IHV01

A PHASE I SAFETY AND IMMUNOGENICITY TRIAL OF IHV01 IN HIV-1 UNINFECTED VOLUNTEERS

DATE: 3/27/17

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MANUFACTURER: Profectus Biosciences

PROTOCOL NUMBER: FLSC-001

IND NUMBER: 16505

CONFIDENTIAL

INVESTIGATOR'S AGREEMENT

I have received, read the Investigator's Brochure for IHV01 and agree to conduct the study as outlined. By my signature below, I attest that I have read, understood, and agree to abide by all conditions, instructions, and restrictions contained in this protocol (including appendices). I will not initiate this study without approval from the appropriate Institutional Review Board (IRB) and I understand that any changes in the protocol must be approved in writing by Profectus BioSciences, Inc. and the IRB before they can be implemented, except where necessary to eliminate immediate hazards to the volunteer. I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

_____

Printed Name of Investigator

_____

Signature of Investigator

_____

Date

PROCEDURES IN CASE OF EMERGENCY

Table 1: Emergency Contact Information

Role in Study	Name	Address and Telephone number
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Responsible Physician	Charles E. Davis Jr., M.D.	Institute of Human Virology 725 West Lombard St, Room N555 Baltimore, MD 21201 410-706-4608
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2. SYNOPSIS

Name of Sponsor/Company: Sponsor: Institute of Human Virology Manufacturer: Profectus Biosciences	
Name of Investigational Product: IHV01	
Name of Active Ingredient: Full Length Single Chain (FLSC), Aluminum Phosphate	
Title of Study: A Phase I Safety and Immunogenicity Trial of IHV01, in HIV-1 Uninfected Volunteers	
Study center(s): Institute of Human Virology	
Principal Investigator: Charles E. Davis Jr., M.D. **Investigators:** Joel Chua, M.D. David J. Riedel, M.D.	
Studied period (years): 1 year Estimated date first patient enrolled: November 2015 Estimated date last patient completed: May 2018	**Phase of development:** I
Objectives: **Primary:** To evaluate the safety and tolerability of intramuscular administration of the IHV01 at three different doses. **Secondary:** To evaluate the immunogenicity of three different doses of the IHV01, a full length single chain (FLSC) gp120-CD4 complex vaccine. This will be assessed by the following parameters: 1) anti-FLSC antibodies, and 2) anti gp-120 (BaL), and 3) competitive titers to CD4i epitopes.	
Methodology: Single center, randomized, dose escalation, placebo-controlled, double-blind trial	
Number of patients (planned): 60	
Diagnosis and main criteria for inclusion: Inclusion/Exclusion Criteria as in Section 7.1 and Section 7.2.	
Investigational product, dosage and mode of administration: IHV01 in sequential dose escalation of 75 ug, 150 ug and 300 ug administered intramuscularly.	
Duration of treatment:	

Volunteers will be immunized with candidate vaccine or placebo on Days 0, 28(week 4), 56(week 8), and 168(week 24). Volunteers will continue to undergo active protocol follow up visits on Days 182(week 26), 196(week 28), 252(week 36). 294 (week 42) and 336 (week 48). The total trial time expected per volunteer is 48 weeks. The protocol recruitment period is expected to be approximately 36 weeks. The total trial duration is estimated to be approximately 72-96 weeks.

Reference therapy, dosage and mode of administration:

Placebo consisting of preservative free normal saline in volumes equal to the investigational product administered intramuscularly.

Criteria for evaluation:

Safety:

1. Monitoring and assessment of hematological, chemical, and immunologic parameters (See Section 11.1.5, Section 12.1, and Table 4).
2. Monitoring participants for local and systemic adverse reactions after each injection and for 12 months after the first injection (Section 11.2).
   a. Local reactogenicity signs and symptoms
   b. Systemic reactogenicity signs and symptoms
   c. Adverse and serious adverse experiences

Immunogenicity:

See Section 12.1 and Section 12.2.

Statistical methods:

Each safety parameter assessed will be compared between vaccine recipients and placebo recipients as determined by Student's T test *and chi-square analyses*. In addition safety parameters with be compared between vaccine dose groups as well as to placebo recipients. Further analyses as described in Section 13.

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3. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

The following abbreviations and specialist terms are used in this study protocol.

Table 2: Abbreviations and Specialist Terms

Abbreviation or Specialist Term	Explanation
Ab	Antibody
ACTG	AIDS Clinical Trials Group
AE	Adverse event
ALT	Alanine aminotransferase
AST	Asparatate aminotransferase
BP	Blood pressure
CBC	Complete Blood Count
CMI	Cell Mediated Immune
CPT	Cell Preparation Tube
CRF	Case Report Form
CTL	Cytotoxic T Lymphocyte
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-Linked ImmunoSorbent Assay
FLSC	Full Length Single Chain gp120-CD4 complex Vaccine
GCP	Good Clinical Practice
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
IB	Investigator's Brochure
ICC	Intracellular Cytokine Cytometry
ICH	International Conference on Harmonization
IHV	Institute of Human Virology
IEC	Independent Ethics Committee
IM	Intramuscular
IRB	Institutional Review Board
OAE	Other significant adverse event
PBMC	Peripheral Blood Mononuclear Cells
PCR	Polymerase Chain Reaction

Abbreviation or Specialist Term	Explanation
PI	Principal Investigator The investigator who leads the study conduct at an individual study center. Every study center has a principal investigator.
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
RBC	Red Blood Cell
RNA	Ribonucleic Acid
SAE	Serious adverse event
SHIV	Simian/HIV virus
SIV	Simian Immunodeficiency virus
SD	Standard deviation
UMMC	University of Maryland Medical Center
WB	Western-Blot

4. INTRODUCTION

4.1. Name and Description of the Investigational Product

The investigational product is IHV01 which consists of the Full Length Single Chain (FLSC) gp120-CD4 chimera subunit vaccine formulated in Aluminum phosphate adjuvant (Alum). The formulation consists of 0.3 mg/ml of FLSC, 2.4 mg/ml of Alum, 5 mM NaOAc, 40 mg/mL mannitol, pH 6.2. The product intended use is as a prophylactic vaccine to prevent infection with HIV. FLSC is encoded by a synthetic gene, which contains a human codon-optimized HIV(BaL) gp120 sequence (an R5-tropic envelope), followed human CD4D1D2, connected together by a flexible 20 amino acid (S-G-A) linker that covalently links the gp120 and CD4 portions. The gp120 sequences are translated as the N terminus of the chimera and the CD4 sequences at the C terminus. This construction allows the gp120 and CD4 moieties to form a stable intrachain binding interaction that forms a transition state structure that presents conserved, conformational domains involved in the early HIV replication process (Fouts, Tuskan et al. 2000).

4.2. Trial Design Overview

This study is designed to evaluate the safety of the IHV01 vaccine and will be a randomized, placebo-controlled, modified double-blinded dose escalation study in 60 healthy adult volunteers. Although there is a great deal of experience with protein vaccines, the optimal dose of IHV01 given intramuscularly is not known. Therefore, this trial is designed as a dose escalation trial in order to determine the optimal dose to move forward with in future clinical trials. This dose will be chosen after reviewing the safety and immunogenicity data from this trial. Alum has been chosen as an adjuvant due to the extensive amount of safety data available. The study will have 3 arms including placebo, 75 µg dose, 150 µg dose, and 300 µg dose and will last for 336 days (48 weeks). Volunteers will be immunized on Day 0, Day 28(4 weeks), Day 56(8 weeks), and Day 168(24 weeks). Each dose group will have 15 vaccine and 5 placebo recipients. The dosage schedule in this trial was based on the animal data and data with other envelope protein vaccines, and is patterned after the schedule utilized in the RV144 trial in Thailand. From this trial, an optimal dose of the candidate vaccine will be chosen for use in future trials. It is anticipated that the trial will be enrolled and completed 18 months after it is open to enrollment.

4.3. Rationale for testing IHV01 vaccine (FLSC) in a phase 1 clinical trial

The challenge for HIV vaccine development stems from evolutionary pressures that abrogate the immunogenicity of conserved, functional epitopes on the envelope spike that are potential targets for cross-reactive antibodies. Large areas are masked by a "glycan shield" of carbohydrate molecules (Leonard, Spellman et al. 1990; Kwong, Wyatt et al. 1998; Wei, Decker et al. 2003) and extensive conformational flexibility (sometimes termed "conformational masking") (Myszka, Sweet et al. 2000; Kwong, Doyle et al. 2002; Chen, Vogan et al. 2005) that dampen immunogenicity of the conserved functional domains. The remaining immunogenic domains ("variable" or "V" loops) tolerate a high degree of sequence variability (Hahn, Gonda et al. 1985; Saag, Hahn et al. 1988; Wyatt, Kwong et al. 1998; Hartley, Klasse et al. 2005) and generate "type-specific" neutralizing antibodies (Putney, Matthews et al. 1986; Rusche, Javaherian et al. 1988; Fung, Sun et al. 1992; Sattentau 1996) that are not cross-reactive. The IHV01 vaccine (FLSC) design approach solves these problems by "locking" the conformation of the envelope into a highly

immunogenic moiety that induces antibodies that target highly conserved epitopes on gp120. Such antibodies would be highly cross-reactive and potentially useful for HIV vaccine development (see Section 4.4 and Section 4.5).

There is a growing body of evidence supporting the FLSC approach. In particular, the anti-CD4i epitope antibodies raised by FLSC have the potential to facilitate "non-neutralizing" control of infection via Fc-Receptor-dependent mechanisms that have been correlated with protection in past studies (Ferrari, Pollara et al. ; Sun, Asmal et al. ; Gomez-Roman, Patterson et al. 2005; Hessell, Hangartner et al. 2007; Florese, Demberg et al. 2009; Hessell, Rakasz et al. 2009; Hidajat, Xiao et al. 2009; Rerks-Ngarm, Pitisuttithum et al. 2009; Xiao, Zhao et al. 2010; Barouch, Liu et al. 2012; Haynes, Gilbert et al. 2012; Robb, Rerks-Ngarm et al. 2012; Barouch, Stephenson et al. 2013; Lewis 2013; Tomaras, Ferrari et al. 2013; Vargas-Inchaustegui and Robert-Guroff 2013). Antibodies to certain CD4i epitopes are especially potent mediators of ADCC activity against freshly targeted host cells and chronically infected cells (Ferrari, Pollara et al. 2011; Guan, Pazgier et al. 2013; Pollara, Bonsignori et al. 2013). Serum antibodies exhibiting Fc-mediated antiviral activity were correlated with the rate of acquiring HIV infection after gp120 immunization in vaccinees from the Vax004 trial (Wilflingseder, Banki et al. 2007); similar correlates were observed in Thai volunteers receiving the ALVAC-HIV/AIDSVAC B/E prime/boost regimen (Rerks-Ngarm, Pitisuttithum et al. 2009; Haynes, Gilbert et al. 2012). Specifically, ADCC activity was correlated with reduced risk of infection in vaccinees with lower IgA responses. Since plasma IgA poorly mediates ADCC, these data suggest that IgG-dependent ADCC is beneficial as long as it is not dampened by competitive plasma IgA responses (Haynes, Gilbert et al. 2012; Tomaras, Ferrari et al. 2013). In accordance with these findings, we have determined that similar responses raised in nonhuman primates by rhFLSC can be correlated with resistance to heterologous virus challenge (DeVico, Fouts et al. 2007; Fouts, Bagley et al. 2015) (see Investigator's Brochure Section 4 and 6.1).

4.4. Findings from nonclinical studies that potentially have clinical significance

4.4.1. Immunological markers of protection

Proof of concept studies show that rhFLSC can elicit protection against SHIV acquisition under experimental conditions that mimic those likely to confront the deployment of an effective HIV vaccine. These include transmitting viruses that are heterologous to the vaccine and are difficult to neutralize. They also show that parenteral immunization can protect against mucosal acquisition, which a probable requirement for an effective HIV vaccine. Proof-of-concept challenge studies also showed that the immune response that provided the best protection in either challenge model was directed to the CD4 induced epitopes that were preferentially presented by the FLSC. These characteristics included a ratio of serum antibodies titers to FLSC/gp120 > 2 and the presence of antibodies titers > 1:100 directed to CD4i epitopes defined by human MAbs 17b, 19e, A32, and N12-i2. Since the FLSC/aluminum phosphate formulation was not evaluated in these studies, a study was performed with cynomolgus monkeys to evaluate the immune response generated with clinical formulation. One group of animals was immunized with rhFLSC also formulated in aluminum phosphate to account for any immunogenicity generated to the heterologous CD4 used in the human FLSC. Cynomolgus macaques were also selected because the region of their CD4 is homologous to the rhesus CD4 used in rhFLSC. The immune response

generated by the aluminum phosphate formulation was robust with antigen specific serum antibody responses directed towards epitopes presented by FLSC. All immunized animals presented FLSC/gp120 ratio >2. Animals immunized with FLSC presented a higher ratio as compared to those immunized with rhFLSC most likely because a portion of the antibody response in the FLSC groups is directed to the heterologous human CD4. All groups also presented competitive serum antibodies directed to CD4i epitopes recognized by 17b, 19e, A32, and N12-i2 that were >200 EU and, in some animals, were >1000 EU. Collectively, these data demonstrate that the immune response generated by the FLSC/ aluminum phosphate formulation will induce a CD4i directed immune response that similar to that generated by FLSC in our proof-of-concept studies.

These proof-of-concept challenge studies and the immunogenicity studies in cynomolgus macaques are summarized in Investigator's Brochure Section 4 and 6.1.

4.4.2. Safety/Toxicity Data

The safety of FLSC absorbed to aluminum phosphate was evaluated in a GLP-compliant, repeat-dose toxicity study in rabbits (Study Number 5000069) and in an immunotoxicity study in cynomolgus monkeys (Study Number VTR-0018).

In the repeat dose toxicity study in rabbits, FLSC was absorbed to aluminum phosphate and dosages of 300 µg/2.4 mg or 900 µg/7.2 mg FLSC/Adjuvant were intramuscularly injected once every three weeks (Days 1, 22, 43, 64 and 85) for 5 total administrations. For the immunotoxicology study, cynomolgus monkeys were repeatedly intramuscularly vaccinated with FLSC or rhesus FLSC (containing HIV gp120 and rhesus CD4). After the third and fourth vaccination, neoantigen KLH was administered. The FLSC contains the D1D2 domains of human CD4 that is only 88% identical to that of cynomolgus and rhesus monkey CD4s. The rhesus CD4 D1D2 amino acid sequence is 100% identical to the cynomolgus CD4 D1D2 sequence, which allows for the evaluation of the induction of antibodies that may cross-react with the endogenous cynomolgus CD4 protein. The rhFLSC was used as a mimic for the test of FLSC in humans. Prior to the pivotal study, a pilot study verified that inhibition of CD4 activity in the cynomolgus monkey negatively affected the immune response against KLH (Study Number VTR-0016 (Study VTR-0018, Appendix 16)). Measures of autoimmune responses to CD4 that may be deleterious included drop in CD4 count, significant impact in the serum response to neoantigen KLH, and drop in *in vitro* proliferation of cells after addition of immune sera in a mix lymphocyte reaction assay.

The toxicology studies showed that repeated intramuscular vaccinations with 300 or 900 µg FLSC (with aluminum phosphate adjuvant) was well tolerated in rabbits (up to 5 administrations) and cynomolgus monkeys (up to 4 administrations). The majority of the effects observed in the rabbit toxicity study can be attributed to general inflammation occurring with intramuscular vaccine administration and/or an active immune response towards the antigen. No deleterious effects on the immune system were detected in the cynomolgus monkey with repeat administration of up to 900 µg FLSC (with aluminum phosphate adjuvant). Of most concern is the potential test article related reduction in platelet count with FLSC; however, this finding only occurred in the rabbit with repeat administration of the high dose of FLSC (900 µg), which is 12-fold greater than the proposed starting dose of 75 µg FLSC in the clinic and 3-fold greater than the proposed maximum clinical dose of 300 µg FLSC. Thus, the safety risk of 4 intramuscular administrations of FLSC (with aluminum phosphate adjuvant) to the patient is low.

4.5. Findings from clinical trials that are relevant to the proposed trial

4.5.1. Correlates of protection

While the need for a preventive HIV vaccine effective worldwide becomes more urgent every year, the correlates of immunity or protection are still not well understood. This makes it difficult to determine the optimal characteristics of an HIV vaccine candidate. At this point, the general consensus view is that a successful HIV vaccine candidate must raise humoral immune responses that recognize conserved domains on the viral envelope and mediate various antiviral effector mechanisms. This view is reinforced by the RV144 clinical trial, which demonstrated modest protection against HIV infection in Thailand that correlated with anti-gp120 antibody responses (Rerks-Ngarm, Pitisuttithum et al. 2009; Haynes, Gilbert et al. 2012). Such mechanisms could include 1) direct neutralizing activity, as passive transfer of broadly neutralizing antibodies affords protection against infection in nonhuman primate models (Mascola, Lewis et al. 1999; Baba, Liska et al. 2000; Hessell, Hangartner et al. 2007); 2) ADCC activity, given its past associations with protection against HIV, SHIV or SIV infection (Ferrari, Pollara et al. ; Sun, Asmal et al. ; Gomez-Roman, Patterson et al. 2005; Hessell, Hangartner et al. 2007; Florese, Demberg et al. 2009; Hessell, Rakasz et al. 2009; Hidajat, Xiao et al. 2009; Rerks-Ngarm, Pitisuttithum et al. 2009; Xiao, Zhao et al. 2010; Barouch, Liu et al. 2012; Haynes, Gilbert et al. 2012; Robb, Rerks-Ngarm et al. 2012; Barouch, Stephenson et al. 2013; Tomaras, Ferrari et al. 2013); and/or a variety of other Fc receptor-dependent effector mechanisms including trogocytosis, phagocytosis and antibody-dependent cell-mediated viral inhibition or ADCVI (Forthal, Landucci et al. 2001; Ackerman and Alter 2013; Kramski, Parsons et al. 2013; Lewis 2013; Pollara, Bonsignori et al. 2013; Vargas-Inchaustegui and Robert-Guroff 2013). As discussed in the Investigator's Brochure Section 4 and 6.1, we have determined that rhFLSC elicits humoral responses against conserved domains, associated with specificities and effector mechanisms that correlate with protection in animal models (DeVico, Fouts et al. 2007; Fouts, Bagley et al. 2015). These findings warrant clinical tests of FLSC in human trials to determine if these and other responses correlated with control or prevention of HIV infection are elicited.

4.5.2. Potential Risks

In theory, it is possible that immunization with gp120-CD4 complexes could induce auto-anti-CD4 responses. These could be antibody responses, T cell responses, or both. It is well established in the older immunological literature that it is possible to break tolerance to self-antigens by adding T helper epitopes. In principle, gp120 T helper epitopes could collaborate with auto-anti-CD4 B cells or CTL precursors to break tolerance to CD4 epitopes. In this regard, it is interesting to note that immunization of a chimp with soluble human CD4 elicited an antibody response against an epitope that is not present on cell surface CD4 although the sera were able to block infection of chimpanzee or human lymphocytes with HIV-1 (Watanabe, Boyson et al. 1992). Similar results were obtained in rhesus macaques immunized with human CD4 (Watanabe, Chen et al. 1991; Watanabe, Levine et al. 1991). These studies used soluble human CD4 that differs in the extracellular domain from chimp CD4 by 4 residues and macaque CD4 by 37 residues raising the question of whether tolerance was actually broken in these studies and whether it would be broken if macaques were deliberately immunized with human CD4. Deliberate immunization of cynomologus macaques with human CD4 showed that the antibody response was selectively directed against human epitopes (Truneh, Frescatore et al. 1990). We were able to verify this result

in our immunotoxicology study using FLSC (Study Number VTR-0018). There was no evidence in this study that immunization of cynomolgus macaques with a homologus CD4 contained in rhFLSC broke tolerance. While these findings strongly indicate that the CD4 moiety of FLSC will be poorly immunogenic in humans, the risk of inducing auto-anti-CD4 antibodies drives the design of the phase 1 safety studies.

It is worthwhile to consider what would happen if an auto-anti-CD4 antibody response occurs in a clinical setting. In this regard, the expanding literature on the therapeutic use of anti-CD4 antibodies is informative. There are two non-exclusive mechanisms whereby passive immunization with anti-CD4 antibodies modulates disease. First, some anti-CD4 antibodies cause depletion of CD4+ T cells that is variable in duration as a function of the protocol and isotype of the antibody (Mourad, Preffer et al. 1998; Mason, Aldrich et al. 2002). Alternatively, anti-CD4 antibodies can coat CD4+ T cells without depleting them from the peripheral circulation (Mourad, Preffer et al. 1998; Mason, Aldrich et al. 2002). In both cases, clinical effects can be observed (Larche, Robinson et al. 2003) but there is evidence in rheumatoid arthritis that coating is more important than depletion for clinical effect (Mason, Aldrich et al. 2002). Very recently, non-depleting anti-CD4 antibodies that block HIV-1 infection in vitro have been developed for passive immunization against HIV-1 (Reimann, Khunkhun et al. 2002). Thus, it is reasonable to expect that if immunization with FLSC were to break tolerance to CD4 both depleting and non-depleting anti-CD4 antibodies are possible outcomes. Each of these possibilities were addressed in the immunotoxicology study (Study Number VTR-0018). No autoimmune responses to CD4 after repeated immunization with FLSC or rhFLSC were observed.

5. TRIAL OBJECTIVES AND PURPOSE

5.1. Purpose

The purpose of this study is to perform a randomized, double-blinded, placebo-controlled dose escalation Phase I clinical trial to evaluate the safety, tolerability and immunogenicity of the full length single chain (FLSC) gp120-CD4 complex vaccine in HIV-1 uninfected healthy adult volunteers.

5.2. Primary Objective - Safety

To evaluate the safety and tolerability of intramuscular administration of the full length single chain (FLSC) gp120-CD4 complex vaccine at three different doses.

5.3. Secondary Objectives - Immunogenicity

To evaluate the immunogenicity of three different doses of the full length single chain (FLSC) gp120-CD4 complex vaccine assessed by the following parameters:1) anti-FLSC antibodies, and 2) anti gp-120 (BaL), and 3) competitive antibodies titers to CD4i epitopes.

6. INVESTIGATIONAL PLAN

6.1. Overall Study Design

This will be a randomized, placebo-controlled, modified double-blinded study in 60 healthy adult volunteers. The study will evaluate the safety and immunogenicity of the full length single chain (FLSC) gp120-CD4 complex vaccine formulated with alum at three different doses. The study will have 3 arms as outlined below in Figure 1 and will last for 336 days (48 weeks). Volunteers will be immunized on Day 0, Day 28(4 weeks), Day 56(8 weeks), and Day 168(24 weeks).

6.1.1. Study Procedures

6.1.1.1. Study Entry

Volunteers who satisfy the entry criteria will be fully informed about the study by the investigator or his/her designee and asked whether they wish to participate. The information given to the patient or his/her legal representative will be in a language understandable to the volunteer or the representative. All questions will be answered prior to the volunteer signing consent. Written consent will be obtained from the volunteer or his/her legal representative prior to performing any study related procedures. This includes obtaining consent for the acquisition and review of the volunteer's prior medical history (and old medical record if appropriate and available). A copy of the informed consent will be given to the volunteer or his/her legal representative.

All volunteers having completed informed consent procedures will undergo a baseline screening evaluation within 30 days of initial immunization with the experimental candidate vaccine (or placebo). The CD4 cell count will be repeated on the day of initial immunization. These two observation points will define protocol baseline evaluation.

6.1.1.2. Study Duration

Each volunteer will complete a pre-immunization screening evaluation within 30 days prior to receiving the immunization series. Study procedures are outlined in Table 4. Volunteers will be immunized with candidate vaccine or placebo on Days 0, 28 (week 4), 56 (week 8), and 168 (week 24). Volunteers will continue to undergo active protocol follow up visits on Days 182 (week 26), 196 (week 28), 252 (week 36), 294 (week 42) and 336 (week 48). The total trial time expected per volunteer is 336 days (48 weeks). The protocol recruitment period is expected to be approximately 16 weeks. The total trial duration is estimated to be approximately 72 weeks (18 months).

6.1.1.3. Study Evaluations

See also the Schedule of Assessments in Table 4.

1. **Study Visit 0, Day –30 to Day –3 (Week –4 to 0): Screening Visit**

A screening evaluation will be performed within 3-30 days of the first immunization to determine eligibility for the study and to collect blood for baseline safety evaluation and immunological exams.

a. Obtain informed consent for study prior to screening
b. Obtain and record medical history including allergies, medications, and concomitant illnesses, and review of systems
c. Counsel volunteer regarding avoiding HIV risk behaviors
d. Perform complete physical examination, including vital signs, temperature, weight and height.
e. Collect blood for CBC with differential, serum chemistries, liver function tests(including AST, ALT, alkaline phosphatase, total bilirubin), PT/PTT, CD4 cell count, Hepatitis B surface antigen, Hepatitis C antibody, HIV-1 ELISA/Western Blot, HIV-1 quantitative RNA PCR, and, in women, serum human chorionic gonadotropin (HCG) assay (See Table 4 and Section 11).
f. Collect urine for urinalysis
g. Collect blood for baseline immunologic exams (See Table 5 and Section 12)

2. **Study Visit 1, Day 0 (Week 0): First Immunization**

a. Obtain interim medical history since screening visit
b. Perform physical examination, including vital signs, temperature, weight and height.
c. Conduct urine pregnancy test (female volunteers).

If volunteer continues to meet inclusion/exclusion criteria he/she will be given a volunteer number, randomized, and the following will be done:

d. Collect blood for safety labs (CBC with differential, serum chemistries, LFT's, CD4 cell count, and Quantitative HIV-1 RNA PCR) (Table 4, Table 5 and Section 11).
e. Collect blood for immunologic studies (Table 4 and Table 5 and Section 12).
f. Administer first injection of the study vaccine as an intramuscular injection in the deltoid muscle as per Section 9.5.
g. Record vital signs and examine injection site for local reactions at 15 and 30 minutes post-injection.
h. Observe and instruct while volunteer takes own temperature and assesses local reactions (as a guide for subsequent evaluations at home).
i. Counsel volunteer regarding avoiding HIV risk behaviors
j. Dispense diary for immunization reactions (Appendix 1) and digital thermometer. Give instructions for diary completion and thermometer use.

3. **Follow-up Phone contact by study nurse (24-48 hours after first immunization)**

a. Obtain interim history including: local and systemic reactions, post-injection oral temperature, Adverse Events, and concomitant medication.

4. **Study Visit 2, Day 14 (Week 2): Follow-up Clinical Visit**
    a. Obtain interim medical history including: review of volunteer diary, adverse events, concomitant medications, and safety labs.
    b. Perform exam of previous injection site and directed physical examination as indicated, including vital signs and temperature.
    c. Counsel volunteer regarding avoiding HIV risk behaviors
    d. Collect blood for safety labs and immunologic studies (Table 4, Table 5 and Section 11).

5. **Study Visit 3, Day 28 (Week 4): Second Immunization**
    a. Obtain interim medical history including:, adverse events, concomitant medications, and safety labs.
    b. Perform exam of previous injection site and directed physical examination as indicated, including vital signs and temperature.
    c. Conduct urine pregnancy test (female volunteers).
    d. Collect blood for safety labs (Table 4, Table 5 and Section 11).
    e. Collect blood for immunologic studies (Table 4 and Table 5 and Section 12).
    f. Administer second injection of the study vaccine as an intramuscular injection in the deltoid muscle as per Section 9.5.
    g. Record vital signs and examine injection site for local reactions at 15 and 30 minutes post-injection.
    h. Observe and instruct while volunteer takes own temperature and assesses local reactions (as a guide for subsequent evaluations at home).
    i. Counsel volunteer regarding avoiding HIV risk behaviors
    j. Dispense diary for immunization reactions (Appendix 1). Give instructions for its completion and for digital thermometer use.

6. **Follow-up Phone contact by study nurse (24-48 hours after second immunization)**
    a. Obtain interim history including: local and systemic reactions, post-injection oral temperature, Adverse Events, and concomitant medication.

7. **Study Visit 4, Day 42 (Week 6): Follow-up Clinical Visit**
    a. Obtain interim medical history including: review of volunteer diary, adverse events, concomitant medications, and safety labs.
    b. Perform exam of previous injection site and directed physical examination as indicated, including vital signs and temperature.
    c. Counsel volunteer regarding avoiding HIV risk behaviors
    d. Collect blood for safety labs and immunologic studies (Table 4 and Table 5 and Section 11 and Section 12).

8. **Study Visit 5, Day 56 (Week 8): Third Immunization**
    a. Obtain interim medical history including:, adverse events, concomitant medications, and safety labs.
    b. Perform exam of previous injection site and directed physical examination as indicated, including vital signs and temperature.
    c. Conduct urine pregnancy test (female volunteers).

d. Collect blood for safety labs (Table 4, Table 5 and Section 11).
e. Collect blood for immunologic studies (Table 4 and Table 5 and Section 12).
f. Administer third injection of the study vaccine as an intramuscular injection in the deltoid muscle as per Section 9.5.
g. Record vital signs and examine injection site for local reactions at 15 and 30 minutes post-injection.
h. Observe and instruct while volunteer takes own temperature and assesses local reactions (as a guide for subsequent evaluations at home).
i. Counsel volunteer regarding avoiding HIV risk behaviors
j. Dispense diary for immunization reactions (Appendix 1). Give instructions for its completion and digital thermometer use.

9. **Follow-up Phone contact by study nurse (24-48 hours after third immunization)**

a. Obtain interim history including: local and systemic reactions, post-injection oral temperature, Adverse Events, and concomitant medication.

10. **Study Visit 6, Day 70 (Week 10): Follow-up Clinical Visit**

a. Obtain interim medical history including: review of volunteer diary, adverse events, concomitant medications, and safety labs.
b. Perform exam of previous injection site and directed physical examination as indicated, including vital signs and temperature.
c. Counsel volunteer regarding avoiding HIV risk behaviors
d. Collect blood for safety labs and immunologic studies (Table 4 and Table 5 and Section 11 and Section 12).

11. **Study Visit 7, Day 84 (Week 12): Follow-up Clinical Visit**

a. Obtain interim medical history including:, adverse events, concomitant medications, and safety labs.
b. Perform directed physical examination as indicated, including vital signs and temperature.
c. Counsel volunteer regarding avoiding HIV risk behaviors
d. Collect blood for safety labs and immunologic studies (Table 4 and Table 5 and Section 11 and Section 12).

12. **Study Visit 8, Day 112 (Week 16): Follow-up Clinical Visit**

a. Obtain interim medical history including:, adverse events, concomitant medications, and safety labs.
b. Perform directed physical examination as
Indicated, including vital signs and temperature.
c. Counsel volunteer regarding avoiding HIV risk behaviors
d. Collect blood for safety labs and immunologic studies (Table 4 and Table 5 and Section 11 and Section 12).

13. **Study Visit 9, Day 168 (Week 24): Fourth Immunization**
    a. Obtain interim medical history including:, adverse events, concomitant medications, and safety labs.
    b. Perform exam of previous injection site and directed physical examination as indicated, including vital signs and temperature.
    c. Counsel volunteer regarding avoiding HIV risk behaviors
    d. Conduct urine pregnancy test (female volunteers).
    e. Collect blood for safety labs (Table 4, Table 5 and Section 11).
    f. Collect blood for immunologic studies (Table 4 and Table 5 and Section 12).
    g. Administer fourth injection of the study vaccine as an intramuscular injection in the deltoid muscle as per Section 9.5.
    h. Record vital signs and examine injection site for local reactions at 15 and 30 minutes post-injection.
    i. Observe and instruct while volunteer takes own temperature and assesses local reactions (as a guide for subsequent evaluations at home).
    j. Dispense diary for immunization reactions (Appendix 1) Give instructions for its completion and for thermometer use.

14. **Follow-up Phone contact by study nurse (24-48 hours after fourth immunization)**
    a. Obtain interim history including: local and systemic reactions, post-injection oral temperature, Adverse Events, and concomitant medication.

15. **Study Visit 10, Day 182 (Week 26): Follow-up Clinical Visit**
    a. Obtain interim medical history including: review of volunteer diary, adverse events, concomitant medications, and safety labs.
    b. Perform exam of previous injection site and directed physical examination as indicated, including vital signs and temperature.
    c. Counsel volunteer regarding avoiding HIV risk behaviors
    d. Collect blood for safety labs and immunologic studies (Table 4 and Table 5 and Section 11 and Section 12).

16. **Study Visit 11, Day 196 (Week 28): Follow-up Clinical Visit**
    a. Obtain interim medical history including:, adverse events, concomitant medications, and safety labs.
    b. Perform directed physical examination as indicated, including vital signs and temperature.
    c. Counsel volunteer regarding avoiding HIV risk behaviors
    d. Collect blood for safety labs and immunologic studies (Table 4 and Table 5 and Section 11 and Section 12).

17. **Study Visit 12, Day 252 (Week 36): Follow-up Clinical Visit**

a. Obtain interim medical history including: adverse events, concomitant medications, and safety labs.
b. Perform directed physical examination as indicated, including vital signs and temperature.
c. Counsel volunteer regarding avoiding HIV risk behaviors
d. Collect blood for safety labs and immunologic studies (Table 4 and Table 5 and Section 11 and Section 12).

18. **Study Visit 13, Day 294 (Week 42): Follow-up Clinical Visit**

a. Obtain interim medical history including: adverse events, concomitant medications, and safety labs.
b. Perform directed physical examination as indicated, including vital signs and temperature.
c. Counsel volunteer regarding avoiding HIV risk behaviors
d. Collect blood for safety labs and immunologic studies (Table 4 and Table 5 and Section 11 and Section 12).

19. **Study Visit 14, Day 336 (Week 48): Follow-up Clinical/End of Study Visit**

a. Obtain interim medical history including: review adverse events, concomitant medications, and safety labs.
b. Perform complete physical examination, including vital signs, temperature, weight and height.
c. Counsel volunteer regarding avoiding HIV risk behaviors
d. Conduct urine pregnancy test (female volunteers).
e. Collect blood for safety labs (Table 4 and Section 11).
f. Collect urine for urinalysis.
g. Collect blood for immunologic studies (Table 4 and Table 5 and Section 12).

6.1.1.4. Missed Visits

A volunteer is considered to have missed a visit if (s)he is not seen in ± 7 days of a scheduled study visit through the first 3 immunizations and within ± 28 days of a scheduled study visit after the third immunization visit. Volunteers who miss one of the immunization visits will be kept on the study if (s)he is willing and it is deemed appropriate by the investigator. Volunteers who miss 2 immunization visits will be removed from the study and may be replaced at the discretion of the investigator.

6.1.1.5. Replacement

A volunteer who drops out of the study for any reason other than toxicity (an SAE related to the study drug) may be replaced. No replacements will be allowed when the enrollment is closed. Volunteers who fail to return for two clinic visits after day 14 may be considered to be dropouts and can be replaced at the discretion of the Investigator. Volunteers who miss one of the first 3 immunization visits can be replaced at the discretion of the Principal Investigator. They will

continue to be evaluated for safety and will be evaluable for immunogenicity up to their last vaccine administration date. Volunteers who miss 2 immunization visits will be removed from the study and may be replaced at the discretion of the investigator. Volunteers with missed vaccination visits will be included in all safety analyses.

6.1.1.6. Pregnancy

In the event of pregnancy while the volunteer is on study, no further immunizations will be given regardless of outcome. The volunteer will be followed for all remaining scheduled visits according to the schedule of procedures for safety evaluation. The site will maintain contact with pregnant volunteers to obtain pregnancy outcome information.

6.1.1.7. Off-study evaluations

In the event a volunteer discontinues participation in the study prematurely, the initial off study evaluation will be treated as an end of study visit and consist of a complete physical examination, follow-up clinical assessment, selected blood chemistry and hematology evaluations, CD4+ count, ultra-sensitive viral load, PBMC and sera for immunogenicity assays (batched), PMBC stored (< -135° C) for assessment of chemokine receptor expression and chemokine production capacity. Patients who withdraw prematurely from the study (prior to the completion of the 32 weeks of study) will have all safety tests performed as soon as possible. Follow up off study evaluations may be conducted if agreed to by volunteer per schedule visit outline in Table 4 to allow for additional safety and immunogenicity evaluation to be completed. However, immunization will not be permitted once a volunteer has discontinued participation in the study. The Investigator will attempt to complete all discharge procedures and will follow each dropout (by telephone if necessary) for adverse experiences through 24 weeks after his/her first vaccine injection.

6.2. Number of Volunteers

The study population will consist of 60 healthy volunteers without HIV-1 infection who are available for at least 48 weeks of follow-up. Individuals of all races and groups may participate as long as the inclusion/exclusion criteria are met. The inclusion/exclusion criteria have been chosen to minimize risk to all participants. Therefore, those individuals with conditions that might place them at increased risk have been excluded.

Pregnant women and children are excluded from the trial as the safety of this vaccine has not previously been evaluated in humans and the effects on children or the unborn child are not known.

6.3. Treatment Assignment

The volunteers in this study will enroll in each group of the study sequentially as this is a dose escalation study. Each volunteer will undergo randomization as described in Section 8.4. Each group will be fully enrolled prior to the enrollment of the next group. The description of the FLSC vaccine is noted in Section 8 and Section 9 and the clinical formulation of the products are listed in Section 9. Volunteers in all arms will be immunized by intramuscular injection on Days 0, 28 (week 4), 28 (week 4), 56 (week 8), and 168 (week 24). Following completion of the immunization schedule, all volunteers will be followed (for an additional 24 weeks) until the end of the trial at 48 weeks as detailed in Section 6.1.1.3.

6.4. Dose Escalation Criteria

Requirements to begin immunizations in the higher dose groups (Groups 2 and 3) are listed below.

1. Safety data including the visit 4 data (2 weeks after the second immunization) in the 20 volunteers in the lower dose group has been collated and then reviewed by the DSMB.
2. DSMB review is completed, no safety concerns are noted, and approval to enroll the next group is granted.

6.4.1. Safety Criteria for Adjustment or Stopping Doses

1. If more than 5 volunteers in any group have an unexplained CD4 cell count decline (confirmed by assays at least 4 weeks apart) of greater than 30 percent and corroborated by a similar CD4% decline (>30%), immunizations in that study group will be stopped. No further immunizations will be allowed until after all data are reviewed by the IHV DSMB and the DSMB authorizes immunizations to continue in this group. Follow-up visits for safety will continue during this time. Note that an unexplained drop in CD4 cell count is defined as a decline that cannot be explained by an intercurrent illness or other event known to cause decreased CD4 cell counts.
2. The entire study will be stopped if five or more vaccine related severe (grade 3 or 4) adverse events occur in any group.
3. The volunteer will have immunizations stopped upon the occurrence of an unexpected Grade IV event felt by the investigator to be related to the study vaccine. No further immunizations will be allowed until after all data are reviewed by the IHV DSMB and the DSMB authorizes immunizations to continue in this group. Follow-up visits for safety will continue during this time.
4. The entire study immunizations will be stopped upon the occurrence of one unexpected serious adverse event felt to be related to the study vaccine.

6.4.2. Pharmacokinetic Criteria for Adjustment or Stopping Doses

Not applicable.

6.5. Criteria for Study Termination

Criteria for Termination of Study immunizations in all volunteers.

1. The entire study will be stopped upon the occurrence of an unexpected Grade 3 or 4 event thought to be related to the study vaccine in 5 volunteers as noted in Section 6.4.1. If after review by the DSMB, these events are determined by the DSMB to be study vaccine related, the volunteers would be continued to be followed on the study for safety, but no further immunizations would occur in any of the groups.
2. Individual volunteers may have immunizations halted as listed in Section 6.4.1.

Table 3: Study Design

Phase 1 FLSC Study Immunization Schedule/Volunteer Allocation							
Group	**Route of Administration**	**N Vaccine/ Control**	**Vaccine Dose**	**Vaccination Schedule in Months (Days)**			
				0	**1(28)**	**2(56)**	**6(168)**
1	IM	15	0.25 ml (75 ug)	FLSC/ ALPO4	FLSC/ ALPO4	FLSC/ ALPO4	FLSC/ ALPO4
		5	0.25 ml	Saline	Saline	Saline	Saline
2	IM	15	0.5 ml (150 ug)	FLSC/ ALPO4	FLSC/ ALPO4	FLSC/ ALPO4	FLSC/ ALPO4
		5	0.5 ml	Saline	Saline	Saline	Saline
3	IM	15	1.0 ml (300 ug)	FLSC/ ALPO4	FLSC/ ALPO4	FLSC/ ALPO4	FLSC/ ALPO4
		5	1.0 ml	Saline	Saline	Saline	Saline
TOTALS		**60**					

Table 4: Schedule of Assessments

STUDY VISIT	**0**	**01**		**02**	**03**		**04**	**05**		**06**	**07**	**08**	**09**		**10**	**11**	**12**	**13**	**14[3]**
STUDY DAY	**-30 TO –3**	**0**	**1-2**	**14**	**28**	**29-30**	**42**	**56**	**57-58**	**70**	**84**	**112**	**168**	**169-170**	**182**	**196**	**252**	**294**	**336**
STUDY WEEK	**-4 TO -0**	**0**		**2**	**4**		**6**	**8**		**10**	**12**	**16**	**24**		**26**	**28**	**36**	**42**	**48**
Informed Consent	X																		
Immunization		**X**			**X**			**X**					**X**						
Complete Medical History and Physical Examination	X																		X
Interval Medical History and Directed Physical Examination(as indicated)		X		X	X		X	X		X	X	X	X		X	X	X	X	
Phone Interview			X			X			X					X					
Dispense diary		X			X			X					X						
Monitor Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood for Screening Labs: CBC with diff Serum Chemistry, LFT's PT/PTT Hepatitis B Surface Antigen Hepatitis C Antibody[1] CD4 cell count HIV-1 RNA PCR HIV-1 ELISA/Western blot	X																		X
Pregnancy Test (serum)	X																		
Pregnancy Test (urine)		X			X			X					X						X
Urinalysis	X																		X

STUDY VISIT	**0**	**01**		**02**	**03**		**04**	**05**		**06**	**07**	**08**	**09**		**10**	**11**	**12**	**13**	**14[3]**
Blood for Safety Labs: CBC with diff Serum Chemistry, LFT's CD4 cell count HIV-1 RNA PCR[2]		X		X	X		X	X		X	X	X	X		X	X	X	X	
Blood for Serologic Assays/Serum storage	X	X[4]		X[4]	X[4]		X[4]	X[4]		X[4]	X	X[4]	X[4]		X[4]	X	X[4]	X	X[4]
Blood for Cellular Immune Response Assays/Storage	X	X[4]		X[4]	X[4]		X[4]	X[4]		X[4]	X	X[4]	X[4]		X[4]	X	X[4]	X	X[4]
Total blood volume(ml)	75 ml	64 ml	X	64 ml	84 ml	X	64 ml	84 ml	X	64 ml	84 ml	64 ml	90 ml	X	64 ml	94 ml	64 ml	64 ml	102 ml
Approximate Visit Duration (minutes)	60	60	10	30	60	10	30	60	10	30	60	30	60	10	30	30	30	30	60

Footnotes:
1. If positive Hepatitis C antibody and documentation of negative Hepatitis C PCR can be provided, the volunteer may enroll.
2. HIV RNA PCR will be performed at the screening and end of study visit. Blood will be stored for the PCR at subsequent visits but the assay will not be performed unless there is a concern for acute infection.
3. Visit 14 procedures will also be carried out in the case of early withdrawal/discontinuation.
4. Initially the planned assay days are those marked with a superscript. The other days will be stored for future studies.

Table 5: Blood Draw Flow Sheet

STUDY VISIT	**0**	**01**		**02**	**03**		**04**	**05**		**06**	**07**	**08**	**09**		**10**	**11**	**12**	**13**	**14[2]**
STUDY DAY	**-30 TO -3**	**0**	**1-2**	**14**	**28**	**29-30**	**42**	**56**	**57-58**	**70**	**84**	**112**	**168**	**169-170**	**182**	**196**	**252**	**294**	**336**
STUDY WEEK	**-4 TO 0**	**0**		**2**	**4**		**6**	**8**		**10**	**12**	**16**	**24**		**26**	**28**	**36**	**42**	**48**
Immunization		X			**X**			**X**					**X**						
Blood for Screening Labs: CBC with diff Serum Chemistry, LFT's, PT/PTT Hepatitis B Surface Antigen Hepatitis C Antibody CD4 cell count HIV-1 RNA PCR	X																		
Blood for Safety Labs: CBC with diff Serum Chemistry, LFT's CD4 cell count HIV-1 RNA PCR[2]		X		X	X		X	X		X	X	X	X		X	X	X	X	X
Pregnancy Test (serum)	3 ml																		
CBC with diff	3 ml	3 ml		3 ml	3 ml		3 ml	3 ml		3 ml	3 ml	3 ml	3 ml		3 ml	3 ml	3 ml	3 ml	3 ml
Serum Chemistry, LFT's,	3 ml	3 ml		3 ml	3 ml		3 ml	3 ml		3 ml	3 ml	3 ml	3 ml		3 ml	3 ml	3 ml	3 ml	3 ml
PT/PTT	3 ml																		3 ml

STUDY VISIT	**0**	**01**		**02**	**03**		**04**	**05**		**06**	**07**	**08**	**09**		**10**	**11**	**12**	**13**	**14[2]**
HIV-1 RNA Quantitative PCR	5 ml	5 ml		5 ml	5 ml		5 ml	5 ml		5 ml	5 ml	5 ml	5 ml		5 ml	5 ml	5 ml	5 ml	5 ml
HBsAg, Hep C Ab	5 ml																		
HIV-1 ELISA/Western blot	5 ml												5 ml						5 ml
CD4 cell count	3 ml	3 ml		3 ml	3 ml		3 ml	3 ml		3 ml	3 ml	3 ml	3 ml		3 ml	3 ml	3 ml	3 ml	3 ml
PBMC stored	30 ml	30 ml		30ml	50 ml		30 ml	50 ml		30 ml	50 ml	30 ml	50 ml		30 ml	50 ml	30 ml	30 ml	50 ml
Serum stored[1]	15 ml	20 ml		20 ml	20 ml		20 ml	20 ml		20 ml	20 ml	20 ml	20 ml		20 ml	30 ml	20 ml	20 ml	30 ml
Total blood volume(ml)	75 ml	64 ml	X	64 ml	84 ml	X	64 ml	84 ml	X	64 ml	84 ml	64 ml	90 ml	X	64 ml	94 ml	64 ml	64 ml	102 ml
Total Study Blood Volume (over 48 weeks):	1125 ml																		

Footnotes:
1. If positive Hepatitis C antibody and documentation of negative Hepatitis C PCR can be provided, the volunteer may enroll.
2. HIV RNA PCR will be performed at the screening and end of study visit . Blood will be stored for the PCR at subsequent visits but the assay will not be performed unless there is a concern for acute infection.
3. Visit 14 procedures will also be carried out in the case of early withdrawal/discontinuation.

7. SELECTION AND WITHDRAWAL OF VOLUNTEERS

7.1. Volunteer Inclusion Criteria

All of the following inclusion criteria must be fulfilled for patients to be eligible for participation in the proposed clinical protocols.

1. Age: 18 to 45 years of age.
2. Sex: Male or Female (female volunteers of child-bearing potential must have a negative serum beta human chorionic gonadotropin(pregnancy) test at time of screening and entry into the study and provide assurance of the use of effective(as judged by the investigator) birth control methods or abstinence beginning at least 60 days prior to the study and during the study
3. Documented HIV-1 seronegative by ELISA
4. Be in good general health without clinically significant medical history, physical examination findings, or clinically significant abnormal laboratory results(i.e., chronic medical conditions as noted in the exclusion criteria such as cancer as well as any conditions that in the opinion of the investigator might pose a risk to the volunteer)
5. No identifiable risk factor for acquisition of HIV infection(i.e., intravenous drug use/needle sharing, unprotected sex with multiple partners)
6. Negative b-HCG pregnancy test on the day of initial vaccination (women only);
7. Negative screen for Hepatitis B surface antigen (HBsAg);
8. Negative screen for antibodies to Hepatitis C virus (Patient may enroll if patient can provide documentation of negative HCV PCR.)
9. Participant must have a CD4 count within the normal range of the clinical laboratory utilized for the study and a CD4 percentage within 20% of the normal range of the clinical laboratory
10. Laboratory parameters must be within pre-specified limits as defined by exclusion criteria.
11. Volunteers must be willing and able to provide written informed consent to participate in the study.
12. Available for at least 48 weeks of follow-up.

7.2. Volunteer Exclusion Criteria

Any volunteer who fulfills any of the following criteria will not be eligible for entry.

1. High risk behavior for acquisition of HIV within 24 weeks of study entry(i.e., intravenous drug use/needle sharing, unprotected sex with multiple partners)
2. Volunteers with an acute and clinically significant medical event (as determined by the investigator) within the past 30 days of screening.
3. Have active tuberculosis or other systemic infectious process by review of systems and physical examination
4. Have a history of immunodeficiency, autoimmune disease, or use of immunosuppressive medications
5. Current treatment for malignancy other than basal or squamous cell carcinoma of the skin or carcinoma in situ of the cervix
6. Is pregnant

7. History of any chronic illness that would interfere with conduct or completion of study(as determined by the investigator)
8. Have evidence of psychiatric, medical and/or substance abuse problems during the past 24 weeks that the investigator believes would adversely affect the volunteer's ability to participate in the trial
9. Have occupational or other responsibilities that would prevent completion of participation in the study
10. Have received any live, attenuated vaccine except rabies vaccine within 60 days of study entry
11. Vaccine (FDA approved; e.g. influenza, pneumovax, etc) administration within 30 days of immunization with the study vaccine. NOTE: Medically indicated subunit or killed vaccines (e.g., Hepatitis A or Hepatitis B) should be given prior to trial initiation or after completion of the study immunizations. If patient requires immunization, injections should be given more than 2 weeks prior or 2 weeks after study immunization
12. Have used experimental therapeutic agents within 30 days of study entry
13. Have received blood products or immunoglobulins in the past 12 weeks
14. Have a history of anaphylaxis or other serious adverse reactions to vaccines
15. Have previously received an HIV vaccine
16. Volunteers with any of the following laboratory parameters at the screening visit (within 30 days of immunization): Hemoglobin <10 (without having received a blood or RBC transfusion within 30 days prior to laboratory test); neutrophil count <750 cells/mm^3; platelet count <50,000/mm^3; serum creatinine > 2.0 mg/dL; aspartate aminotransferase (AST) or alanine aminotransferase (ALT) > 3 times the upper limits of normal; total bilirubin > 1.5 mg/dL
17. Pregnant women or women who are breast-feeding; female volunteers of childbearing potential who are not using or willing to use effective (as judged by the investigator) contraceptive methods or abstinence while enrolled in this study.
18. Use of any immune modulators or suppressors within 45 days of study entry including but not limited to agents such as interleukins(e.g. IL-2), interferons (e.g. IFN-α), high dose systemic steroids(e.g. $\geq$ 20 mg prednisone equivalent/day) for > 30 days, thalidomide, filgrastim (G-CSF), sargramostim (GM-CSF), dinitrochlorobenzene (DNCB), thymosin alpha, thymopentin, inosiplex, polyribonucleoside, ditiocarb sodium, cyclosporin, mycophenolate mofetil, methotrexate, and cancer chemotherapy.
19. No other investigational agent within 30 days of study entry
20. Any other condition which, in the opinion of the investigator, might interfere with completion of the study or evaluation of the results
21. Have active Hepatitis B virus infection (positive HBsAg) or Hepatitis C infection(defined as positive antibodies. Patient may enroll if patient can provide documentation of negative HCV PCR.))

7.3. Volunteer Withdrawal Criteria

7.3.1. Reasons for Withdrawal for Study

Volunteers have the right to withdraw from the study at any time for any reason.

7.3.1.1. Conditions mandating withdrawal from the Study

Individual volunteers will be withdrawn from further immunizations on the study for the following reasons:

- Experiencing a grade 4 toxicity/adverse event or serious adverse event considered by the principal investigator to be related to study vaccine
- Becoming pregnant during the study
- Requiring cytotoxic chemotherapy or immunomodulatory therapy during the course of the study
- Missing 2 immunization visits (injections)

Note that those withdrawn for toxicity reasons will continue to be followed for safety as described in Section 6.1.1.7.

7.3.1.2. Conditions permissible to withdraw from the Study

- Protocol violations
- Intercurrent illness
- Administrative reasons
- Adverse events requiring discontinuation of any study drug;
- At the discretion of the investigator in situations where the volunteer is unable to comply, including situations involving unmanageable drug or substance abuse; patients lost to follow-up.

7.3.2. Withdrawal Procedures

In the event that the volunteer withdraws from the study, the investigator will record the reason for and the date of the withdrawal and make every effort to complete an off study evaluation as noted below. The procedures outlined for Reporting of Adverse Experiences must be followed if the patient is withdrawn due to a serious adverse event. Volunteers may be withdrawn from the vaccine or from the study at any time without prejudice to their subsequent medical care. The sponsor should be notified of all study withdrawals within 24 hours. Study completion is defined as completion of protocol-specified procedures through 48 weeks. See Section 6.1.1.7 as well.

7.3.2.1. Volunteer Withdraws

If the volunteer withdraws consent, he/she will be asked to complete an end of study visit. If he/she agrees, the end of study visit will be performed as described in Section 6.1.1.7. If he/she does not agree, no further study activity will occur.

7.3.2.2. Volunteer Withdrawn by Investigator

If the volunteer is withdraw by the investigator, he/she will be asked to complete an end of study visit. If he/she agrees, the end of study visit will be performed as described in Section 6.1.1.7. If he/she does not agree, no further study activity will occur.

7.3.2.3. Volunteer Withdrawn for Vaccine Related Toxicity

Should a volunteer be discontinued from the study for toxicity reasons, the volunteer will continue to have safety visits performed until the end of the study as in Section 6.1.1.7.

7.3.2.4. Volunteer Withdrawn by Investigator for other reasons

The Volunteer will be asked to undergo an end of study visit. If he/she agrees, the end of study visit will be performed as in Section 6.1.1.7. If he/she does not agree, no further study activity will occur.

7.3.3. Reasons to Halt Study

If 5 or more volunteers in any group have an unexplained CD4 cell count decline (confirmed by assays at least 4 weeks apart) of greater than 30 percent and corroborated by a similar CD4% decline (>30%), immunizations in that study group will be stopped. No further immunizations will be allowed until after all data are reviewed by the IHV DSMB and the DSMB authorizes immunizations to continue in this group. Follow-up visits for safety will continue during this time. Note that an unexplained drop in CD4 cell count is defined as a decline that cannot be explained by an intercurrent illness or other event known to cause decreased in CD4 cell counts. Other reasons are noted in Section 6.4.1 and Section 6.5.

7.3.4. Reasons for Discontinuation of all Immunizations in the Study

All immunizations across the entire study will be stopped upon the occurrence of 5 or more unexpected Grade 3 or 4 events felt to be related to the study vaccine in any group or a serious adverse event felt to be related to study vaccine in one volunteer as noted in Section 6.4.1 and Section 6.5. Follow-up visits for safety will continue to occur as per study protocol. No further enrollments will occur.

8. TREATMENT OF VOLUNTEERS

8.1. Description of Study Drug

The FLSC final product consists of 0.3 mg/ml of purified FLSC Drug Substance formulated with Aluminum Phosphate ($AlPO_4$) at 2.4 mg/mL in binding buffer (mannitol 40 mg/mL; sodium acetate 5 mM, pH 6.2. Aluminum ion concentration is 1.2 mg/mL and 1.3 mL product is vialed in 2cc glass vials.

Table 6: Investigational Product

	Investigational Product	
Product Name:	IHV01	0.9% Sodium Chloride Inj., USP
Dosage Form:	1 ml single dose vial	Liquid
Unit Dose	300 ug/vial	10 ml/ single dose vial
Route of Administration	Intramuscular	Intramuscular
Physical Description	FLSC 0.3 mg/ml, Aluminum Phosphate 2.4 mg/ml, Mannitol 40 mg/ml, Sodium Acetate 5 mM	NaCl 9 mg/ml Preservative Free
Manufacturer	Profectus Biosciences	Hospira, Inc.*

*The company the saline is purchased from may change depending on what vendor the institution is using at the time that the trial begins.

8.2. Concomitant Medications

Concurrent medications (with the exception of those noted in the Exclusion Criteria) will be permitted as medically necessary during the study. Changes in medication treatment during this protocol will be permitted if clinically indicated at the discretion of the patient's primary care physician. All changes in medications will be recorded. Medically indicated vaccines (FDA approved; e.g. influenza, pneumococcal, etc.) should be administered either 30 days prior to or following study vaccine administration to minimize potential confusion of adverse experiences due to the study vaccine vs the other vaccine.

8.3. Treatment Compliance

Adherence with the protocol will be assessed by the study personnel at each clinic visit. Careful review of the volunteers' interim medical history will be performed. If appropriate, corroboration of any outside medical clinic or hospital visits will be obtained with the volunteer's consent.

8.4. Randomization and Blinding

Volunteers will be randomized using block randomization in a 3:1 ratio of FLSC:placebo. They will be block randomized in groups of 4 with each study group. This will be done by the research pharmacist utilizing random # generator called Stat Trek. There will be 5 groups of 4 volunteers in each randomized block (each block will have 3 FLSC recipients and 1 placebo recipient).

8.4.1. Blinding

Both the investigator and the volunteer will be blinded to the study drug/placebo but not to the group assignment as it is a dose escalation trial. The syringes containing the study drug/placebo will be covered by the study pharmacist to insure blinding of the study.

8.4.2. Blinding and Code Breaking Procedures

Dose labels will not allow for identification of treatment. The investigator and volunteer will not know which treatment is administered. The pharmacist responsible for product preparation will prepare the appropriate product

for the volunteer as indicated by the block randomization and cover the syringe to prevent identification. The nurse/physician responsible for injection and the volunteer will not know which product is injected as the syringes will be blinded (a film will be affixed on each syringe by the staff responsible for product preparation).

Consequently, two sealed envelopes for each volunteer containing emergency decoding information for each volunteer will be issued by the research pharmacist. The emergency decoding information indicates the treatment corresponding to each randomization number. One envelope will be retained by the investigator and the other one by the research pharmacy/medical monitor.

The code can be broken by opening the individual envelope for the volunteer. The vaccine/diluent injected will be indicated in the envelope. The code may be broken only in case of a Serious Adverse Event and if identification of the treatment can influence the treatment of the adverse event.

In case of code breaking, the name of the person who requests it, the date and reason must be written on the list. The event must be immediately reported on the CRF. The sponsor will immediately be informed by phone or by fax.

At the end of the trial the emergency decoding information will be returned to the sponsor and checked. In the absence of any problem, codes will be broken at the time of the analysis, when the data base is validated and locked.

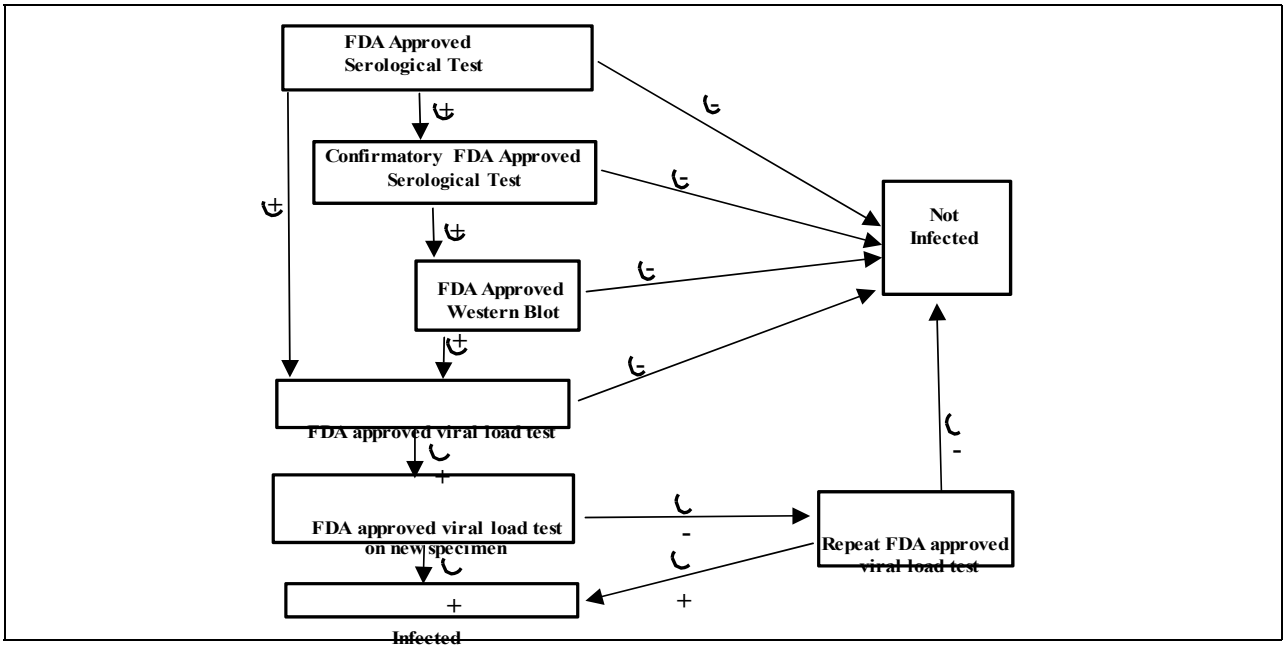
8.5. Identification of participants as volunteers in HIV vaccine trial

All volunteers will receive an individual identification card indicating their status as an HIV-1 vaccine trial participant. All volunteers will be counseled periodically regarding the potential for testing positive on routine screening tests for HIV-1 as a consequence of participation in this trial and receiving these vaccine products. All volunteers will be offered further confirmatory testing and certification as to the nature of their vaccine trial participation whenever needed to address complications arising at home, at work or in the community that could arise from routine screening from HIV-1.

8.6. Detection of inter-current HIV-1 infections.

Each volunteer will be counseled at each visit and an algorithm followed to identify inter-current HIV-1 infections. The algorithm is shown in Figure 1 and uses FDA approved serological and nucleic acid diagnostic kits at each step. If an individual is determined to have an inter-current infection with HIV-1 they will be excluded from further immunizations and counseled about their medical options. Enrollment in the trials does not include subsequent care for an inter-current HIV-1 infection. The volunteer will be counseled on how to access that care if they choose to do so.

Figure 1: Algorithm to detect inter-current HIV-1 infections



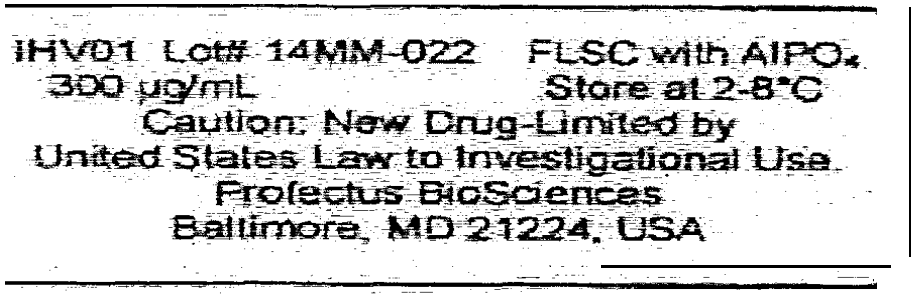
9. STUDY DRUG MATERIALS AND MANAGEMENT

9.1. Study Drug

The FLSC final product consists of 0.3 mg/ml of purified FLSC Drug Substance formulated with Aluminum Phosphate ($AlPO_4$) at 2.4 mg/mL in binding buffer (mannitol 40 mg/mL; sodium acetate 5 mM, pH 6.2. Aluminum ion concentration is 1.2 mg/mL and 1.3 mL product is vialed in 2cc glass vials.

9.2. Study Drug Packaging and Labeling

The information to appear on each study medication vial label will be:



The placebo will be preservative free saline provided by the IHV research pharmacy.

9.3. Study Drug Storage

FLSC will be stored between +2°C and +8°C as directed by the manufacturer.

9.4. Study Drug Preparation

The product preparation and the injection will be performed in different places. The product preparation must be performed in the absence of the investigator and of the nurse responsible for injection. This will be performed by a pharmacist or nurse. If it is a nurse, the nurse must not be performing other duties with the study.

9.4.1. FLSC 75 ug (Group 1)

Before injection, the FLSC vial will be removed from the refrigerator and swirled until a uniform solution results. Once the FLSC is in a uniform solution, 0.25 ml (75ug) of FLSC will be withdrawn from the viral using a 22-25 gauge, 25 mm (1 inch) needle with a 1-mL sterile disposable syringe. The syringe will then be covered with a film/aluminum foil to prevent visualization of the product.

9.4.2. FLSC 150 ug (Group 2)

Before injection, the FLSC vial will be removed from the refrigerator and swirled until a uniform solution results. Once the FLSC is in a uniform solution, 0.5 ml (150ug) of FLSC will be withdrawn from the viral using a 22-25 gauge, 25 mm (1 inch) needle with a 1-mL sterile disposable syringe. The syringe will then be covered with a film/aluminum foil to prevent visualization of the product.

9.4.3. FLSC 300 ug (Group 3)

Before injection, the FLSC vial will be removed from the refrigerator and swirled until a uniform solution results . Once the FLSC is in a uniform solution, 1.0 ml (300ug) of FLSC will be withdrawn from the viral using a 22-25 gauge, 25 mm (1 inch) needle with a 1-mL sterile disposable syringe. The syringe will then be covered with a film/aluminum foil to prevent visualization of the product.

9.4.4. Placebo (Groups 1-3)

Before injection, depending on the group (see Table 7), the saline will be withdrawn using a 22-25 gauge, 25 mm (1 inch) needle with a 1-mL sterile disposable syringe. The syringe will then be covered with a film/aluminum foil to prevent visualization of the product.

Table 7: Placebo Groups

Group Number	Volume of Saline
1	0.25 ml
2	0.5 ml
3	1.0 ml

9.5. Administration

Immunizations will be administered on an outpatient basis on Day 0, Day 28, Day 56, and Day 168. Evaluations listed on the study schema will be conducted prior to immunization with the study vaccine. Total time required in the clinic for an immunization visit will be approximately one hour, unless the subject experiences an Adverse Event which requires additional treatment. The volunteer will be instructed to call a designated staff member immediately if an Adverse Event should occur after the volunteer leaves the clinic.

The intramuscular route will be used in all cases. At all stages of the preparation, aseptic procedures must be followed. After cleansing the site of injection with a suitable antiseptic, the injections will be given as an intramuscular injection in the deltoid muscle of the volunteer's non-dominant arm (unless preferred otherwise by the patient) with a 22 gauge 25 mm (1 inch) needle. All of the product contained in the syringe will be injected. As noted in Section 9.4, according to the group number, drug product or diluent will be given as an intramuscular injection in the deltoid muscle of the volunteer's non-dominant arm per institutional protocol. The study vaccine may be given in the deltoid muscle of the dominant arm at the investigator's discretion (e.g., open wound near/on the proposed injection site, request of the volunteer, etc.). Immediately after injection, the subject will be observed closely for 30 minutes. Complete vital signs will be recorded prior to administration, at 15 minutes and 30 minutes post-injection. Injection site evaluations will be recorded at 15 minutes and 30 minutes post-injection. The volunteer will be instructed in the evaluation of these local reactions; study personnel will observe and instruct while the volunteer takes his/her own temperature at Visit 1 (as guide for subsequent evaluations. Volunteer diary card for immunization reactions will be dispensed and instructions for its completion will be given.

If there are no clinically meaningful abnormalities and the volunteer has no complaints which are considered cause for concern by the Principal Investigators or his designee, he/she may leave the clinic with instructions to immediately call a designated staff member should any complications arise. If there are any local or systemic reactions present, the volunteer will be asked to return to the clinic the following day for a follow-up visit.

Treatment of AEs will be at the discretion of the Investigator and the clinic staff. Medication (epinephrine and Benadryl) and equipment for treatment of emergencies will be available during injection and for 30 minutes immediately following to allow a rapid response to any acute reactions. At all stages of the administration, aseptic procedures must be followed. It is important to use a separate sterile syringe for each individual study participant to prevent transmission of infectious agents. Once taken out of the refrigerator, prepare as soon as products have reach room temperature, and once reconstituted, use as soon as possible, but within 1 hour. Discard reconstituted unused vaccine.

After reconstitution and preparation, inspect the vaccine for particulate matter and discoloration. If either of these conditions exist, the vaccine should not be administered and another syringe/replacement dose should be selected. The staff in charge of product preparation will affix an opaque cover on each syringe.

9.6. Study Drug Accountability

The IHV Research pharmacy will be responsible for study drug accountability. The Research Pharmacist will utilize the University of Maryland Medical Center Policy 06-05, Investigational Drug Accountability to ensure complete and accurate records of investigational drug accountability. (See Appendix 3)

9.7. Study Drug Handling and Disposal

All vaccine products will be prepared by an investigational pharmacist who will provide the product to research study personnel in a blinded fashion in a syringe pre-packaged and prepared with vaccine antigen and diluent, vaccine antigen and adjuvant, or placebo (diluent) and diluent mixed together. All materials for this study must be stored under recommended storage conditions in an area free of environmental extremes with controlled limited access. The FLSC will be kept at +2°C to +8° Celsius; and brought out to room temperature no earlier than one hour prior to administration of vaccine. The placebo (preservative free normal saline) will be stored as per package insert.

10. CLINICAL SAMPLE COLLECTION

10.1. Blood Sample Collection

Blood sample collection will occur as outlined in Table 4 and in Section 6.1.1. Blood volumes for these assessments will be as outlined in Table 5.

10.2. Urine Sample Collection

Urine sample collection will occur as outlined in Table 4 and in Section 6.1.1.

10.3. Sample Analysis

All safety laboratory assessments will be analyzed by the University of Maryland Medical Center Clinical Laboratory as noted in Section 15.2

All immunogenicity laboratory assessments will be analyzed at one of the laboratories outlined in Section 15.2 and per description in Section 12.2 and Section 15.3.

11. ASSESSMENT OF SAFETY

Although no safety concerns have been identified with this vaccine product in animal studies to date, there is a theoretical risk of the development of anti-CD4 antibodies. Antibodies against CD4 have been shown to occur in HIV infected individuals and their significance is not known. However, the presence of these antibodies has not been demonstrated to be deleterious in these individuals. Therefore, we do not believe that this vaccine poses a risk to the individuals enrolling in the trial. In order to ensure volunteer safety, we are monitoring CD4 cell count frequently throughout the trial. Any individual who experiences any of the criteria noted in Section 7.3.1 will be withdrawn from the study.

11.1. Safety Parameters

The safety parameters to be utilized in this study are listed below. The schedule for their assessment is listed in Table 4 and Section 6.1.1.

1. Local and systemic vaccine reactogenicity.
2. Laboratory Safety Evaluation
3. Medical History and Physical Examination

11.1.1. Demographic/Medical History

A complete medical history will be conducted on all volunteers prior to enrollment. The medical history will include a review of body systems, determination of any current symptoms or conditions, and documentation of any concurrent medications and other treatments that might preclude the volunteer from participation in the study. It will also include a review of the volunteer's prior medical record. A complete physical examination will consist of an exam including evaluations of the following: general appearance, skin, HEENT, extremities, heart, lungs, lymph nodes, and abdomen; additional examination based on history and symptoms. A directed physical examination will be performed at interim visits as noted in the protocol and Table 4 Schedule of Assessments and will include examinations of the injection site(s) and lymph nodes with special attention to the axillary lymph nodes. In addition, physical manifestations of any previously noted condition or adverse experience will be evaluated.

11.1.2. Vital Signs

Vital signs will be taken at the study visits as outlined in Section 6.1.1.

11.1.3. Weight and Height

Weight and height will be taken at the study visits as outlined in Section 6.1.1.

11.1.4. Physical Examination

A complete physical examination will be conducted on all volunteers prior to enrollment. A complete physical examination will consist of evaluations of the following: general appearance, skin, HEENT, extremities, heart, lungs, lymph nodes, and abdomen; additional examination based on history and symptoms. A directed physical examination will be performed at interim visits as noted in the protocol (Section 6.1.1 and Table 4) and will include examinations of the injection site(s) and lymph nodes with special attention to the axillary lymph nodes. In addition, physical manifestations of any previously noted condition or adverse experience will be evaluated.

11.1.5. Laboratory Assessments

All abnormal laboratory values will be scored utilizing the ACTG toxicity grading system (see Appendix 2) where applicable or other appropriate criteria. Hematology and clinical laboratory evaluations will be performed by the

UMMS clinical laboratory facility. The following safety tests will be performed as outlined in the Schedule of Assessments (Table 4).

- Hematology: CBC, including differential, platelets
- Clinical Chemistries: serum electrolytes, liver function tests, renal function tests, PT/PTT
- Urinalysis: routine and microscopic, pregnancy test on women
- CD4 + T cell count
- HIV viral load by Roche-Amplicor ultra-sensitive RNA PCR

11.1.5.1. Hematology

CBC, including differential, platelets. The schedule is as outlined in Section 6.1.1 and Table 4 .

11.1.5.2. Blood Chemistry

Serum electrolytes, liver function tests, renal function tests, PT/PTT will be performed as outlined in Section 6.1.1 and Table 4.

11.1.5.3. Urinalysis

Urinalysis will be performed as outlined in Section 6.1.1 and Table 4.

11.1.5.4. Virus Serology

Serologies for HIV, Hepatitis B and Hepatitis C will be performed as outlined in Section 6.1.1 and Table 4.

11.1.5.5. Pregnancy Screen

Serum pregnancy test will be performed at the screening visit. Subsequently urine pregnancy tests will be performed prior to each immunization and at the end of the study. It will also be performed if there is concern for pregnancy in a volunteer during the study.

11.1.5.6. Assessment of Vaccine Reactogenicity

The safety evaluation will include an evaluation of both local and systemic reactions to the candidate immunotherapeutic vaccines. Assessment of local reactogenicity will include erythema, induration, swelling, itching, pain and tenderness, skin discoloration, skin breakdown, and presence of subcutaneous nodules at the site of injection. Assessment of systemic reactions will include assessment of fever, chills, nausea, vomiting, arthralgia, myalgia, malaise, urticaria, wheezing, dizziness, headache. Each volunteer will complete a self-administered questionnaire/diary daily for 7 days after each immunization to assess reactogenicity. In addition, follow up telephone contact or clinic visit will be conducted by research nurse 24 to 48 hours following each immunization to record reactogenicity/adverse events. The study personnel will also conduct a careful assessment of possible adverse experiences at each clinic visit (See Table 4). Both the volunteer diary and the elicited adverse experiences will be used to determine the occurrence of adverse events. Any discrepancies between these sources will be resolved by careful review of the data with the volunteer. All adverse events will be recorded in the CRFs as noted in Section 11.4.

11.2. Adverse and Serious Adverse Events

11.2.1. Definition of Adverse Events

11.2.1.1. Adverse Event (AE)

An adverse event is defined as any unfavorable or unintended change in body structure (signs), body function (symptoms) or laboratory result associated temporally with the use of study treatment, whether or not considered related to the study treatment. As defined by 21 CFR 312.32 (a), an unexpected adverse drug experience is:

"An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure listed only cerebral vascular accidents. "Unexpected," as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation."

All AEs that occur after any volunteer has signed consent or within 84 days following the cessation of immunizations and while being followed on the study protocol, whether or not they are related to the study, must be recorded on forms provided by the Institute of Human Virology.

11.2.1.2. Serious Adverse Event (SAE)

A serious adverse event is an AE occurring during any study phase (i.e., baseline, treatment, washout, or follow-up), and at any dose of the investigational product, comparator or placebo, that fulfills one or more of the following:

- Results in death
- It is immediately life-threatening (i.e., the volunteer was, in the opinion of the Investigator, at immediate risk of death from the event as it occurred).
- It requires in-patient hospitalization or prolongation of existing hospitalization
- It results in persistent or significant disability or incapacity (i.e., the event causes a substantial disruption of a person's ability to conduct normal life functions).
- Results in a congenital abnormality or birth defect
- Is an important and significant medical event that, based upon appropriate medical judgment, may jeopardize the patient or volunteer and may require medical or surgical intervention to prevent one of the other outcomes defining serious.

All SAEs that occur after any volunteer has signed consent or within 84 days following the cessation of treatment, and while on study protocol, whether or not they are related to the study, must be recorded on forms provided by the Institute of Human Virology.

Any serious adverse event (including death) due to any cause must be reported immediately to the principal investigator and the sponsor.

11.2.1.3. Other Adverse Event (OAE)

OAEs will be identified by the Drug Safety Physician and if applicable also by the Clinical Study Team Physician during the evaluation of safety data for the Clinical Study Report. Significant adverse events of particular clinical importance, other than SAEs and those AEs leading to discontinuation of the volunteer from the study, will be classified as OAEs. For each OAE, a narrative may be written and included in the Clinical Study Report.

11.3. Relationship to Study Drug

An Investigator who is qualified in medicine must make the determination of relationship to the investigational product for each AE. The Investigator should decide whether, in his or her medical judgment, there is a reasonable possibility that the event may have been caused by the investigational product. The relationship of an adverse event to the study medication will be determined by the investigator based on the following criteria.

1. Definitely Related:
    a. It follows a reasonable temporal sequence from vaccine administration
    b. It follows a known pattern of response to the vaccine
    c. Adverse event is most likely explained by vaccination than by other mechanisms. (It cannot be reasonably explained by the known characteristics of the volunteer's clinical state, environmental or toxic factors, or other modes of therapy administered to the volunteer)
    d. It appears on re-challenge with the vaccine or there is a local reaction at the injection site.
2. Probably Related:
    a. It follows a reasonable temporal sequence from vaccine administration
    b. It follows a known pattern of response to the vaccine
    c. Adverse event is more likely explained by vaccination than by other mechanisms. (It cannot be reasonably explained by the known characteristics of the volunteer's clinical state, environmental or toxic factors, or other modes of therapy administered to the volunteer)
3. Possibly Related:
    a. It follows a reasonable temporal sequence from vaccine administration.
    b. It follows a known pattern of response to the vaccine
    c. Adverse event is explained equally well by causes other than vaccination. (It may have been produced by the volunteer's clinical state, environmental or toxic factors, or other modes of therapy administration to the volunteer)
4. Not Related:
    a. It is clearly and incontrovertibly due to causes other than vaccination (e.g. disease, environment, etc.)
    b. It does <u>not</u> follow a reasonable temporal sequence from vaccine administration.
    c. It does not meet any of the criteria for vaccine relationship as listed under remotely related, possibly related or probably related.

If the relationship between the AE/SAE and the investigational product is determined to be “possible” or “probable” the event will be considered to be related to the investigational product for the purposes of expedited regulatory reporting.

11.4. Recording Adverse Events

Adverse events spontaneously reported by the volunteer and/or in response to an open question from the study personnel or revealed by observation will be recorded during the study at the investigational site. However, abnormal values that constitute an SAE or lead to discontinuation of administration of study drug must be reported and recorded

as an AE. Information about AEs will be collected from signing of consent until the end of the study. Serious Adverse Event information will be collected from signing of consent until 24 weeks following the last dose of study drug. The AE term should be reported in standard medical terminology when possible. For each AE, the investigator will evaluate and report the onset (date and time), resolution (date and time), intensity, causality (as defined in Section 11.3), action taken, serious outcome (if applicable), and whether or not it caused the patient to discontinue the study.

All adverse events occurring during the trial must be evaluated by the investigator and graded according to the adverse experience grading table (Appendix 2).

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria under Section 11.2.1.2. An AE of severe intensity may not be considered serious.

Should a pregnancy occur, it must be reported and recorded on the Institute of Human Virology's pregnancy form. Pregnancy in itself is not regarded as an AE unless there is a suspicion that an investigational product may have interfered with the effectiveness of a contraceptive medication. However, it is a criterion for stopping immunizations in a volunteer as noted in Section 6.1.1.6 and Section 7.3.1.1.

The outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth or congenital abnormality) must be followed up and documented even if the patient was discontinued from the study.

All reports of congenital abnormalities/birth defects are SAEs. Spontaneous miscarriages should also be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs.

11.5. Reporting Adverse Events

11.5.1. Adverse Events

All adverse events, including both observed and volunteered problems, complaints, signs or symptoms, and any death occurring while on study are to be recorded (whether or not the event is related to vaccine administration). The duration and intensity of the event as well as whether or not the event might be associated with the study medication or other causes will be evaluated.

The severity of each adverse event will be graded according to the common toxicity criteria of the AIDS Clinical Trials Group (ACTG) as listed in Appendix 2. When intensity changes occur more frequently than once a day, the maximum severity for the event should be listed. If the intensity category changes over a number of days, then these changes should be recorded separately, having distinct onset and stop dates for each grade.

If the investigator has evidence that the adverse event is not caused by the vaccine, and after consultation with the study Principal Investigator, then the study vaccine may be continued. All adverse events, irrespective of causality, must be recorded in the CRF. All changes in study regimen must be accurately recorded on the study drug pages of the CRF. All adverse events occurring during the trial must be evaluated by the investigator and graded according to the adverse experience grading table (Appendix 2). **Any serious adverse event (including death) due to any cause must be reported immediately to the principal investigator and the sponsor.** The decision to remove a patient for an adverse event, whether laboratory or clinical, will depend on the severity of the event and its relation to the vaccine and study requirements and therefore, will require judgment. The guiding principal will be to insure the safety of participants and integrity of the study. It is expected that all vaccine related grade 4 events will require discontinuation as will most grade 3 events. Vaccines will be discontinued for any Grade event or at the discretion of the investigator or wish of the volunteer. If a volunteer is withdrawn due to an adverse event follow-up will be conducted as outlined in Section 6.1.1.7.

11.5.2. Serious Adverse Events

All SAEs (related and unrelated) will be recorded while on study protocol. Any SAEs considered possibly or probably related to the investigational product and discovered by the Investigator at any time after the study should be reported. All SAEs must be reported to the Institute of Human Virology through the Medical Monitor within one business day of the first awareness of the event. The Investigator must complete, sign and date the SAE pages, verify the accuracy of the information recorded on the SAE pages with the corresponding source documents, and send a copy by fax to the Institute of Human Virology. If a volunteer is withdrawn due to a serious adverse event, follow-up will be conducted as outlined in Section 6.1.1.7.

Additional follow-up information, if required or available, should all be faxed to the Institute of Human Virology within one business day of receipt and this should be completed on a follow-up SAE form and placed with the original SAE information and kept with the appropriate section of the CRF and/or study file.

The Institute of Human Virology is responsible for notifying the relevant regulatory authorities of certain events. It is the Principal Investigator's responsibility to notify the IRB of all SAEs that occur at his or her site. Investigators will also be notified of all unexpected, serious, drug-related events (7/15 Day Safety Reports) that occur during the clinical trial.

11.6. Data Safety Monitoring Board

The Clinical Research Unit is experienced in establishing DSMBs in single-site protocols in the participation in single and multi-site clinical trials. Adverse Event Reporting requirements of the IRB, FDA, the NIH Biotechnology Activities, and the industry/private sponsor will be followed. For this trial, IHV has established a DSMB. The DSMB plan will be approved by the IRB prior to implementation.

The Data Safety Monitoring Board (DSMB) will review this trial for safety beginning with the enrollment of the first volunteer into the study. The Board will meet prior to advancement to the next higher dose group as outlined in the protocol to insure that there are no safety concerns. It will also meet approximately every 3 months but can be called together on an ad-hoc basis if a particular patient event calls for immediate attention. At the regularly scheduled (3 month) meetings and the advancement to the higher dose group meetings, the board will review the following:

- All Adverse Events
- Update on all Serious / Study Related Events
- All safety related amendments
- Protocol deviations / exceptions
- Laboratory tests, enrollment numbers, clinical summaries
- Require changes in Standard Operational Procedures for Adverse Events Reporting and Chart Audit as necessary.

All reviews will be of blinded data. However, the DSMB will have access to the envelopes with the treatment assignment of the volunteers to unblind the data should this be needed to ensure the safety of study participants. The DSMB may recommend or mandate changes to the study, and has the power to halt the study if it feels that a volunteer's safety is compromised. The DSMB will provide the Principal Investigator, with a written report detailing its review of the study. The investigator(s) will have the opportunity to respond to the DSMB's report.

Board members will be notified of all internal serious study related, drug related, and/or device related Adverse Events in this study within 48 hours of occurrence via email. Each board member should respond via e-mail, or fax within 48 hours.

A DSMB member who is a physician will be called upon via fax, e-mail, or phone call to discuss any deviations/exceptions a PI may be thinking of requesting prior to approaching the sponsor. This will help to determine if the deviation/exception is necessary and will not compromise the safety of the patient.

The IHV DSMB will have at least 4 members not including the Medical Monitor. The DSMB will actively monitor the trial. The members of the Board will not be directly involved with the execution of this study, have conflicts of interest with the conduct of this study, or with study personnel. The Board will be composed of at least the following members:

1. A Statistician
2. At least two physician-investigators

DSMB includes the following members: John Bartlett MD from Johns Hopkins SOM who will also serve as committee Chair; Alan Cross MD from the University of Maryland SOM; Philip Mackowiak MD from the University of Maryland SOM; Kirsten Lyke MD (Medical Monitor) from the University of Maryland SOM; and Lawrence Moulton PhD from Johns Hopkins SOM (statistician).

12. Assessment of Immunogenicity

12.1. Endpoint Evaluations of Immunogenicity

12.1.1. Immunogenicity Endpoint

The primary evaluation of immunogenicity will consist of the generation of antibodies to FLSC as determined by antibody titration using FLSC as the detection antigen. A positive vaccine response will be defined as a 4-fold increase in antibody directed against native FLSC protein by ELISA. In addition geometric mean antibodies titers will be assessed and compared among groups.

12.1.2. Secondary Immunogenicity Endpoints

The secondary evaluation of immunogenicity will include the generations of antibodies to gp120 and human CD4, components of FLSC, determined by antibody titration using gp120 and human CD4 as the detection antigen.

12.1.3. Exploratory/Developmental Immunogenicity Endpoints

1. Antigen-specific CD4+ T cell responses measured by peptide stimulated intracellular cytokine production coupled with surface markers to identify CD4+ T cell subsets, particularly those of the T-follicular helper cell lineage.
2. Assess the presence of neutralizing antibodies;
3. Antibody-Dependent Cellular Cytotoxicity activity;
4. To assess and compare the induction of T-helper and cytotoxic T-lymphocyte activity by ELISPOT and intracellular cytokine staining;
5. Additional developmental assays might be performed on de-identified specimens for antigen-specific B cell responses based on emerging literature

12.2. Immunogenicity Assays

12.2.1. Endpoint Assay

ELISA based assay using FLSC protein will be used. A positive vaccine response will be defined as a 4-fold increase in antibody directed against native FLSC protein by ELISA.

The human serum samples collected before and after immunization will be analyzed, after a 1 hour heating step at 60°C, for the presence of anti-$FLSC_I$ antibodies (total IgG) by the ELISA technique. To determine whether the development of anti-FLSC antibodies is really linked to a specific immune stimulation following vaccination and not to a general immune activation, all sera will be also tested against an irrelevant HIV antigen, p24, that is not cross-reactive with FLSC. In this case, the titers against p24 should remain constant. The results will be expressed as half-maximal antibody titers in arbitrary units (log).

The ELISA assays will be conducted as follows: 6 to 12 serial three-fold dilutions of the serum samples, starting at 1/10, will be tested in 96-well ELISA plates pre-coated with the antigen of interest (*ie* FLSC or p24). The presence of specific antibodies will be revealed by addition of an anti-human total IgG peroxidase-conjugate, followed of a chromogenic hydrogen peroxide-specific substrate. Finally, each well will be read by an automatic plate reader at an optical density (OD) of 450-650 nm.

The background will be given by the blanks (*ie* 6 to 12 wells with all the reagents except sera) which OD values should not exceed 0.2 in average. The OD values from all samples will be subtracted from this mean blank OD. One negative control, a serum from an HIV-negative volunteer, will be present on each ELISA plate. Its OD value, subtracted from the mean blank OD, should not exceed 0.2 at the lowest dilution (1/100).

Two anti-FLSC (or anti-p24) human sera will be on on each ELISA plate: one, with a predetermined antibody titer (see below*), will serve as the standard: *ie* all sample titers will be calculated from the regression curve of this standard (OD = f(inverse dilution)) using the SoftMaxPro software (Molecular Devices). The second positive serum will be used as the positive control: its mean titer (m) and the corresponding standard deviation (sd), will have to be calculated from the standard after several independent experiments. The assay will be considered as valid if the titer of this positive control measured on the ELISA plate is included in the titer range: m $\pm$ 3 sd (log).

*The titer of the standard will predetermined in several independent experiments: it will represent the average of the values obtained after all these experiments, each value being the inverse serum dilution giving an OD of around 1 (subtracted from the mean blank OD).

The person responsible for the ELISA assays will be Dr. Timothy Fouts, at Profectus BioSciences.

12.2.2. Secondary Endpoint Assay

ELISA based assay using gp120 and soluble human CD4 will be carried out as described in Section 12.2.1.

12.2.3. Developmental Assays

1. Antigen-specific CD4+ T cell responses will be measured by peptide stimulated intracellular cytokine production coupled with surface markers to identify CD4+ T cell subsets, particularly those of the T-follicular helper cell lineage. These responses will be assessed by culturing 3×10^6 PBMC in 1 mL of R10 medium containing brefeldin A (10 ug/mL) in the absence or presence of FLSC peptide pools (15mers overlapping by 11 residues), or 1 ug/mL SEB (Sigma) for 6 hours. Cells will be surface stained with titrated amounts of anti-CD3, anti-CD4, anti-CD27, anti-CD45RO, anti-CCR7, anti-CXCR5, anti-CD150, anti-CCR6, anti-PD-1 and anti-CD19 antibodies. The surface-stained cells will be permeabilized (Cytofix/Cytoperm kit; BD Biosciences) and stained with anti-IFN-γ, anti-IL-2, anti-IL-17a, anti-IL-21 and anti-CD154 antibodies. Events will be collected on a FACSFortessa equipped with four lasers and a high-throughput module. Electronic compensation will employ antibody capture beads (BD Biosciences) and the data analyzed using FlowJo, Version 10.6.
2. Assess the presence of neutralizing antibodies by TZM-bl cells (Seaman, Janes et al. 2010) and A3R5 assays (Sarzotti-Kelsoe, Daniell et al. 2014).
3. Antibody-dependent Cellular Cytotoxicity activity (ADCC);
4. To assess and compare the induction of T-helper and cytotoxic T-lymphocyte activity by ELISPOT and intracellular cytokine staining;
5. Additional developmental assays might be performed on de-identified specimens for antigen-specific B cell responses based on emerging literature

12.2.4. Assay Timepoints

The scheduled assay timepoints are detailed in Table 4.

12.2.5. Data Analysis

Immunogenicity parameters will be compared using response and non-response as well as mean medium and geometric mean titers between vaccine recipients and placebo recipients (i.e. Arms 1 and 2 compared to Arm 3) as determined by Student's T test *and chi-square analyses*. Immunogenicity parameters will be compared between FLSC in Arms 1, 2, and 3 as well as between the placebo recipients (Arms 1 to 3 and 2 to 3). Exploratory analyses will be performed (e.g., examination of frequency distributions, modality of distributions, kurtosis and skewness, etc.) for all variables collected before subjecting the values to statistical analysis. Cross-sectional parametric and non-parametric analyses will be conducted on variables exhibiting normal and non-normal distributions, respectively, to examine differences and trends among treatment categories.

The dose selected will be that which provides the optimal CD4i epitope immunogenicity and antiviral activity and safety profile. Based on our results in macaques, we believe that the FLSC will be safe even when administered at the highest dose of 300 μg. Titers will be evaluated for normality (Kolmogorov-Smirnov test) then compared via a parametric test such as one-way analysis of variance (ANOVA). If the data fails the normality test we will use the Kruskal-Wallis test (3-group comparison) and Wilcoxon rank-sum test (2-group comparison) to compare groups. A p value < 0.05 will indicate that one of the vaccination regimens elicits significantly higher response in that measure versus the others.

13. STATISTICS

13.1. Determination of Sample Size

The sample size was set to 60 (15 active vaccine recipients in each group and 5 placebo recipients in each group) to allow adequate identification of potential toxicities and document safety. The study design was developed as a compromise between the costs of vaccine manufacturing and regulatory clearance and the need to detect safety. Within each group: The study is planned with 15 experimental volunteers and 5 control volunteers. With expectation that significant adverse event in the control volunteers to be very small (<0.1%), we will be able to detect a minimal SAE rate of 73.5% among experimental volunteers with power of 0.8. The Type I error probability associated with this test of the null hypothesis that the SAE rates for experimental and control subjects are equal is 0.05. We used Fisher's exact test to evaluate this null hypothesis. Pooled across groups: The study is planned with 45 experimental volunteers and 15 control volunteers. With expectation that significant adverse event in the control volunteers to be very small (<0.1%), we will be able to detect a minimal SAE rate of 36.8% among experimental volunteers with power of 0.8. The Type I error probability associated with this test of the null hypothesis that the SAE rates for experimental and control volunteers are equal is 0.05. We used Fisher's exact test to evaluate this null hypothesis.

13.2. Statistical Methods

Each safety parameter assessed will be compared between vaccine recipients and placebo recipients as determined by Student's T test *and chi-square analyses*. In addition safety parameters with be compared between vaccine dose groups as well as to placebo recipients. In addition each volunteer will serve as their own control for the occurrence of toxicity. Adverse reaction as defined in Appendix 1 and 3 will be reported independent of a statistical association.

Immunogenicity parameters will be compared using response and non-response as well as mean medium and geometric mean titers between vaccine recipients and placebo recipients as determined by Student's T test *and chi-square analyses*. Immunogenicity parameters will be compared between vaccine dose groups as well as between each to placebo recipients. Exploratory analyses will be performed (e.g., examination of frequency distributions, modality of distributions, kurtosis and skewness, etc.) for all variables collected before subjecting the values to statistical analysis. Cross-sectional parametric and non-parametric analyses will be conducted on variables exhibiting normal and non-normal distributions, respectively, to examine differences and trends among treatment categories.

13.3. Volunteer Population

Sixty (60) volunteers. Volunteers that do not complete the study may be replaced at the discretion of the principal investigator. Volunteers will be recruited from the healthy population in the Baltimore community and the metropolitan Baltimore/Washington area.

13.3.1. Population analyzed

13.3.1.1. Definitions of Populations

Included patients: volunteers performing at least V0 (screening) visit.

Randomized patients: volunteers assigned a randomization number

Injected patients: volunteers administered at least one dose of product

13.3.1.2. Full Analysis Set

As the sample size was small, immunogenicity data will be analyzed on the population of injected volunteers in an "intent to treat" analysis. This population will be identified as the Full analysis set.

Volunteers' data will be analyzed in the treatment group allocated by randomization.

No per protocol analysis will be performed.

A complete description of protocol violations will be performed in order to investigate the possible impact of protocol violations on the immunogenicity evaluation.

Volunteers included and not injected (i.e., not in the full analysis set) will not be taken into account.

13.3.1.3. Safety Analysis Sets

The safety data will be analyzed for all volunteers who receive at least one injection (= Full analysis set). Only safety information collected after at least one injection will be taken into account. Adverse events which occur between V0 and the first injection will be listed.

In case of randomization error, a volunteer will be analyzed according to the treatment he/she actually received.

For safety evaluation "after any injection" all the volunteers injected and evaluated at least once will be considered as assessable. In case of withdrawal, it may lead to an underestimation of the occurrence rate.

Exploratory analyses will be performed (e.g., examination of frequency distributions, modality of distributions, kurtosis and skewness, etc.) for all variables collected before subjecting the values to statistical analysis.

13.3.1.4. Immunogenicity Analysis Sets

The immunogenicity data will be analyzed for all volunteers who receive at least one injection (= Full analysis set). Only safety information collected after at least one injection will be taken into account.

In case of randomization error, a volunteer will be analyzed according to the treatment he/she actually received.

Exploratory analyses will be performed (e.g., examination of frequency distributions, modality of distributions, kurtosis and skewness, etc.) for all variables collected before subjecting the values to statistical analysis.

13.4. Primary Criterion Analysis

The primary reactogenicity (safety) endpoints are occurrence of severe systemic and severe local reactions. Separate assessments of systemic and local reactions will be performed. Each safety parameter assessed will be compared between vaccine recipients and placebo recipients as determined by Fisher exact test with a Type I error of no greater than 5%.

13.5. Secondary Criterion Analysis

Immunogenicity parameters will be compared using response and non-response as well as mean medium and geometric mean of titers between vaccine recipients and placebo recipients as determined by Wilcoxon matched pairs test or the Sign test and the Fisher exact test. Immunogenicity parameters will be compared between dose groups of the FLSC vaccine as well as between each to placebo recipients. Exploratory analyses will be performed (e.g., examination of frequency distributions, modality of distributions, kurtosis and skewness, etc.) for all variables collected before subjecting the values to statistical analysis.

13.6. Level of significance

Level of significance is not pertinent to this trial as it is a Phase 1 safety and immunogenicity trial.

13.7. Criteria for Termination of the Trial

The Institute of Human Virology and the Principal Investigator reserve the right to discontinue this study for administrative reasons at any time. The trial may also be discontinued if there is insufficient product to continue or if it is determined that the study medication may not be safe for further administration to volunteers. See Section 6.4.1 , Section 6.5 and Section 7.3.

13.8. Procedures for Missing or Spurious Data

Missing or spurious data will be reviewed by appropriate study personnel and the IHV monitors. These personnel will attempt to determine the cause of the missing or spurious data and to clarify the data. Any information that is not obtained as specified in the protocol should be indicated by the designation ND for not done or NA for not applicable. An explanation should be noted on the case report form as to why the required information was not obtained. If illegible or uncertain entries require clarification, the clarification may be printed above the original entry, initialed and dated.

13.9. Changes in Statistical Methods

Any deviation from the original statistical plan will only be done after consultation with a statistician and approval of the sponsor. This is not expected to occur except if it is determined that the original statistical plan is not sufficient or appropriate for the data analyzed.

14. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

14.1. Study Monitoring

Before an investigational site can enter a patient into the study, a representative of Institute of Human Virology will visit the investigational study site to:

- Determine the adequacy of the facilities
- Discuss with the investigator(s) and other personnel their responsibilities with regard to protocol adherence, and the responsibilities of the Institute of Human Virology or its representatives. This will be documented in a Clinical Study Agreement between the Institute of Human Virology and the investigator.

During the study, a monitor from the Institute of Human Virology or representative will have regular contacts with the investigational site, for the following:

- Provide information and support to the investigator(s)
- Confirm that facilities remain acceptable
- Confirm that the investigational team is adhering to the protocol, that data are being accurately recorded in the case report forms, and that investigational product accountability checks are being performed
- Perform source data verification. This includes a comparison of the data in the case report forms with the patient's medical records at the hospital or practice, and other records relevant to the study. This will require direct access to all original records for each patient (e.g. clinic charts).
- Record and report any protocol deviations not previously sent to Institute of Human Virology.
- Confirm AEs and SAEs have been properly documented on CRFs and confirm any SAEs have been forwarded to Institute of Human Virology and those SAEs that met criteria for reporting have been forwarded to the IRB.

The monitor will be available between visits if the investigator(s) or other staff needs information or advice.

14.2. Audits and Inspections

Authorized representatives of the Institute of Human Virology, a regulatory authority, an Independent Ethics Committee or an Institutional Review Board may visit the site to perform audits or inspections, including source data verification. The purpose of an Institute of Human Virology 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, Good Clinical Practice guidelines of the International Conference on Harmonization, and any applicable regulatory requirements. The investigator should contact Institute of Human Virology immediately if contacted by a regulatory agency about an inspection.

14.3. Institutional Review Board (IRB)

The Principal Investigator must obtain IRB approval for the investigation. Initial IRB approval, and all materials approved by the IRB for this study including the patient consent form and recruitment materials must be maintained by the Investigator and made available for inspection.

15. Quality Control and Quality Assurance

To ensure compliance with Good Clinical Practices and all applicable regulatory requirements, the Institute of Human Virology may conduct a quality assurance audit. Please see Section 14.2 for more details regarding the audit process.

15.1. Study Monitoring

The study will be monitored by the Sponsor or its representatives in compliance with government regulations and according to good clinical practices and standard operating procedures. Regular telephone contacts and site visits will be made throughout the course of the study. These individuals will have access, both during the trial and after trial completion, to review and audit all records (including original volunteer records, drug accountability and storage, and general study documentation and execution) necessary to ensure integrity of the data, and will periodically review progress of the study with the principal investigator. Every attempt will be made to follow the protocol and to obtain and record all data requested for each volunteer at the specified times. However, ethical reasons may warrant the failure to obtain and record certain data, or to record data at the times specified. If this becomes necessary, the reasons for such must be clearly documented on the case report form.

Monitoring visits will be made on a frequent basis with advanced notification of the study center by the sponsor or its representatives. Study center personnel will be informed of the study documents/records which will be reviewed at each visit. It is expected that monitoring visits will be made approximately every 4 to 12 weeks dependent upon the rapidity of enrollment.

15.2. Laboratory

All safety laboratories will be performed at the University of Maryland Medical Center Clinical laboratories, a CLIA certified laboratory.

All immunology laboratory studies are planned to occur at the IHV Immunology Laboratory, Profectus Biosciences or one or more of the Bill and Melinda Gates Core Laboratories which currently include the Vaccine Research Center, NIH; the Duke Human Vaccine Center, Durham, North Carolina; Harvard's Ragon Institute, Cambridge, MA, or Dartmouth University, Hanover,NH.

15.3. Handling of Biologic Samples

The transmission of retroviruses may occur through contact with contaminated needles and blood or blood products. Consequently universal blood and body fluid precautions should be employed by all personnel involved in drawing of blood and handling of specimens in clinic and laboratory locations. For samples that have to be shipped to other sites, specific regulations regarding intersFLSCe/international shipment apply and these procedures must be adhered to. It is the responsibility of the investigator to ensure that all study specimens are appropriately handled.

1. *Virology Evaluations:* For HIV PCR and other research virology assays, plasma and cells must be collected and processed within 6 hours, and either stored at < –135 C° for PBMC and < –70° C for serum or used by the laboratory investigator in an appropriate time frame.
2. 2) *Immunology Evaluations:* All clinical and research immunological studies must be performed on samples collected and processed within a 6 hour period and stored at <–135 C° for PBMC and <–70° C for serum or provided to the appropriate laboratory

16. Ethics

16.1. Ethics Review

The final study protocol, including the final version of the Informed Consent Form, must be approved or given a favorable opinion in writing by an IRB which is constituted and operates in accordance with Part 56 of Title 21 of the Code of Federal Regulations. The investigator must submit written approval to the Institute of Human Virology before he or she can enroll any patient/subject into the study.

The Principal Investigator is responsible for informing the IRB or IEC of any amendment to the protocol in accordance with local requirements. In addition, the IRB or IEC must approve all advertising used to recruit patients for the study. The protocol must be re-approved by the IRB or IEC upon receipt of amendments and annually, as local regulations require.

The Principal Investigator is also responsible for providing the IRB with reports of any reportable serious adverse drug reactions from any other study conducted with the investigational product. Institute of Human Virology will provide this information to the Principal Investigator.

Progress reports and notifications of serious adverse drug reactions will be provided to the IRB or IEC according to local regulations and guidelines.

17. Ethical Conduct of the Study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/Good Clinical Practice, applicable regulatory requirements and the Institute of Human Virology's policy on Bioethics.

Investigator Responsibilities

The execution responsibility of this protocol rests with the Principal Investigator. The Principal Investigator is responsible for the submission of the AEs, and an assessment of the causal relationship with the treatment. AEs will be recorded using an adverse experience grading system adapted from the ACTG and AVEG tables; all AEs must be recorded in the case report forms with details of the duration and grade of each episode, and the action taken for each event. A serious AE is considered one that results in death, a life threatening adverse drug experience, inpatient hospitalization, a persistent disability/incapacity, or a congenital abnormality/birth defect. All serious adverse experiences, including death, on study or within 30 days of withdrawal, must be recorded on a serious adverse events (SAE) form and sent to the study sponsor.

Protocol Modification

It is essential to the success of the study that the Investigator adhere to both the spirit and the letter of the protocol. Except in the event of a medical emergency or where necessary to protect the safety, rights or welfare of the study volunteer(s), any changes to the protocol will require the prior written approval of the sponsor, the IHV. In the case of a minor deviation from protocol, a letter from the Sponsor will suffice. Changes to the protocol affecting study objectives, study design, volunteer population, study procedures or significant administrative aspects will require that the protocol be formally amended. Protocol amendments will be made only after having been agreed to by the investigators and IHV. All formal amendments must be approved by the Institutional Review Board (IRB) prior to implementation.

17.1. Written Informed Consent

Volunteer

The volunteer will be informed of the nature of the study agent and its intended purpose; study procedures and associated risks will be explained as well as possible adverse experiences. Written informed consent shall then be obtained from each volunteer, in accordance with FDA regulations set forth in Part 50 of Title 21 of the Code of Federal Regulations, prior to entering the volunteer into the trial or prior to performing any unusual or non routine procedure that involves risk to the volunteer. The investigator shall provide a copy of the current IRB-approved informed consent to the volunteer and a signed copy shall be maintained in the volunteer's record file. The informed consent will be written in accordance with US Federal Regulations for Protection of Human Subjects (21 CFR 50.25[a] and 21 CFR 50.25[b]).

Investigator

The Principal Investigator(s) at each center will ensure that the patient is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study. Patients must also be notified that they are free to discontinue from the study at any time. The patient should be given the opportunity to ask questions and allowed time to consider the information provided.

The patient's signed and dated informed consent must be obtained before conducting any study procedures.

The Principal Investigator(s) must maintain the original, signed Informed Consent Form. A copy of the signed Informed Consent Form must be given to the patient.

18. DATA HANDLING AND RECORDKEEPING

18.1. Inspection of Records

Institute of Human Virology will be allowed to conduct site visits to 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, study drug stocks, drug accountability records, volunteer charts and study source documents, and other records relative to study conduct. In addition, representatives of the Food and Drug Administration (FDA) and the Institutional Review Board (IRB) shall have access to the source data and documents when requested. During any document reviews, volunteer anonymity will be maintained with strict adherence to professional standards of confidentiality.

18.2. Retention of Records

The Principal Investigator must maintain all documentation relating to the study for a period of 2 years after the last marketing application approval, or if not approved 2 years following the discontinuance of the test article for investigation. If it becomes necessary for Institute of Human Virology or the Regulatory Authority to review any documentation relating to the study, the Investigator must permit access to such records.

18.3. Data Recording

Source documents of all data will be maintained in the volunteer's research clinical record. In addition Case Reports Forms (CRF) will be created and maintained for each volunteer utilizing REDCap(Research Electronic Data Capture) software. All study data will be recorded on the Case Report Forms (CRFs). REDCap tracks all data corrections made by authorized users, providing audit trails for monitoring or query.

Any information that is not obtained as specified in the protocol should be indicated by the designation ND for not done or NA for not applicable. An explanation should be noted on the case report form as to why the required information was not obtained.

18.4. Data Control Methods

In order to assure adequate control and provide study data which are consistent and of the highest quality, the following measures will be employed:

- Each clinical procedure (*e.g*. physical examination) for a particular volunteer will be conducted by the same person, if possible, throughout the volunteer's study participation;
- Data automatically generated will be acceptable only after review by the appropriate specialist; for example, computer-generated EKG interpretation must be signed off by a cardiologist.
    - Adverse Events will be both volunteered by the volunteer and specially elicited by questioning. Careful unbiased monitoring of volunteers for development of adverse experiences will be essential throughout the study. Volunteers will be questioned at every clinical visit in order to detect toxicity, however mild. Volunteer interviews must be conducted in a non-leading way (e.g. "How have you been feeling since your last clinic visit; have you noticed anything out of the ordinary?"). Questions may be specific to symptoms previously reported in order to follow ongoing complaints.

19. PUBLICATION POLICY

All personnel involved in the study shall not disclose or use for any purpose any data, records, and other information related to the study until approved by Principal Investigator. The data shall be confidential until publication. Any publication or presentation related to the trial must be approved by the Institute of Human Virology before submission of the manuscript.

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21. APPENDICES

APPENDIX 1. DETAILED REACTOGENICITY FORM

(Note these are the items which will be on the diary card given to each volunteer after each immunization to record any events occurring in the first 7 days following each immunization.)

PATIENT NAME: _____ DATE: _____
STUDY NUMBER:_____STUDY DAY:_____ NURSE:_____

PLEASE ENTER ANY SYMPTOMS YOU MAY HAVE WITHIN 48 HOURS AFTER YOUR VACCINE INJECTION IN THE COLUMN BESIDE IT. MAIL IT IN THE ENVELOPE PROVIDED (CIRCLE Y OR N)

SYMPTOM	YES/NO		ONSET (m/d/y)	DURATION (hours)
FEVER	Y	N		
CHILLS	Y	N		
NAUSEA	Y	N		
VOMITING	Y	N		
JOINT PAINS (arthralgia)	Y	N		
MUSCLE ACHES (myalgia)	Y	N		
FATIGUE (malaise)	Y	N		
HIVES OR RASH (urticaria)	Y	N		
WHEEZING	Y	N		
HEADACHES	Y	N		
ITCHING	Y	N		
PAIN	Y	N		
DIARRHEA	Y	N		
OTHER	Y	N		

*PLEASE CALL YOUR PROTOCOL NURSE/PHYSICIAN IF YOU SHOULD HAVE ANY OF THE SIGNS AT THE INJECTION SITE ON THIS LIST (CIRCLE Y OR N).

SYMPTOM	YES/NO		ONSET (m/d/y)	DURATION (hours)
REDNESS (erythema)	Y	N		
ROUGHNESS (induration)	Y	N		
SWELLING	Y	N		
NODULE (subcutaneous)	Y	N		
TENDERNESS	Y	N		
SKIN BREAKDOWN	Y	N		
SWOLLEN OR TENDER LYMPH NODES UNDER YOUR ARM	Y	N		
INABILITY TO MOVE OR USE ARM	Y	N		
OTHER	Y	N		

Reviewed by_____(Protocol Nurse)

Reviewed by_____(Study MD)

APPENDIX 2. TABLE FOR GRADING SEVERITY OF ADULT ADVERSE EXPERIENCES

(Adapted from the ACTG and AVEG tables)

ABBREVIATIONS: Abbreviations utilized in the Table:

ULN = Upper Limit of Normal	LLN = Lower Limit of Normal
Rx = Therapy	Req = Required
Mod = Moderate	IV = Intravenous
ADL = Activities of Daily Living	Dec = Decreased

ESTIMATING SEVERITY GRADE

For abnormalities NOT found elsewhere on the Table, use the scale below to estimate grade of severity:

GRADE 1 Mild	Transient or mild discomfort; no limitation in activity; no medical intervention/therapy required
GRADE 2 Moderate	Mild to moderate limitation in activity - some assistance may be needed; no or minimal medical intervention/therapy required
GRADE 3 Severe	Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalizations possible
GRADE 4 Life- threatening	Extreme limitation in activity, significant assistance required; significant medical intervention/therapy required, hospitalization or hospice care probable

SERIOUS OR LIFE-THREATENING AEs

ANY clinical event deemed by the clinician to be serious or life-threatening should be considered a grade 4 adverse experience. Clinical events considered to be serious or life-threatening include, but are not limited to:

seizures, coma, tetany, diabetic ketoacidosis, disseminated intravascular coagulation, diffuse petechiae, paralysis, acute psychosis

MISCELLANEOUS

> When two values are used to define the criteria for each parameter, the lowest values will occur first.

> Parameters are generally grouped by body system.

> Some protocols may have additional protocol specific grading criteria.

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE THREATENING
HEMATOLOGY				
Hemoglobin	8.0 g/dL - 9.4 g/dL	7.0 g/dL - 7.9 g/dL	6.5 g/dL - 6.9 g/dL	<6.5 g/dL
Absolute Neutrophil Count	1000 - 1500/mm^3	750 - 999/mm^3	500 - 749/mm^3	<500/mm^3
Platelets	75,000 - 99,000/mm^3	50,000 - 74,999/mm^3	20,000 - 49,999/mm^3	<20,000/mm^3
Prothrombin Time (PT)	>1.0 - 1.25 x ULN	>1.25 - 1.5 x ULN	>1.5 - 3.0 x ULN	>3 x ULN
PTT	>1.0 - 1.66 x ULN ULN	>1.66 - 2.33 x ULN	>2.33 - 3.0 x ULN	>3.0 x ULN
Methemoglobin	5.0 - 10.0%	10.1 - 15.0%	15.1 - 20.0%	>20%
CHEMISTRIES				
SODIUM				
Hyponatremia	130 - 135 meq/L	123 - 129 meq/L	116 - 122 meq/L	<116 meq/L
Hypernatremia	146 - 150 meq/L	151 - 157 meq/L	158 - 165 meq/L	>165 meq/L
POTASSIUM				
Hypokalemia	3.0 - 3.4 meq/L	2.5 - 2.9 meq/L	2.0 - 2.4 meq/L	<2.0 meq/L
Hyperkalemia	5.6 - 6.0 meq/L	6.1 - 6.5 meq/L	6.6 - 7.0 meq/L	>7.0 meq/L
PHOSPHATE				
Hypophosphatemia	2.0 - 2.4 mg/dL	1.5 - 1.9 mg/dL	1.0 - 1.4 mg/dL	<1.0 mg/dL
CALCIUM - (corrected for albumin)				
Hypocalcemia	7.8 - 8.4 mg/dL	7.0 - 7.7 mg/dL	6.1 - 6.9 mg/dL	<6.1 mg/dL
Hypercalcemia	10.6 - 11.5 mg/dL	11.6 - 12.5 mg/dL	12.6 - 13.5 mg/dL	>13.5 mg/dL
MAGNESIUM				
Hypomagnesemia	1.2 - 1.4 meq/L	0.9 - 1.1 meq/L	0.6 - 0.8 meq/L	<0.6 meq/L
BILIRUBIN				
Hyperbilirubinemia	>1.0 - 1.5 x ULN	>1.5 - 2.5 x ULN	>2.5 - 5 x ULN	>5 x ULN

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE THREATENING
GLUCOSE				
Hypoglycemia	55 - 64 mg/dL	40 - 54 mg/dL	30 - 39 mg/dL	<30 mg/dL
Hyperglycemia (nonfasting and no prior diabetes)	116 - 160 mg/dL	161 - 250 mg/dL	251 - 500 mg/dL	>500 mg/dL
Triglycerides	------	400 - 750 mg/dL	751 - 1200 mg/dL	>1200 mg/dL
Creatinine	>1.0 - 1.5 x ULN	>1.5 - 3.0 x ULN	>3.0 - 6.0 x ULN	>6.0 x ULN
URIC ACID				
Hyperuricemia	7.5 - 10.0 mg/dL	10.1 - 12.0 mg/dL	12.1 - 15.0 mg/dL	>15.0 mg/dL
LIVER TRANSAMINASE (LFTs)				
AST (SGOT)	1.25 - 2.5 x ULN	>2.5 - 5.0 x ULN	>5.0 - 10.0 x ULN	>10.0 x ULN
ALT (SGPT)	1.25 - 2.5 x ULN	>2.5 - 5.0 x ULN	>5.0 - 10.0 x ULN	>10.0 x ULN
GGT	1.25 - 2.5 x ULN	>2.5 - 5.0 x ULN	>5.0 - 10.0 x ULN	>10.0 x ULN
Alk Phos	1.25 - 2.5 x ULN	>2.5 - 5.0 x ULN	>5.0 - 10.0 x ULN	>10.0 x ULN
PANCREATIC ENZYMES				
Amylase	>1.0 - 1.5 x ULN	>1.5 - 2.0 x ULN	>2.0 - 5.0 x ULN	>5.0 x ULN
Pancreatic amylase	>1.0 - 1.5 x ULN	>1.5 - 2.0 x ULN	>2.0 - 5.0 x ULN	>5.0 x ULN
Lipase	>1.0 - 1.5 x ULN	>1.5 - 2.0 x ULN	>2.0 - 5.0 x ULN	>5.0 x ULN
CARDIOVASCULAR				
Cardiac Arrhythmia	-----	Asymptomatic; transient dysrhythmia, no Rx req	Recurrent/persistent dysrhythmia; symptomatic Rx req	Unstable dysrhythmia, hospitalization and Rx req

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE THREATENING
Hypotension	Transient orthostatic hypotension, no Rx (with heart rate increased by >20 beats/min correctable with OR decreased by <10 mm Hg systolic BP)	Symptoms (OR BP decreased by <20 mm Hg systolic) oral fluid Rx	IV fluid req, no hospitalization req	Hospitalization req
Hypertension	Transient, increase >20 mm/Hg; no Rx	Recurrent; chronic increase, >20 mm/Hg, Rx req	Acute Rx req; outpatient hospitalization possible	Hospitalization req
Pericarditis	Minimal effusion	Mild/mod asymptomatic effusion, no Rx	Symptomatic effusion, pain, EKG changes	Tamponade OR pericardio centesis OR surgery req
Hemorrhage, blood Loss	------	Mildly symptomatic, no Rx required	Gross blood loss OR 1-2 units transfused	Massive blood loss OR >2 units transfused
GASTROINTESTINAL				
Nausea	Mild OR transient; reasonable intake maintained	Mod discomfort OR intake decreased for <3 days	Severe discomfort OR minimal intake for ≥3 days	Hospitalization req
Vomiting	Mild OR transient; 2-3 episodes per day OR mild vomiting lasting <1 week	Mod OR persistent; 4-5 episodes per day; OR vomiting lasting ≥1 week	Severe vomiting of all food/fluids in 24 hrs OR orthostatic hypotension OR IV Rx req	Hypotensive shock OR hospitalization req for IV Rx req
Diarrhea	Mild OR transient; 3-4 loose stools per day OR mild diarrhea lasting <1 week	Mod OR persistent; 5-7 loose stools per day OR diarrhea lasting ≥1 week	Bloody diarrhea; OR orthostatic hypotension OR >7 loose stools/day OR IV Rx required	Hypotensive shock OR hospitalization req

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE THREATENING
Oral Discomfort/ Dysphagia	Mild discomfort, no difficulty swallowing	Difficulty swallowing but able to eat and drink	Unable to swallow solids	Unable to drink fluids; IV fluids req
Constipation	Mild	Moderate (abdominal pain for 78 hours with impaction, require output prescription	Severe (Requiring disimpaction or hospital treatment)	Distention with vomiting(OR obstipation)
RESPIRATORY				
Cough (for aerosol studies)	Transient; no Rx	Treatment associated cough; inhaled bronchodilator	Uncontrolled cough;systemic Rx req	------
Bronchospasm Acute	Transient; no Rx; FEV1 70% - <80% (or peak flow)	Rx req; normalizes with bronchodilator; FEV1 50%-<70% (or peak flow)	No normalization with bronchodilator; FEV1 25% - <50% (or peak flow), retractions	Cyanosis; FEV1 <25% (or peak flow) OR intubated
Dyspnea	Dyspnea on exertion	Dyspnea with normal activity	Dyspnea at rest	Dyspnea requiring O_2 therapy
NEUROLOGIC				
Neuro-cerebellar	Slight incoordination OR dysdiadochokinesia	Intention tremor OR dysmetria OR slurred speech OR nystagmus	Ataxia requiring assistance to walk or arm incoordination interfering with ADLs	Unable to stand
Neuro-psych/mood medical	------ hospitalization	------	Severe mood changes requiring intervention	Acute psychosis req (suicidal gesture/attempts)

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE THREATENING
Paresthesia (burning, tingling, etc)	Mild discomfort; no Rx req	Mod discomfort; non-narcotic analgesia req	Severe discomfort;OR narcotic analgesia with symptomatic req with symptomatic improvement	Incapacitating; OR not responsive to narcotic analgesia
Neuro-motor	Mild weakness in muscle of feet but able to walk and/or mild increase or decrease in reflexes	Mod weakness in feet(unable to walk on heels and/or toes), mild weakness in hands, still able to do most hand tasks and/or loss of previously present reflex or development of hyperreflexia and/or unable to do deep knee bends due to weakness	Marked distal weakness (unable to dorsiflex toes or foot drop), and mod proximal weakness e.g., in hands interfering with ADLs and/or req assistance to walk and/or unable to rise from chair unassisted	Confined to bed or wheel chair because of
Neuro-sensory	Mild impairment (dec sensation, e.g., vibratory, pinprick, hot/cold in great toes) in focal area or symmetrical distri-bution	Mod impairment (mod dec sensation, e.g., vibratory, pinprick, hot/cold to ankles) and/or joint position or mild impairment that is not symmetrical	Severe impairment (dec or loss of sensation to knees or wrists) or loss of sensation of at least mod degree in multiple different body areas (i.e., upper and lower extremities)	Sensory loss involves limbs and trunk.
MUSCULOSKELETAL				
Arthralgia/Arthritis	Arthralgia	Arthralgia with joint effusion or moderate impairment of activity	Frank arthritis with or without effusion OR resulting in severe impair-ment of activity	------

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE THREATENING
Myalgia	Myalgia without limitation of activity	Muscle tenderness at other than injection site or with moderate impairment of activity	Frank myonecrosis OR with severe impairment of activity	-----
URINALYSIS				
Proteinuria				
Spot urine	1+	2 - 3+	4+	Nephrotic Syndrome
24 hour urine	200 mg-1 g loss/day OR <0.3% OR <3 g/l	>1 - 2 g loss/day OR 0.3 - 1.0% OR 3 - 10 g/l	>2 - 3.5 g loss/day OR >1.0% OR >10 g/l	Nephrotic syndrome OR >3.5 g loss/day
Gross Hematuria	Microscopic only	Gross, no clots	Gross plus clots	Obstructive OR transfusion req
MISCELLANEOUS				
Fever oral >12 hours	37.7 - 38.5C OR 100.0 - 101.5F	38.6 - 39.5C OR 101.6 - 102.9F	39.6 - 40.5C OR 103 - 105F	>40.5C OR >105F
Headache	Mild; no Rx req	Mod; OR non-narcotic analgesia Rx	Severe; OR responds to initial narcotic Rx	Intractable; OR requiring repeated narcotic Rx
Allergic Reaction	Pruritus without rash	Localized urticaria	Generalized urticaria angioedema	Anaphylaxis

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE THREATENING
Cutaneous/Rash/ Dermatitis	Erythema, pruritus	Diffuse maculopapular rash OR dry desquamation	Vesiculation OR moist desquamation	ANY ONE: mucous membrane involvement, OR ulceration suspected Stevens-Johnson(TEN), erythema multiforme, necrosis req surgery, exfoliative dermatitis
Local Reaction (2^o parenteral Rx - not vaccination or skin test)	Erythema	Induration <10mm OR inflammation OR phlebitis	Induration >10mm OR ulceration	Necrosis of skin
Fatigue	Normal activity reduced <25%	Normal activity reduced 25-50%	Normal activity reduced >50%; cannot work	Unable to care for self
Eye	-----	Mild pain, visual changes, conjunctival erythema, abnormal slit lamp	Loss of vision, clinically diagnosed uveitis, moderate to severe pain, glaucoma	-----

APPENDIX 3. INVESTIGATIONAL DRUG ACCOUNTABILITY POLICY/PROCEDURE

Section 06
Policy No. 05
PAGE 1 of 4

UNIVERSITY OF MARYLAND MEDICAL CENTER **DEPARTMENT OF PHARMACY SERVICES** **POLICY AND PROCEDURE MANUAL**	**POLICY NO. 06-05** Effective Date: 08/06 Revision Date: 08/07
P&P SECTION: Investigational Drug Services SUBJECT: Investigational Drug Accountability	**Policy Owner: Assistant Director,** Clinical Pharmacy Services Site Application: UMMC Distribution: Pharmacy Leadership
APPROVALS: Director, Department of Pharmacy Services_____	

1. PURPOSE

The purpose of this policy is to ensure complete and accurate records of investigational drug accountability.

2. SCOPE

This policy applies to all pharmacists and technicians involved in preparation and dispensing of investigational drugs. The National Cancer Institute (NCI) accountability form is the preferred drug accountability record form (DARF) for all investigational studies, unless the sponsor requires different dispensing documentation. The procedure defined below pertains to the NCI DARF.

3. RESPONSIBILITY

All pharmacists and technicians involved in preparation and dispensing of investigational drugs are responsible for maintaining accurate and complete records of drug accountability.

4. POLICY

Investigational drug accountability records will meet defined requirements for completeness and accuracy.

5. PROCEDURES

5.1 A separate accountability log will be maintained for each drug in each protocol. If there is more than one strength or dosage form for a drug for the same protocol, a separate DARF is required for each product (e.g. a drug with a 1 mg vial and a 5 mg vial requires a different DARF for the 1 mg vial and for the 5 mg vial).

5.2 The accountability log will be completed accurately.

5.2.1 Each line will be completed for all spaces.

5.2.2 Corrections will be performed by placing one line through the error, then initialing, and dating the error. Erasures and "whiteouts" are not permitted.

5.2.3 A black ball point pen will be used to record all entries on the logs.

5.2.4 All agent transactions will be noted on the DARF (e.g. receipt of agent, broken vials, returns, transfer of agent).

5.2.5 No clinical notes will be placed on the DARF.

5.3 To begin a new DARF, complete the upper portion of the form as follows:

Page Number - Record the page number consecutively on the forms for each drug used on the protocol.

Control Record- Check this box if the record is being used to account for research drug stored at the control pharmacy.

Satellite Record – Check this box if the record is being used to account for research drug not at the control pharmacy or primary site.

Name of Institution (the name of the location to which the drug is shipped from NCI)

Protocol Number (NCI) (may add the institutional protocol number if necessary)

Drug Name, Dose Form and Strength

Protocol Title - May use abbreviations if necessary.

Dispensing Area (the location where the drug is dispensed, e.g. infusion center pharmacy, satellite location, central pharmacy)

Investigator (name of the investigator whose name the drug is ordered from NCI)

Balance Forward- Enter zero if the current page is the first page or bring the balance forward from the previous page.

5.4 Upon receiving a drug shipment, enter the following information on the DARF **:**

Date (Date the drug is received including year)

Patient's Initials, Patient's ID Number and Dose - Enter "Received from [name of the study sponsor]"

Quantity Dispensed or Received (" + " and # of vials, ampoules, tablets, or kits indicating # to be added to balance)

Balance (Balance total)

Manufacturer and Lot Number - If the drug shipment contains more than one lot number, make separate entry for optimal record keeping.

Recorder's Initials

5.5 When preparing drugs for dispensing, enter the following information on the DARF**:**

Date (the date of dispensing/preparation including year)

Patient's Initials - Use the same initials as for the protocol registration.

Patient's I.D. Number (patient's study number)

Dose (actual dose administered)

Quantity Dispensed ("-" and # of vials, ampoules, tablets, or kits dispensed indicating the # to be subtracted from balance)

Balance - Balance total should equal quantity on hand.

Manufacturer and Lot Number - If there is more than one lot number being used, make separate entries for optimal record keeping.

Recorder's Initials

5.6 When transferring investigational drugs to a satellite location, enter the following information on the DARF**:**

Date (the date of transfer including year)

Patient's Initials, Patient's I.D. Number, and Dose ("transfer to [name of location]")

Quantity Dispensed and Received ("-" and # of vials, ampoules, tablets or kits dispensed indicating the # to be subtracted from the balance; provide sufficient supply for at least one treatment; also document receipt of returned drug with "+" and the # received from the satellites indicating an addition to the balance)

Balance - Balance total should equal quantity on hand.

Manufacturer and Lot Number - If there is more than one lot number being used, make separate entries for optimal record keeping.

Recorder's Initials

5.7 Upon receiving the drugs, the satellite should enter the following information on the DARF**:**

Date (the date of transfer including year)

Patient's Initials, Patient's I.D. Number, and Dose ("Received or transferred from Control Pharmacy")

Quantity Received ("+" and # received indicating # to be added to the balance)

Balance (balance total)

Manufacturer and Lot Number If there is more than one lot number being used, make separate entries for optimal record keeping.

Recorder's Initials

5.8 To return drugs to NCI or the sponsor, complete the Return Drug List Form and enter the following information in the DARF:

Date (the date of return including year)

Patient's Initials, Patient's I.D. Number, and Dose – Enter "Returned to NCI or [name of sponsor]"

Quantity Received ("-" and # returned indicating # to be subtracted from the balance)

Balance (balance total)

Manufacturer and Lot Number- If there is more than one lot number being used, make separate entries for optimal record keeping.

Recorder's Initials

5.9 When transferring DCTB supplied study drugs to another DCTD approved protocol, enter the following on the DARF

Date (the date of transfer including year)

Patient's Initials, Patient's I.D. Number, and Dose ("transfer to [name of protocol]")

Quantity Dispensed and Received ("-" and # of vials, ampoules, tablets or kits transferred)

Balance (balance total should equal quantity on hand)

Manufacturer and Lot Number - If there is more than one lot number being used, make separate entries for optimal record keeping.

Recorder's Initials

5.10 When subject specific multi-dose investigational agent kit is dispensed, all transactions will be recorded following:

Subject specific investigational agent kit may contain multi-dose investigational agents.

Investigational drug service pharmacy must keep master and subject specific drug accountability logs for multi-dose investigational agent kit.

Upon receipt of multi-dose subject specific kit from the sponsor, it will be logged in into a master drug accountability log as "Received from (sponsor's name)".

When the multi-dose subject specific kit is dispensed to the specific study subject, entire assigned investigational drug kit will be logged out from the master drug accountability log on the date of the first treatment as "Transferred to (subject's initials) drug accountability log".

The entire assigned multi-dose subject specific kit will be logged in into the subject specific drug accountability log on the date of the first treatment as "Received from master drug accountability log".