm HIV status. They will provide this information to the persons involved. In previous studies, very few participants (0.30%) had a falsely positive test for 6 months after the last vaccine [85]. All were certified negative for HIV virus infection by additional tests. Currently people who have received experimental HIV vaccines are deferred from blood donation, even though they do not have HIV infection.

The study staff will take appropriate action to assist volunteers with any discrimination they may experience by their participation in this study. Such measures will be accompanied with appropriate counselling. An additional risk includes stigmatization regarding volunteers' practices. In this study some volunteers may test HIV-positive.

5.3.1.2 Local Reactions

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

5.3.1.3 Systemic Reactions

Participants may exhibit general signs and symptoms associated with administration of a vaccine injection, including fever, tiredness, chills, rash, myalgia, arthralgia, nausea, headache, and dizziness. These side effects will be monitored, but are generally of short duration and rarely require treatment. Should such reaction persist and require treatment, the volunteer will be referred to appropriate medical care services.

5.3.1.4 Non-Invasive and Invasive Mucosal samplings

The non-invasive mucosal secretion samples collected in the clinic (rectal sponges or rectal lavage, cervico-vaginal cups) will be obtained by trained study personnel. Alternately, for the collection of cervico-vaginal secretions, women may elect to insert and remove cervical cups themselves. Inserting an instrument (speculum, anoscope, sponge, cup) into the anus or the vagina may cause discomfort and slight irritation. There is no evidence of rectal sponge or rectal lavage or cervical cup sampling contributing to risk of HIV or other sexually transmitted infection. Semen will be collected by masturbation. 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 3 days prior to semen collection.

Cervical biopsies may cause minor bleeding and discomfort during the collection of mucosa biopsies. A slight breach or irritation of the cervical mucosa may facilitate the risk of HIV infection, should the volunteer engage in risky sexual behavior. As a precautionary measure, however, all volunteers will be counseled to avoid sexual intercourse for 3 days prior and one week after cervical biopsies.

Sigmoid biopsies will be performed through a flexible sigmoidoscopy. Brief cramping and gas pains may be felt as air is inserted or as the scope advances. The passing of gas is necessary and should be expected after the procedures are terminated. After the procedure, the physician will make every effort to remove the gas. Volunteers may choose to receive sedation prior and during the procedure in order to ameliorate the discomfort and anxiety they may feel. There may be slight bleeding from the biopsy site which generally stops spontaneously. In rare cases, interventional endoscopic technique to stop bleeding is needed. There is a remote possibility that a biopsy may result in significant bleeding or even perforation)that may allow fluid and bacteria to enter the abdominal space) requiring emergency medical care. A gastroenterologist will perform this procedure in order to minimize these risks. As a precautionary measure, however, all volunteers will be counseled to avoid sexual rectal intercourse for 3 days prior to and 7 days following sigmoid biopsies.

5.3.1.5 Bone marrow aspiration

Risks or discomforts associated with this procedure include pain and pressure when the needle is inserted into the hip bone although local anesthesia will ameliorate the pain. The volunteer may feel a quick, sharp pain down the leg as the sample is taken and soreness at the procedure site for several days. Serious, but

rare problems include excessive bleeding at the site, and infection of the bone. Extremely rarely, excessive bleeding may necessitate blood transfusion. The potential risk factors most often associated with hemorrhage are a diagnosis of myelo-proliferative disorder, aspirin therapy or both [86]. Other potential risk factors include warfarin therapy, disseminated intravascular coagulation and obesity. Before the procedure, blood tests will be performed according to the site procedures to ensure that there is no safety risk for the volunteer. To mitigate these risks, an experienced and trained physician will perform the procedure at the King Chulalongkorn Memorial Hospital and Hospital for Tropical Diseases, in facilities and access to emergency or critical care whereas available.

5.3.1.6 Leukapheresis

Adverse reactions to leukapheresis procedure are rare and include vaso-vagal episodes related to needle insertions and transient volume shifts, peri-oral paresthesias, chills, nausea, and heartburn caused by the citrate anticoagulant used during the procedure. Vaso-vagal reactions are handled by postural manipulation and fluid administration. Volunteers will be observed closely by an experienced blood bank technician during the procedure. Citrate reactions are usually relieved by slowing the rate of the anticoagulant infusion and by administering oral calcium carbonate tablets. Approximately 50 mL of red blood cell volume will be lost as residual loss in the machine during the procedure. In addition, 150 mL of plasma will be removed at the end of each leukapheresis procedure for storing with the white blood cells. Those volunteers who undergo leukapheresis will be exempted from the immunogenicity blood draw scheduled at the same visit. Leukapheresis will be performed on participants whose platelet counts or hemoglobin counts meet the protocol inclusion criteria.

5.3.1.7 Pregnancy

The effect of the candidate HIV vaccines on pregnancy and fetus are unknown, female volunteers should avoid getting pregnant during the immunization phase and the following 3 months by using adequate birth control. These candidate HIV vaccines have not been specifically studied in pregnant women. Limited data on pregnancy outcomes available from RV144 do not suggest a significant increase of abnormal pregnancy outcomes in women who become pregnant after vaccination compared to those in the placebo group. A pregnancy test will be performed at screening, prior to each vaccination (same day), and prior to any of the invasive procedures. Pregnant women will be excluded from further vaccination but will be followed for safety through the end of the study and the pregnancy will be followed to term.

Possible genetic effect to the offspring of a male volunteer

The possible effect of these vaccines to the offspring of a male volunteer is unknown.

5.3.1.8 Lactation

These candidate HIV vaccines have not been specifically studied in pregnant and lactating women. No data on lactating women are available from RV144. There is no information about harm to an unborn child or a child who is breastfeeding. Breastfeeding women will not be enrolled. Should a female participant decide to breastfeed during the vaccination period, she will be excluded from further vaccination but will be followed for safety until the end of the study.

5.3.1.9 Venipuncture

Blood sampling carries a minimal risk of minor discomfort and bruising at the site of the needle puncture and, rarely, vaso-vagal reactions and the possibility of infection at the needle puncture site. Trained and experienced personnel will mitigate the risks of this procedure.

5.3.1.10 Allergic Reaction

As with any Investigational New Drug (IND) product administration and no matter what precautions are taken, there is always the risk of a serious, or even life-threatening, allergic reaction. Medical emergency equipment is located at each study clinic. This is available to handle emergencies, such as anaphylaxis and cardiac arrest. No such allergic reaction has been described so far with the HIV vaccines used in this study.

5.3.1.11 Guillain-Barré Syndrome

No vaccine-related Guillain-Barré syndrome has been described following administration of the HIV vaccines used in this study.

5.3.1.12 Unknown Risks

The participants of this study randomized to the vaccine groups will receive the primary series vaccinations used in RV144 and booster doses of either the ALVAC-HIV/AIDSVAX® B/E combination or AIDSVAX® B/E alone. As with any investigational vaccine, it is not known whether the vaccine may be effective at attenuating or preventing disease. No enhancement on HIV acquisition or HIV infection has been observed in RV144.

5.3.1.13 Risk of genetic tests and HLA testing

The greatest risk associated with genetic testing concerns privacy of volunteers. Test results can be used to provide information about how susceptible individuals are to certain diseases. Used inappropriately, this information could be discriminatory (for example, by insurance companies). HLA typing can also be used to determine who the true parent of a child is. However, the blood samples obtained in this study will not be used for this purpose; they will be used only to provide study investigators information about immune responses. The results will be coded to protect the volunteer's identity. The HLA type will only be connected to volunteer by the coded study number and not by name or other personal information. Neither the volunteer nor his/her private doctor will be given the results of the HLA test unless there is any medical requirement.

5.3.2 Alternative to these IND HIV Vaccines

At this time there are no known alternatives to these vaccines to afford the same potential protection from acquiring HIV.

5.3.3 Potential Benefit for Subjects

Study participants may receive no direct benefit from study participation. They will however have access to their medical records. Female participants who choose to undergo cervical mucosal samplings will benefit from the early detection of cervical cancer by Pap smear. Participants who choose to undergo

sigmoid biopsies may benefit from early detection of lower bowel cancer through the evaluation of suspicious polyps that may be found during these biopsies. Findings of medical concern will be referred for appropriate care and treatment. The volunteer will receive education and risk reduction counselling about HIV/AIDS. Volunteers will receive counselling in a confidential manner and additional important clinical information may be provided to their physicians. Others may benefit from knowledge gained in this study that may contribute to the development of an HIV vaccine.

5.3.4 Risks to the Study Personnel and the Environment

The principal risk in any clinical setting is in the handling of needles that may be contaminated and the attendant risks including hepatitis, human immunodeficiency virus (HIV), and other human pathogens. Adherence to standard operating procedures (SOP) for working with infectious agents and universal precautions will reduce the risk of exposure.

There are no known risks to the environment other than those associated with the generation of biohazardous waste attendant to vaccination of humans. All biohazardous waste will be disposed of as stipulated by local regulations and in accordance with study site SOPs.

5.4. Route of Administration, Dosage Regimen, Treatment Period, and Justification

Refer to section 7.4, Table 7 and Table 8.

5.5. Compliance Statement

The study will be conducted according to the protocol and in compliance with International Conference on Harmonization (ICH) Good Clinical Practice (GCP), Belmont Principles, CIOMS guidelines, Declaration of Helsinki and other applicable regulatory and Department of Defense (DoD) and the Ministry of Public Health and Thai Medical Council requirements. All identified study personnel will be trained to perform their roles and will carry out their responsibilities in accordance with ICH GCP guideline and clinic site SOPs. Roles and responsibilities of study staff are presented in Appendix G.

5.6. Study Population

Healthy, HIV-uninfected volunteers between age 20 and 40, weighing over 45 kilograms and available for follow up in the next 24 months will be enrolled. This study is exploratory in nature. A total of 360 volunteers will be enrolled and randomized in four groups: 27 vaccine recipients and 3 placebo recipients in Group I, 100 vaccine recipients and 10 placebo recipients each from Groups II, III and IV (divided in IVa and IVb with 50 vaccine recipients and 5 placebo recipients each, for a total of 327 vaccine recipients and 33 placebo recipients). As much as possible an equivalent proportion of male and female participants (50%) will be enrolled in each arm.

Refer to section 12.2 for a statistical justification of the sample size.

5.7. Study Sites

Clinical activities will be conducted at:

- The Vaccine Trial Centre, Faculty of Tropical Medicine, Mahidol University, Bangkok.
- The Royal Thai Army Clinical Research Center, AFRIMS, Bangkok

- The Research Institute for Health Sciences (RIHES), Chiang Mai University, Chiang Mai.

For invasive procedures:

- The Thai Red Cross AIDS Research Centre, Bangkok: Cervical biopsy
- King Chulalongkorn Memorial Hospital, Bangkok: Sigmoid biopsy, Bone marrow aspiration and Leukapheresis
- Hospital for Tropical Diseases, Mahidol University, Bangkok: bone marrow aspiration.

6. Trial Objectives and Purpose

6.1. Primary Objectives

- Characterize and compare cellular and humoral immune responses in the systemic compartment and humoral responses in the mucosal compartments in accordance with the RV144 primary vaccination series and following booster vaccination with either the ALVAC-HIV/AIDSVAX®B/E combination or AIDSVAX®B/E alone or placebo.

- Assess the safety and tolerability of the vaccination regimens.

6.2. Secondary Objectives

- Evaluate and compare the lymphoproliferation responses of participant PBMCs to HIV antigens and mitogens between vaccination regimens

- Characterize and compare innate immune responses (characterization of NK cells and APOBEC anti-retroviral factor) between the vaccination regimens

6.3. Exploratory objectives

- Characterize and compare cellular immune responses in the mucosal compartment in accordance with the RV144 primary vaccination series and following booster vaccination with either the ALVAC-HIV/AIDSVAX®B/E combination or AIDSVAX®B/E alone or placebo.

- Characterize B cell functional specificities in the systemic and bone marrow compartments for each vaccination regimen

- Assess the local B-cell responses in ex-vivo cervical mucosal explants for each vaccination regimen

- Assess the HIV mobility in mucosal secretions for each vaccination regimen

- Assess the innate and early adaptive responses to vaccination by gene activation for Groups I, II, and III

- Evaluate systemic and mucosal activation of T cell targets for HIV infection for each vaccination regimen

7. Trial Design

7.1. Study Endpoints

7.1.1 Immunogenicity Study Endpoints

Vaccine-induced immune responses will be assessed in study participants at weeks 4, 12, 14, 24, 26, 36, 48, 50, 60, 62, 72, 74,and 96, with a focus on peak immunogenicity time points at week 26 (Groups I to IV), week 50 (Groups II and III), week 62 (Group IVa) and week 74 (Group IVb).

Measurement of CD8+ T-cell effector function will be evaluated by using surrogate markers of CD8+ effector function including IFN-γ ELISPOT and intra-cellular cytokine staining (ICS). The CD4+ function will be assessed by the lymphoproliferation responses as measured by the 3H-thymidine incorporation assay and/or the functional CFSE assay. Innate immunity (mostly NK cells) will be assessed by measuring antibody-dependent cellular toxicity (ADCC) and antibody-dependent cellular viral inhibition (ADCVI) assays, by flow cytometric panels to phenotype the different type of NK cells and a cytokine array assay to characterize the type of cytokines elicited by this vaccine regimen, by the assessment of APOBEC expression and gene activation by DNA microarray technique at Day 3 and 2 weeks post fourth and boost vaccinations and 6 months post boost (will not be performed in Group IV). Results will be expressed as frequency of positive responders and magnitude of the response for each of the different assays above mentioned.

Leukapheresis will be performed at weeks 26 (Group I) and 50 (Group II, III) in a subset of willing volunteers for in-depth investigations of the T-cell responses and establishment of T-helper cell lines.

Humoral responses will be assessed by HIV-specific serum binding antibody assays and neutralizing antibody assays, ADCC and ADCVI assays (see innate immunity above). Humoral mucosal immune responses in the rectal, semen and cervico-vaginal compartments will be assessed using non-invasive sampling methods (sponge or lavage, cup, and masturbation) at baseline and weeks 14, 26, 48 (except Groups IVa and IVb), 50, 60 (Group IVa only), 62 (Group IVa only), 72, 74 (Group IVb only), and 96. Cell-mediated immune responses will be assessed utilizing invasive sigmoid and cervical biopsies that will be performed for Group I at week 26, Groups II and III at week 50, Group IVa at week 62, and Group IVb at week 74. Results will be expressed as frequency of positive responders and magnitude of the response for each of the different assays above mentioned. Results of immunology assessments through invasive collection methods in the mucosal compartment are exploratory and will be mostly descriptive as performed in a limited number of participants.

In addition, B cell functional specificities will be characterized in peripheral blood, bone marrow (provided by bone marrow aspiration) and sigmoid compartments (provided by sigmoid biopsies) in a subset of volunteers from Group II. Flow cytometric studies will identify and characterize the gut B cells. Ig levels will be assayed by sensitive ELISA and surface plasmon resonance assays. B specificities will be characterized by tetramer assays. Both microarray expression RNA analysis and proteomic profiling will be done on B cell populations. Panel of cytokines including IFN-α, TNF-α, IL-10, TGF-β, as well as B regulatory cytokines, and plasma microparticles will be quantitated and as well phenotyped by flow cytometry for source of cells of origin. Plasma TRAIL, FAS Ligand and TNFRII will be determined to monitor apoptosis levels. Phage-displayed antibody libraries from peripheral blood and bone marrow aspirates will be developed.

Bone marrow aspirations will be performed at week 50 on a subset of willing volunteers from Group II only. Results will be exploratory and descriptive.

In the context of unknown immune correlates of protection, additional assessments may be performed as new scientific technologies and assessment tools are made available.

For further details, see Section 10.1.

7.1.2 Safety Study Endpoints

Post-vaccination reactions will be assessed including local (at the injection site) reactions such as erythema, induration, pain/tenderness, swelling and limitation of arm movement, and systemic reactions such as fever, tiredness, chills, myalgia, arthralgia, headache, nausea, dizziness, and rash. They will be recorded on diary card during the 3 days following each vaccination. The study staff will document any post-vaccination reaction(s) and all related information (severity, frequency, etc) concerning such a reaction in the subject's clinic source documents. Results will be expressed as frequency of the overall and specific post-vaccination reactions.

Serious Adverse Events will be recorded until the end of the trial. All adverse events occurring through visit 11 (Groups I, II and III), visit 12 (Group IVa) and visit 14 (Group IVb) will be elicited and recorded. After these visits, only AEs that are "medically significant" events, defined as requiring multiple visits (two or more) to a physician for the same condition, or that result in hospitalization or an emergency room visit, will be captured. Results will be expressed as frequency of the AEs and SAEs and individual descriptions will be tabulated according to MedDRA organ class system.

7.2. Overall Study Design

The trial design is illustrated in Table 3 and Table 4 and detailed below.

This Phase II study is an in-depth assessment of the immunogenicity profile in participants receiving the ALVAC-HIV/AIDSVAX®B/E vaccination regimen used in RV144 and the safety and immunogenicity of an additional booster dose of either the ALVAC-HIV/AIDSVAX®B/E combination or AIDSVAX®B/E alone compared to no boost.

Volunteers will be evaluated at a screening visit for HIV status, general health, medical history, and undergo a physical exam and laboratory evaluations.

All groups will receive ALVAC-HIV or ALVAC placebo at Day 0 and weeks 4, followed by ALVAC-HIV or ALVAC-HIV placebo + AIDSVAX®B/E or AIDSVAX placebo at weeks 12 and 24 with an additional boost or placebo as follows:

- Group I will receive no boost at week 48
- Group II will receive ALVAC-HIV/AIDSVAX®B/E or ALVAC placebo/AIDSVAX placebo at week 48
- Group III will receive AIDSVAX®B/E or AIDSVAX placebo at week 48
- Group IVa will receive ALVAC-HIV/AIDSVAX®B/E or ALVAC placebo/AIDSVAX placebo at week 60
- Group IVb will receive ALVAC-HIV/AIDSVAX®B/E or ALVAC placebo/AIDSVAX placebo at week 72

Safety and tolerability will be assessed with both clinical and laboratory monitoring. Vaccine-related reactions will be solicited with the aid of a diary card and interview with volunteers three days after each vaccination. The information gained from the review of the diary card and the interview with the volunteers will documented in the clinical study chart. In addition, adverse events will be documented at each clinical encounter after the first vaccination through weeks 72 or visit 11 (Groups I, II and III), visit 12 (Group IVa) and visit 14 (Group IVb). Adverse events will be graded for seriousness, severity and relationship to the experimental product. Pregnancy testing will be conducted at screening, prior to each injection, and prior to any invasive procedure. Pregnant and lactating women will be excluded from enrollment or further vaccination if enrolled.

Mucosal secretions will be collected at intervals specified in the Schedule of Events (Table 4) in the rectal

and/or semen, and cervico-vaginal compartments to assess humoral immune responses. In addition, sigmoid and cervical biopsies will be performed 2 weeks after final vaccination at weeks 26 (Group I) and 50 (Groups II and III), week 62 (Group IVa), and week 74 (Group IVb) on a subset of willing volunteers to assess T-cell responses (and B cell characteristics for sigmoid biopsies at week 50 in Group II only) and activation of potential T-cell targets for HIV infection at the mucosal surface. Leukapheresis performed at weeks 26 (Group I) and 50 (Groups II, III only) on a subset of willing volunteers will be utilized to obtain sufficient cells to map HIV-specific responses with given antigens. Bone marrow aspirations will be performed at week 50 on a subset of willing volunteers from Group II only, to study B cell characteristics. Mucosal biopsies, leukapheresis, and bone marrow aspirations will follow standard procedures for clinical care.

Table 3: Study Design

Vaccination Schedule						
GROUP	**NUMBER OF SUBJECTS VACCINE/ PLACEBO**	**Week of Study**				
		0	**4**	**12**	**24**	**48**
		ALVAC-HIV or placebo, 1mL, IM, left deltoid muscle **AIDSVAX® B/E or placebo**, 1 mL, IM, right deltoid muscle				
I	**27**	**ALVAC-HIV**	**ALVAC-HIV**	**ALVAC-HIV + AIDSVAX® B/E**	**ALVAC-HIV + AIDSVAX® B/E**	
	3	**ALVAC-HIV placebo**	**ALVAC-HIV placebo**	**ALVAC-HIV placebo + AIDSVAX® B/E placebo**	**ALVAC-HIV placebo + AIDSVAX® B/E placebo**	
II	**100**	**ALVAC-HIV**	**ALVAC-HIV**	**ALVAC-HIV +**	**ALVAC-HIV +**	**ALVAC-HIV +**

				AIDSVAX® B/E	**AIDSVAX® B/E**	**AIDSVAX® B/E**
	10	**ALVAC-HIV placebo**	**ALVAC-HIV placebo**	**ALVAC-HIV placebo + AIDSVAX® B/E placebo**	**ALVAC-HIV placebo + AIDSVAX® B/E placebo**	**ALVAC-HIV placebo + AIDSVAX® B/E placebo**
III	**100**	**ALVAC-HIV**	**ALVAC-HIV**	**ALVAC-HIV + AIDSVAX® B/E**	**ALVAC-HIV + AIDSVAX® B/E**	**AIDSVAX® B/E**
	10	**ALVAC-HIV placebo**	**ALVAC-HIV placebo**	**ALVAC-HIV placebo + AIDSVAX® B/E placebo**	**ALVAC-HIV placebo + AIDSVAX® B/E placebo**	**AIDSVAX® B/E placebo**
IVa		**0**	**4**	**12**	**24**	**60**
	50	**ALVAC-HIV**	**ALVAC-HIV**	**ALVAC-HIV + AIDSVAX® B/E**	**ALVAC-HIV + AIDSVAX® B/E**	**ALVAC-HI.V + AIDSVAX® B/E**
	5	**ALVAC-HIV placebo**	**ALVAC-HIV placebo**	**ALVAC-HIV placebo + AIDSVAX® B/E placebo**	**ALVAC-HIV placebo + AIDSVAX® B/E placebo**	**ALVAC-HIV placebo + AIDSVAX® B/E placebo**
IVb		**0**	**4**	**12**	**24**	**72**
	50	**ALVAC-HIV**	**ALVAC-HIV**	**ALVAC-HIV + AIDSVAX® B/E**	**ALVAC-HIV + AIDSVAX® B/E**	**ALVAC-HIV + AIDSVAX® B/E**
	5	**ALVAC-HIV placebo**	**ALVAC-HIV placebo**	**ALVAC-HIV placebo +**	**ALVAC-HIV placebo + AIDSVAX® B/E placebo**	**ALVAC-HIV placebo +**

				AIDSVAX® B/E placebo		**AIDSVAX® B/E placebo**

Table 4: Schedule of Events

Visit Number	S1	1	2	3	4	5a	5b	6	7	8a	8b	8c	9	10	11	12	13	14	15**Exit**
Visit Day	-45 to -3	0	28	84	98	168	171	182	252	336	339	343	350	420	434	504	518	672	686
Visit week		0	4	12	14	24	+3d	26	36	48	+3d	49	50	60	62	72	74	96	98
Visit Window			-3+7d	+/-7d	-3+7d	+/- 7d		-3+7d	+/- 7d	+/- 14d		-3+ 3d	-3+ 7d	+/- 14d	-3+ 7d	+/- 14d	-3+ 7d	+/- 28d	
CLINICAL	X	X	X	X	X	X	X[0]	X	X	X[0]	X[0]	X[0]	X	X[0]	X[0]	X	X[0]	X	X
Demographic and contact information	X																		
Informed Consents (Main, mucosal	X																		
Test of Understanding	X																		
Enrollment & Randomization		X																	
Vaccinations		**X**	**X**	**X**		**X**				**X[16]**				**X[16]**		**X[16]**			
Examine injection site 30-minutes post-injection		X	X	X		X				**X[16]**				X[16]		X[16]			
Physical exam [1]	X	X	X	X		X	X[0]	X	X	X[0]	X[0]		X	X[0]	X[0]	X	X[0]	X	
Medical history/review eligbility[2] Vital Signs	X	X	X	X	X	X	X[0]	X	X	X[0]	X[0]		X	X[0]	X[0]	X	X[0]	X	
Concomitant Medications		X	X	X	X	X		X	X	X[0]			X	X[0]	X[0]	X	X[0]	X	
Adverse Event Documentation			X	X	X	X		X	X	X[0]			X	X[0]	X[0]	X	X[0]	X	
Diary Card [3]		X	X	X	X	X		X		X[0]			X	X[0]	X[0]	X	X[0]		
HIV Risk Counselling[1]	X	**X**	X	X	X	X	X[0]	X	X	X[0]	X[0]	X[0]	X	X[0]	X[0]	X	X[0]	X	X
Counselling on HIV; Pregnancy (pre and post)[1]	X	X	X	X	X	X	X[0]	X	X	X[0]	X[0]	X[0]	X	X[0]	X[0]	X	X	X	X
Pregnancy Test	X	X	X	X		X		X [15]		X[0]			X[15]	X[0]	X[0, 15]	X	X[0,15]		
Urinalysis (blood, protein and glucose)	X																	X	
CBC with differential	X							X					X					X	
Creatinine, ALT/AST	X							X					X					X	
RPR serology and HSV testing	X																		
STI diagnostic tests [5]	X																		
Pap smear (women) [6]	X																		
HIV EIA/WB	X	X						X		X[0]			X[0a]					X	

Visit Number	S1	1	2	3	4	5a	5b	6	7	8a	8b	8c	9	10	11	12	13	14	15**Exit**
Visit Day	-45 to -3	0	28	84	98	168	171	182	252	336	339	343	350	420	434	504	518	672	686
Visit week		0	4	12	14	24	+3d	26	36	48	+3d	49	50	60	62	72	74	96	98
Visit Window			-3+7d	+/-7d	-3+7d	+/- 7d		-3+7d	+/- 7d	+/- 14d		-3+ 3d	-3+ 7d	+/- 14d	-3+ 7d	+/- 14d	-3+ 7d	+/- 28d	
RESEARCH																			
Vaccinations		**X**	**X**	**X**		**X**				**X** [16]				**X**[16]		**X**[16]			
HIV neutralizing antibody assays		5			5			10		5 [0,13a]			10	5 [0]	10 [0]	5	10 [0]	5	
HIV binding antibody		X[17]			X[17]			X[17]		X[0,17]			X[17]	X[0,17]	X[0,17]	X[17]	X[0,17]	X[17]	
ADCC, ADCVI, NK, APOBEC		X[17]			X[17]			X[17]		X[0,13a,17]			X[17]	X[0,17]	X[0,17]	X[17]	X[0,17]	X[17]	
B-cell ELISPOT		X[17]			X[17]			X[17]					X[17]		X[0,17]		X[0,17]		
ELISPOT, ICS, LPA		25.5	25.5	25.5	25.5	25.5		25.5 [13]	25.5	25.5 [13a]			25.5 [13,]	25.5 [0]	25.5 [0]	25.5	25.5 [0]	25.5	
DNA Microarray (for Gr I, II, III with Bx or BM)							10+3[13]	10+3[13]			10+3[13]		10+3[13]			10+3[13]			
Mucosal secretions [7] (sponges or rectal lavage, cups, semen)		X[8]			X[8]			X[8]		X [13a]			X[0,8]	X[0,8a]	X[0,8a]	X[8]	X[0,8b]	X	
Leukapheresis[9]								X[9]					X[9]						
Plasma LH and FSH [cervical biopsies and secretions only]		X[17]			X[17]			X[17]		X[0,13a,17]			X[17]	X[0,17]	X[0,13b,17]	X[17]	X[0,13b,17]	X[17]	
Sigmoid and cervical biopsies [10]								X					X		X		X		
Bone marrow aspiration [11]													X						
B cell characterization in sigmoid								X					X		X		X		
HIV transport in mucosal secretions		X			X			X		X[0,13a]			X	X[0]	X[0,13b]	X	X[0,13b]	X	
Ex-vivo mucosal B-cell response and resistance to HIV infection								X[17]					X[17]		X[17]		X[17]		
Additional blood draw for sigmoid biopsy and BM[10]						30 [13]						30 [13]		30 [0,13]		30 [0,13]			
Storage & additional immunogenicity testing (mL)[12]		100	50	50	50	50		100 [13]	50[13a]	50[13a]			50 [13]	40 [0]	100 [0]	50	100 [0]	50	
Invasive Procedure Safety Labs[14]								10					10[13a]		10[13b]		10[13b]		

Daily volume (mL) [14]	16.5	130.5	75.5	75.5	80.5	105.5	13	166.5	75.5	80.5	13	30	166.5	100.5	145.5	110.5	145.5	88.5	
Cumulative volume for groups I, II, III[14]	16.5	130.5	75.5	75.5	80.5	105.5	13	166.5	75.5	8.05	13	x	156.5	x	x	x	x	88.5	1171
Cumulative volume for group IVa[14]	16.5	130.5	75.5	75.5	80.5	75.5	x	143.5	25.5	x	x	x	93.5	100.5	145.5	x	x	88.5	1131.5
Cumulative volume for group IVb[14]	16.5	130.5	75.5	75.5	80.5	75.5	x	143.5	25.5	x	x	x	93.5	x	x	110.5	145.5	88.5	1061

0. Clinic visits 5b and 8b are only required for volunteers participating in bone marrow aspiration, sigmoid, cervical biopsy and are optional for those participating in leukapheresis.

   Visit 8c is only for volunteers undergoing bone marrow aspiration, sigmoid, cervical biopsy in Groups II, III. Visit 10 and 11 are for Group IVa participants only, Visit 13 for Group IVb participants only. Volunteers in Groups IVa and IVb do not participate in Visit 8a.

   0a. HIV EIA is performed at Week 50 for Groups IVa and IVb only.

1. Physical exam at entry and at week 96 before study end with targeted physical only as required for interim visits. Targeted HIV counseling and reproductive health education will be conducted.

2. Full history at entry and at week 96 before study end with directed, interval history at all other visits.

3. Diary cards and instructions on how to complete the 3-day reactogenicity diary will be provided at vaccination visits (Weeks 0, 4, 12, 24 for Group I, II, III and IV, Week 48 for Groups II and III, Week 60 for Group IVa, and Week 72 for Group IVb). Diary cards will be collected and reviewed at the next study visit following vaccination. The diary card will be used as memory tools for better identification of reactions.

4. To provide adequate time for informed consent process and completing screening procedures, screening can occur over the course of two visits. Volunteers will be offered to participate in the optional invasive procedures (cervical biopsy, sigmoid biopsy, bone marrow aspiration and leukapheresis) but can participate in only one procedure. Informed consents for mucosal biopsies, bone marrow aspiration and leukapheresis can be administered at a later time during the study prior to the procedure.

5. HSV and syphilis serology test will be checked on all participants enrolled in the study. Individuals agreeing to optional mucosal collections (semen, rectal, and cervico-vaginal secretions, cervical and sigmoid biopsies) will undergo additional testing to evaluate for sexually transmitted infections. These tests include gonorrhea/Chlamydia genital infections (by urine NAAT). Additionally rectal

swabs (for those individuals practicing receptive anal intercourse in the past 6 months) will be collected to test for GC/CT infection. Women will additionally undergo a pelvic examination that includes a PAP smear testing and visual inspection to evaluate for further abnormalities. Individuals with abnormalities noted on examination and laboratory testing not explained by these conditions may undergo additional testing (e.g. wet prep for *Trichomonas vaginalis* in women*)* or be referred for further care and treatment (e.g. abnormalities on PAP smear). Men with abnormalities on urinalysis suggestive of STI not explained by testing above may be referred for further care and evaluation**.**

6. Pap smears will be performed only on women who agree to mucosal secretion collections and or cervical biopsy. It can be performed at any time point before the mucosal sample collection.

7. Mucosal secretion collections will be obtained from all willing volunteers from all clinical sites (Bangkok, Chiang Mai):

   Male volunteers: semen collection by masturbation and/or rectal secretions collected by sponge or lavage
   Female volunteers: cervico-vaginal secretions collected by cups
   For menstruating females, mucosal collection window can be extended to -3 +14days

8. Mucosal secretion collection will be collected for all groups.
   8a. Mucosal secretion collection for Group IVa only.
   8b. Mucosal secretion collection for Group IVb only.

9. Leukapheresis will be performed on a subset of willing male and women volunteers, (total of 25 vaccine and 4 placebo recipients)- See Table 6. During leukapheresis visits, blood for immunogenicity assays will not be drawn. Group I volunteer will undergo leukapheresis at week 26 (peak immunogenicity) and Groups II and III will be done at week 50. No leukapheresis will be performed in Groups IVa and IVb and at RIHES, Chiang Mai.

10. Cervical biopsies will be performed in a subset of willing female volunteers who agree to undergo this optional procedure. (targeted total of 35 vaccine and 6 placebo recipients). For Group I, 6 volunteers (5 vaccine and 1 placebo) will undergo biopsy at week 26. The invasive procedures will be performed at week 50 for Groups II and III. Group IVa will undergo biopsy at week 62 and Group IVb at week 74. See Table 5.

    Sigmoid biopsies will be performed in a subset of willing male and female volunteers (targeted total of 35 vaccine and 6 placebo recipients). For Group I, 6 volunteers (5 vaccine and 1 placebo) will undergo biopsy at week 26. The invasive procedures in Groups II and III will be performed at week 50. Invasive procedures for Group IVa will be performed at Week 62. Invasive procedures for Group IVb will be performed at Week 74. See Table 5. Subjects undergoing sigmoid biopsy will be studied for B cell characteristics

and all have an additional blood draw of 30 mL (Week 24 for Group I, Week 49 for Groups II and III, Week 60 for Group IVa, and Week 72 for Group IVb).

No biopsy (sigmoid or cervical) will be performed at RIHES Chiang Mai.

11. Bone marrow aspiration will be performed in a subset of willing volunteers in Group II only: 5 (men or women) vaccine recipients and 5 (men or women) placebo recipients (total of 10 volunteers). No bone marrow aspiration will be performed at RIHES Chiang Mai. See Table 6.

12. Immunogenicity Assessments (Section 10) describes in detail the specific laboratory investigations that will be conducted with these samples. Additionally plasma aliquots from female volunteers who consent to providing mucosal or biopsy samples will be evaluated for LH and FSH. All excess samples will be stored and archived.

13. The immunogenicity blood draws will not be collected at weeks 26 (Group I) and 50 (Groups II, III) for those subjects undergoing leukapheresis at these visits.

    13a. These samples will not be collected for Groups IVa and IVb.
    13b. These samples will be collected only for Group IVa at week 62 and IVb at week 74

    Microarray blood draws will be performed only those who agree to bone marrow aspiration, cervical and sigmoid biopsies. There will be no microarray blood draw for Groups IVa and IVb.

14. The blood volumes drawn are in accordance with the Thai National Blood Bank policy: donation every three months but no more than 4 times a year; the amount of blood for each donation is according to weight that applies to both male and female: 50 Kg, 450 mL; 45-50 Kg, 350 mL. In this study, only volunteers who weighing over 45 kilograms will be enrolled in this study. The maximum amount drawn in any 12 weeks interval is week 4 to 14 where **339.5** mL are drawn. The maximum draw is **166.5** mL. Pre-procedure labs will be drawn according to the requirements of the institutions performing the respective invasive procedures. Totals listed include the maximum for any volunteer, although this total will only be drawn in a subset of volunteers.

15. Pregnancy testing performed on women willing to undergo cervical and sigmoid biopsies, leukapheresis or bone marrow aspiration. Test must be negative prior to any of these procedures.

16. Vaccination and post-injection examination at Week 48 is for Groups II and III only, Vaccination at Week 60 is for Group IVa, vaccination at Week 72 is for Group IVb only.

17. Additional sample not required as plasma or cells will be aliquoted from ‘storage and additional immunogenicity collection’ samples. Additional immunogenicity testing may be necessary to further characterize the immune responses detected in the initial testing. These will use stored blood samples at time points mentioned in the Schedule of Events.

Table 5: Sigmoid and Cervical Biopsies

Group	Sigmoid biopsies (targeted number) 35 vaccine (V) + 6 placebo (P) male or female				Cervical biopsies (targeted number) 35 vaccine (V) + 6 placebo (P) female			
	Week 26	Week 50	Week 62	Week 74	Week 26	Week 50	Week 62	Week 74
I	5 V + 1 P				5 V + 1 P			
II		10 V + 1 P				10 V + 1 P		
III		10 V + 2 P				10 V + 2 P		
IVa			5 V + 1 P				5 V + 1 P	
IVb				5 V + 1 P				5 V + 1 P

Table 6: Leukapheresis and Bone Marrow Aspirates

Group	Leukapheresis (targeted number) 25 vaccine (V) + 4 placebo (P) male or female		Bone Marrow Aspirate (targeted number) 5 vaccine (V) + 5 placebo (P) male or female
	Week 26	Week 50	Week 50
I	5 V + 1 P		
II		10 V + 1 P	5 V + 5 P
III		10 V + 2 P	

7.3. Measures Taken to Minimize/Avoid Bias

7.3.1 Randomization

A randomization schedule will be centrally generated with fixed sized strata for RIHES Chiang Mai (n=60) and combined Bangkok clinics (n=300). The biopsies, leukapheresis and bone marrow aspirations will be performed only at Bangkok sites; RIHES Chiang Mai will not perform these procedures. The study will recruit approximately equal numbers of males and females but formal targets will not be established. Recruitment slots will be identified consistent with the above stratification plan and within strata, randomization will be performed creating a random ordinal listing. Sets of contiguous recruitment slots will be assigned to each clinic.

Only the independent statistician and the database developer will have a complete set of randomization lists for biopsies, leukapheresis and bone marrow aspiration. The individual site lists will be kept under lock and key by the pharmacy staff at the respective clinical sites. At the end of the study after unblinding of the volunteers, the lists will be returned to the study sponsor.

Pharmacy staff will be trained in Good Clinical Practices (GCP) and instructed not to discuss randomization lists, assignments, or participant assignments with study personnel. Pharmacy staff will be required to sign a confidentiality agreement and will be the only person(s) on site who will know the randomization assignment. The randomization assignment will not appear on any label or source document leaving the pharmacy.

7.3.2 Blinding

The PI, study staff and volunteers will be blinded as to receipt of active vaccine or placebo, but will not be blinded to group allocation. Since the ALVAC-HIV vaccine and the ALVAC placebo products are not identical in appearance, to preserve blinding the material inside the syringe will be masked. In addition, the pharmacy staff preparing the vaccine syringes will not be involved in the clinical assessment of participants and will be instructed not to comment on the appearance of experimental agent to study staff. For all participants, the volume of injection will be consistent.

Pharmacy staff will be trained in Good Clinical Practices (GCP) and instructed not to discuss the vaccine randomization lists, codes, or participant assignments with study personnel. Pharmacy staff will be required to sign a confidentiality agreement and will be the only person(s) on site who will know the randomization assignment. The randomization assignment will not appear on any label or source document leaving the pharmacy.

Samples will be labeled on site using labels containing participant study numbers, protocol number and visit number. The samples will be accompanied by a specimen tracking form (per participant) that records the study number, date and time of sample collection.

Any request for unblinding, with its rationale, must be forwarded through the PI. The PI will evaluate the request and will notify the HIV Vaccine Product Manager and the Study Medical Monitor (SMM). The SMM will evaluate the request and will advise the Sponsor regarding a course of action. The Sponsor will decide whether to approve the request for unblinding.

In the case of a decision to unblind, the Sponsor will authorize the independent statistician to provide this information to the PI, who must notify the participating IRBs and provide the study assignment (vaccine vs. placebo) to the site physician. It should be noted that there are very few circumstances in which unblinding will be essential to the medical management of a vaccine (or placebo) recipient.

In case of vaccine-related death or life-threatening serious adverse events (SAEs), knowledge of whether a participant received vaccine or placebo can be critical for the interpretation of the significance of clinical findings and thus impact decisions regarding continuation of study participation. In such cases, the assignment of a participant may be unblinded by the Pharmacist staff at the request of the PI in consultation with the SMM.

The site investigator will report episodes of accidental unblinding, with an explanation, to the HIV Vaccine Product Manager at AFRIMS who will inform participating IRBs and Sponsor.
Follow-up of unblinded participants will continue through the duration of the trial.

Since HIV-1 binding antibody and Western Blot results (see Section 11.1.5.4) may reflect vaccine or placebo assignments, access to such data will be limited to the laboratory personnel who are performing the tests and managing the data, and the independent statistician. If diagnostic testing of original and verification samples reveal true HIV infection, the participant will be notified by the clinic staff / physician. The PI, SMM, and Sponsor will be informed of HIV infection by the laboratory. HIV-infected participants will be informed about their HIV infection, but the participant, study site, investigators, Sponsor, SMM, and manufacturer staff will remain blinded as to their treatment assignment until the study is closed and the database is locked.Some participants may be tempted to know their assignment to vaccine or placebo through voluntary HIV testing. Participants will be actively discouraged from having HIV testing outside of the trial protocol. If specific needs arise, the research team will provide HIV testing and assist participants who need HIV status determination. Further, participants will be counseled that if they have engaged in behaviour that may have increased risk of exposure to HIV, they should have HIV testing done and this should be done through the vaccine trial system.

7.4. Investigational Products

The investigational products to be utilized in this trial include ALVAC-HIV (vCP1521), ALVAC placebo, AIDSVAX® B/E, and the AIDSVAX® B/E placebo.

ALVAC-HIV (vCP1521), manufactured by IDT Biologika, Germany, for Sanofi Pasteur (formerly Aventis Pasteur), is a recombinant canarypox vector vaccine that has been genetically engineered to express subtype E HIV-1 gp120 (strain 92TH023) linked to the transmembrane anchoring portion of gp41 (strain LAI), and HIV-1 gag and protease (LAI strain). vCP1521 is grown in chicken embryo fibroblasts (CEB) and formulated at a dose of 10^6CCID50 with 10 millimole (mM) TrisHCl, pH 9 and lactoglutamate. ALVAC-HIV (vCP1521) is formulated as a lyophilized vaccine for injection and is reconstituted with 1 mL of sterile sodium chloride (0.4% NaCl) for a single dose. Administration of ALVAC-HIV is a 1 mL intramuscular injection into the left deltoid muscle.

ALVAC Placebo manufactured by IDT Biologika, Germany, for Sanofi Pasteur (formerly Aventis Pasteur), is supplied as a sterile, lyophilized product that consists of a mixture of virus stabilizer, and freeze drying medium and is reconstituted in 1 mL of sterile sodium chloride (0.4% NaCl). Administration of ALVAC Placebo is a 1 mL intramuscular injection into the left deltoid muscle.

AIDSVAX® B/E manufactured by Genentech Inc for Global Solutions for Infectious Diseases GSID, in the United States, is a bivalent HIV gp120 glycoprotein vaccine with subtype B (MN) and subtype E (A244) proteins absorbed onto a total of 600 μg aluminum hydroxide gel adjuvant. The recombinant gp120s are produced in a genetically engineered Chinese hamster ovary (CHO) cell line. The envelope glycoproteins of MN and A244 are co-formulated with aluminum hydroxide adjuvant at a dose of 600μg (300μg each). The combined dose of 600μg (300μg of each antigen) is a sterile suspension, administered as a 1mL intramuscular injection into the right deltoid muscle.

AIDSVAX® B/E placebo is manufactured by Hollister-Stier Laboratories LLC for GSID, in the United States, is a sterile suspension of 600μg of aluminum hydroxide adjuvant, administered as a 1mL intramuscular injection into the right deltoid muscle.
Table 7 and Table present a summary description of the investigational products and respective placebos.

Table 7: Investigational Products

Product Name	ALVAC-HIV (vCP1521)	AIDSVAX® B/E
Dosage Form	2 mL vial, containing lyophilized product to be reconstituted with 1 mL of diluent 0.4% NaCl	2 mL vial
Unit Dose	1 mL per injection containing 10^6 CCID50 per dose administered	1 mL per injection (300 µg dose/antigen for a total of 600 µg per dose administered absorbed on 600 µg of aluminum hydroxide adjuvant)
Route of Administration	Injection in left deltoid muscle	Injection in right deltoid muscle
Physical Description	After reconstitution, limpid to slightly opalescent solution, colorless with possible presence of particles or filaments	White to slightly grey suspension
Manufacturer	IDT Biologika, Germany, for Sanofi Pasteur (formerly Aventis Pasteur)	Genentech Inc. for GSID, in the United States
Product Indication	Prevention of HIV infection or disease	Prevention of HIV infection or disease
Storage Conditions	Refrigerated at +2°C to +8°C DO NOT FREEZE	Refrigerated at +2°C to +8°C DO NOT FREEZE

Table 8: Investigational Placebos

Product Name	ALVAC Placebo	AIDSVAX® B/E Placebo
Dosage Form	2 mL vial, containing lyophilized product to be reconstituted with 1 mL of diluent 0.4% NaCl	2 mL vial
Unit Dose	1 mL per injection	1 mL per injection containing a sterile suspension of 600 µg of aluminum hydroxide adjuvant
Route of Administration	Injection in left deltoid muscle	Injection in right deltoid muscle
Physical Description	After reconstitution, clear, colorless	White to slightly grey suspension
Manufacturer	IDT Biologika, Germany, for Sanofi Pasteur (formerly Aventis Pasteur)	Hollister-Stier Laboratories LLC for GSID, in the United States
Product Indication	Placebo	Placebo
Storage Conditions	Refrigerated at +2°C to +8°C DO NOT FREEZE	Refrigerated at +2°C to +8°C DO NOT FREEZE

7.4.1 Investigational Product Packaging and Labeling

The investigational product, ALVAC-HIV or ALVAC Placebo, is covered under an Investigational New Drug (IND) application. This IND product will be labeled as indicated below:

ALVAC-HIV active

- RV306 / S-11-0002
- 1 vial, 1 dose Intramuscular
- Store between +2º and +8º C (Do not freeze)
- Expiration date:
- Manufactured by IDT Biologika for Sanofi Pasteur
- Sponsor: Surgeon General's Office, U.S. Army, Falls Church, VA, USA
- Caution: New Drug – Limited by United States law to investigational use only.

ALVAC-HIV placebo

- RV306 / S-11-0002
- 1 vial, 1 dose Intramuscular
- Store between +2º and +8º C (Do not freeze)
- Expiration date:
- Manufactured by IDT Biologika for Sanofi Pasteur
- Sponsor: Surgeon General's Office, U.S. Army, Falls Church, VA, USA
- Caution: New Drug – Limited by United States law to investigational use only.

Diluent 0.4% NaCl

- RV306 / S-11-0002
- 1 vial, 1 dose Intramuscular
- Store between +2º and +8º C (Do not freeze)
- Expiration date:
- Manufactured by IDT Biologika for Sanofi Pasteur
- Sponsor: Surgeon General's Office, U.S. Army, Falls Church, VA, USA
- Caution: New Drug – Limited by United States law to investigational use only.

The investigational product, AIDSVAX® B/E or AIDSVAX® B/E Placebo, is covered under an Investigational New Drug (IND) application. This IND product will be labeled as indicated below:

AIDSVAX® B/E active

- RV306 /S-11-0002
- 1 vial, 1 dose Intramuscular
- Store between +2º and +8º C (Do not freeze)
- Expiration date:

- Manufactured by Genentech, Inc. for GSID
- Sponsor: Surgeon General's Office, U.S. Army, Falls Church, VA, USA
- Caution: New Drug – Limited by United States law to investigational use only.

AIDSVAX® placebo

- RV306 /S-11-002
- 1 vial, 1 dose Intramuscular
- Store between +2° and +8° C (Do not freeze)
- Expiration date
- Manufactured by Hollister-Stier Laboratories LLC for GSID
- Sponsor: Surgeon General's Office, U.S. Army, Falls Church, VA, USA
- Caution: New Drug – Limited by United States law to investigational use only.

7.4.2 Investigational Product Storage

The investigational products will be stored in a secure pharmacy refrigerator at the clinical sites. Doses of ALVAC-HIV, ALVAC Diluent 0.4% NaCl, or ALVAC Placebo must be kept at +2-8°C until preparation (DO NOT FREEZE). The vaccine should be given within 2 hours of reconstitution of vaccine.

Doses of AIDSVAX® B/E or AIDSVAX® B/E Placebo must be kept at +2-8°C until preparation (DO NOT FREEZE OR SHAKE). The vaccine should be given within 2 hours of being drawn into a syringe.

If deviation in storage temperature occurs outside of the allowable excursions defined in the Pharmacy Manual of Operation, the pharmacy staff must report the storage temperature excursion promptly to USAMMDA and to the Principal Investigator. The excursion must be evaluated and investigated and action must be taken to restore and maintain the desired temperature limits. Any use of these products must be approved by USAMMDA before further use.

7.4.3 Investigational Product Preparation

Each vaccine will be supplied as a 2 mL glass vial. Vials are intended for single use only and thus do not contain a preservative.

ALVAC-HIV (vCP1521) Vaccine and Placebo

Allow vial to equilibrate to room temperature. Add 1 mL of 0.4% sodium chloride from the diluent vial. Allow the material to dissolve. Gently swirl the mixture in the vial, do not shake the vial. Withdraw 1 mL ALVAC-HIV (vCP1521). The ALVAC Placebo will be prepared in the same manner. ALVAC-HIV (Vcp1521) or ALVAC Placebo is administered intramuscularly into the left deltoid muscle by syringe within 2 hours of reconstitution.

AIDSVAX® B/E Vaccine and Placebo
Allow vial to equilibrate to room temperature. Gently roll the mixture in the vial, do not shake. Withdraw 1 mL AIDSVAX® B/E. The AIDSVAX Placebo will be prepared in the same manner. AIDSVAX® B/E or AIDSVAX Placebo is administered intramuscularly into the right deltoid muscle by syringe within 2 hours of reconstitution.

Members of the research team who have received specific training in detection and treatment of anaphylaxis will give all vaccine injections. Each clinical site where injections occur will be supplied with appropriate medications for emergency use if anaphylaxis occurs.

7.5. Duration of Subject Participation

The total duration of subject participation is 96 weeks from enrollment.

7.6. Dose-adjustment Criteria

No modification of dosage for any of the vaccine products will be allowed for this study.

7.6.1 Safety Criteria for Stopping Doses

The ALVAC and AIDSVAX products alone and in combination have been safely administered to over 9,000 volunteers. The RV306 study will adopt a standard of pharmacovigilance commensurate with the extant experience.

Site investigators will notify the PI who will notify the Study Medical Monitor or the Pharmacovigilance Committee Chair by phone and/or email of any grade 4 (potentially life-threatening) or 5 (deaths) events which are thought to be possibly, probably, or definitely related to vaccination, within 24 hours of learning of the event. This information should include subject ID, diagnosis, severity, relation to product, clinical description of event to the extent possible and actions taken, if any.

Upon notification of the Study Medical Monitor, of such an event as described above, the SMM will notify Pharmacovigilance Committee Chair will convene a teleconference to review the event and determine appropriate responses. The Pharmacovigilance Committee may pause vaccination at any time but will use as a guide, based upon an abundance of data supporting safety of these vaccinations, the occurrence of one grade 5 or one grade 4 event judged to be possibly, probably or definitely related to vaccination.

The Pharmacovigilance Committee will include the following:

- Pharmacovigilance Committee Chair or designee
- Study Principal Investigator or designee
- Study Medical Monitor or designee
- Walter Reed Army Institute of Research Representative
- HIV Vaccine Product Manager or designee
- DAIDS Medical Officer
- Local (Independent) Medical Research Monitor
- Sponsor's Medical Monitor

Additional Pharmacovigilance Committee participants may include, as needed, the following:

- Senior Investigators
- Associate Investigators
- Clinical research nursing staff
- Laboratory directors
- Data management and regulatory staff
- Site investigators

Pharmacovigilance Committee conference calls or meetings will require at a minimum the participation of PVC Chair and Co-chair, the PI or designee, the HIV Vaccine Product Manager or designee, and SMM or designee. Pharmacovigilance Committee members will be responsible to notify their respective IRBs (MOPH EC, RTA IRB, Mahidol Faculty of Tropical Medicine Ethical Review Board, RIHES Human Experimentation Committee, Chulalongkorn IRB, WRAIR IRB) of decisions taken by the committee. The Product Manager will notify USAMMDA and USAMRMC ORP HRPO. USAMMDA will be responsible for safety reporting to the U.S. FDA.

7.6.2 Pharmacokinetic Criteria for Dose Adjustment or Stopping Doses

Not applicable

7.6.3 Study Termination Criteria

The U.S. Food and Drug Administration (FDA), the Thai FDA, the study sponsor, USAMRMC Human Research Protection Office (ORP HRPO), the WRAIR IRB or MOPH Ethical Review Committee may stop the trial or suspend the use of this product at any time. The PI and SMM may, after consultation with the Sponsor, may recommend that the study be paused. In such instances, sufficient justification in writing or by email should be provided to the sponsor and the IRBs. The RTA IRB, Mahidol Faculty of Tropical Medicine Ethical Review Board, RIHES Human Experimentation Committee, or Chulalongkorn IRB may limit the further participation of investigators under their jurisdiction.

7.7. Investigational Product Accountability

Once the vaccine products are received in Thailand the Sponsor's Liaison is responsible for overseeing the distribution of the investigational product to the study sites. The sponsor's representative has delegated drug accountability responsibility for this product to the Principal Investigator. The PI may delegate, in writing, this responsibility to another individual, but the PI is ultimately responsible for the investigational product and its proper storage on receipt at the study site until it is transferred back to the sponsor's representative or designee or is destroyed, as directed by the sponsor's representative.

After the investigational product is distributed, the PI or her designee will be responsible for maintaining logs documenting product receipt, storage, reconstitution, and administration to subjects and amount of investigational product remaining at the end of the study and prior to final disposition in the secured Pharmacy area.

All unused or partially used investigational product and empty vials will be returned or destroyed and disposed as biohazardous waste as per local site SOP as directed by the Sponsor and as stipulated by applicable local, state, and Federal regulations.

7.8. Trial Treatment Randomization Assignments

See Section 7.3.1.

7.9. Identification of Data to be Recorded on the Case Report Forms

Source data will be collected in the study site. For more information on data handling, refer to section 16.

8. Selection and Withdrawal of subjects

8.1. Recruitment of Subjects

Volunteers will be recruited from Chiang Mai and Bangkok study sites through flyers, advertisements, word-of-mouth, bulletins, or equivalent IRB-approved materials. It is anticipated that there a screening to enrollment ratio of 3:1 is needed in order to obtain enough volunteers for enrollment in order to meet protocol objectives and who would be willing to undergo invasive procedures. Therefore, we anticipate screening approximately 1080 potential volunteer to enroll 360 study subjects.

Refer to section 5.6 for a detailed description of the study population.

8.2. Informed Consent Process

Study potential volunteers may participate in an optional group information session about vaccines from the PI or designee during which the study will be explained and participation requirements outlined. The session will take place at the clinical sites and the site staff will make all necessary provisions to assure the privacy of these discussions with all potential volunteers participating in these information sessions. The optional group information session may be conducted at all clinical sites as necessary. Volunteers requesting one-on-one consenting may receive individualized sessions. Information sessions may take up to two hours .These information sessions will be followed by an opportunity for questions from the volunteers.

For those individuals who express an interest in continuing with the consent process, a study team member will review the consent form privately in detail with the potential volunteers and answer any questions. A potential volunteer may wish to discuss the study with family or friends before making any decision as to whether or not to participate in the study and come back later to inform the PI or designee of his/her decision. After review with the study team member, an Informed Consent Form will be signed by all volunteers prior to enrollment in the study and a Test of Understanding (TOU) will be completed. To pass the TOU, the volunteer must answer 80% or 8 out of 10 of the questions correctly including two compulsory questions answered correctly. If the volunteer is unable to do so, he or she will be given two more opportunities to repeat the TOU. If after three attempts to pass the TOU the volunteer is still unable to do so, the volunteer will become ineligible for study participation. Separate and specific informed consent forms will be administered for each of the optional mucosal collection and invasive procedures including vaginal and cervical secretion, semen and/or rectal secretion, leukapheresis, sigmoid or cervical biopsies, and bone marrow aspiration, prior to the undertaking of these investigations. A signed copy of the informed consent will be provided to the volunteer.

8.3. Eligibility Screening

Each subject must meet all inclusion and no exclusion criteria. Only eligible subjects will be given the investigational product.
Volunteers who have passed the TOU and have given written informed consent will be asked for proof of identification and undergo a complete medical history, physical examination, and screening laboratory assessments to confirm eligibility for trial participation. Screening assessments as described in Table 4, including syphilis by RPR serology, will be completed after the informed consent process has been completed. HSV and syphilis serology test will be checked on all participants enrolled in the study. Individuals agreeing to optional mucosal collections (semen, rectal, and cervico-vaginal secretions,

cervical and sigmoid biopsies) will undergo additional testing to evaluate for sexually transmitted infections. These tests include gonorrhea/Chlamydia genital infections (by urine NAAT). Additionally rectal swabs (for those individuals practicing receptive anal intercourse in the past 6 months) will be collected to test for GC/CT infection. Women will additionally undergo a pelvic examination that includes a Pap smear testing and visual inspection to evaluate for further abnormalities. Individuals with abnormalities noted on examination and laboratory testing not explained by these conditions may undergo additional testing (e.g. wet prep for *Trichomonas vaginalis* in women*)* or be referred for further care and treatment (e.g. abnormalities on PAP smear). Men with abnormalities on urinalysis suggestive of STI not explained by testing above may be referred for further care and evaluation.

General eligibility for clinical trials will be dependent on the results of laboratory tests and answers to the interview questions. Counselling related to the potential risks of becoming pregnant during this trial will be provided. Pre-HIV test counselling and post-HIV counselling will be provided during the screening process by trained counsellors.

Findings of medical concern from any of these screening evaluations will be referred for appropriate medical care and treatment. For women with an abnormal Pap smear result (any finding other than "normal") no cervical biopsy will be performed. Their continuation or discontinuation from the study will be left to the medical judgment of the Principal Investigator and/or Senior Site Investigator, and they will be referred for appropriate medical care and treatment if warranted.

Clinical data and specimens collected during screening from volunteers who are subsequently found to be ineligible for participation in the study will become part of the study records and specimens will be evaluated as described in the protocol.

8.4. Subject Inclusion Criteria

Subjects must meet all of the following criteria to be included in the study:

- Healthy, HIV-uninfected male and female volunteers between age 20 and 40, weighing over 45 kilograms, available for a period of 24 months and having a Thai identity card
- Must be at low risk for HIV infection per investigator assessment
- Must be able to understand and complete the informed consent process
- Must be capable of reading Thai
- Must successfully complete a Test of Understanding prior to enrollment as described in section 8.2
- Must be in good general health without clinically significant medical history
- HIV-uninfected per predefined algorithm within 45 days of enrollment
- Laboratory screening analysis
    - Hemoglobin: Women ≥12.0 g/dL, Men ≥12.5 g/dL
    - White cell count: 4,000 to 11,000 cells/mm3
    - Platelets: 150,000 to 450,000/mm3
    - Normal liver function: ALT/AST ≤1.25 institutional upper limit of reference range
    - Creatinine: ≤1.25 institutional upper limit of reference range

- Urinalysis (dipstick) for blood and protein no greater than 1+ and negative glucose
- Female-Specific Criteria:
    - Negative pregnancy test for women at screening and prior to each vaccination (same day) and prior to any of the invasive procedures. Test must be negative for women subjects to proceed
    - Be using an adequate birth control method for 45 days prior to the first vaccine/placebo vaccination and will continue to be followed for at least 3 months 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, diaphragms, intrauterine device (IUD), or abstinence

8.5. Subject Exclusion Criteria

Subjects meeting any of the following criteria will be excluded from the study:

- Asplenia: any condition resulting in the absence of a functional spleen

- Bleeding disorder diagnosed by a medical doctor (e.g., factor deficiency, coagulopathy, or platelet disorder requiring special precautions)

- Therapeutic anticoagulation resulting in history of abnormal prothrombin (PT) / international normalized ration (INR) of partial thromboplastin time (PTT)

- Women breast-feeding or pregnant (positive pregnancy test) or planning to become pregnant during the window between study enrollment and 3 months after the last vaccination visit

- 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 eggs, egg products, streptomycin, or neomycin

- Subject has received any of the following substances:

    - Chronic use of therapies that may modify immune response, such as IV immune globulin and systemic corticosteroids (in doses of $\geq$ 20 mg/day prednisone equivalent for periods exceeding 10 days), and use of experimental vaccines
    - 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 (duration of 10 days or less, or a single injection) of corticosteroid

for a non-chronic condition (based on investigator clinical judgment) at least 2 weeks prior to enrollment in this study
  - Blood products within 120 days prior to HIV screening
  - Immunoglobulins within 30 days prior to HIV screening
  - Any licensed vaccine within 14 days prior to initial study vaccine administration in the present study
  - Receipt of any investigational HIV vaccine
  - Investigational research agents or vaccine within 30 days prior to initial study vaccine administration in the present study
  - Anti-tuberculosis prophylaxis or therapy during the past 90 days prior to enrollment

- Active sexually transmitted infection confirmed by clinical exam and diagnostic test

- Any medical, psychiatric, social condition, occupational reason, or other responsibility that, in the judgment of the investigator, is a contraindication to protocol compliance or impairs a subject's ability to give informed consent

- Psychiatric condition that precludes compliance with the protocol; past or present psychoses; past or present bipolar disorder; disorder requiring lithium; or within 5 years prior to enrollment, a history of suicide ideation or attempt

- Study site employees who are involved in the protocol and/or may have direct access to study related area

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

8.6. Subject Withdrawals and Discontinuation of Vaccinations

A participant may withdraw his/her consent to participate in the study at any time without prejudice. In those cases where a "withdrawal of consent" is requested by the volunteer, documentation of the withdrawal of consent and the reason(s) for the request will be captured in the subject's clinical research records. The subject will be asked if she/he will agree to complete the clinical assessments listed for the study termination/early discontinuation visit (Visit 14, Section 9). If she/he declines, this will end the subject's interaction with the study team for this protocol. The study team will engage in no further communication with the volunteer except as directed by an IRB and/or USAMRMC in regards to information concerning participant safety.

Only data and samples already collected will be analyzed according to protocol. The study team will not utilize samples or data from this volunteer for any future use and will discard residual samples when the study is completed. Counseling about any issue will be provided if he/she decides to discontinue participation in the study. Medical advice regarding what is in the best interest of the subject will also be provided. Reasons for withdrawing will be documented in the subject's study records.

8.6.1 Investigator Withdrawal of Subjects

The PI may withdraw a participant if, in his/her clinical judgment, it is in the best interest of the participant. The PI may discontinue the subject's activity without the subject's consent if any of these criteria is met:

- A subject fails to comply with study procedures,
- A subject's safety or health may be compromised by further participation,
- The sponsor decides to terminate or suspend the study,
- If a subject is withdrawn by an investigator, the reason should clearly be stated in the source documents and on the status change electronic case report form (eCRF).

When a subject withdraws due to an AE or is withdrawn by the PI due to an AE, the USAMRMC Regulatory Affairs (usarmy.detrick.medcom-usammda.mbx.sae-reporting@mail.mil) must be notified promptly (within 24 hours). Investigators must also follow the specific policies at each institution regarding the timely reporting of withdrawals due to AEs to the relevant IRBs (as listed in the study schema). If a participant does not complete the vaccination schedule secondary to an adverse event (including SAEs) or toxicity, he or she will continue to be followed according to the protocol visit schedule for safety, and, at a minimum, until the adverse event/toxicity is resolved and/or chronicity is established. In all cases, the PI will make a reasonable effort to complete study exit visit procedures (Visit 14)

8.6.2 Discontinuation of Scheduled Vaccinations

If pregnancy or HIV infection is diagnosed, no further vaccinations will be given, however participants will be encouraged to continue their follow-up visits for safety assessments. Whenever possible, the tests and evaluations listed for Visit 14 should be carried out for all subjects who decline follow-up according to the protocol visit schedule. All pregnancies will be followed to term regardless whether or not the subject declines to participate in her remaining protocol visit schedule. The site will maintain contact with pregnant participant in order to obtain this pregnancy outcome information.

Pregnancies beginning between the 45 days prior to the first vaccine/placebo vaccination and 3 months after the final vaccine/placebo vaccination will be reported as protocol deviations.

If a participant misses a vaccination visit outside of the visit windows listed by visit in Table 4, no further vaccinations will be administered. However, the participant will continue to be followed according to the protocol visit schedule. If the subject declines to participate in his/her remaining protocol visit schedule

they will be asked to complete the tests and evaluations listed for Visit 14. With the approval of the SMM, vaccination outside the window prescribed and continuation in the trial may be allowed in exceptional situations.

8.6.3 Data Collected for Withdrawn Subjects

All data collected up to the time of withdrawal will be reported. The status change eCRF will be completed, with the reason for withdrawal specified.

8.6.4 Replacement of Subjects

If a subject withdraws from the study, the next available participant will be placed in the vacated randomization slot. Replacements for participants who withdraw from vaccination or the study will be permitted only while enrollment is open. To minimize bias, only the study pharmacist or pharmacist technician will know whether the replacement has been assigned to placebo or vaccine.

8.6.5 Follow-up for Withdrawn Subjects

For those subjects who fail to return to the clinic for study visits or vaccinations, the site investigator and/or site staff will make a reasonable effort to determine the reason for the withdrawal from the study and to complete termination procedures (as described for Visit 14). If possible, telephone calls, registered letters, email correspondence or home visits according to permission granted by the volunteer will be used to contact volunteer to determine the reason(s) why the volunteer fails to continue their study participation. All attempts to contact study volunteers will be document in the study/clinical source records.

If contact is made, they will be invited to return for a final visit wherein a targeted examination may be performed although they have the right to refuse this visit. In all cases, the PI will make a reasonable effort to complete study termination procedures for these volunteers.

9. Study Visit Procedures

9.1. Vaccination and Study Visits

See Table 4: Schedule of Events

Participants will be queried using a predetermined review of systems/reactogenicity worksheet. If adverse effects experienced by the participant are deemed "severe", he/she will be asked to return to the clinic for evaluation by the investigator.

Post-test counselling and provision of HIV test result will be conducted at the visit following those visits requiring HIV serology or earlier as per the Schedule of Events, Table 4.

Biological samples such as serum, plasma, PBMCs, whole blood, mucosal secretion, mucosal biopsies and bone marrow aspiration will be stored in a quality-controlled environment. Transport and storage of these biological samples will be handled according to GLP standards. Any future use of these biological samples that are not specified in the protocol will require additional IRB review and approval. In addition, a subject may decide at any point to withdraw consent for the future use of his/her samples and in this case his/her samples will be destroyed according to the biohazard disposal regulations at the applicable site. However, all data generated from these samples up to the time of the volunteer withdrawing his/her consent will remain part of the study database and analysis.

9.1.1 Leukapheresis Procedure

Leukapheresis is essential to map HIV-specific functional responses within a given antigen, where in excess of 30 million cells are usually required for each antigen. Minimally, three antigens will be investigated requiring 90 million thawed viable cells (40-60% recovery from cryopreserved cells) in both vaccine and placebo recipients. To obtain this same amount of cells would otherwise require 450 mL of peripheral blood draw.

Prior to the start of the leukapheresis procedure, a pregnancy test will be performed on women (only proceed if the test is negative). During leukapheresis visits, blood for immunogenicity assay will not be drawn. Leukapheresis will be performed by qualified medical personnel at the Blood Bank Unit at King Chulalongkorn Memorial Hospital and according to the policies and procedures of the Chulalongkorn Blood Bank.

9.1.2 Mucosal Samplings

The assessment of innate and adaptive mucosal immune responses is essential as rectal and cervico-vaginal mucosae are the entry point of HIV-1 during sexual exposure. The assessment of cell-mediated immune responses requires isolating cells from mucosal tissues. Non-invasive sampling methods will be utilized for collections of secretions from the rectal, cervico-vaginal and semen compartments.

In order to obtain immune cells in greater quantities (3-6 million cells) for more advanced immunological assays, tissue sigmoid and cervical biopsies will be obtained from subsets of male and female participants

who have agreed to provide consent for these procedures. The laboratory investigations that will be conducted utilizing these mucosal secretion and biopsy samples are detailed in section 10.1.3.3 and 10.1.3.4.

Plasma LH and FSH will be evaluated on women using stored blood samples collected at the time point of the sampling (cervico-vaginal secretions or cervical biopsies).

9.1.2.1 Mucosal secretion collections

The non-invasive mucosal secretion sample collections in the clinic (rectal, cervico-vaginal) will be done by trained study personnel. Alternately, for the collection of cervico-vaginal secretions, women may elect to insert and remove cervical cups themselves. Inserting an instrument (speculum, anoscope, sponge, cup) into the anus or the vagina may cause discomfort and slight irritation. Male participants may also provide semen collections, which will be obtained by masturbation. Female volunteers providing cervico-vaginal secretions will also be asked to avoid receptive vaginal intercourse or insertion of objects intravaginally (tampons, douching, etc) 3 days before specimen collection. If menstruating they will be asked to return for sample collection (as per SOP). Male volunteers providing semen secretions will also be asked to avoid masturbation to ejaculation 3 days prior to specimen collection and volunteers providing rectal secretions will also be asked to avoid receptive anal intercourse or insertion of objects into the anus (enema, etc) 3 days before specimen collection.

9.1.2.2 Mucosal biopsies

Sigmoid biopsy will be performed at King Chulalongkorn Memorial Hospital - Bangkok. Cervical biopsy will be done at Thai Red Cross AIDS Research Center. The mucosal biopsies will be performed by qualified medical personnel.

Prior to undertaking cervical or sigmoid biopsies, a pregnancy test will be performed (and must be negative to proceed).

Prior and during sigmoid biopsy, the volunteers will be offered by a licensed physician sedative and pain medications that normally will provide relaxation and produce a drowsy feeling such as pethidine 25mg IV and/or midazolam 2.5 mg IV administered over at least 2 minutes. Medication will be provided to the volunteer free of charge by the study team. Sigmoid biopsies will be performed through a colonoscope. A gastroenterologist will perform this procedure.

In addition, for both cervical and sigmoid biopsies, the use of aspirin and non-steroid anti-inflammatory drugs by participants will be prohibited 7 days before and 1 day after the procedure. The volunteers who

undergo sigmoid biopsies will also be asked to avoid receptive anal intercourse 3 days before and 7 days after the biopsy. Women undergoing cervical biopsies will also be asked to avoid sexual vaginal intercourse 3 days before and 7 days after the biopsy.

In all participants willing to undergo sigmoid biopsy, a peripheral blood draw of 30 mL will be performed at week 24 for Group I, week 49 for Groups II and III, week 60 for Group IVa, and week 72 for Group IVb. This will allow us to compare immune responses between the blood and the sigmoid colon. In addition, B cells will be isolated from sigmoid biopsy samples and frozen until testing.

9.1.3 Bone Marrow Aspiration

Bone marrow (BM) aspiration biopsies are carried out principally to permit cytological assessment but also for immuno-phenotypic, cytogenetic, molecular genetic, and other specialized investigations. The study of B cells in the peripheral blood, BM and the gut of vaccine recipients may help elucidate the rapidly waning antibody response observed in RV144 [87].

BM aspiration removes a small amount of BM fluid through a needle put into a hip bone. The procedure is classically done in the iliac crest under sterile conditions and local anesthesia [88]. A needle will be inserted into the numbed area of the bone and approximately 2 mL of marrow will be removed with a sterile syringe. This procedure will take about 20-30 minutes. Volunteers willing to undergo this procedure will be asked to sign a specific informed consent. Prior to undertaking bone marrow aspiration, a pregnancy test will be performed (and must be negative to proceed)

BM aspirates will be performed at a single time point at week 50 in a subset of 10 (5 vaccine and 5 placebo recipients) willing male or female volunteers from Group II only. All participants that have BM aspirations performed will also have 30 mL of peripheral blood drawn the same day to be able to compare, at the time of BM aspirate, the B cell populations in peripheral blood and in BM. In all participants willing to undergo BM aspiration, a peripheral blood draw of 30 mL will be performed at week 49 to enable comparison of immune responses between the blood and the bone marrow.

9.2. Concomitant Medications

Information regarding concomitant medications used in association with an adverse event will be collected and recorded in the source documents. In addition information pertaining to non-HIV vaccines, immunoglobulin preparations, immunosuppressive medication, and antiretroviral drugs will be elicited at study visits and recorded in source documents.

9.3. Procedures for Monitoring Subject Compliance

All vaccinations and study procedures will be conducted under the direct supervision of the investigational staff.

10. Immunogenicity Assessment

10.1. Specification of Immunogenicity Endpoints

RV306 IMMUNOLOGY ASSAYS			
Cellular Assays	**Performance of Fresh vs. Frozen Samples (Peripheral blood or leukapheresis)**	**Function Measurement**	**Laboratory**
CD4: Lymphocyte proliferation	Frozen cells	Measures full helper cell recognition and expansion Characterize the function of proliferating cells	MHRP
CD4+ and CD8+ T cell lines	Frozen cells	T cell lines will be used for epitope mapping of the response to vaccine and for viral infectivity and inhibition studies.	MHRP
CD4+, CD8+, and other T cell characterization	Frozen cells	Defines phenotypic characteristics of T cells recognizing the antigen by high throughput multicolor flow cytometry. Surrogate measure of CD8+ effector activity	MHRP University of Essen
PBMC ELISPOT IFN-γ B-cell ELISPOT	Frozen cells	Measures cytokine secretion after stimulation with Ag and T-cell epitope mapping	MHRP

HLA subtyping	Frozen cells	DNA sequencing of individual HLA types	MHRP Illumina, Inc. Macrogen Clinical Lab Macrogen Inc.
Flow cytometry for innate immune cell phenotyping and a cytokine array assay, APOBEC	Frozen PBMC Fresh mucosal cells	Phenotyping NK cells and characterize the cytokines elicited by the different booster regimens	MHRP
DNA Microarray: gene expression to vaccine antigens and APOBEC	Frozen PBMC	Host gene expression profile and signature to vaccine antigens	Case Western Reserve University MHRP

Humoral Assays	**Serum or plasma**	**Function Measurement**	**Laboratory**
ADCC, ADCVI, and other non-neutralizing antibody functions	Frozen Plasma/Serum	Measures lysis of HIV expressing targets mediated by HIV specific antibodies Mapping of ADCC activity	MHRP Duke University Ragon Institute
B cell characterizations	Bone marrow Peripheral blood	Phage-displayed antibody libraries, repertories of VH and VL B cell receptors	Duke University MHRP NIH Vaccine Research Center
HIV-specific binding	Frozen serum or plasma	Binding antibody to vaccine antigens	MHRP Duke University BRIA Lab TRCARC
HIV-specific neutralizing antibodies	Frozen serum	Neutralizing activity against circulating HIV strains in Thailand	MHRP Duke University

Immunoglobulin isolation	Frozen Plasma	Isolation of mixed Ig from plasma	MHRP
Monoclonal antibody	Frozen PBMC	Isolation of anti-HIV monoclonal antibodies from peripheral B cells	Duke University NIH Vaccine Research Center

Mucosal Assays	**Mucosal secretions**	**Function Measurement**	**Laboratory or *Clinical Trial Site***
HIV-specific binding antibodies	Semen Rectal secretions Cervico-vaginal secretions	Binding antibody to vaccine antigens	MHRP Duke University Ragon Institute
Localised B-cell response induced by vaccination: IgG and IgA ELISA and neutralizing responses, cytokine secretion, microarrays and *ex vivo* HIV infection Monoclonal antibody production and memory B cell isolation	Sigmoid biopsies Cervical biopsies	Localized vaccine-induced B-cell responses Impact of infection on vaccine B-cell responses Effect of localized vaccine responses on viral infection kinetics Isolation and characterization of HIV-specific monoclonal antibodies and memory B cell	MHRP Imperial College Duke University
B cell characterization	B cell isolated from sigmoid tissues	Phage-displayed antibody libraries, repertoires of VH and VL B cell receptors	Duke University
Viscoelasticity and HIV transport	Cervico-vaginal rectal secretions, semen	Inhibition of HIV migration in mucosal secretions	MHRP Northwestern University
CD4+ , CD8+, and other T cell characterization	Fresh cells from sigmoid biopsies	Defines phenotypic characteristics of T cells recognizing the antigen by multicolor flow cytometry	MHRP

10.1.1 Primary Immunogenicity Endpoints

Immunogenicity will be assessed using the following assays at time points defined in section 7.1.1.

Cellular immunology assays
Peripheral blood for all cellular and potential humoral assays will be collected for immunologic studies according to the Schedule of Events. PBMC will be separated standard Ficoll. Novel validated assays developed by others with potential application to this study will be evaluated on HIV seropositive cells first and if minimum performance guidelines are met will also be used to evaluate the vaccinees. The remaining cells will be cryopreserved for future immunologic assays. Undiluted plasma will be stored and for humoral immunogenicity assays. Table 4 lists the various assays by visit schedule. For visits specified in the Schedule of Events, whole blood will also be collected in serum tubes.

Intra-cellular cytokine staining assay (ICS)
Cryopreserved PBMC will be stimulated with HIV-specific antigens and tested using standard (but not limited to) ICS assay.

IFN-γ ELISPOT Assay
Cryopreserved PBMC will be stimulated with HIV-specific antigens and tested using standard ELISPOT assay.

Humoral antibody studies

HIV-specific binding antibody assays
Binding antibody assays will be performed to detect serum or plasma binding antibodies to HIV-1 antigens. Serum and plasma will be also tested for their ability to block HIV binding to the α4β7 receptors and other possible HIV coreceptors.

Mucosal IgG and IgA binding antibody assays
Binding antibody assays will be performed to detect binding antibodies to HIV-1 antigens in mucosal secretions. Semen will be self-collected and rectal and cervical mucosal secretions will be collected by a non-invasive method using sponges or cups. For women, plasma LH and FSH will be evaluated on stored samples collected at the same time points.

Antibody-dependent Cell Mediated Cytotoxicity (ADCC) Assay and Antibody-dependent Cell Mediated Viral Inhibition (ADCVI) Assays

ADCC assays will be performed on plasma collected at visits specified in the schedule of events according to the protocol described [89,90] using rapid fluorometric (RF) ADCC assay and/or whole blood (WB) ADCC. Other non-neutralizing antibody assays such as ADCP (antibody dependent cellular phagocytosis) may be utilized as novel assays develop.

Neutralizing Antibody Assays
Current neutralization assays performed for HIV vaccine trials are cell line-based and PBMC assays for HIV subtype B and E viruses and possibly other HIV subtypes or CRFs [91].

10.1.2 Secondary Immunogenicity Endpoints

Lymphoproliferation assays (LPA)
The proliferative responses of participant's PBMC to HIV-specific antigens and mitogens will be measured according to a standard protocol [92] and/or by the CFSE assay to characterize the function of proliferating cells. The following panel of antibodies will be used but will not be limited to: IL-2, TNF-α, IFN-γ, and CD107 [93].

Innate Immunity

HLA subtyping
HLA subtyping will be determined by PCR on cryopreserved PBMCs.

Characterization of innate immune and T cells
Flow cytometric panels will be used to study the phenotype and function of different types of innate immune and T cells, including but not limited to CD4+, CD8+, Th17, NK, NKT, and dendriticcells and a cytokine array assay to characterize the type of cytokines elicited by the vaccine regimens. Cells may be sorted by specific phenotype for further assessment of function and gene expression.

APOBEC anti-retroviral factor

An emerging paradigm in the prevention of HIV-1 infection is the development of a rapid innate immune response to the virus, in view of the infection and destruction of CD4+CCR5+memory T cells within 2–3 weeks of HIV-1 transmission, mostly in the mucosally associated lymphoid tissue. Assessment of APOBEC expression within CD4+memory T cells will be conducted as previously described [94,95].

B-cell ELISPOT
The HIV-1 envelope glycoprotein (Env) functional spike has evolved multiple immune evasion strategies, and only a few broadly neutralizing determinants on the assembled spike are accessible to antibodies. Serological studies, based upon antibody binding and neutralization activity *in vitro,* suggest that vaccination with current Env-based immunogens predominantly elicits antibodies that bind non-neutralizing or strain-restricted neutralizing epitopes. However, the fractional specificities of the polyclonal mixture of antibodies present in serum, especially those directed to conformational Env epitopes, are often difficult to determine. Furthermore, serological analyses do not provide information regarding how repeated antigen inoculation impacts the expansion and maintenance of Env-specific B-cell subpopulations. A highly sensitive Env-specific B-cell ELISPOT system was developed, which allows the enumeration of antibody-secreting cells from diverse anatomical compartments directed against different structural determinants of Env. B-cell ELISPOT assays will be performed at multiple time points using stored cells.

10.1.3 Exploratory Immunogenicity Endpoints Justification

Leukapheresis is essential to establish CD4+ or CD8+ antigen-specific T-cell lines that will be used as a tool to map HIV-specific functional responses within a given antigen, where in excess of 30 million cells are usually required for each antigen. Minimally, three antigens will be investigated requiring

90 million thawed viable cells (40-60% recovery from cryopreserved cells) in both vaccine and placebo recipients. To obtain this same amount of cells would otherwise require 450 mL of peripheral blood draw. Plasma from apheresis procedure will be used to isolate immunoglobulin for related immunological studies.

The assessment of innate and adaptive mucosal immune responses is essential to understand, as rectal and cervico-vaginal mucosae are the entry point of HIV-1 during sexual exposure. The assessment of cell-mediated immune responses requires isolating cells from mucosal tissues. These tissues can only be obtained by performing tissue biopsies to get immune cells in sufficient quantity (3-5 million cells) to perform advanced immunological assays. Sigmoid biopsies are a proxy surrogate of rectal tissues (the rectal mucosa being thinner and fragile compared to sigmoid mucosa). Cervical biopsies can be easily performed in women and will be used for special investigations detailed in section 10.1.3.4. B cells characteristics are important to explore in different compartments including peripheral blood, gut and bone marrow in order to better understand the mechanisms of antibody induction, their persistence and memory recall.

10.1.3.1 Selection of volunteers

The specific immunology investigations described below require biological samples that are collected by non-invasive methods (sponge or lavage, cup) and invasive procedures including sigmoid and cervical biopsies, bone marrow aspirations or leukapheresis. At enrollment the main consent form that must be signed by all volunteers will mention the possibility of mucosal secretion collection and invasive sampling procedures including mucosal biopsies, bone marrow aspiration and leukapheresis. Separate consents for each of these secretion collection and invasive procedures will be provided to those subjects who elect to participate in these additional procedures:

- Cervical biopsies will be performed in a subset of willing female volunteers, targeted total of 35 vaccine and 6 placebo recipients (see Table 5).
- Sigmoid biopsies will be performed in a subset of willing male and female volunteers, targeted total of 35 vaccine and 6 placebo recipients (see Table 5).
- Leukapheresis will be performed in a subset of willing male and female volunteers, targeted total of 25 vaccine and 4 placebo recipients (see Table 6).
- Bone marrow aspirations will be performed at week 50 in a subset of willing volunteers from Group II only, in a targeted total of 5 vaccine recipients and 5 placebo recipients, total of 10 bone marrow aspirations (see Table 6).

Only participants who have signed the informed consent for each specific invasive procedure (can be signed at anytime between enrollment and the scheduled procedure) and selected subject to the case sampling targets will undertake these investigations.

Volunteers will be referred by the Thai Red Cross AIDS Research Centre to King Chulalongkorn Memorial Hospital, Bangkok, for sigmoid biopsies, bone marrow aspiration and leukapheresis. Cervical biopsy will be performed at the Thai Red Cross AIDS Research Centre. Bone marrow aspiration can also be performed at the Hospital for Tropical Diseases, Mahidol University.

Transportation will be organized by the study staff at no cost to the volunteer, and accommodation. Accommodation will be offered for volunteer's convenience and not for medical need as all procedures are done as outpatient procedure.

No mucosal biopsies or leukapheresis or bone marrow aspirations will be collected from volunteers enrolled at RIHES, Chiang Mai.

10.1.3.2 Establishment of T helper cell lines

T helper (Th) cells can be a useful tool in the understanding of vaccine immunogenicity and also understanding the mechanisms governing the Th response to HIV-1 is probably a key element in unveiling the pathogenesis of the disease because of the primary role the CD4+ T cell plays. Determining the fine specificity of T cells in vaccine recipients can be a powerful tool that could lead to the definition, in combination with other assays of correlates of protection or for an HIV infected individual it can be correlated to a patient's outcome. The classic approach that has been used to study the interaction between the virus and its target cells has been the use of transformed CD4+ T cell lines. Although easy to grow and maintain in culture these T cell lines do not reflect the exact same behaviour of the primary CD4+ lymphocytes [96,97]. Therefore non-transformed CD4+ antigen specific T cell lines can be more informative because they are more similar to the *in vivo* status. We have previously demonstrated the capability to establish and characterize antigen specific T cell lines [98]. State-of-the-art immuno-monitoring assays and epitope mapping may reveal a deeper underlying complexity to the immune responses generated by ALVAC-based vaccines and the efficacy observed in RV144 may be related to the proliferative capacity of T cells generated by the vaccine. It has already been established that the ALVAC-HIV (vCP1521) generates robust proliferative T cell responses against both Env and Gag insert gene products, but that classical effector T cells (IFN-γ producers) are infrequent. To further study these proliferating T cells we plan to use a CFSE-based assay to first label and identify the dividing T cells and then determine the surface phenotype, function repertoire and infectivity (with HIV) of the insert- and vector-specific T cells. T cell lines will be generated from subjects displaying robust proliferative

responses and these lines will be used for epitope mapping, surface phenotyping, functional assessment, and MHC-restriction analysis.

10.1.3.3 Ex vivo mucosal model of local B-cell responses elicited after vaccination

The early antibody responses to HIV-1 infection have been characterized in extracellular fluids, plasma, and in mucosal secretions. The earliest autologous-virus-specific neutralizing antibodies are detected around 80 days after infection [99]. During the first 6-8 months, antibody responses evolve, mature by increasing their avidity and decreasing their conformational dependence until reaching a plateau [100]. In addition to the delayed B-cell responses, HIV-1 [101,102] can induce severe damage to the mucosal B-cell population within the first 80 days of infection. Therefore a vaccine candidate needs to prime very early and durable antibody responses to have a chance to effectively control early establishment of viral infection. Analysis of antibodies elicited during RV144 has shown absence of neutralizing antibodies despite partial protection. Furthermore, analysis of B-cell responses in clinical trial participants has been limited to biological fluids, and the possibility that these responses can modulate and/or are modulated by *in vivo* infection has not been addressed in mucosal tissues. This emphasizes the significant absence of models for B-cell responses in the field of anti-HIV/SIV vaccine development. *Ex vivo* mucosal explants models could provide a surrogate endpoint that might have been predictive of protection/susceptibility in the RV144 study.

Modeling tissue infection in explant cultures and analyzing local B-cell dependent immune responses, an *ex vivo* model of mucosal humoral responses has been developed by establishing a series of correlations between B-cell responses and susceptibility to *ex vivo* infection of human tissue explants. For this purpose we will obtain mucosal tissue samples including cervical and sigmoid specimens from a subset of study volunteers.

To assess mucosal B-cell responses and their potency in mucosal tissue biopsies from humans, we will determine (i) localized vaccine-induced B-cell responses, (ii) the impact of infection on vaccine B-cell responses, and (iii) the potential effect of localized vaccine responses on viral infection kinetics after *ex vivo* challenge.

Experimental design

In addition to blood and mucosal samples collected within the protocol to assess both immune responses and activation of potential T cell targets for HIV infection, cervical and sigmoid tissue biopsy samples will be obtained. Biopsies will be taken after the second boost at weeks 26 and 50.

i) *Localized B-cell responses induced by vaccination*. Levels of IgG and IgA locally expressed will be measured at different time points in culture supernatants from biopsies kept in culture for 15 days. Tissue explants will be cultured and supernatants harvested from all tissue explant and biopsy cultures at different time points. Samples will be analyzed for total and specific IgG and IgA responses using established ELISA assay. Neutralization capacity of antibodies secreted in culture supernatants at different time points will be determined *ex vivo* using cell line-based assay. The profile of secreted cytokines in supernatants will also

be assessed using multiplex ELISA array. Tissue samples will be frozen and processed for microarray analysis to better characterize the immune responses at the genomic level.

Ex vivo neutralization results will be correlated with neutralizing titers in blood, and the *ex vivo* production of immunoglobulins as well as multiplex ELISA array and microarray data will supplement the T cell studies performed on mucosal surfaces.

(ii) *Impact of infection on vaccine B-cell responses*. The explant model could be an *ex vivo* surrogate to assess efficacy of *in vivo* vaccination of humans. Tissue taken after immunization will be infected *ex vivo* and supernatant samples harvested at different time points. Infection of the cultures will be confirmed by measurement of p24 antigen content of culture supernatants by p24 ELISA at each harvest time point. To determine if viral infection has an effect on local mucosal B-cell responses, several parameters will be measured in harvested culture supernatants. Modulation of cytokine secretion in culture supernatants due to *ex vivo* infection will be measured by multiplex ELISA array technology. This cytokine profile will be correlated with the profile assessed in section (i) with samples obtained after immunization but not infected *ex vivo*. We will also quantify total and specific IgG and IgA secreted into explant culture supernatants at the five harvest time points, using established ELISA. These levels will be compared with those elicited post-vaccination and pre-*ex vivo* infection.

Assessment of the influence of *ex vivo* viral infection on local B-cell responses will supplement with key information the *in vivo* efficacy results of the clinical studies and will further validate the tissue explant model as fundamental tool for vaccine studies.

(iii) *Effect of localized vaccine responses on viral infection kinetics*. The *ex vivo* infection of biopsies described above will allow us to determine the level of local protection induced by *in vivo* vaccination and if there is any effect on viral replication kinetics. Tissue explants will be infected as described above and the level of p24 antigen in culture supernatants at different time points will be assessed by p24 ELISA.

The correlations established with tissue explants will be key to further validate this *ex vivo* model as a surrogate of vaccine efficacy.

In addition, mucosal monoclonal antibodies will be isolated and characterized. Mucosal tissue B cells will be tease from sigmoid and cervical biopsy and transformed with Epstein Barr virus prior to freezing them for shipment to Duke University for single cell sorting and/or memory B cell cultures. In addition, fresh mucosal biopsies will be snap frozen for mounting on a cryostat for making frozen section that will be stained for Env binding to B cells, and the Env specific B cells laser dissected with a laser dissection (Zeiss) microscope system for isolation of Env-specific B cell VH and VL genes for rescuing antibodies from mucosal sites.

10.1.3.4 HIV mobility in mucosal secretions

Cervico-vaginal mucus (CVM) and semen in HIV-infected individuals contain cell-free and cell-associated HIV. Both forms of virions are plausible mediators of infection, and both, to be infectious, must penetrate the mucus barrier that coats and adheres to vaginal and penile epithelia during coitus. To the extent that mucus can limit the amount of virus that contacts the epithelium, the mucus layer can reduce the probability

of infection. Prior research has not revealed whether cell-free HIV can penetrate human CVM and rectal mucus. To directly determine whether cell-free HIV can diffuse through CVM rectal mucus and semen, an HIV replication defective virus-like particle, with CCR5 HIV envelope, and labeled with green fluorescent protein will be used. The translational movements of individual HIV virions in each sample will be observed and compared among the different vaccination groups using high-resolution multiple-particle tracking with confocal florescence microscope [103].

10.1.3.5 Innate immunity and early adaptive responses to vaccination by gene activation assessment

The DNA microarray assay is a new and powerful tool used to better define, understand or predict immune responses to specific antigens. It enables simultaneous analysis of the activation of a large number of genes on a single chip. This technology may permit the identification of novel differential markers expressed or co-expressed given a specific antigen or genotype associated with disease. These markers may later be utilized in flow cytometric assays. DNA microarray assays have been used to evaluate the quality of vaccines and/or provide information in the design of novel vaccines. For example based on the expression profiles of 76 genes in the rat lung one day after inoculation of influenza vaccine, the whole-virion influenza vaccine (pandemic influenza vaccine and whole virion-particle vaccine) and sub-virion vaccine (HA vaccine) from saline were found to be indistinguishable, i.e. there was no difference in vaccine quality [104]. DNA microarrays have been used to evaluate the gene expression profile in human peripheral blood mononuclear cells following smallpox and yellow fever vaccination, and naturally occurring upper respiratory infection [105]. Specific host gene expression signatures were identified that distinguished one or a small number of viral agents. In all cases, the DNA microarray was found to be a rapid, informative and highly sensitive method in vaccine evaluation.

In order to understand better the expression profile of a protective vaccine like the Yellow fever YF 17D, DNA microarrays have been used in North and South America [106,107]. The study demonstrated the complexity of the immune system in providing long lasting protection involving not only genes associated with antibodies but a highly integrated response including T-cells, natural killer cells (NK) and macrophages. DNA microarrays will explore the innate and early adaptive immune responses to vaccination by screening genomic expression.

10.1.3.6 B cell Characterization and Cytokine Secretion Profile

Preliminary analysis of RV144 showed that binding antibodies to clade B and E gp120 were present in 99% of vaccinated subjects but titers waned 15-fold over 20 weeks. ADCC with gp120-coated targets were present in approximately 75% of vaccinees for clade B but titers were not stable and waned over 20 weeks. Neutralizing antibodies targeted as subset of Tier 1 and 2 viruses and were less potent than the failed Vax003 and Vax004 trials. One hypothesis is that a correlate of protection in RV144 might be a short-lived antibody response than can neutralize or block an early of the HIV-1 mucosal transmission [108]. The mucosal immune response to vaccination and the effect of vaccination on acute HIV mucosal pathogenesis is likely to be a critical metric of vaccine efficacy. The study of B cells in the peripheral blood (PB), bone

marrow (BM) and gut of vaccine recipients may therefore help elucidate the rapidly waning antibody response in RV144 [87].

CHAVI scientists tried to understand why broadly reactive neutralizing antibodies are not made in HIV infection [109,110]. The hypothesis is that certain specificities of broadly reactive neutralizing antibodies are not made because they cannot be made. Specifically, the observation was made that 2F5 and 4E10 broadly reactive neutralizing antibodies are anti-cardiolipin as are other lipid reactive auto-antibodies (2F5 and 4E10), while 1b12 is a dsDNA autoantibody [111]. Haynes found that in human PB B cell subsets, using antigen-specific B cell tetramers for identification of B cells specific for the 2F5 BCR, there are few if any of these B cells present in HIV-infected patients' blood. The data to date indicate that B cells capable of making 2F5-like antibodies are not in the PB. The critical question is whether they in the IgM+ IgD-, CD19+ population of immature B cells, or in CD10 + CD19+ transitional B cells in BM before deletion or receptor editing mechanisms occur.

A critical need for the field is to obtain sufficient gut material for generation of neutralizing gut IgG and IgA monoclonal antibodies that not only neutralize in conventional neutralizing antibody assays (i.e., pseudovirus assays) but also protect against HIV infection at mucosal surfaces in non-traditional assays of virus penetrating epithelial surfaces and infecting dendritic cells and other non-T cell types.

Flow cytometric studies will identify and characterize the gut B cells. Immunoglobulin levels will be assayed by sensitive ELISA and surface plasmon resonance assays. B specificities will be characterized by tetramer assays. Both microarray expression RNA analysis and proteomic profiling will be done on B cell populations. The CHAVI panel of cytokines including IFN-α, TNF-α, IL-10, TGF-β, as well as a panel of B regulatory cytokines, and plasma microparticles will be quantitated and as well phenotyped by flow cytometry for source of cells of origin. Plasma TRAIL, FAS Ligand and TNFRII will be determined to monitor apoptosis levels.

Phage-displayed antibody libraries from PB and BM of HIV-infected patients are studying the repertoires of VH and VL B cell receptors that are deleted in the BM. It has recently been found that highly potent cross-reactive human monoclonal antibodies from viruses causing acute infections, as SARS CoV, Hendra and Hipah viruses, can be selected from naïve libraries (constructed from healthy uninfected unimmunized humans) and contain only few mutations compared to the germline [112]. In contrast, all broadly cross-reactive HIV neutralizing antibodies selected from the BM of HIV-infected long-term non-progressors contained numerous mutations compared to the germline. It has been hypothesized that HIV-1 has evolved a strategy to reduce or eliminate the immunogenicity of the highly conserved epitopes of such antibodies by using "holes" (absence or very weak binding to these epitopes of germline antibodies that is not sufficient to initiate and/or maintain an efficient immune response) in the human germline B cell receptor (BCR) repertoire. Screening of naïve libraries against HIV envelope glycoprotein (Env) is underway to test the hypothesis that cross-reactive antibodies against HIV do not exist in the natural repertoire of humans.

To further test this hypothesis, this study proposes to develop novel libraries from vaccinated individuals who undergo BM aspirations. BM and PB mononuclear cells will be taken and libraries will be prepared

and screened against recombinant Env oligomers prepared by Bart Haynes' group (Duke University). The results should provide significant amount of data which in combination with direct testing by flow cytometry for the existence of B cells with surface associated Env-specific IgGs could suggest whether such HIV Env + B cells exist. If such B cells don't exist for HIV (but do exist for the SARS CoV S glycoprotein which will be used as a positive control), this will provide additional evidence of lack of B cells with IgG against conserved broadly neutralizing epitopes on Env.

Bone marrow aspirates will be performed at one single time point at week 50 in a subset of willing volunteers from all study groups. All participants that have BM aspirations performed will also have 30 mL of PB drawn to be able to compare, at the time of BM aspirate, the B cell populations in PB (presumably after deletion) and the B cell populations in BM (presumably before deletion).

10.1.4 QA/QC for Immunogenicity Assays

This program was originally implemented for the RV144 trial and will continue operationally for this study. Prior to the conduct of all assays, technicians are trained on the assay theoretically and practically and all training documented. Standardized SOPs are used along with standardized data collection methods. All reagents are tested on HIV seropositive and seronegative individuals (at least 5 of each) prior to using the reagents. This includes HIV peptides for the ELISpot and ICS assays and common recall antigens for the lymphocyte proliferation assays (LPA). The impact of cryopreservation on cell viability and function are monitored using HIV-uninfected donor PBMC stored under identical conditions to the study samples [113]. PBMC will be thawed and tested every 6 months (3-5 vials per interval from the same donor) in the ELISpot and antigen-specific LP assay.

The tritiated thymidine and/or CFSE LPA, extra-cellular-based p24 neutralization and ICS assays have been qualified at the MHRP laboratories in Rockville, MD, USA. The ELISPOT assays are now validated at USAMC-AFRIMS laboratory in Thailand.

10.1.5 Specimen Archiving and Transfer

Biological samples such as serum, plasma, PBMCs, whole blood, mucosal secretion (cervico-vaginal secretion, semen, rectal secretion), mucosal biopsies (cervical biopsy and sigmoid biopsy) and bone marrow aspiration remaining after all assays described in this protocol have been completed will be bar coded and archived using electronic specimen storage and tracking system. PBMC will be stored at -125^{o}C or lower and plasma/sera will be stored at -70^{o}C or lower. Samples will be archived in the HIV Vaccine Research Center of Excellence (HVRC), Royal Thai Army Medical Department, Thailand for 5 years from protocol closure with possible extension after request to IRBs and Ethics Committees. If no archiving renewal is requested or approved, these specimens will be destroyed per applicable SOP's of the archiving institutions.

The Principal Investigator and the Sponsor and authorized regulatory bodies may have access to the data regarding the archived specimens.

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

After completion of laboratory work to meet with the study objectives, a small amount of leftover samples, including plasma and PBMCs, will be provided to the National Serum Bank at the Department of Medical Sciences, Thai MOPH.

10.2. Methods/Timing for Assessing, Recording, and Analyzing Immunogenicity Endpoints

Study-specific procedures for investigational assay(s) are presented in the Laboratory Manual of Operations (MOP).

11. Safety Assessment

Safety monitoring will be conducted throughout the study; therefore safety concerns will be identified by continuous review of the data by the PI, clinic staff, SMM, and United States Army Medical Materiel Development Activity (USAMMDA) Clinical Support Services Division.

Study Safety Management: A data safety monitoring board (DSMB) is not required for this study.

Study Medical Monitor: The SMM will ensure uniformity of clinical determinations made by investigators at clinical sites. The SMM will be responsible for assessing medical issues related to protocol conduct, adherence to inclusion/exclusion criteria and protection of all human subjects participating in the study. The SMM will provide clinical input to questions from investigators, site physicians and coordinators. All deaths and serious or unexpected adverse events will be reported to the Study Medical Monitor by the Principal Investigator. The SMM will participate with other members of the clinical operations project team to evaluate and appropriately process serious adverse events (SAEs). The Study Medical Monitor will ensure that standards for safety reporting follow all US and Thai government and DoD guidelines and regulations. The SMM will review all safety data summaries for submission to institutional review boards and any applicable external advisory boards.

Local (Independent) Medical Research Monitor: The Local (Independent) Medical Research Monitor will serve as an independent physician who can be approached for medical information by volunteers, act as their advocate and assess their medical care for events which occur during the course of the trial. He/she will also collaborate with the Study Medical Monitor to oversee the progress of the clinical trial and ensure that it is conducted, recorded, and reported in accordance with the protocol, standard operating procedures (SOPs), GCP, and the applicable regulatory requirements.

The LMRM shall have the following specified human subject safety-related authorities at the site that they represent: stop research activities, remove human subjects from the study, review monitoring plans, and participate in event assessment and reporting.

USAMMDA Clinical Support Services Division: The Clinical Support Services Division will be responsible for coordinating and integrating the review of safety data regarding OTSG-sponsored products. The Product Safety Surveillance Branch will review each SAE report and other immediately reportable event reports for medical consistency, accuracy, and completeness and will follow each event until it is satisfactorily resolved.

11.1. Specification of Safety Endpoints

11.1.1 Demographic/Medical History

Age, gender, date of birth (DOB), birth place, level of education, occupation and baseline medical history of volunteers will be recorded.

11.1.2 Vital Signs

Blood pressure and body temperature will be monitored 30-60 minutes post each vaccination

11.1.3 Physical Examination

For the purpose of assessing adverse events, symptoms and directed medical examination will be performed based on the medical judgment of the investigator. In addition, volunteers will be asked to record their temperature and complete a diary card at home six hours after the vaccination and each day for the next three days. Study staff will provide thermometers and volunteers will be trained to correctly use the thermometer and read the temperature.

11.1.4 Electrocardiogram

Not applicable

11.1.5 Laboratory Assessments

11.1.5.1 Hematology

Complete blood cell count will be performed on whole blood as per the Schedule of Events

11.1.5.2 Blood Chemistry

Creatinine, aspartate amino-transferase, alanine amino-transferase, will be tested on plasma or serum according to the Schedule of Events.

11.1.5.3 Urinalysis

Urinalysis by dipstick for blood, protein and glucose will be performed as per the Schedule of Events.

11.1.5.4 HIV Diagnostic Algorithm

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

An HIV-1 EIA assay will be utilized throughout the course of the study. If the EIA is reactive, the test will be repeated in duplicate. If repeatedly reactive, an HIV Western blot will be performed. If the Western blot is positive, HIV nucleic acid testing (NAAT) will be performed on the plasma specimen stored at –70ºC. If the NAAT is positive, a diagnosis of HIV infection is suspected. Notification from the lab that a verification specimen is required is sent to the CRC at the research site and the participant is called back for counselling and repeat blood draw. A second blood specimen (verification specimen) will be obtained for complete repeat HIV diagnostics. If the second plasma specimen is positive both serologically and by NAAT, a diagnosis of HIV infection is considered established. If the verification specimen is not positive (negative or Western blot indeterminate), the participant will be counseled that one additional blood collection (second verification specimen) for retesting will be necessary. If results of this repeat verification specimen are positive, infection is established; if not positive, the participant will be informed that he/she is not HIV infected and will return to the protocol’s regular visit schedule. This process is outlined below in the HIV Testing Algorithm.

HIV Testing Algorithm

Diagnostic specimen (plasma)

↓

EIA → non-reactive → "Uninfected"

↓

Reactive

↓

EIA x 2 → both non-reactive → "Uninfected"

↓

Either reactive

↓

Western blot → not positive → "Uninfected"

↓

Positive*

↓

Nucleic Acid Test → negative → "Uninfected"

↓

Positive → perform quantitative RNA assay

↓

"Suspicious of HIV infection"

↓↓

NEW PARTICIPANT APPOINTMENT(S) FOR VERIFICATION SPECIMEN(S):

A. Repeat testing algorithm, as above.

B. Blood also collected for CD4 count and HIV RNA quantitation.

Verification specimen: Negative (x 2) → "HIV-uninfected"; return to routine follow-up

Verification specimen: Positive (x 1) → "HIV-infected"; inform site physician

1) Appointment for counselling and blood collection for CD4 count and HIV viral load.
2) Refer to treatment unit.

*If the first verification specimen is found to be not HIV positive (negative or indeterminate), a second verification specimen will be collected and fully tested. If this repeat verification specimen is also negative, the volunteer is diagnosed as HIV uninfected; if positive, the diagnosis of HIV infection is considered established.

Laboratory assays
HIV EIA and HIV Western blot: Both Thai and U.S. Food and Drug Administration (FDA)-approved kits may be used.

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

Samples that are Western blot indeterminate may also be subject to supplemental nucleic acid testing at the discretion of the principal investigator or designee.

11.1.5.5 Pregnancy Screen

A pregnancy test will be performed at screening, prior to each vaccination, and prior to invasive procedures (cervical / sigmoid biopsies, leukapheresis or bone marrow aspiration) as indicated in the Schedule of Events. No vaccine or invasive procedure will be performed if the pregnancy test is positive.

11.2. Adverse and Serious Adverse Events

11.2.1 Adverse Event

An AE, as defined by the ICH guideline for GCP, is:
"Any untoward medical occurrence in a patient or clinical investigations subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom,

or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product."

An AE is considered to be any adverse change or exacerbation from a baseline condition that occurs following the initial administration of an investigational product whether or not the event is considered related to the investigational product. Examples of this include but are not limited to the following:

- Adverse changes including new signs and symptoms, intercurrent illness modifying the clinical course, or the worsening of a baseline condition including the increased frequency of an event or an increased intensity of a condition.
- Concomitant disease with onset or increased severity after the start of investigational product administration
- A new pattern in a pre-existing condition, occurring after the receipt of investigational product that may signal a clinically meaningful change.
- Clinically significant changes in laboratory values.

11.2.2 Solicited Adverse Event

A solicited AE is a predetermined event, identified in the Investigator's Brochure, which may reflect safety concerns related to the investigational product. Adverse events that will be solicited and assessed for this study include localized post-vaccination reactions such as erythema, induration, pain and tenderness, swelling and limitation of arm movement, and systemic reactions such as fever, tiredness, chills, myalgia, arthralgia, headache, nausea, dizziness, and rash. These reactogenicity events will be recorded during the 3 days following each vaccination.

11.2.3 Serious Adverse Event

As defined by the Code of Federal Regulations (CFR), a serious adverse event (SAE) is any adverse drug experience occurring at any dose that results in any of the following outcomes:

- Death,
- Life-threatening adverse drug experience defined as any adverse drug experience that, in the opinion of the investigator, places the patient or subject at immediate risk of death from the reaction as it occurred. It does not include a reaction that, had it occurred in a more severe form, might have caused death.
- In-patient hospitalization for >24 hours or prolongation of existing hospitalization. Hospitalization for either elective surgery related to a pre-existing condition that did not increase in severity or frequency following initiation of the study or for routine clinical procedures will not be considered an SAE. Hospitalizations for the performance of biopsy procedures are not considered SAEs.
- Persistent or significant disability/incapacity, defined as: "A substantial disruption of a person's ability to conduct normal life functions."

- Congenital anomaly/birth defect (in the offspring of a subject).
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered SAEs when, based upon appropriate medical judgment, they jeopardize the patient or subject and require medical or surgical intervention to prevent one of the outcomes 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 in-patient hospitalization, or the development of drug dependency or drug abuse.

11.2.4 Unexpected Adverse Drug Experience

As defined by 21 CFR 312.32(a), an unexpected adverse drug experience is:

"Any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure" An unexpected adverse event for this study refers to an adverse vaccine experience that has not been previously observed (not included in the investigator brochure) rather than from the perspective of such experience not being anticipated from the pharmacological properties of the vaccine. For example, under this definition, although erythema at the site of injection is an expected adverse event, a generalized rash following vaccination would not be expected.

11.2.5 Other Adverse Events

All other adverse events (those that do not fall under the categories of solicited, an SAE or unexpected) that are identified by site staff, the PI and the Medical Research Monitors, will be documented in the subject's clinic records and entered in the study eCRFs.

11.3. Relationship to Investigational Product

The investigator must assign a relationship of each AE to the investigational product. The investigator will use clinical judgment in conjunction with the assessment of a plausible biologic mechanism, a temporal relationship between the onset of the event in relation to receipt of the investigational product, and identification of possible alternate etiologies including underlying disease, concurrent illness or concomitant medications. The relationship of vaccination to adverse event (AE) will be determined based on the following definitions:

Not Related:
No relationship to investigational product. Applies to those events for which evidence exists that there is an alternative etiology.

Unlikely Related:

Likely unrelated to the investigational product. Likely to be related to factors other than investigational product, but cannot be ruled out without certainty.

Possibly Related:

An association between the event and administration of investigational product cannot be ruled out. There is a reasonable temporal association, but there may also be an alternative etiology such as the subject's clinical status or underlying factors including other therapy.

Probably Related:

There is a high degree of certainty that a relationship to the investigational product exists. There is a reasonable temporal association, and the event cannot be explained by known characteristics of the subject's clinical state or factors including other therapy.

Definitely Related:

An association exists between the receipt of investigational product and the event. An association to other factors has been ruled out.

11.4. Recording Adverse Events

11.4.1 Methods and timing for assessing, recording, and analyzing safety parameters

Adverse events, solicited AEs, and SAEs will be assessed at all study visits, documented in the source records, and recorded on the eCRFs using accepted medical terms and/or the diagnoses that accurately characterize the event. When a diagnosis is known, the AE term recorded on the eCRF will be the diagnosis rather than a constellation of symptoms. The investigator will assess all AEs for seriousness, relationship to investigational product, severity, and other possible etiologies.

The timeframe for the collection of AEs and SAEs begins at the first administration of investigational product through the end of the trial. All adverse events occurring through visit 11 (Groups I, II and III), visit 12 (Group IVa) and visit 14 (Group IVb) will be elicited and recorded After these visits, only AEs that are "medically significant" events, defined as requiring multiple visits (two or more) to a physician for the same condition, or that result in hospitalization or an emergency room visit, will be captured on source documents/eCRFs. When an event has not resolved by study closure, it will be documented on the AE eCRF as "ongoing".

11.4.2 Duration of Follow-Up of Subjects after Adverse Events

Non-clinically significant AEs still ongoing at the end of the study will be listed as "continuing". SAEs ongoing at the end of the study will be followed to resolution or stabilization of the condition with the probability that it will become chronic. All SAE outcomes will be reported to the sponsor's representative using the Serious Adverse Event Report Form.
If at any time following the completion of the study, a former subject brings to the attention of the investigator, an SAE that is considered to be related to the investigational product, the event will be reported as defined in section 11.3.

11.4.3 Severity Assessment

All AEs will be assessed for severity by the investigator. Inherent in this assessment is the medical and clinical consideration of all information surrounding the event including any medical intervention required. Each event will be assigned one of the following categories: mild, moderate, severe, or potentially life-threatening. Refer to the DAIDS table for grading severity of adverse events in Appendix H for further guidance in the assignment of severity. The criteria below may be used for any symptom not included in the grading scale. Any grade 4 (potentially life-threatening) AE or grade 5 must be reported as an SAE.

- **Mild (Grade 1):** Symptoms causing no or minimal interference with usual social and functional activities.
- **Moderate (Grade 2):** Symptoms causing greater than minimal interference with usual social and functional activities.
- **Severe (Grade 3):** Symptoms causing inability to perform usual social and functional activities.
- **Potentially Life threatening (Grade 4):** Symptoms causing inability to perform basic self-care functions OR medical or operative intervention indicated to prevent permanent impairment, persistent disability or death.
- **Death (Grade 5):** Death

11.5. Reporting Adverse Events

The PI, co-investigators, site investigators and site staff will exercise due diligence in ascertaining, accurately recording and promptly entering data on the eCRF for all adverse events for all study subjects. As data becomes available from the subject, the clinics and the laboratories, adverse events should be recorded and entered by the site staff on a daily basis. Site investigators will review, in a timely manner, this adverse event source data and determine the severity of the events and their relation to the study vaccines. Site investigators and the LMRM are encouraged to contact the SMM for consultation regarding adverse events.

The PI will report all AEs to the sponsor's representative (USAMRMC Division of Regulated Activities and Compliance) and the local IRBs in the appropriate safety, annual, and/or final reports. The study site will provide data files to the sponsor's representative for preparation of annual and final reports to the FDA.

11.5.1 Reporting Serious and Unexpected Adverse Events

Contact information for reporting SAEs is provided in Table 9

11.5.1.1 Reporting to the Sponsor

All unexpected AEs that are considered related to the product must be reported within 72 hours **and SAEs** whether or not the event is considered related to study product must be reported promptly (within 48 hours) to the sponsor's representative as per 21 CFR 312.64. Further, the investigator should comply with relevant study site SOPs on reporting AE's judged to be related to the investigational product and SAEs.

The minimal information that the investigator will provide to USAMMDA Clinical Services Support Division (CSSD) is specified in Table 10. The sponsor's representative may request additional information for purposes of the study. The Sponsor's Safety Pharmacovigilence physician will review all reported information and make the Sponsor's assessment of causality.

In order to comply with regulations mandating sponsor notification of specified SAEs to the US FDA within 7 calendar days, investigators must submit additional information as soon as it is available. The sponsor's representative will report unexpected SAEs associated with the use of the drug to the FDA as specified at 21 CFR 312.32 (c).

Investigators must follow all relevant regulatory requirements as well as specific policy at each institution regarding the timely reporting of SAEs to the WRAIR IRB, MOPH EC, RTA IRB, RIHES Human Experimentation Committee, Chulalongkorn IRB, Mahidol Faculty of Tropical Medicine Ethical Review Board, SMM, LMRM, and the USAMRMC ORP HRPO.

Reporting to the sponsor's representative does not fulfill the investigator's duty to report all unanticipated problems involving risk to human subjects or others to the IRB. The PI or his designee will notify the WRAIR IRB, MOPH EC, RTA IRB, Mahidol Faculty of Tropical Medicine Ethical Review Board, Chulalongkorn IRB, RIHES Human Experimentation Committee, the ORP HRPO, LMRM, and the SMM.

Table 9: Study Contacts for Reporting Serious Adverse Events

Sponsor's Representative Clinical Services Support Division	U.S. Army Medical Research & Materiel Command Clinical Services Support Division ATTN: MCMR-UMR 1430 Veterans Drive Fort Detrick, MD 21702-9232 Fax: +1 301 619 0197 Tel: +1 301 619 0317 usarmy.detrick.medcom-usammda.mbx.sae-reporting@mail.mil
Institutional Review Board	Ministry of Public Health EC Office of the Secretary, the Ethical Review Committee of Research in Human Subjects 3rd Floor, Dept. of Medicine Services Building Tiwanon Road, Nonthaburi 11000 Tel: +66 2 591 8251, 590 6171-2 Fax: +66 2 591 8251 Royal Thai Army Medical Department IRB 5th floor, Phramongkutklaovejvithaya Building Phramongkutklao Medical School 315 Rajvithi Road, Bangkok 10400 Tel: +66 2 354 7600, Ext 94270; 2 354 9011 Fax: +66 2 354 9011 research_pcm@yahoo.com Ethics Committee of the Faculty of Tropical Medicine, Mahidol University c/o Research and Academic Services 4th Floor, The 60th Anniversary of His Majesty the King's Accession to the Throne Building Faculty of Tropical Medicine, Mahidol University 420/6 Ratchawithi Road, Bangkok 10400, Thailand Tel: +66 2 354 9100-19, Ext. 1349 Fax: +66 2 306 9126 pornpimon.ada@mahidol.ac.th Chulalongkorn University IRB

	Faculty of Medicine, Chulalongkorn University AnandaMahidol Building, 3rd Floor Bangkok, Thailand Tel: +6622564455 Fax: +6622554493 research_affairs@gmail.com Human Experimentation Committee (HEC) Research Institute for Health Sciences (RIHES) Chiang Mai University 110 Intavaroros Road, Chiang Mai 50200, Thailand Tel: +66 5394 5055, 5394 6148, ext. 360 Fax: +66 5322 1849 jwipasa@chiangmai.ac.th WRAIR IRB US Army Garrison-Forest Glen Human Subjects Protection Branch 503 Robert Grant Avenue, Silver Spring, Maryland 20910-7500. USA Tel: +1 3013199940 Fax: +1 301 319 9961 usarmy.detrick.medcom-wrair.mbx.hspb@mail.mil
USAMRMC Office of Research Protection Human Research Protection Office	U.S. Army Medical Research and Materiel Command, ATTN: MCMR-RP 504 Scott Street Fort Detrick, Maryland 21702-5012. Fax: +1 3016197803 Tel: +1 3016192165 HRPO@amedd.army.mil
Study Medical Monitor	Jean-Louis Excler, MD US Military HIV Research Program 6720-A Rockledge Drive, Suite 400 Bethesda, MD 20817, USA Tel: +63 947 893 7459 jexcler@hivresearch.org
Local (Independent) Medical Research Monitor	Prof. Emer. Swangjai Pungpak

	Consultant Department of Clinical Tropical Medicine Faculty of Tropical Medicine Tel: +66 8 1920 8405

Table 10: SAE Information to be reported to the Sponsor's Representative

Notification Method	**Information to be Provided**
Email or Telephone	IND number, HRPO log number, sponsor study number, name of the investigational product, and investigator name and contact number
	Subject identification number and initials
	SAE, onset date, date of investigational product administration, severity, relationship, and subject's current status
AND	
Email or Fax	Cover sheet or letter
	Serious adverse event report form
	Supplemental Serious Adverse Event form
	Concomitant medication case report form or a list of concomitant medications
	Medical record progress notes including pertinent laboratory/diagnostic test results
NOTE: When submitting SAE reports via email, the subject line of each email notification will read as follows: **SAFETY REPORT – IND # _____, Sponsor Study #_____, Subject# _____, Event term: _____**	

11.5.1.2 Reporting to the IRB

Unanticipated events and social harms may occur during the course of the study. When such events are related to study participation, the local IRBs and WRAIR IRB will be informed. The study staff, informed of these events, will inform the PI or his/her designee. The PI or designee will then prepare a narrative summary of the event and report it to the IRBs and SMM.

All unanticipated problems related to the study and involving risk to subjects or others and all subject deaths should be promptly reported by phone (+1 301 319 9940) or by facsimile (+1 301 319 9961) or email (usarmy.detrick.medcom-wrair.mbx.hspb@mail.mil) to the WRAIR IRB. A complete written report should follow the initial notification within 10 business days. The complete report should be sent to the Director, Human Subjects Protection Branch (HSPB), Walter Reed Army Institute of Research (WRAIR), 503 Robert Grant Avenue, Silver Spring, Maryland 20910-7500.

Investigators are required to forward safety information provided by the sponsor's representative to the IRB.

When a previously enrolled human subject becomes a prisoner and the relevant research protocol was not reviewed and approved by the IRB in accordance with the requirements of DODI 3216.02 subparagraphs 7.b (1) and (2) to include prisoners as research subjects, the principal investigator shall promptly notify the IRB.

11.5.1.3 Reporting to ORP HRPO

Unanticipated problems and social harms reported to the WRAIR IRB through the Human Subjects Protection Branch (HSPB) will be forwarded to USAMRMC ORP HRPO in accordance to the WRAIR SOP "Reporting requirements to USAMRMC for Headquarters – Level review (SOP UWZ-C-636)".

11.5.2 Reporting Additional Immediately Reportable Events to the Sponsor's Representative and ORP HRPO

11.5.2.1 Pregnancy

Each pregnancy beginning during the first 36 weeks of the trial must be reported promptly (within 72 hours of identification) by email or fax to the IRBs and, the sponsor's representative and the ORP HRPO.

Subjects who become pregnant after Day 0 will be followed to term, and the following information will be gathered for outcome, date of delivery, health status of the mother and child including the child's gender, height and weight. Complications and or abnormalities should be reported including any premature terminations. A pregnancy is reported as an AE or SAE only when there is suspicion that the investigational product may have interfered with the effectiveness of contraception or there was a serious complication in the pregnancy including a spontaneous abortion or an elective termination for medical rationale.

11.5.2.2 Reporting AE-related Withdrawal of Consent

Any AE-related withdrawal of consent during the study must be reported promptly (within 72 hours of identification) by email or fax to the sponsor's representative (usarmy.detrick.medcom-usammda.mbx.sae-reporting@mail.mil) and ORP HRPO. Investigators must also follow the specific policies at each institution regarding the timely reporting of withdrawals due to AEs to the IRBs. In all cases, the PI will make a reasonable effort to complete study exit visit procedures.

11.5.2.3 Additional Immediately Reportable Event

See section 15.1.3.

11.5.2.4 Pending Inspections/ Issuance of Reports

The knowledge of any pending compliance inspection/visit by the FDA, Office for Human Research Protections (Department of Health and Human Services), or other government agency concerning clinical investigation or research, the issuance of Inspection Reports, FDA Form 483, warning letters, or actions taken by any Regulatory Agencies including legal or medical actions and any instances of serious or continuing noncompliance with the regulations or requirements will be reported immediately to USAMRMC ORP HRPO and the sponsor's representative.

11.5.3 IND Annual Report to the FDA

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

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

11.5.4 Final Report

A report will be prepared in accordance with ICH E3 Guideline "Structure and Content of Clinical Study Reports" and provided to the sponsor's representative for review and approval. The sponsor's representative will use this report to prepare the final clinical study report for submission to the FDA. A summary of the final study report will be provided to the IRB listed in the study schema.

11.6. Referral and Management of HIV-infected Volunteers

If an HIV infection is suspected in a volunteer according to the diagnostic algorithm (Section 11.1.5.4), the laboratory will inform the site investigator. The volunteer will have an appointment 2-3 weeks after original blood draw for a verification visit (and a second one, if the first is not positive). At the verification visit(s), the volunteer will be counseled based on the need for repeat testing to determine results.

Respecting confidentiality, the volunteer will be counseled to voluntarily inform his/her potentially exposed partner(s) of the HIV test result; the vaccine trial team will offer assistance in counselling and HIV testing for the partner(s). Risk behaviour will be assessed and focused counselling provided. A CD4 count, RNA viral load will be performed as clinical service to facilitate referral. The volunteer will be referred to medical services where s/he can get support and medical care and treatment according to National Health Care

Coverage Scheme s/he is entitled to. Laboratory results of HIV diagnosis, CD4 count and RNA viral load will be sent in a confidential manner to the site investigator who will provide the results to the volunteer's physician.

Volunteers who become HIV-infected during the vaccination phase of the trial (which may be detected via other health care interactions outside the trial's planned visits) will have no further vaccination, and will be counseled by the research team and referred to medical services where they can get support and medical care and treatment according to National Health Care Coverage Scheme to which they are entitled.

The medical services include clinical examination by a physician, necessary laboratory tests, and standard medications in accordance with the most current National Guidelines for Clinical Management of HIV/AIDS in Adults and Children issued by the MOPH.

12. Statistics

12.1. Description of Statistical Methods

12.1.1 Analysis Overview

The study intends to characterize immune responses in individuals vaccinated with the ALVAC-HIV (vCP1521) and AIDSVAX® B/E primary series used in the RV144 protocol and given a booster injection at week 48 with either the ALVAC-HIV/AIDSVAX® B/E combination or AIDSVAX® B/E alone for Groups II and III, at week 60 for Group IVa and week 72 for Group IVb, compared to no boost. This protocol design represents an evaluation of 6 vaccination regimens, 5 of which are active:

- Placebo (n=33) vs.
- Primary series with no boost (n=27) vs.
- Primary series + ALVAC-HIV (vCP1521) and AIDSVAX® B/E boost (n=100) vs.
- Primary series + AIDSVAX® B/E boost (n=100) vs.
- Primary series + ALVAC-HIV (vCP1521) and AIDSVAX® B/E boost (n=50) (Group IVa) vs.
- Primary series + ALVAC-HIV (vCP1521) and AIDSVAX® B/E boost (n=50) (Group IVb)

Subjects in the placebo group will receive single or dual placebo injections and this approach will assist in blinding subjects as to whether active product vaccination has occurred. The placebo recipients will also serve as a source of blinded samples for the set of immunology assays that are the focus of this study. The main interest is in determining whether immunologic differences can be detected between the 5 antigen-containing regimens. Response rates and where applicable titer level distributions with appropriate summary statistics will be estimated for each immunologic assay performed. The study will evaluate a

series of cellular, humoral and mucosal immune assays. In many cases, trial specimens will be used to further evaluate the practicality of sampling, specimen processing and storage procedures combined with assay performance characteristics.

12.1.2 Primary Immunogenicity Analysis and Multiple Endpoints

- Primary immunogenicity assays are the ICS analysis and IFN-γ ELISPOT assay, both of which were performed as immunogenicity assessments of RV144 and earlier studies. For the ICS results, positive response definition is provided by the laboratory and historically a response is defined by the % gated events ≥ 3 x background (unstimulated cells) and the corrected % gated events ≥ 0.05%. A series of control and HIV peptides are examined for the CD4+ and CD8+ fractions and separate assessment of Env and Gag responses performed. Overall and subclass response rates are summarized and tested. It is anticipated that pre-boost staining will be essentially null, but change from baseline will be examined if relevant. Magnitude of staining is also compared. Results will be reported at the time of the final boost and repeated measurements analyses will incorporate within-person correlation effects via GEE (generalized estimating equation) estimation for binary endpoint models and unstructured correlation matrix estimation in linear models with assumed normal error distribution. Similar analytic methods will be used for the ELISPOT assay where the historic definition of positive response is peptide pool SFC/Million PBMC ≥ 4xbackground (unstimulated cells) and the uncorrected (peptide pool SFC/Million PBMC) ≥ 55 SFC/Million PBMC. For both assays, the definition of positive response will be finalized before treatment groups are uncoded.

- Multiple Time points: While immunogenicity samples are collected throughout the study, all active treatment arms are identical through week 48 after which assessments are made at 3 time points (50, 72 and 96 weeks). The principal timepoint is week 50; it is expected that if differences exist they should be detectable at that timepoint. Temporal response profiles will also be compared.

- Contrast Multiplicity: Differences between the week 48 boosted arms and the unboosted arm are anticipated. Global tests of equivalence of the 2 active Week 48 boosted treatment arms (Group II, III) will be made and followed by pairwise contrasts when the global test is significant. The modification to Group IV (50 subjects receive a combined boost at week 60 and 50 subjects receive a combined boost at week 72) adds an additional exploratory analysis. Groups IVa and IVb will still be compared as described above.

- The frequency and titer of HIV-specific antibodies including IgG and IgA measured in mucosal secretions will be compared between timepoints and between vaccine groups using standard statistical methods.

- Safety analysis will include data collected from all randomized subjects. Adverse event data will be listed individually and summarized by body system and preferred terms within a body system for each treatment group. Serious and/or unexpected AEs will also be discussed on a case-by-case basis. For the tabulation of the AEs by body system, a subject will be counted only once in a given body system. For example, a subject reporting nausea and diarrhea will be reported as one subject, but the symptoms will be listed as two separate AEs within the class. Therefore the total number of AEs reported within a body system may exceed the number of subjects within the body system reporting AEs. The total number of AEs reported within body systems between each treatment

group and the control group will be compared using Fisher's exact test. Vaccination reactions will be examined and compared to historical rates from the RV144 protocol.

12.1.3 Analysis Addressing the Secondary and Exploratory Study Objectives

Secondary objectives include estimation of treatment effects and contrasts among the treatment arms. A large set of assay classes are described in Section 10. Consistent with the under replication of the placebo arm, comparisons of the three active boost vaccination regimens are of particular interest. Comparison between groups will be mostly descriptive. Standard parametric and nonparametric estimation and testing methods will be used whenever applicable. Typical statistical methods will be comparable to those described for the primary immunogenicity endpoints.

12.1.4 Subgroup Analysis

Not applicable

12.1.5 Clinical Laboratory Data Analyses

Not applicable

12.2. Planned Enrollment and Reason for Sample Size

The study will include a total of 360 individuals i.e. 327 vaccine recipients and 33 placebo recipients (see Table 3 Study Design). The study sample size will permit detection of large differences in boost vaccination arm response rates at week 50 (>20 percentage points) with adequate power (80%) across the expected response range for selected assays (e.g. ICS with p1=0.30, p2=0.60, power=99%, for 0.4 vs. 0.6 power=83%). Losses to follow-up or non-adherence are not expected to occur in more than 5% of the cases resulting in a small, expected power loss (e.g. ICS with p1=0.30, p2=0.60, power=98%, for 0.4 vs. 0.6 power=79%). The study is also designed to detect differences in response rates of greater than 30% (power=81%) between a boost arm (n=100) and the non-boost arm (n=27). Sample size was derived using a pooled Z-test and, given the explorative nature of the study, a 2-tailed 5% level test for the pairwise comparisons. In addition differences in mean assay levels of up to 0.40 standard deviations for the pairwise comparison of the active boost regimens will be detectable (with power=80%). For directional contrasts with the combined placebo recipients, large differences in response rate for several antibody measures are anticipated. If true rates after placebo injection are 5%, then increases to >20% can be detected with 80% power.

The pairwise comparisons of Group IVa and IVb (50 subjects per group) will have at least 80% power for a two-tailed 5% level test to detect an approximate 30% difference in response rates

between the arms at week 62, week 74, and week 96 as well approximately 0.6 standard deviation differences between the two groups. Given the exploratory nature of the comparison, no adjustments for loss to follow-up or multiple comparisons are incorporated.

12.3. Level of Significance to be Used

Five percent level two-sided tests will be used throughout when comparing the active boost regimens.

12.4. Statistical Criteria for the Termination of the Trial

12.4.1 Interim Analysis and Stopping Rules

There are no planned interim analyses for purpose of trial termination and no statistical criteria for study termination in this clinical trial.

12.5. Accounting for Missing, Unused, and Spurious Data

Non-analyzable data will be documented in the deviations.

12.6. Procedures for Reporting Alterations from the Original Statistical Plan

Any alteration(s) from the original statistical plan as indicated in the protocol will be described in an amendment to the protocol and the SAP. The protocol amendment will be submitted to all IRBs.

12.7. Selection of Subjects to be Included in Analyses

To the extent possible given available data all randomized subjects will be included in the safety analyses while immunogenicity analyses will focus on treated subjects with product administration.

13. Direct Access to Source Data/Documents

Subjects will be identified on eCRFs by a unique subject identification number and on source documents. This number will be automatically generated by the Clinical Appointment Scheduling and Tracking System and will contain no personal associations. Each site will have a site-specific first digit in the numerical code. No personal identifier will be used in any publication or communication used to support this research study. The subject identification number will be used if it becomes necessary to identify data specific to a single subject. Representatives of USAMRMC, the sponsor's representative, the IRBs listed in the study schema, and the FDA are eligible to review medical and research records related to this study as a part of their responsibility to protect human subjects in clinical research. Personal identifiers will be removed from photocopied medical and research records.

13.1. Study Monitoring

Study monitoring will be the responsibility of the USAMMDA Clinical Support Services Division. Upon successful approval of the protocol and establishment of the Regulatory File, the clinical monitor will establish a clinical monitoring plan. To ensure that the investigator and the study staff understand and accept their defined responsibilities, the clinical monitor will maintain regular correspondence with the site and may be present during the course of the study to verify the acceptability of the facilities, compliance with the investigational plan and relevant regulations, and the maintenance of complete records.

Monitoring visits by a sponsor's representative-designated clinical monitor will be scheduled to take place at the initiation of the study, during the study at appropriate intervals, and after the last subject has completed the study. A report of monitoring observations will be provided to the principal investigator (for corrective actions), USAMRMC Division of Regulated Activities and Compliance, and the product manager.

All clinical and research records must be available for review by the sponsor's representative, USAMRMC, WRAIR, representatives of the USAMRMC ORP, representatives of the FDA, OHRP, local IRB representatives, and other regulatory agencies as part of their responsibilities for insuring the protection of research participants.

13.2. Audits and Inspections

Authorized representatives of the sponsor, the FDA, the independent ethics committee or institutional review board may visit the site to perform audits or inspections, including source data verification. The purpose of the audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, GCP guideline of the ICH, and any applicable regulatory requirements.

The investigator should contact the sponsor's representative and ORP HRPO immediately if contacted by a regulatory agency about an inspection.

13.3. Institutional Review Board

The principal investigator must obtain IRB approval for the investigation. Initial IRB approval, and all materials approved by the IRBs for this study including the subject consent form and recruitment materials must be maintained by the investigator and made available for inspection.

The PI will be responsible for preparing and submitting continuing review reports per institution and IRBs' policies. The PI or a designee will submit the approved continuing review reports and the WRAIR IRB, MOPH EC, RTA IRB, Mahidol Faculty of Tropical Medicine Ethical Review Board, RIHES Human Experimentation Committee, Chulalongkorn IRB approval notifications to HRPO as soon as the documents are available.

The PI or designee will transmit the approved final study report and the WRAIR IRB, MOPH EC, RTA IRB, RIHES Human Experimentation Committee, Chulalongkorn IRB and Mahidol Faculty of Tropical Medicine Ethical Review Board approval notification to the USAMRMC ORP HRPO as soon as the documents are available.

14. Quality Control and Quality Assurance

To ensure compliance with GCP and all applicable regulatory requirements, the sponsor's representative may conduct quality assurance audits. Refer to section 14 for more details regarding the audit process.

Auditing of the clinical trial may be conducted at any time during the study to ensure continued compliance with regulations, policies and procedures. Auditing will be undertaken, as needed, by independent personnel designated by the Quality Office, USAMRMC. Audit findings will be documented in a formal audit report that will detail the conduct of the audit and summarize the observations noted.

15. Ethics

15.1. Ethics Review

The study is based on suitably performed laboratory and animal experimentation; the study will be conducted under a protocol reviewed by the study site institutional review boards (or ethics committee or review committee); the study is to be conducted by scientifically and medically qualified persons; the benefits of the study are in proportion to the risks; the rights and welfare of the subjects will be respected; the physicians conducting the study will ensure that the hazards do not outweigh the potential benefits; the results to be reported will be accurate; subjects will give their informed consent and will be competent to do so and not under duress; and all study staff will comply with the ethical principles in 21 CFR Part 50 and the Belmont Principles.

15.1.1 Review/Approval of Study Protocol

Before a clinical study can be initiated, the study protocol and other required documents will be submitted to the following departments in the order listed for review and/or approval, with the final review by the FDA:

- Integrated Product Team
- Combined DAIDS -WRAIR Scientific Review Committee
- Sponsor's Representative Team (Division of Regulated Activities and Compliance, USAMMDA, DRAC, CSSD, SRAA)
- WRAIR IRB, RTA IRB, Chulalongkorn IRB, RIHES Human Experimentation Committee, Mahidol Faculty of Tropical Medicine Ethical Review Board
- Commander, WRAIR, if applicable
- Office of Research Protections, Human Research Protection Office (ORP HRPO)
- Sponsor's Representative (acting for The Office of the Surgeon General of the Army)
- USAMRMC Commanding General, if applicable

Enrollment in this protocol may not begin until all approvals have been obtained and the formal authorization letter is received by the PI from the sponsor's representative.

15.1.2 Protocol Modifications

All modifications to the protocol and supporting documents (informed consent, recruitment materials, etc) must be reviewed and approved prior to implementation. Any protocol amendment will be agreed upon and approved by the sponsor's representative prior to submission to the IRBs and prior to implementation of said change or modification. The informed consent document must be revised to concur with any

amendment as appropriate and must also be reviewed and approved with the amendment. Any subject already enrolled in the study will be informed about the revision and asked to sign the revised informed consent document if the modification directly affects the individual's participation in the study. A copy of the revised, signed, and dated informed consent document will be given to the subject. All original signed versions of the informed consent document will be retained in the volunteer's study folder.

Any modification that could potentially increase risk to subjects must be submitted to the HRPO for approval prior to implementation. Documentation that the IRBs reviewed and approved the modifications also will be submitted. All other amendments will also be submitted to the HRPO for inclusion in the HRPO study file.

15.1.3 Protocol Deviation Procedures

A protocol deviation is defined as an isolated occurrence involving a procedure that did not follow the study protocol. The timeline for reporting protocol deviations to the Human Subjects Protection Branch (HSPB) and WRAIR Institutional Review Board (IRB) is determined by the categorization of the deviation.

Emergent/significant deviations are defined as those that jeopardize the safety or rights of a subject or the scientific integrity of the study. These deviations will be brought to the protocol team for review and concurrently the PI will report these deviations to the local IRBs. The HIV Vaccine Product Manager will report these deviations to the Sponsor, to the HSPB, the WRAIR IRB, and the ORP HRPO by email and in writing within 10 business days of becoming aware of the deviation.

Non-emergent/non significant deviations will be reported annually in the continuing review report to all IRBs and HRPO and in the final study report. This report will include a description of the deviation, any actions taken in response to the deviation and an assessment of the impact of the deviation.

15.2. Ethical Conduct of the Study

This study will be conducted in accordance with 21 CFR Part 50 and the Belmont Principles and local regulations of respect for persons, beneficence, and justice.

The procedures set out in this study are designed to ensure that the sponsor's representative and all study personnel abide by the principles of the ICH GCP Guideline and the CFR. The PI confirms this by signing this study protocol and FDA Form 1572.

15.2.1 Confidentiality

In this research, the subject's health information will be collected and used to conduct the study; to monitor the subject's health status; to measure effects of the investigational product; to determine research results, and possibly to develop new tests, procedures, and commercial products. Health information is used to report results of research to the sponsor's representative and Federal regulators and may be reviewed during study audits for compliance with study plans, regulations, and research policies.

No personal identifier will be used in any publication or communication used to support this research study. The subject's identification number will be used in the event it becomes necessary to identify data specific to a single subject.

15.2.2 Compensation for Participation

Information on compensation is described in the participant consent form. Participants will be compensated for each study visit, including the post-test counselling visits, for time lost from work, travel expenses, and meals. In addition, specific compensation will be provided for optional procedures including mucosal samplings, bone marrow aspiration and leukapheresis.

- For each normal scheduled including screening visit: 1000 Baht

In addition:

- For each mucosal secretion collection: 1000 Baht
- For biopsy procedure: 2000 Baht
- For leukapheresis procedure: 2000 Baht
- For bone marrow aspiration procedure: 3500 Baht

15.2.3 Redress of Research-Related Injury

The US DoD and DAIDS/NIAID/NIH are funding this protocol. As stated in the consent form, participants who experience illness or injury arising from participation in the study will receive medical care as 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 cost associated with this study, there is a limit to the amount of coverage available. Other than medical care, and other payments as stated in the consent form, there is no other compensation available from this research study.

15.3. Written Informed Consent

The informed consent process and document will be reviewed and approved by the IRBs and sponsor's representative prior to initiation of the study. The consent document contains a full explanation of the possible risks, advantages, and alternate treatment options, availability of treatment in the case of injury, in accordance with 21 CFR 50, and agreement that samples can be analyzed for future use. The consent document indicates that by signature, the subject, or where appropriate, legal guardian, permits access to relevant medical records by the sponsor's representative and by representatives of the FDA. The sponsor's representative will submit a copy of the initial IRB- and sponsor's representative-approved consent form to the FDA and will maintain copies of revised consent documents that have been reviewed and approved by the IRBs.

A written informed consent document, in compliance with 21 CFR Part 50, 32 CFR Part 219, and the Belmont Principles, will be signed by all subjects before any study-related procedures are initiated for each subject. This consent document will be retained by the investigator as part of the study records. The investigators or their designees will present the protocol in lay terms to individual subjects. Questions on the purpose of the protocol, protocol procedures, and risks to the subjects will then be solicited. Questions will be answered by the appropriate member of the study team. No subject should grant consent until questions have been answered to his/her satisfaction. The subject should understand that the investigational product is an investigational drug and is not licensed by the US or Thai FDA for commercial use, but is permitted to be used in this clinical research. Informed consent includes the principle that it is critical the subject be informed about the principal potential risks and benefits. This information will allow the subject to make a personal risk versus benefit decision and understand the following general principles:

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

Should the protocol be modified, the subject consent document must be revised to reflect the changes to the protocol. If a previously enrolled subject is directly affected by the change, the subject will receive a copy of the revised informed consent document. The approved revision will be read, signed, and dated by the subject. A description of this clinical trial will be available on http://www.ClinicalTrials.gov. The data stored in this website will be an aggregate summary of the trials results and will not contain individual demographic information or personal identifiers.

Volunteers may withdraw from the study at any time during the study.

15.4. End of Study Unblinding

Once all study visits have been completed, the site investigators will contact study participants inviting them to come back to the clinic to find out whether they received the study vaccines or placebo. When the

participant returns to the clinic for unblinding, they will be given another letter, which indicates their study arm.

Clinic visits may occur following the final visit for the purpose of providing unblinding or other information, if needed.

15.5. Social Harm Reporting

Unanticipated events and social harms may occur during the course of the study. When such events are related to study participation, the study staff, informed of these events, will inform the PI or his/her designee. The PI or designee will then prepare a narrative summary of the event and report to the IRBs and MHRP ROC. The Pharmacovigilance Committee including DAIDS Medical Officer and WRAIR, Human Subjects Protection Branch (HSPB), will then be informed. The local medical 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 USAMRMC ORP HRPO.

16. Data Handling and Recordkeeping

The primary source documents for this study will be the volunteer's study folder. If separate research records are maintained by the investigator(s), the medical record and the research records will be considered the source documents for the purposes of auditing the study. The source documents will be retained at the site.

For this study, an iDataFax database system will be used for the collection of the study data in an electronic format. The iDataFax database system will be designed based on the protocol requirements, the approved eCRF layouts and specifications, and in accordance with 21 CFR Part 11. The eCRF layouts and specifications define and identify the applicable source data that will be collected and captured into the iDataFax database system. The applicable source data will be electronically transcribed by the site designee onto the (eCRF in the iDataFax database system. No source data will be recorded directly in the eCRF without a prior written record of the data. The investigator is ultimately responsible for the accuracy of the data entered into the eCRF. Data monitoring and management will be performed in the iDataFax database system by the study monitor and the designated Data Management group.

A detailed data management plan will be written and approved by the Sponsor, the study team and the PI. The plan will be drafted prior to study initiation but will be finalized before study closeout and database lock.

16.1. Inspection of Records

The sponsor’s representative or designee will be allowed to visit the investigation facilities for the purpose of monitoring any aspect of the study. The investigator agrees to allow the monitor to inspect the drug storage area, investigational product stocks, drug accountability records, subject charts, study source documents, and other records relative to study conduct.

Subjects' health information is used to report results of research to the sponsor's representative and regulators and may be reviewed during study audits for compliance with study plans, regulations, and research policies. The consent document indicates that by signature, the subject permits access to relevant medical records by the sponsor's representative and by representatives of the US and Thai regulatory bodies.

16.2. Retention of Records

For this study, an eCRF will be used for study data collection and monitoring. Completed, monitored eCRFs will be electronically stored with password protection in a secure location by the sponsor's representative or designee. A copy of each completed eCRF will be retained by the investigator. If it becomes necessary for the sponsor's representative or designee or the FDA to review any documentation relating to the study, the investigator must permit access to such records. The clinical trial information will be entered into a database maintained by the National Institutes of Health/National Library of Medicine (NIM/NLM).

Federal regulations require that the PI retain a copy of all records that support eCRFs for this study (i.e., ICFs, clinical laboratory results, source documents, IP dispensing records) for whichever of the following is the shortest:

- Two years following the date of approval by the FDA of the IP for the purposes that were the subject of the investigation; or
- Five years following the date on which the results of the investigation were submitted to the FDA in support of, or as part of, an application for a research or marketing permit for the IP for the purposes that were the subject of the investigation.

If the investigation does not result in the submission of data in the support of, or as part of, an application for a research or marketing permit, records must be retained for 2 years following notification by the Office of the Surgeon General, or his representative, that the entire clinical investigation (not merely the PI's portion) is completed, terminated or discontinued; or for 2 years following the withdrawal of an Investigational New Drug Application.

If a PI retires, relocates or for other reasons withdraws from the responsibility of keeping the study records, custody must be transferred to a person who will accept this responsibility. The Office of the Surgeon General, or his representative, must be notified in writing of the name and address of the new custodian.

The PI will be responsible for retaining sufficient information about each subject, i.e., name, address, telephone number, and subject identifier in the study, so that the sponsor's representative, the WRAIR IRB, MOPH EC, RTA IRB, Chulalongkorn IRB, Mahidol Faculty of Tropical Medicine Ethical Review Board, RIHES Human Experimentation Committee, the FDA, employees of USAMRMC, or other regulatory authorities may have access to this information should the need arise.

It is the policy of the USAMRMC that Volunteer Registry Data Sheets are completed on all volunteers participating in greater than minimal risk research for entry into the Command's Volunteer Registry database. The Volunteer Registry Database will collect the following data on volunteers:

- Names (first and last name)
- Date of birth
- Contact information, both permanent and local
- Study name and study dates, and dates of individual's participation
- Serious adverse event and unexpected adverse events related to the vaccines experienced during the time of trial participation.
- Details of the product received

The intent of the database is two-fold: first, to readily answer questions concerning an individual's participation in research sponsored by USAMRMC; and second, to ensure that the USAMRMC can exercise its obligation to ensure research volunteers are adequately warned (duty to warn) of risks and to provide new information as it becomes available. The information will be stored in Thailand for 75 years under the responsibility of AFRIMS in coordination with Faculty of Tropical Medicine, Mahidol University.

Clinical trial information from this study will be kept at the database at the National Medical Library in the United States of America/ the National Institute of Health of the United States of America (www.clinicaltrials.gov). The data stored in this website will be an aggregate summary of the trials results and will not contain individual demographic information or personal identifiers.

17. Publication Policy

All data collected during this study will be used to support this IND. All data may be published in the open medical or military literature with the identity of the subjects protected. Anyone desiring to publish or present data obtained during the conduct of the study will conform to study site policies and then forward the publication for review to the Commander, USAMMDA and USAMRMCCLEARANCES@amedd.army.mil prior to submission.

18. Appendices

Appendix A. Test of Understanding (TOU)

Appendix B. Main Informed Consent Form

Appendix C. Cervical Biopsy for Female Participant – Informed Consent Form

Appendix D. Sigmoid biopsy – Informed Consent Form

Appendix E. Leukapheresis - Informed Consent Form

Appendix F. Bone Marrow Aspiration- Informed Consent Form

Appendix G. Study Personnel Roles and Responsibilities

Principal Investigator: Responsible for the study design and will serve as a liaison between the sites, vaccine developer and Sponsor, and contribute support to overall project management and the analysis and reporting of the study data. The PI will take all necessary precautions to ensure that the study obtains the proper clearance for all publication and abstracts, and maintain a study regulatory file as instructed by the study IND sponsor.

Senior Site-Investigators: Will oversee the conduct of the study at the clinic, and are responsible for local IRB submission and approval, study conduct, and reporting all unanticipated problems and adverse events to the protocol team and the IRBs. They may conduct the participant study visits, assist with AE assessment and reporting, report any findings and study status directly to the study PI, and coordinate with the PI in the planning, design, and execution of the study.

Site Investigators: Will assist the Senior Site Investigators in the conduct of the study. They will conduct participant study visits, assist with AE assessment and reporting and coordinate with all clinic staff in the planning of participants study visits and study procedures.

Community Advisory Board Chairperson: Liaise between the Principal Investigator and communities where the volunteers are recruited and conveys questions, concerns, advises and recommendations regarding volunteers' participation in the study.

Study Medical Monitor: will ensure uniformity of clinical determinations made by investigators at clinical sites. The SMM will be responsible for assessing medical issues related to protocol conduct, adherence to inclusion/exclusion criteria and protection of all human subjects participating the study. The SMM will provide clinical input to questions from investigators, site physicians, and coordinators. All deaths and serious or unexpected adverse events will be reported to the SMM by the Principal Investigator. The SMM will participate with other members of the clinical operations project team to evaluate and appropriately process serious adverse events (SAEs). The SMM will ensure that standards for safety reporting follow all US and Thai government and DoD guidelines and regulations. The SMM will review all safety data summaries for submission to institutional review boards and any applicable external advisory boards.

Local (Independent) Medical Research Monitor (LMRM): The LMRM will serve as an independent physician who can be approached for medical information by volunteers, act as their advocate and assess their medical care for events which occur during the course of the trial. He/she will also collaborate with the Study Medical Monitor to oversee the progress of the clinical trial and ensure that it is conducted, recorded, and reported in accordance with the protocol, standard operating procedures (SOPs), GCP, and the applicable regulatory requirements.

The LMRM shall have the following specified human subject safety-related authorities at the site that they represent: stop research activities, remove human subjects from the study, review monitoring plans, and participate in event assessment and reporting.

US Army HIV Vaccine Product Manager:

A qualified physician stationed at the MHRP, WRAIR, chartered by the Principal Assistant for Acquisitions at MRMC who is responsible for cost, schedule, and performance of all HIV vaccine advanced development activities, including this trial. Activities include protocol development, oversight of study operations, and primary liaison with the WRAIR IRB and regulatory personnel at USAMMDA.

Counsellors: Counsellors can be physician, nurse or any specifically trained study personnel to perform HIV pre- and post-test HIV counselling at clinical study site.

Consultants: Protocol consultants are responsible for providing input for the study design, protocol development, and serve as technical advisors and subject matter experts for study execution.

Data Manager: Responsible for overall data management and providing a final data transfer of all clinical trial data to the Sponsor no later than 20 business days following final database closure including, but not limited to, the data dictionary, formats, assignments, and any accompanying memorandums. The media format and transfer specifications will be agreed upon with the Sponsor at the time of the database prior to closure.

Statistician: Will receive coded data from the data manager and use those data to perform all investigational data analyses in collaboration with MHRP and the study team.

Appendix H. DAIDS Table for Grading Severity of Adverse Events

19. List of References

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