HIV VACCINE
TRIALS NETWORK

# PROTOCOL

# HVTN 119

## A phase 1 clinical trial to evaluate the safety and immunogenicity of pDNA vaccines expressing HIV M Group $p24^{Gag}$ conserved elements and/or $p55^{Gag}$, administered with *IL-12* pDNA by intramuscular electroporation, in healthy, HIV-uninfected adult participants

**DAIDS DOCUMENT ID 12061**

**BB IND *TBD* HELD BY DAIDS**

**CLINICAL TRIAL SPONSORED BY**

Division of AIDS (DAIDS)
National Institute of Allergy and Infectious Diseases (NIAID)
National Institutes of Health (NIH)
Department of Health and Human Services (DHHS)
Bethesda, Maryland, USA

**STUDY PRODUCT(S) PROVIDED BY**

DAIDS, NIAID, NIH, DHHS
Bethesda, Maryland, USA

Profectus BioSciences, Inc.
Tarrytown New York, USA

Ichor Medical Systems, Inc.
San Diego, California, US

**February 17, 2017**
FINAL
HVTN 119, Version1.0

# Contents

# 1 Ethical considerations

Multiple candidate HIV vaccines will need to be studied simultaneously in different populations around the world before a successful HIV preventive vaccine is found. It is critical that universally accepted ethical guidelines are followed at all sites involved in the conduct of these clinical trials. The HIV Vaccine Trials Network (HVTN) has addressed ethical concerns in the following ways:

- HVTN trials are designed and conducted to enhance the knowledge base necessary to find a preventive vaccine, using methods that are scientifically rigorous and valid, and in accordance with Good Clinical Practice (GCP) guidelines.

- HVTN scientists and operational staff incorporate the philosophies underlying major codes [1-3], declarations, and other guidance documents relevant to human subjects research into the design and conduct of HIV vaccine clinical trials.

- HVTN scientists and operational staff are committed to substantive community input—into the planning, conduct, and follow-up of its research—to help ensure that locally appropriate cultural and linguistic needs of study populations are met. Community Advisory Boards (CAB) are required by DAIDS and supported at all HVTN research sites to ensure community input.

- HVTN clinical trial staff counsel study participants routinely on how to reduce HIV risk. Participants who become HIV-infected during the trial are provided counseling on notifying their partners and about HIV infection according to local guidelines. Staff members will also counsel them about reducing their risk of transmitting HIV to others.

- Participants who become HIV-infected during the trial are referred to medical practitioners to manage their HIV infection and to identify potential clinical trials they may want to join.

- The HVTN provides training so that all participating sites similarly ensure fair participant selection, protect the privacy of research participants, and obtain meaningful informed consent. During the study, participants will have their wellbeing monitored, and to the fullest extent possible, their privacy protected. Participants may withdraw from the study at any time.

- Prior to implementation, HVTN trials are rigorously reviewed by scientists who are not involved in the conduct of the trials under consideration.

- HVTN trials are reviewed by local and national regulatory bodies and are conducted in compliance with all applicable national and local regulations.

- The HVTN designs its research to minimize risk and maximize benefit to both study participants and their local communities. For example, HVTN protocols provide enhancement of participants' knowledge of HIV and HIV prevention, as well as counseling, guidance, and assistance with any social impacts that may result from research participation. HVTN protocols also include careful medical review of each

research participant's health conditions and reactions to study products while in the study.

- HVTN research aims to benefit local communities by directly addressing the health and HIV prevention needs of those communities and by strengthening the capacity of the communities through training, support, shared knowledge, and equipment. Researchers involved in HVTN trials are able to conduct other critical research in their local research settings.

- The HVTN values the role of in-country Institutional Review Boards (IRBs), Ethics Committees (ECs), and other Regulatory Entities (REs) as custodians responsible for ensuring the ethical conduct of research in each setting.

# 2 IRB/EC review considerations

US Food and Drug Administration (FDA) and other US federal regulations require IRBs/ECs/REs to ensure that certain requirements are satisfied on initial and continuing review of research (Title 45, Code of Federal Regulations (CFR), Part 46.111(a) 1-7; 21 CFR 56.111(a) 1-7). The following section highlights how this protocol addresses each of these research requirements. Each HVTN Investigator welcomes IRB/EC/RE questions or concerns regarding these research requirements.

## 2.1 Minimized risks to participants

**45 CFR 46.111 (a) 1 and 21 CFR 56.111 (a) 1: Risks to subjects are minimized.**

This protocol minimizes risks to participants by (a) correctly and promptly informing participants about risks so that they can join in partnership with the researcher in recognizing and reporting harms; (b) respecting local/national blood draw limits; (c) performing direct observation of participants postvaccination and collecting information regarding side effects for several days postvaccination; (d) having staff properly trained in administering study procedures that may cause physical harm or psychological distress, such as blood draws, vaccinations, HIV testing and counseling and HIV risk reduction counseling; (e) providing HIV risk reduction counseling and checking on contraception use (for women); and (f) providing safety monitoring.

## 2.2 Reasonable risk/benefit balance

**45 CFR 46.111(a) 2 and 21 CFR 56.111(a) 2: Risks to subjects are reasonable in relation to anticipated benefits, if any, to subjects, and the importance of the knowledge that may reasonably be expected to result.**

In all public health research, the risk-benefit ratio may be difficult to assess because the benefits to a healthy participant are not as apparent as they would be in treatment protocols, where a study participant may be ill and may have exhausted all conventional treatment options. However, this protocol is designed to minimize the risks to participants while maximizing the potential value of the knowledge it is designed to generate.

## 2.3 Equitable participant selection

**45 CFR 46.111 (a) 3 and 21 CFR 56.111 (a) 3: Subject selection is equitable**

This protocol has specific inclusion and exclusion criteria for investigators to follow in admitting participants into the protocol. Participants are selected because of these criteria and not because of positions of vulnerability or privilege. Investigators are required to maintain screening and enrollment logs to document volunteers who screened into and out of the protocol and for what reasons.

## 2.4 Appropriate informed consent

**45 CFR 46.111 (a) 4 & 5 and 21 CFR 56.111 (a) 4 & 5: Informed consent is sought from each prospective subject or the subject's legally authorized representative as required by 45 CFR 46.116 and 21 CFR Part 50; informed consent is appropriately documented as required by 45 CFR 46.117 and 21 CFR 50.27**

The protocol specifies that informed consent must be obtained before any study procedures are initiated and assessed throughout the trial (see Section 9.1). Each site is provided training in informed consent by the HVTN as part of its entering the HVTN. The HVTN requires a signed consent document for documentation, in addition to chart notes or a consent checklist.

## 2.5 Adequate safety monitoring

**45 CFR 46.111 (a) 6 and 21 CFR 56.111 (a) 6: There is adequate provision for monitoring the data collected to ensure the safety of subjects.**

This protocol has extensive safety monitoring in place (see Section 11). Safety is monitored daily by HVTN Core and routinely by the HVTN 119 Protocol Safety Review Team (PSRT). In addition, the HVTN Safety Monitoring Board (SMB) periodically reviews study data.

## 2.6 Protect privacy/confidentiality

**45 CFR 46.111 (a) 7 and 21 CFR 56.111 (a) 7: There are adequate provisions to protect the privacy of subjects and maintain the confidentiality of data.**

Privacy refers to an individual's right to be free from unauthorized or unreasonable intrusion into his/her private life and the right to control access to individually identifiable information about him/her. The term "privacy" concerns research participants or potential research participants as individuals whereas the term "confidentiality" is used to refer to the treatment of information about those individuals. This protocol respects the privacy of participants by informing them about who will have access to their personal information and study data (see Appendix A). The privacy of participants is protected by assigning unique identifiers in place of the participant's name on study data and specimens. In the United States, research participants in HVTN protocols are protected by a Certificate of Confidentiality from the US NIH, which can prevent disclosure of study participation even when that information is requested by subpoena. Participants are told of the use and limits of the certificate in the study consent form. In addition, each staff member at each study site in this protocol signs an Agreement on Confidentiality and Use of Data and Specimens with the HVTN and each study site participating in the protocol is required to have a standard operating procedure on how the staff members will protect the confidentiality of study participants.

# 3 Overview

### Title

A phase 1 clinical trial to evaluate the safety and immunogenicity of pDNA vaccines expressing HIV M Group $p24^{Gag}$ conserved elements and/or $p55^{Gag}$, administered with *IL-12* pDNA by intramuscular electroporation, in healthy, HIV-uninfected adult participants

### Primary objectives

- To evaluate the safety and tolerability of the HIV-1 pDNA vaccines p24CE1/2 and $p55^{gag}$ administered with *IL-12* pDNA by intramuscular injection with electroporation
- To compare the two pDNA prime/boost strategies with respect to breadth (defined as the number of targeted CEs) of $CD4^+$ and $CD8^+$ T-cell responses to conserved regions of the $p24^{Gag}$ protein

### Study products and routes of administration

- p24CE1/2 is a DNA plasmid (pDNA) that encodes 2 cassettes, p24CE1 and p24CE2, each consisting of 7 conserved element (CE) sequences from HIV-1 $p24^{Gag}$ separated by short amino acid spacers. The p24CE1 and p24CE2 proteins differ by 7 amino acids and together cover >98% of HIV-1 Group M sequences worldwide. The p24CE1 cassette is under the control of the human CMV promoter/enhancer and the bovine growth hormone (BGH) polyadenylation signal. The p24CE2 cassette is in the opposite transcriptional orientation and is under the control of the simian CMV promoter and the simian virus 40 (SV40) polyadenylation signal.

- $p55^{gag}$ refers to a DNA plasmid encoding an expression-optimized full length HIV-1 $p55^{Gag}$ protein from the HIV-1 molecular clone HXB2 (Genbank NP_057850).

- *IL-12* refers to GENEVAX® IL-12 DNA Plasmid (Profectus BioSciences, Inc., Tarrytown, New York, USA). The *IL-12* pDNA adjuvant is a dual promoter plasmid that expresses the genes encoding human IL-12 proteins p35 and p40 under separate regulatory control. The p35 subunit is under the control of the human CMV promoter/enhancer and the SV40 polyadenylation signal. The p40 subunit is under the control of the simian CMV promoter and the BGH polyadenylation signal. The plasmid vaccines will be admixed with *IL-12* pDNA to produce a single injectable solution delivered by intramuscular (IM) injection followed by electroporation.
- Placebo: Sodium Chloride for Injection, USP 0.9%.
- Intramuscular TriGrid Delivery System (TDS-IM) (Ichor Medical Systems, San Diego, California), a device for electroporation mediated intramuscular administration of DNA based-biologic candidates.

**Table 3-1 Schema**

| Study arm | Number | Dose (total) | Month 0 (Day 0) | Month 1 (Day 28) | Month 3 (Day 84) | Month 6 (Day 168) |
|---|---|---|---|---|---|---|
| Group 1 | 25 | 4 mg | p24CE1/2 | p24CE1/2 | p24CE1/2+ $p55^{gag}$ | p24CE1/2+ $p55^{gag}$ |
| | | 2 mg | *IL-12* | *IL-12* | *IL-12* | *IL-12* |
| | 3 | - | Placebo | Placebo | Placebo | Placebo |
| Group 2 | 25 | 4 mg | $p55^{gag}$ | $p55^{gag}$ | $p55^{gag}$ | $p55^{gag}$ |
| | | 2 mg | *IL-12* | *IL-12* | *IL-12* | *IL-12* |
| | 3 | - | Placebo | Placebo | Placebo | Placebo |
| Total | 50+ 6 = 56 | | | | | |

*Notes*

All injections will be delivered IM followed by electroporation (IM/EP). The doses shown will be divided between 2 injection sites, such that 2 mg p24CE1/2 or $p55^{gag}$ pDNA vaccine, or a mixture of 1 mg each, and 1 mg *IL-12* pDNA, or placebo, will be given in each deltoid at every vaccination timepoint.

Enrollment will proceed in both groups simultaneously and will be restricted to US HVTN Clinical Research Sites (CRSs). Across the participating CRSs, enrollment will be restricted to a maximum of 1 participant per day until 10 participants have been enrolled. The HVTN 119 Protocol Safety Review Team (PSRT) will review the cumulative safety data on each of these 10 participants, including at minimum local and systemic reactogenicity data reported for the first 72 hours postvaccination, and will determine whether it is safe to proceed with full enrollment in that group.

### Participants

56 healthy, HIV-uninfected volunteers aged 18 to 50 years; 50 vaccinees, 6 placebo recipients

### Design

Multicenter, randomized, placebo controlled, double-blind trial

### Duration per participant

18 months (12 months of scheduled clinic visits (main study) and 1 follow-up contact)

### Estimated total study duration

26 months (includes enrollment, planned safety holds, and follow-up)

### Investigational New Drug (IND) sponsor

DAIDS, NIAID, NIH, DHHS (Bethesda, Maryland, USA)

### Study product providers

- **p24CE1/2 pDNA and p55$^{gag}$ pDNA**: DAIDS, NIAID, NIH, DHHS (Bethesda, Maryland, USA)
- ***IL-12* pDNA adjuvant**: Profectus BioSciences, Inc. (Tarrytown, New York, USA)
- **Intramuscular TriGrid Delivery System (TDS-IM) EP Device**: Ichor Medical Systems, Inc. (San Diego, California, USA)

### Core operations

HVTN Vaccine Leadership Group/Core Operations Center, Fred Hutchinson Cancer Research Center (FHCRC) (Seattle, Washington, USA)

### Statistical and data management center (SDMC)

Statistical Center for HIV/AIDS Research and Prevention (SCHARP), FHCRC (Seattle, Washington, USA)

### HIV diagnostic laboratory

University of Washington Virology Specialty Laboratory (UW-VSL) (Seattle, Washington, USA)

### Endpoint assay laboratories

- Duke University Medical Center (Durham, North Carolina, USA)
- FHCRC/University of Washington (Seattle, Washington, USA)
- Vanderbilt University (Nashville, Tennessee, USA)

### Study sites

HVTN Clinical Research Sites to be specified in the Site Announcement Memo

### Safety monitoring

HVTN 119 PSRT; HVTN Safety Monitoring Board (SMB)

## 3.1 Protocol Team

### Protocol leadership

| | | | |
|---|---|---|---|
| *Chair* | Spyros Kalams<br>Vanderbilt University<br>615-322-8688<br>spyros.a.kalams@vanderbilt.edu | *Statistician* | Allan DeCamp<br>SCHARP, FHCRC<br>206-667-6047<br>adecamp@scharp.org |
| *Co-chair* | Hyman Scott<br>Bridge HIV, SFDPH<br>415-437-7438<br>hyman.scott@sfdph.org | *Medical officer* | Mary Allen<br>DAIDS, NIAID<br>240-627-3036<br>mallen@niaid.nih.gov |
| *Protocol Team leader* | Marnie Elizaga<br>HVTN Core, FHCRC<br>650-854-4984<br>melizaga@fredhutch.org | *Laboratory lead* | Nicole Frahm<br>HVTN Laboratory Program<br>206-667-6268<br>nfrahm@fredhutch.org |

### Other contributors to the original protocol

| | | | |
|---|---|---|---|
| *Core medical monitor* | Marnie Elizaga<br>HVTN Core, FHCRC | *DAIDS protocol pharmacist* | Nayri Khairalla<br>DAIDS, NIAID<br>301-761-6659 |
| *Vaccine developer representatives* | James I. Mullins<br>University of Washington | *Clinical safety specialist* | Jill Zeller<br>HVTN Core, FHCRC |
| | Barbara K. Felber<br>National Institutes of Health | *Clinical trials manager* | Marianne Hansen<br>HVTN Core, FHCRC |
| | George N. Pavlakis<br>National Institutes of Health | *Clinical data manager* | Huguette Redinger<br>SCHARP, FHCRC |
| *Adjuvant developer representative* | Susan Sciotto-Brown<br>Profectus Biosciences | *HVTN program manager* | Gina Escamilla<br>SCHARP, FHCRC |
| *Device developer representative* | Drew Hannaman<br>Ichor Medical Systems | *DAIDS Project Officer* | Chris Butler<br>DAIDS, NIAID |
| *Laboratory protocol operations manager* | On Ho<br>HVTN Laboratory Program, FHCRC | *Protocol development manager* | Meg Trahey<br>HVTN Core, FHCRC |
| *Regulatory affairs* | Laurie Rinn<br>HVTN Core, FHCRC | *Community educator/recruiter* | Laura Michelson<br>Fenway CRS |
| *Clinic coordinator* | Naomi Prashad<br>Nashville CRS | *Community engagement unit representative* | Jim Maynard<br>HVTN Core, FHCRC |
| *Community Advisory Board (CAB) members* | Ebony Gordon<br>Nashville CAB | *Statistical research associate* | Erika Thommes<br>SCHARP, FHCRC |
| | Fred Mazyck<br>Boston-Fenway CAB | *Technical editor* | Erik Schwab<br>HVTN Core, FHCRC |

# 4 Background

## 4.1 Rationale for trial concept

The variability of HIV is a major stumbling block in vaccine design, since successful vaccines must protect against an ever-expanding universe of viral antigens. The plasticity of HIV allows for an enormous number of mutant strains that retain viability, which in turn allows the virus to escape from immune responses while preserving protein and overall viral function. The task of summarizing this diversity into immunogens of a manageable size is formidable. As a result, we are currently unable to produce HIV vaccines that predictably elicit broad, durable protective immunity.

HIV vaccine immunogens have generally been derived from individual viral isolates. To maximize immunologic breadth, investigators have employed consensus and deduced ancestral sequences [4-6], as well as created antigenic combinations of common variants that evolve as a result of immune pressure (eg, mosaics [7-10] and similar structures [11,12]), with the goal of blocking common escape forms of viruses and thus effectively block immunological escape pathways. However, it may not be possible to vaccinate with all of the viral antigenic diversity required to block viable or transition escape forms of the virus—as rapid, sequential epitope evolution, including uncommon intermediates, is commonplace early in infection [13,14]. For example, a study of the recognition of the most common mutant forms of the HIV Nef protein (representing ~65% of the known variation of this protein), found that peptides were not recognized based on their frequency in the virus population [15]. Thus, the immunogen sequence diversity that may be required to block viable or transition escape mutants may be too large to accommodate for a single or a manageably sized variation inclusive vaccine.

Another important concern is that presentation of variable epitopes, such as provided by a mosaic-like vaccine, would result in immunodominance (ie, hierarchical preference for recognition of one epitope of the vaccine over another, magnified by its presentation in different variant forms) or immunodominating responses (ie, suppression of some responses by others) by non-protective epitopes. Non-protective epitopes are those whose recognition is associated with a lack of virologic containment in vivo (eg, high viral load) or those that can readily escape without impairing viral fitness. Immunodominant epitopes with a high degree of conservation do not necessarily confer immune control of viral replication, as they may represent viral adaptation to human HLA types at the population level, and at minimal fitness cost [16-18]. It seems likely that if HIV segments were capable of mutating without limiting virus functionality, they would not contribute substantially to a vaccine's protective response. We further suggest that variable epitopes can serve as immunodominant "decoys" that can absorb immune reactivity and potentially preclude responses against protective epitopes. For example, the most significant difference between HIV strains infecting vaccine vs. placebo recipients in the Step (HVTN 502) vaccine trial occurred within a region including the SLYNTVATL epitope in Gag [19], an immunodominant epitope, responses to which are not associated with diminution of viral load [20]. Although unproven, SLYNTVATL reactivity may therefore represent an immunodominant decoy response that is detrimental to vaccine efficacy [21].

This trial will evaluate the safety and immunogenicity of a novel vaccine encoding "conserved elements" (CE) of the HIV-1 Gag core protein, $p24^{Gag}$. The p24CE1/2 pDNA vaccine is composed exclusively of two gene segments (p24CE1 and p24CE2, on one

plasmid, referred to as p24CE1/2 pDNA) expressing carefully selected portions of the HIV-1 capsid protein, $p24^{Gag}$, the most abundant protein in HIV virions (Figure 4-1). $p24^{Gag}$ was chosen because of its availability as an immune target during infection, and because CTL responses to Gag, and in particular the $p24^{Gag}$ component of Gag, have been associated in numerous studies with greater control of HIV viremia [22-32]. The sequences expressed by p24CE1/2 pDNA correspond to the portions of $p24^{Gag}$ found in nearly every HIV-1 (M group) strain observed to date throughout the world. Unlike most of the HIV-1 genes and their encoded proteins, CE have rarely if at all been observed to mutate (for the most part they are composed of amino acids conserved in at least 98% of all HIV-1 infections), and represent components that are enriched with those essential to virus infectivity.

The following observations guided the design of the p24CE1/2 pDNA vaccine:

1. HIV mutates to sequences that contain ancestral amino acids (AA) when transmitted to a new host, essentially recovering a more fit state in the absence of the specific immune responses that shaped the viral genome in the previous host [33-35]. Hence, the p24CE1/2 vaccine is composed of conserved, ancestral, center of tree [6] sequences.
2. CTL that target specific viral proteins have reproducible effects on viral load. Numerous studies have shown that robust Gag-specific CTL contain infection [22-32], whereas CTL responses against the HIV-1 Pol protein generally do not correlate with levels of viremia, and responses against Env and the accessory proteins correlate with a lack of control of viremia [16-18,22,23].
3. Changes in conserved amino acids, especially in Gag, often but not always will debilitate or inactivate the virus [36-40]. In contrast, the majority of escape mutations in Env [36,39] or Nef [41] have a neutral or an enhancing impact on viral replication fitness. Hence, the p24CE1/2 pDNA vaccine is composed exclusively of conserved Gag protein elements.
4. CTL recognition of some conserved epitopes of the virus are preferentially detected in long-term non-progressors and in those with strong virologic control, most notably those with HLA B*57, B*58 [42-47], and B*27 [42,46,48-51] alleles. These same epitopes are present in the p24CE1/2 vaccine, and importantly, they are also targets of immune responses in virologic "controllers" that do not possess these HLA proteins [52]. This illustrates the criticality of the viral protein sequence being targeted by the immune system, as opposed to the HLA type, in controlling infection.
5. Viruses from "elite controllers" (ie, plasma HIV RNA <50 copies/mL in the absence of antiretroviral therapy) have impaired replication capacity both in acute/early [47,53] and in chronic infection [47,54].
6. Immunodominance of some epitopes can obscure or prevent reactivity against other, potentially protective epitopes, whereas responses to subdominant epitopes have been associated with better virologic control [55-57]. This suggests that an immunogen should not mimic the immunodominance elicited in most natural infections (B*57, B*58 and B*27 individuals being a notable exception). Hence, a focus on careful exclusion of these epitopes is likely to assist targeting of critical epitopes while removing potential immunodominant decoy responses.
7. Some AA segments in HIV proteins are conserved throughout a given HIV-1 subtype, the entire M group of HIV-1, and in some instances also in HIV-2 and SIV [56,58]. This is especially true for AA conserved since the beginning of the HIV pandemic, as opposed to those that have had HLA imprinting occur, with

immunologic escape mutants now comprising the most conserved AA state [37]. These segments make up the p24CE1/2 pDNA vaccine, are generally critical to viral function, and thus represent the Achilles' heel of the virus.

Recently a similar approach focusing on conserved elements is being taken, albeit with a heavy emphasis on Pol protein segments, in the European community [59]. Inclusion of conserved element approaches into the HVTN portfolio significantly broadens its scientific agenda.

The focus of the study is a pDNA prime-boost vaccine regimen, comprising p24CE1/2 pDNA prime followed by p24CE1/2+ $p55^{gag}$ pDNA booster vaccination. This regimen focuses the priming response to highly conserved, but subdominant epitopes, which we hypothesize will then be boosted, and possibly broadened by a combination of p24CE1/2 vaccine, and full length *gag* in the booster vaccination. This strategy is expected to achieve significantly broader epitope recognition compared to a regimen of full-length *gag* pDNA vaccine given alone.

This trial will be composed of 2 groups receiving different prime-boost regimens. Group 1 will receive p24CE1/2 pDNA prime followed by co-delivery of p24CE1/2 pDNA and full-length *gag* pDNA (CE/CE+*gag*). Group 2 will receive prime and boost with the full-length *gag* pDNA (*gag*/*gag*). The vaccines will be co-administered with *IL-12* pDNA, and given with electroporation. Four vaccinations will be given in the series, with priming vaccinations at 0 and 1 month, and boost vaccinations given at 3 and 6 months. Cellular and humoral immune responses in vaccine recipients will be compared to a control set of individuals given placebo injections given with electroporation on the same schedule.

In summary, we hypothesize that by targeting regions of the HIV-1 $p24^{Gag}$ protein that are invariable across Group M viruses, this vaccine approach may elicit specific immune responses linked to protection and viral control, rather than immune responses that may include decoy responses with detrimental effects or limited antiviral potential.

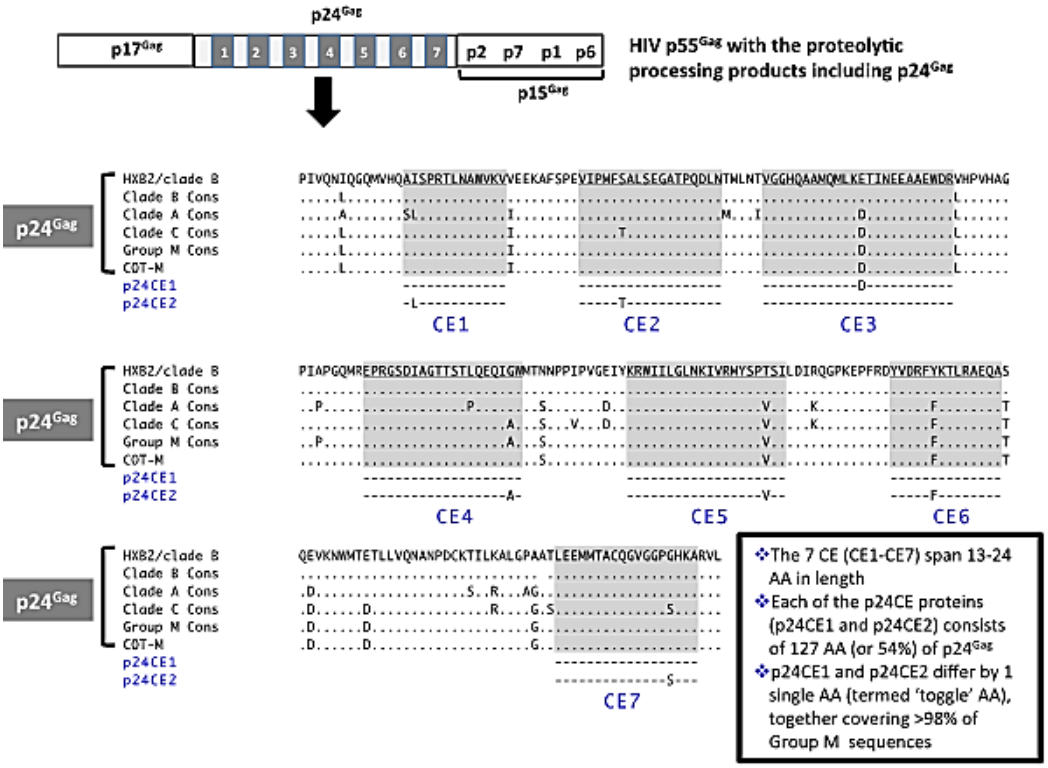



**Figure 4-1 Alignment of p24CE1, p24CE2 and p24$^{Gag}$ proteins.** The schematic representation at the top of the figure shows the p55$^{Gag}$ and its proteolytic processing products including p24$^{Gag}$ protein. Below is the alignment of the amino acid (AA) sequences of the consensus p24$^{Gag}$ sequences of different HIV-1 clades and of the seven CE encoded by the p24CE1/2 pDNA vaccine. The AA sequences of the p24$^{Gag}$ of different HIV-1 clades (A, B, C), group M consensus, COT-M, and of the p24CE1 and p24CE2 proteins are shown. The CE1 to CE7 sequences with the single AA change per CE are indicated.

## 4.2 p24CE1/2 pDNA

p24CE1/2 pDNA (Figure 4-2) is a dual promoter plasmid generated to express the p24CE1 gene from the human CMV promoter and the p24CE2 gene from the simian CMV promoter in the opposite transcriptional orientation. There are 7 CE segments of 13, 18, 24, 20, 20, 14 and 18 amino acids in the p24CE pDNA vaccine[58]. One "toggled" amino acid is included in each of the 7 CE segments, resulting in two protein coding elements (p24CE1 and p24CE2) differing by a total of 7 codons, each sharing 127 amino acids with p24$^{Gag}$. Coding sequences were optimized to enhance expression in human cells [60-65]. The p24CE sequences represent 54% of p24$^{Gag}$, including most extended coiled regions of the protein. CE regions include amino acids at p24$^{Gag}$ hexamer interfaces, which provide a structural rationale for their conservation, and CE are enriched with AA sites that if mutated to the second-most common AA found in the HIV database, nonetheless result in noninfectious virus [37,38].

The plasmid also contains two polyadenylation signals (bovine growth hormone polyA signal for p24CE1 and the simian virus 40 polyA signal for p24CE2). Of note, the promoter and polyA signal for the p24CE1 gene are identical to the single gene expression vector used to express the p55$^{Gag}$ protein. Furthermore, the promoters and poly A signals are the same as used in the dual expression *IL-12* pDNA plasmid to be used in the trial (although promoters and poly A signals were combined differently).

The CE segments are separated by linkers of 0-3 amino acids in length composed of Alanine, or Alanine and Lysine, or Alanine and Glycine. The total length of the proteins spans 157 AA including 17 AA of the GM-CSF signal peptide and 13 AA of linker sequences. It is known that inclusion of protease cleavage sites in DNA vaccines can enhance immunogenicity and preserve epitopes for HIV antigens [66]. The length and sequence of the linker sequences were therefore set based on the existing knowledge of cleavage specificities and peptide availability [67], as well as to avoid fortuitous junctional homologies with HIV and the human proteome, the latter determined by searching against the HIV and human proteome databases [68].

Production levels of p24CE proteins from the dual expression plasmid were indistinguishable from the combination of the two single vectors (unpublished). In studies performed in macaques, no differences in the levels of induced immune responses were noted using the single p24CE1/2 pDNA expressing p24CE1 and p24CE2 genes (N=10) versus the combination of two vectors, each expressing one of the two respective genes (N=6) (see Figure 4-5) [69-71].

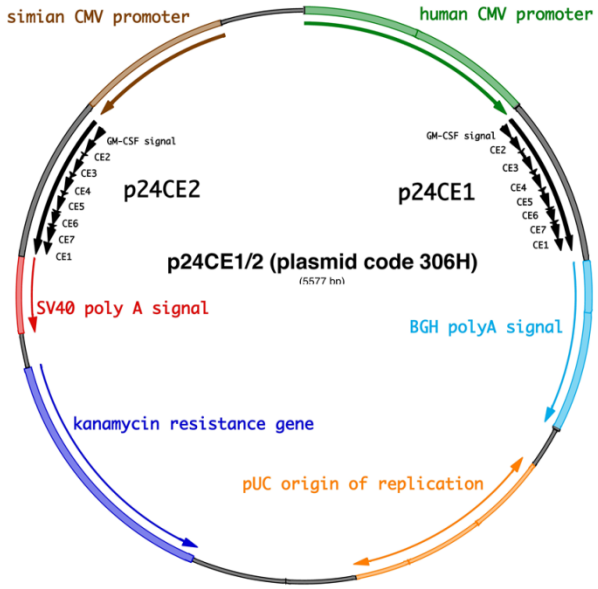



**Figure 4-2 Schematic of the p24CE1/2 pDNA (plasmid code 306H)**

For the clinical trial, the p24CE1/2 pDNA is produced at a concentration of 4 mg/mL, and is formulated in 30 mM citrate buffer pH 6.5 containing 150 mM NaCl, 0.01% ethylenediamine tetraacetic acid (EDTA) and 0.25% bupivacaine-HCl. The pDNA was manufactured by Ajinomoto Althea, San Diego, CA. The pDNA is formulated in bupivacaine, a local anesthetic that forms stable liposomal-like structures upon direct mixing with pDNA and further protects pDNA from degradation [72]. The p24CE1/2 pDNA will be admixed with *IL-12* pDNA, or with $p55^{gag}$ and *IL-12* pDNA, to produce a single injectable solution. The p24CE1/2 pDNA has not been tested in humans. p24CE1/2 pDNA vaccine formulated in water given IM with EP has been tested in macaques monitored for >2 years without any adverse effects.

The p24CE1/2 (plasmid 306H) and $p55^{gag}$ (plasmid 114H, see Section 4.3) pDNAs both use a plasmid backbone that is derived from pVR1012 [73] (Figure 4-3). The pVR1012 plasmid backbone has also been used in DNA vaccine studies including the following:

- the *gag-pol-nef* plasmids VRC4413, VRC4305, VRC4311 (Vaccine Research Center, NIAID) for which biodistribution and toxicity studies were performed (BB-IND10681 SN000);
- the p55$^{gag}$ plasmid DNA VRC4401 (Vaccine Research Center, NIAID; GenBank CS070894) that has been used in a clinical trial (NCT00123968) as part of the DNA vaccine HIVDNA016-00-VP.

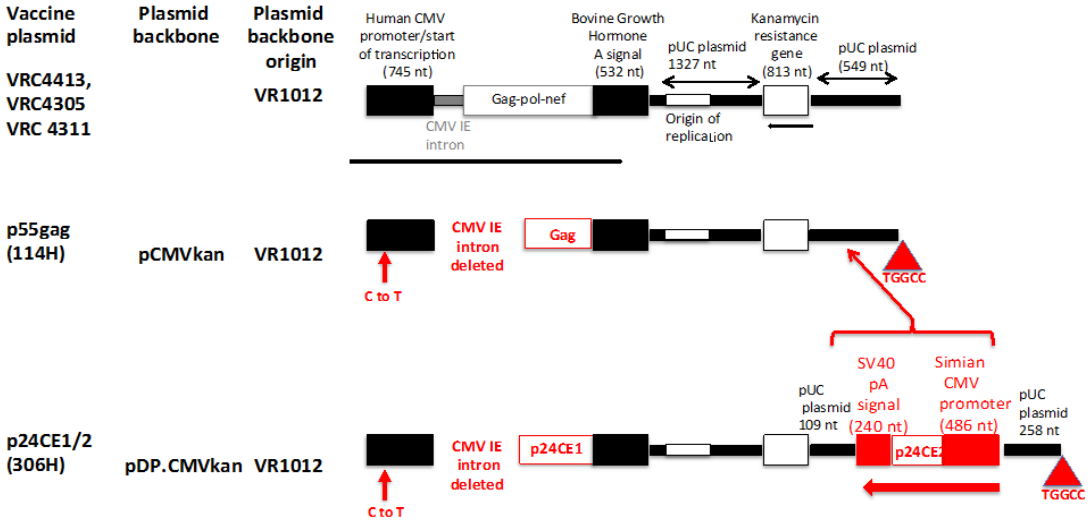



**Figure 4-3 Comparison of plasmids derived from pVR1012.** Plasmids VRC4413, VRC4305 and VRC4311 express *gag-pol-nef* using mammalian expression vector pVR1012. The plasmid backbone of p55$^{gag}$ (plasmid 114H) and p24CE1/2 (plasmid 306H) are also derived from pVR1012. Differences in pDP.CMVkan and pCMV.kan backbones are indicated in red (single nt change in the hCMV promoter, lack of CMV IE intron, 5 nt insertion between pUC and CMV promoter; pDP.CMVkan contains a cassette inserted within pUC that includes the simian CMV promoter and the SV40polyA signal sequence. Each vector has unique inserts.

In summary, the pCMVkan and pDP.CMVkan plasmid backbones used in p24CE1/2 and p55$^{gag}$ pDNAs, respectively, and that of pVR1012 can be compared as follows:

- identical BGH polyA signal,
- identical kanamycin resistance gene
- identical origin of replication, colE1
- identical pUC nucleotide sequence between backbone segments

with these differences:

- one nucleotide difference in the human CMV promoter (745 nt): thymidine at position 362 in p55$^{gag}$ and p24CE1/2 pDNA instead of cytosine.
- lack of the intronic sequence; according to our studies, splicing is not necessary for efficient expression of RNA-optimized genes; also, the presence of splice sites may generate alternatively spliced mRNAs encoding aberrant proteins
- insertion of 5 nucleotides (TGGCC) at the junction of pUC plasmid and human CMV promoter in pCMVkan and pDP.CMVkan
- presence of simian CMV promoter in the pDP.CMVkan derived plasmid p24CE1/2 pDNA

- presence of SV40 polyA signal in the pDP.CMVkan derived plasmid p24CE1/2 pDNA

A variety of approaches have been taken to assure that CE immunogens present relevant T-cell epitopes to the immune system:

1. To ensure that the epitopes in the CE vaccine could be processed appropriately for presentation by HLA, 40-mer peptides encompassing each CE as well as junctional regions were generated and subjected to proteolytic processing by human cell extracts. Fifteen of 16 known epitopes were identified as optimal or 1-3 AA extended degradation products in these assays (Le Gall et al., unpublished).
2. To determine whether CE are conserved because they are immunologically silent (due to impaired presentation on HLA), or because they have functional significance, T-cell responses to $p24^{Gag}$ were evaluated in 50 HIV-1 infected subjects without protective HLA class I alleles [52]. These 50 individuals consisted of HIV-1 controllers (<2,000 copies RNA/mL) and non-controllers (>50,000 copies RNA/mL) and excluded those with HLA*B27/57/58 alleles in order to focus development on immunogens for the majority of people without such favorable genetic backgrounds and, potentially, without the perhaps unique mechanisms of immune control [74] exhibited by HLA*B27/57/58 expressing individuals. For those without the aforementioned protective HLA alleles, recognition of HIV may be more likely to be obscured by immunodominant decoys and may benefit from directed targeting to structurally critical segments of the virus such as the CE. p24CE were found to be rich in epitopes, with >30 epitopes targeted by >40 different HLA class I molecules in the 50 subjects [52]. Interestingly, >70% of all responses to specific 10-mer peptides and their embedded optimally defined epitopes were observed in individuals not expressing the known restriction element [52], further supporting our approach to use CE rather than only known epitopes as immunogens. The 25 controllers tested preferentially recognized the TW10 and KK10 epitope regions, which were previously associated with control in B*57/B*58, and B*27 individuals, respectively, even though these controllers did NOT possess these "protective" alleles. Differential reactivity was also focused on a known B*14 DA9 epitope, again targeted by individuals that did not possess the B14 allele. In conclusion, the specific epitopes recognized, and not necessarily the HLA type, are critical for the control of infection.

As the vaccine plasmids as well as the *IL-12* plasmid are formulated in 0.25% bupivacaine, there is a risk of allergic reaction, including rash, urticaria, angioedema, bronchospasm, or anaphylaxis. Significant adverse experiences including cardiac arrest and death have occurred following intravenous delivery of bupivacaine. In most cases, this has followed use of bupivacaine at a dose of 1.6 mg/kg. Bupivacaine in this study is being administered IM at a dose of 2.5 mg/ml (maximum 2 ml, or 5 mg). A 50 kg person would receive a dose of 0.1 mg/kg per dose administered. Participants with a history of allergic reaction to amide-type local anesthetics (bupivacaine [Marcaine], lidocaine [Xylocaine], mepivacaine [Polocaine/Carbocaine], etidocaine [Duranest], prilocaine [Citanest, EMLA® cream]) will be excluded.

## 4.3 $p55^{gag}$ pDNA

The $p55^{gag}$ pDNA (plasmid 114H) contains expression-optimized full length HIV-1 $p55^{gag}$ gene from the HIV-1 molecular clone HXB2 (Genbank NP_057850) cloned into the pCMVkan plasmid between the human CMV promoter and the bovine growth

hormone polyA signal (Figure 4-4). The p55$^{gag}$ pDNA and the p24CE1/2 pDNA share the same plasmid backbone which is described above in Section 4.2. Like the p24CE1/2 pDNA, the p55$^{gag}$ pDNA is formulated in bupivacaine.

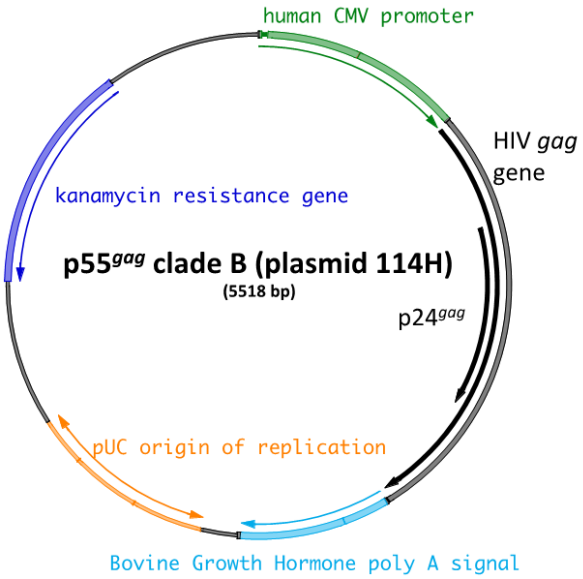



**Figure 4-4 Schematic of p55$^{gag}$ pDNA**

p55$^{gag}$ pDNA is produced at a concentration of 4 mg/mL, in 30 mM citrate buffer pH 6.5 containing 150 mM NaCl, 0.01% EDTA and 0.25% bupivacaine-HCl. The pDNA was manufactured by Ajinomoto Althea, San Diego, CA. The p55$^{gag}$ pDNA will be admixed with *IL-12* pDNA or with p24CE1/2 and *IL-12* pDNA to produce a single injectable solution.

Related plasmids expressing HIV p55$^{Gag}$ have been tested in several clinical trials, including HVTN 060, 063, 070, 080, 087, and no significant adverse effects related to vaccine were reported.

## 4.4 *IL-12* pDNA adjuvant

The GENEVAX® IL-12 DNA plasmid is a dual promoter expression plasmid which expresses the genes encoding human IL-12 subunits p35 and p40 under separate regulatory control. The p35 subunit is under the control of the hCMV promoter/enhancer and the SV40 polyadenylation signal. The p40 subunit is under the control of the sCMV promoter and the BGH polyadenylation signal. The plasmid contains a chimeric kanamycin resistance gene and a pUC bacterial origin of replication. The *IL-12* pDNA adjuvant is be produced at a concentration of 2 mg/mL and is formulated in 30 mM citrate buffer pH 6.5 containing 150 mM NaCl, 0.01% EDTA, and 0.25% bupivacaine-HCl. The plasmid is formulated in bupivacaine to enhance stability and facilitate entry into cells [72]. It was manufactured by Ajinomoto Althea, San Diego, CA.

The *IL-12* pDNA adjuvant will be given together with the p24CE1/2 pDNA, the p55$^{gag}$ pDNA and the p24CE1/2 and p55$^{gag}$ pDNA combination, respectively and will be delivered with electroporation (EP). HVTN 080, a phase 1 clinical trial, has demonstrated

the safety and immunogenicity of 3 mg PENNVAX™-B (*gag, pol, env*) vaccine (Inovio Pharmaceuticals, Inc.) with 1 mg *IL-12* pDNA (Profectus BioSciences, Inc.) given by IM/EP [75]. In addition, recent work by Pavlakis, Felber and colleagues has shown induction of immunity with the proposed vector given with *IL-12* pDNA EP in rhesus macaques, with superior $CD4^+$ and $CD8^+$ T-cell as well as Ab responses [76-78]. Other clinical trials (HVTN 087, IAVI B004) have shown reduced $CD4^+$ T-cell response rates in groups given *IL-12* pDNA, while $CD8^+$ T-cell responses were equivalent or improved, compared to study arms without *IL-12* pDNA [79,80]. The *IL-12* pDNA adjuvant, GENEVAX® IL-12 DNA Plasmid, has been tested by the HVTN in 5 previous trials: HVTN 060 (DAIDS-ES ID 10057, BB IND#12367), HVTN 063 (DAIDS-ES ID 10058, BB IND#12439), HVTN 070 (DAIDS-ES ID 10490, BB IND#13449), HVTN 080 (DAIDS-ES ID 10741, BB IND#14116), and HVTN 087 (DAIDS-ES ID 11673, BB IND#14976). Please refer to the Investigator's Brochure (IB) for additional information.

## 4.5 Electroporation (EP)

EP has been shown to be an efficient means to introduce DNA into cells [81]. EP is a technology in which a transient electric field is applied to the target tissue in order to enhance the cellular uptake of large molecules such as DNA. EP works by temporarily increasing the permeability of cell membranes. Although EP has been used experimentally in humans [82,83], EP remains investigational. Preclinical and phase 1 studies have suggested that EP enhances the potency of pDNA vaccines [80,84-87]. Ichor Medical Systems has focused on the development of EP technology where the means for agent administration and electric field application are integrated into a fully automated administration device. The overarching goal of this approach, embodied in the Ichor TDS-IM, is to enable agent administration, placement of electrodes, and timing of EP delivery to be completed in a simple, user independent fashion. Each TDS-IM device consists of three components, a single use Application Cartridge housing the EP electrodes and the agent of interest, a handheld, multi-use Integrated Applicator, and a Pulse Stimulator. The TriGrid™ electrode array for intramuscular delivery consist of four electrodes arranged in two triangles to form a diamond shape around an integrated central injection needle. Correlation of the electrode configuration with the agent distribution pattern intrinsic to the target tissue ensures that the EP effect is induced only in tissues where the agent of interest has been distributed, improving tolerability and minimizing tissue disruption. To date, the TDS-IM has been used for IM delivery of DNA vaccines or placebo in over 20 human clinical studies totaling in more than 700 clinical trial participants. The device has been used for administration of pDNA doses of up to 4.0 mg per site and 8.0 mg overall. Use of the device has been associated with mild to moderate injection site reactions resolving within 24-72 hours. Other mild or moderate transient adverse events have included paresthesia or hypoesthesia in the injected limb, cutaneous bleeding, bruising, or hematoma, vasovagal reactions, flu-like symptoms, headache, fever, nausea, chills, dizziness, malaise, fatigue, arthralgia, myalgia, neuralgia, and CPK increase. One event of brief syncope has been reported in a person with pre-existing bradycardia. No serious pDNA or device related adverse events have been reported during these studies. Although the use of EP administration is associated with substantially more acute discomfort than a conventional injection, to date, study subjects have exhibited a high degree of compliance.

HVTN 112 is a phase 1 trial to evaluate the safety, tolerability, and immunogenicity of a prime-boost regimen of HIV-1 *nef/tat/vif, env* pDNA vaccine delivered intramuscularly with electroporation and HIV-1 rVSV *envC* vaccine in healthy HIV-uninfected adult

participants (BB IND #16783, DAIDS-ES ID 11988). In HVTN 112, an event occurred in which the EP device was difficult to remove from a person's arm, causing severe pain and stress. The participant was receiving injection with EP for the first time. Pain was managed with local anesthesia to allow device removal. After the event, the person had some pain, tenderness, and bruising, but did not have any serious adverse reactions, and quickly resumed normal activities. After investigation, the conclusion is that the device had functioned normally, but that in this case, there may have been an unusual mismatch between the device setting specified for the person's arm skinfold measurement, and the person's arm physique (ie, skin and deltoid muscle thickness) which led to contact between the injection needle and the underlying bone. This happened in one participant out of 15 in this study, and has not been previously reported with this device before, in over 700 other subjects, and over 3000 administrations.

Please refer to the TDS-IM Investigator's Brochure for additional information regarding the TDS-IM device.

## 4.6 Trial design rationale

One major goal of the proposed phase 1 trial is to demonstrate that a DNA plasmid expressing the CE regions of $p24^{Gag}$, given with *IL-12* pDNA adjuvant and IM/EP delivery, has an acceptable safety profile.

The proposed trial is also designed as a proof-of-concept demonstration that conserved elements of the viral $p24^{Gag}$ protein, normally subdominant in HIV infections, can be immunogenic, can induce levels of immune response comparable to full-length Gag, can potently shift the immunodominance of Gag epitopes from variable to conserved regions of the $p24^{Gag}$ protein when used in a CE/CE+*gag* prime/boost vaccination regimen, and can induce increased levels of polyfunctional and cytotoxic T cells directed to CE compared to the full-length Gag antigen administered as a pDNA vaccine. *IL-12* pDNA delivered with plasmid DNA vaccines with EP may have an adjuvant effect on CD8+ responses and provide an advantage to the evaluation of immunogenicity of the p24CE1/2 and $p55^{gag}$ pDNA vaccines.

### 4.6.1 Dose (amount and number)

The DNA vaccines (p24CE1/2, $p55^{gag}$) will be given at 4 mg total dose per vaccination (2 mg per deltoid). Doses of DNA vaccines previously given in HVTN studies with IM/EP have ranged from 3 mg to 8 mg. Multiple priming vaccinations are proposed to increase the likelihood of robust immune responses.

GENEVAX® IL-12 DNA plasmid will be given at 2 mg total dose per vaccination (1 mg per deltoid). Doses of *IL-12* pDNA adjuvant previously given in HVTN studies with IM/EP have ranged from 0.25 mg to 1.5 mg, with up to 1 mg given at a single injection site.

In HVTN 080 (see Section 4.10.3), which showed an increase in ICS response rates (not statistically significant, possibly due to small sample sizes), 1 mg *IL-12* pDNA was given at a single site, and well-tolerated. In other studies, the local dose of *IL-12* pDNA was 0.25 to 0.75 mg. An adjuvant effect appears to be dose dependent on the local IM dose, and 1 mg per injection site has been chosen for this study, to try to replicate the adjuvant effect seen in HVTN 080. *IL-12* pDNA has not been associated with severe

reactogenicity or serious adverse events with up to 1.5 mg total dose per vaccination visit in these studies. We expect the minimal increase to 2 mg total dose (1 mg per injection site) to be safe and well tolerated both at the injection sites and systemically.

### 4.6.2 Prime-boost regimen

A prime-boost regimen of 4 vaccinations will be given, with injections at 0, 1 (priming immunogen) and 3, 6 months (booster immunogen(s)). This schedule allows for comparison with other HVTN studies of DNA vaccines given with IM/EP, which vaccinated participants on schedules of 0, 1 and 3 months or 0, 1, 3 and 6 months. Group 1 (CE/CE+*gag*) receives a combination of 2 immunogens for the boost. Group 2 (*gag*/*gag*) receives one immunogen as both the prime and the boost. Partially heterologous boost vaccinations are proposed in Group 1 to further increase the likelihood of robust immune responses.

### 4.6.3 Choice of control

Sodium Chloride for Injection, USP 0.9%, will serve as the placebo for the p24CE1/2 pDNA vaccine, the p55$^{gag}$ pDNA vaccine, and *IL-12* pDNA. Sodium Chloride for Injection, USP 0.9% is nonreactogenic and well tolerated. It will be delivered by IM/EP as a control for blinding purposes. It is possible that the presence of bupivacaine in the vaccines and adjuvant may reduce the pain of injection reported on the VAS relative to the saline control. This should not adversely affect the efficiency of blinding.

## 4.7 Plans for future product development and testing

Positive results would prompt at least two avenues of future trials, towards evaluation of efficacy, either alone or in conjunction with other immunogens against other regions of the viral proteome, and for the development of additional CE immunogens corresponding to other regions of HIV-1 proteins and a therapeutic vaccine to target the highly conserved less exhausted epitopes of the HIV.

## 4.8 Preclinical safety studies

During pre-IND review, the Center for Biologics Evaluation and Research concurred that preclinical safety studies with the p24CE1/2 pDNA and p55$^{gag}$ pDNA study products were not necessary based on previous nonclinical and clinical experience with similar products. DAIDS has developed significant nonclinical and clinical experience with pDNAs formulated in bupivacaine that contain *gag* inserts (multiple investigational products), and with *IL-12* pDNA, including the use of intramuscular administration with electroporation to deliver a *gag* pDNA with *IL-12* pDNA. In addition, the 3 investigational plasmids to be studied have been evaluated in a macaque study that extended more than 2 years, using endotoxin-free DNA preparations purified with the Qiagen kit in the laboratory. This study not only demonstrated that the animals developed durable cellular and humoral immune responses, but also that no adverse effects were observed, either after the initial vaccination series or after an additional booster vaccination at approximately 2 years. Furthermore, the cGMP p24CE1/2 pDNA and p55$^{gag}$ pDNA preparations have been tested in an immunological and observational study in C57BL/6 mice (N=5/group). Mice were administered 20 micrograms of pDNA/mouse (2 vaccinations at 0 and 4 months and sacrificed 2 weeks later) via IM/EP. No adverse

effects were observed and the mice developed similar levels of antigen-specific cellular immune responses compared to mice vaccinated in parallel arms within the same study with the laboratory-produced endotoxin-free pDNAs.

## 4.9 Preclinical immunogenicity studies

A variety of model systems were used to demonstrate that delivery of p24CE pDNA would induce strong immune responses. A series of proof-of-concept studies (Table 4-1), detailed in Sections 4.9.2 and 4.9.3, were conducted in mice and macaques. The vaccine concept aiming to focus the immune responses to the highly conserved CE sequences of the Gag capsid protein was developed and tested for HIV, and, using an analogous design, for SIV, which will allow in vivo testing of vaccine efficacy in a macaque challenge model. Briefly, these studies demonstrated that vaccination with CE pDNA induces potent immune responses to subdominant epitopes, which could not be achieved by vaccination with pDNA expressing the full-length Gag. Vaccine regimens including booster vaccinations with *gag* pDNA or co-delivery of CE+*gag* pDNA greatly augment the cytotoxic CE-specific responses, however the latter booster regimen maximizes the breadth of the responses to the otherwise subdominant CE epitopes. Thus, in preclinical trials, the regimen consisting of p24CE1/2 pDNA priming followed by co-delivery of CE+*gag* pDNA booster vaccination results in immune responses with greatest magnitude, breadth and cytotoxicity. This vaccine regimen has been selected for the clinical trial.

**Table 4-1 Summary of preclinical immunogenicity studies**

| Study number | Product | Schedule (months) | Animal | N (sex) | pDNA Dose groups | References | Assay |
|---|---|---|---|---|---|---|---|
| 1 | HIV: p24CE1, p24CE2; p24CE1 + p24CE2; or $p55^{gag}$; cGMP p24CE1/2; or cGMP $p55^{gag}$ | 0, 4 or 0, 3, 6 weeks | C57BL/6, Balb/c mice | ~300 (F) | 20 µg | [88] | ICS, ELISA, Western |
| 2 | HIV: p24CE1+p24CE2+*IL-12*(P) | 0, 2* | Indian (5)*, Chinese (1)* RM | 4 (M) 2 (F) | 2 mg CE, 0.2 mg *IL-12* | [69,70] | ICS, ELISA, Western |
| 3 | HIV: p24CE1/2+*IL-12*(P) | 0, 2 | Indian RM | 4 (M) | 2 mg CE, 0.2 mg *IL-12* | [69,70] | ICS, ELISA, Western |
| 4 (continued from 2) | HIV: $p55^{gag}$+*IL-12 (*B) | 4 (B) | | | 1 mg *gag*, 0.2 mg *IL-12* (B) | [89] | ICS, ELISA, Western |
| 5 (continued from 3) | HIV: $p55^{gag}$+*IL-12 (*B) | 4 (B) | | | 1 mg *gag*, 0.2 mg *IL-12* (B) | [69,70] | ICS, ELISA, Western |
| 6 | HIV: p24CE1/2+*IL-12* (P)- p24CE1/2+$p55^{gag}$+*IL-12* (B) | 0, 1 (P)- 4, 6 (B) | Indian RM | 6 (M) | 4 mg CE, 0.2 mg *IL-12* (P)– 2 mg CE, 2 mg *gag*, 0.2 mg *IL-12* (B) | [71] | ICS |
| 7 | SIV: p27CE1+p27CE2+*IL-12*(P) | 0, 2, 4 | Indian RM | 13 (M) 1 (F) | 2 mg CE, 0.2 mg *IL-12* | [71] | ICS, ELISA, Western |
| 8 (6 animals, continued from 7) | SIV: *gag*+*IL-12* (B) | 0, 2, 4 (P)- 6 (B) | Indian RM | 5 (M) 1 (F) | 2 mg CE, 0.2 mg *IL-12* (P)– 1 mg *gag*, 0.2 mg *IL-12* (B) | [71] | ICS, ELISA, Western |
| 9 | SIV: p27CE1+p27CE2+*IL-12* (P)- p27CE1+p27CE2+ *gag*+*IL-12* (B) | 0 (P)- 2, 4, 6 (B) | Indian RM | 6 (M) | 2 mg CE, 0.2 mg *IL-12 (P)*– 2 mg CE, 2 mg *gag*, 0.2 mg *IL-12 (B)* | [71] | ICS |
| 10 | HIV: $p55^{gag}$+*IL-12* | 0, 2 | Indian RM | 4 (3 M; 1 F) | 2 mg $p55^{gag}$, 0.2 mg *IL-12* | [69,70] | ICS, ELISA, Western |
| 11 (continued from 10) | HIV: p24CE1+p24CE2+*IL-12(B)* | 4 | | | 2 mg CE, 0.2 mg *IL-12* | [69,70] | ICS, ELISA, Western |
| 12 | HIV: $p37^{gag}$ | 0, 1, 2, 5.5, 6, 6.5 | Indian RM | 3 (2 M; 1 F) | 0.25 mg $p37^{gag}$ (0.5 mg at 5th vaccination) | [76] | ICS, ELISA, Western |
| 13 | HIV: $p55^{gag}$ | 0, 1, 3.5, 6, 9 | Indian RM | 4 | 2 mg $p55^{gag}$ | [89] | ICS, ELISA, Western |
| 14 | SIV: *gag*+*IL-12* | 0, 2 | Indian RM | 24 (19 M; 5 F) | 0.25 mg $p57^{gag}$ 0.25 mg MCP-$p39^{gag}$ 0.1 mg *IL-12* | [71] | ICS, ELISA, Western |
| 15 | SIV: *gag*+*IL-12* | 0, 2, 4, 6 | Indian RM | 2 (F) | 0.5 mg $p57^{gag}$ 0.5 mg MCP-$p39^{gag}$ 0.1 mg *IL-12* | [71] | ICS, ELISA, Western |
| 16 | SIV: *gag*+*IL-12* | 0, 1, 4.5 | Indian RM | 3 (M) | 0.5 mg $p57^{gag}$ 0.5 mg MCP-$p39^{gag}$ 0.1 mg *IL-12* | [71] | ICS, ELISA, Western |
| 17 | SIV: *gag*+*IL-12* | 0, 3, 6 | Indian RM | 2 (M) | 0.5 mg $p57^{gag}$ 0.5 mg MCP-$p39^{gag}$ 0.1 mg *IL-12* | [71] | ICS, ELISA, Western |

(P) prime; (B) boost; (RM) rhesus macaque; *IL-12,* macaque *IL-12* pDNA. All product administrations were at 2 sites with IM/EP except study 13 which was IM only.

*One animal in each group (M437, P314) had earlier been vaccinated with a poorly immunogenic variant of p24CE pDNA which expressed an unmodified p24CE protein without the GM-CSF leader sequence, and then received 3 priming vaccinations at 0, 1 and 2 months with p24CE1 and p24CE2 plasmids as used for the other animals, but without macaque *IL-12* pDNA adjuvant. These animals received the *gag* pDNA booster vaccinations at 8 and 9 months.

### 4.9.1 Immunogenicity of p24CE ex vivo

Transfection of CE-encoding RNA into human dendritic cells derived from HIV-seronegative individuals was used to present p24CE1 antigen to autologous naïve T cells in order to generate primary human immune T-cell responses to CE ex vivo. Levels of $CD4^+$ and $CD8^+$ T-cell responses comparable to that of the full-length Gag protein were generated, even though only the p24CE1 protein (without p24CE2) was used as immunogen [90] (and unpublished).

### 4.9.2 Immunogenicity of p24CE DNA in mice

Study 1 (Table 4-1): Balb/c, C57BL/6, and HLA-transgenic C57BL/6 mice (expressing human HLA A*02 or B*07) [88] were immunized with pDNA expressing CE or full-length Gag. Different leader sequences were used to alter intracellular trafficking of the CE immunogens, thus manipulating protein localization, stability, and also the nature of elicited immune responses. The GM-CSF leader was chosen to move forward into human studies. Immunization of C57BL/6 mice with full-length $p55^{gag}$ pDNA induced poor $CD4^+$ T-cell mediated responses against CE, and to only 2 of the 7 CE segments. In contrast, vaccination with p24CE1 and p24CE2 pDNAs induced cross-clade reactive, robust T-cell responses to 4 of the 7 CE. The CE-induced responses were multifunctional and composed of both $CD4^+$ and $CD8^+$ T cells with mature cytotoxic phenotypes. In contrast, $p55^{gag}$ pDNA induced immune responses recognized the CE only weakly or not at all. p24CE pDNA vaccination also induced humoral immune responses similar in magnitude to those induced by $p55^{gag}$ pDNA, which recognize the virus encoded $p24^{Gag}$ protein. Similarly, $p55^{gag}$ pDNA induced humoral immune responses did not recognize the CE. Together, p24CE pDNA vaccine induces cellular and humoral immune responses that are only poorly or not induced by full-length *gag* DNA vaccination.

In addition to testing laboratory-purified plasmids, C57BL/6 mice were also vaccinated with the Althea produced cGMP p24CE1/2 and $p55^{gag}$ pDNA preparations. This study showed the expected induction of antigen-specific T cell responses, recapitulating previously reported findings [83]. The immune responses induced with the cGMP DNAs paralleled those obtained with laboratory-produced pDNA preparations. These data support qualification of these cGMP plasmid preparations for the clinical trial.

### 4.9.3 Immunogenicity of p24CE DNA in macaques

Strong immunogenicity induced by the p24CE1/2 pDNA was also demonstrated in Rhesus macaques [69-71], and novel prime-boost regimens were developed to maximize magnitude, breadth and the generation of cytotoxic T cell responses recognizing the CE.

**Studies 2 and 3 (**Table 4-1**):** These studies were conducted in macaques which received vaccinations with a combination of the two HIV p24CE1and p24CE2 single expression vectors (study 2) or with the dual expression vector p24CE1/2 (study 3) to examine the immunogenicity of the CE immunogen. The animals received 2 vaccinations (0 and 2 months), except for 2 animals (M437, P314) that were primed at 0, 2, and 4 months. Cellular immune responses were measured in blood at 2 weeks after the last vaccination by stimulation of PBMC with peptides spanning the 7 CE (Figure 4-5), and compared with results from study 6, in which animals were primed with the dual expression vector p24CE1/2 pDNA at months 0 and 1 (Table 4-1).

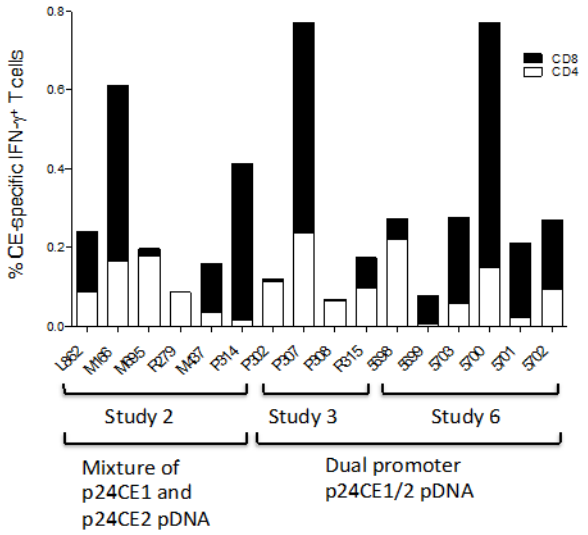



**Figure 4-5 p24CE pDNA vaccine is immunogenic in macaques.** Macaques (N=16) were vaccinated with a mixture of p24CE1&p24CE2 pDNA (N=6) or the dual expression vector p24CE1/2 (N=10; 4 animals from study 3 and 6 animals from study 6). The pDNA vaccine for all animals, except M437 and P314, also contained 0.2 mg macaque *IL-12* pDNA. The frequency of CE-specific IFN-$\gamma^+$ T cells was measured two weeks after the last priming vaccination using CE-specific peptide pools composed of a mixture of 15-mer overlapping by 11 AA and 10-mer peptides overlapping by 9 AA covering both p24CE1 and p24CE2 proteins. The CE-specific $CD4^+$ (open bars, labeled CD4) and $CD8^+$ (filled bars, labeled CD8) T cells are shown.

All animals developed CE-specific cellular responses, as measured by IFN-γ production, with a frequency ranging from 0.1% to 0.8% of total T cells in blood (Figure 4-5). These responses were elicited by both $CD4^+$ and $CD8^+$ T cells, of both central ($CD28^+$, $CD95^+$) and effector memory phenotype ($CD28^-$, $CD95^+$), and included cytotoxic and polyfunctional CE-specific T cells, as defined by their granzyme B content, ability to secrete two cytokines (IFN-γ and TNF-α) and ability to degranulate (CD107a). Both the combination of individual p24CE1 and p24CE2 pDNAs (study 2) and the dual expression plasmid p24CE1/2 (studies 3 and 6) induced similar CE-specific responses.

Mapping of the CE-specific responses showed a response breadth of 1-3 CE per animal (median = 3). Thus, the p24CE pDNA vaccine induced a broader response than the *gag* DNA vaccine, which elicited CE-specific T cell responses in only ~50% of the animals with a narrower response breadth of 0-3 CE per animal (median = 0). The breadth of the CE-specific responses obtained by the 2 vaccine regimens is depicted in Figure 4-6, where the percentage of the vaccinated animals recognizing each specific CE is shown.

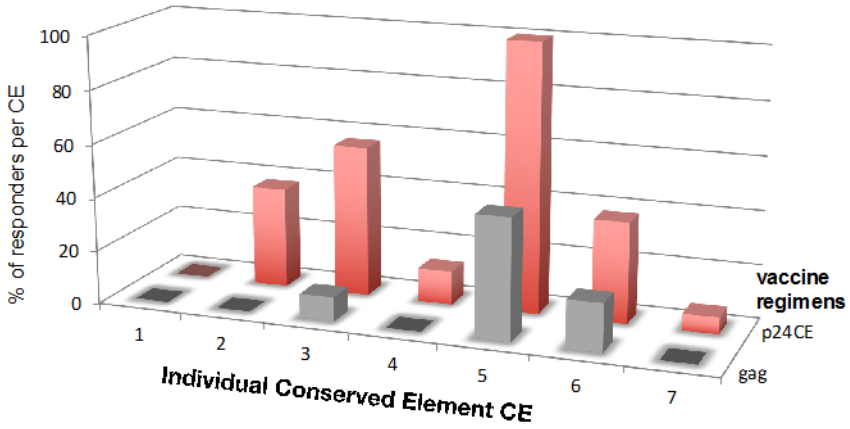



**Figure 4-6. Increased breadth of immune responses in the p24CE pDNA vaccinated macaques.** Animals were vaccinated with p24CE pDNA (N=16; Table 4-1 studies 2, 3, and 6 after 2 vaccinations, except 2 animals which received 3 vaccinations) or *gag* pDNA (N=11; Table 4-1 studies 10, 12, 13; 2-6 vaccinations) and T cell responses to individual CE were mapped. The plot shows the % responders to each CE induced by the *gag* pDNA (grey bars) and CE pDNA (red bars). The bars show the percentage of the vaccinated animals recognizing each specific CE.

Furthermore, analysis of humoral responses mimics those of the cellular responses, with only the CE pDNA vaccine being able to induce responses recognizing the CE.

**Studies 4 and 5 (**Table 4-1): The animals from studies 2 and 3 were enrolled into follow-up studies (studies 4 and 5, respectively) where they received a $p55^{gag}$ pDNA booster vaccination administered 2 months after the final priming vaccination. Despite the poor ability to induce de novo CE-specific immunity, $p55^{gag}$ pDNA booster vaccination significantly increased (p=0.0115) the IFN-$\gamma^+$ CE-specific T cells (reaching up to ~3% of the total T cell population) (Figure 4-7) and the CE-specific polyfunctional cytotoxic T cells (as measured by 4 functions; p = 0.0068; Figure 4-8).

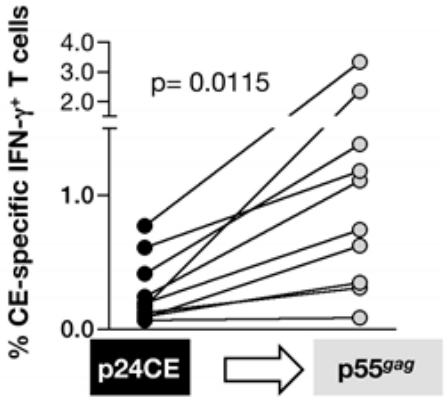



**Figure 4-7 Boosting of p24CE pDNA-primed macaques with p55gag pDNA increases CE-specific cellular responses.** The p24CE pDNA primed macaques received a $p55^{gag}$ pDNA booster vaccination. Frequency of total CE-specific IFN-$\gamma^+$ T cells before and after the boost is shown. CE-specific responses in the ten p24CE pDNA vaccinated animals (studies 2 and 3) were measured 2 weeks after the last prime and 2 weeks after receiving a single booster vaccination with 1 mg of $p55^{gag}$ pDNA. PBMC were stimulated with a mixture of 15-mer peptides (overlapping

by 11 AA) and 10-mer peptides (overlapping by 9 AA) spanning the 7 CE. P value is from paired t test.

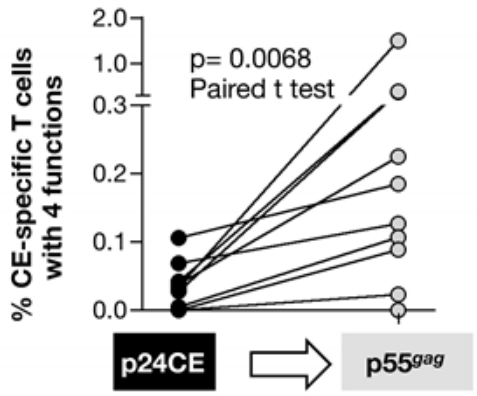



**Figure 4-8 Increased frequency of polyfunctional cytotoxic CE-specific T cell responses by the p24CE pDNA prime-p55$^{gag}$ pDNA booster vaccination regimen.** Frequency of total CE-specific polyfunctional (4 functions: IFN-$\gamma^+$ TNF-$\alpha^+$ CD107a$^+$ GzmB$^+$) CE-specific T cells before and after the p55$^{gag}$ pDNA booster vaccination. Polyfunctional (granzyme B$^+$, IFN-$\gamma^+$ TNF-$\alpha^+$ and CD107a$^+$) CE-specific responses were measured 2 weeks after the prime and 2 weeks after the boost using a mixture of peptides spanning the seven CE. P value is from paired t test.

This observation supports the notion that CE-containing peptides are indeed processed from the p55$^{Gag}$ protein and are presented as peptides to the immune system, but are poorly immunogenic, potentially due to immunological interference with dominant responses from other Gag epitopes in variable regions. Therefore, these data demonstrate that the immunodominance hierarchy can be significantly altered by p24CE pDNA priming vaccination, focusing responses onto otherwise subdominant epitopes. A vaccine regimen that includes p24CE pDNA prime and p55$^{gag}$ pDNA boost vaccination results in CE-specific T cell responses characterized by increased magnitude and cytotoxicity but no increase in the CE response breadth. Furthermore, p55$^{gag}$ pDNA boost expanded the response and included responses against both highly conserved and variable regions outside of CE with a skewing to the highly conserved regions.

In addition to cellular immune responses, vaccination with p24CE plasmids induced robust antibody responses in macaques [70]. These antibody responses recognized linear CE epitopes and the intact p24CE proteins, whereas the full-length Gag immunogen failed to elicit responses that recognized CE (studies 2 and 3). Furthermore, humoral responses to CE were greatly enhanced by a full-length Gag booster vaccination (studies 4 and 5). Thus, the CE pDNA vaccine induces potent cellular and humoral immune responses to subdominant epitopes that are greatly augmented by the p55$^{gag}$ pDNA booster vaccination.

Together, studies 4 and 5 demonstrate that the immunodominance exerted by variable Gag epitopes was lost in the presence of pre-existing CE-specific responses. Thus, p24CE pDNA as the priming immunogen resulted in broadened responses, including against the subdominant highly conserved epitopes, and avoided eliciting immunodominant responses against potentially “decoy” epitopes, while focusing responses to CE, for which few viable escape pathways may exist.

**Study 6 (**Table 4-1**):** This study was modeled after study 9 (see below), the latter of which was performed earlier, using analogous molecules from SIV, and corresponded to the most effective protocol found to induce the broadest recognition of SIV Gag CE. Study 6 included 2 priming vaccinations (0, 1 month) with HIV p24CE1/2 pDNA (Figure 4-5) followed by 2 booster vaccinations (4, 6 months) with co-delivery of p24CE1/2+p55$^{gag}$ pDNA (Figure 4-9). All vaccinations included macaque *IL-12* pDNA. The priming vaccination induced robust CE-specific responses (median 0.3% of T cells), which were significantly increased by each booster vaccination, reaching maximal responses after the 2$^{nd}$ booster vaccination (median 1.5% of T cells) (Figure 4-9).

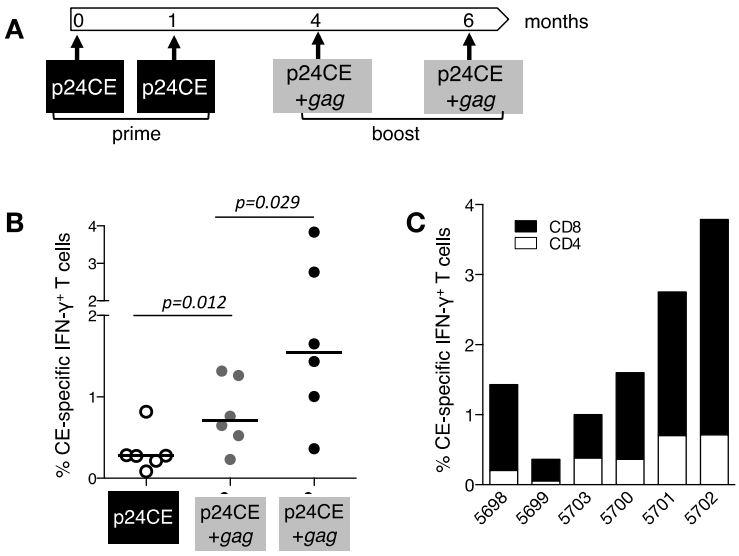



**Figure 4-9 CE pDNA prime-CE+*gag* pDNA boost.** (A) The cartoon depicts the HIV CE pDNA prime/CE+*gag* pDNA booster vaccination regimen. (B) Cellular immune responses were measured 2 weeks after the 2$^{nd}$ prime and 2 weeks after each booster vaccination. P values are from paired t test. (C) Frequency of CE-specific IFN-γ$^{+}$ T cell responses for individual macaques is shown for CD4$^{+}$ (open bars) and CD8$^{+}$ (filled bars) T cells after the 2$^{nd}$ CE+*gag* pDNA booster vaccination.

The CE-specific responses were mediated both by CD4$^{+}$ and CD8$^{+}$ T cells with a skewing towards CD8$^{+}$ T cell responses (Figure 4-9). This vaccination regimen also induced highly cytotoxic CE-specific IFN-γ$^{+}$ T cells with a frequency of >89% of granzyme B$^{+}$.

The responses to individual CE were measured using CE-specific peptide sub-pools. The CE+*gag* pDNA booster vaccine showed that all CE were recognized and induced a broader response compared to the *gag* pDNA only booster vaccine (Figure 4-10).

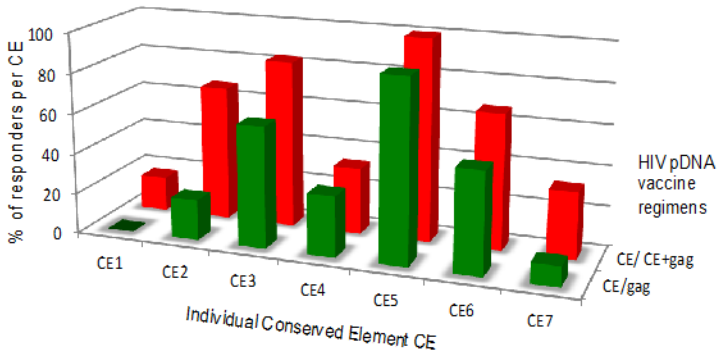
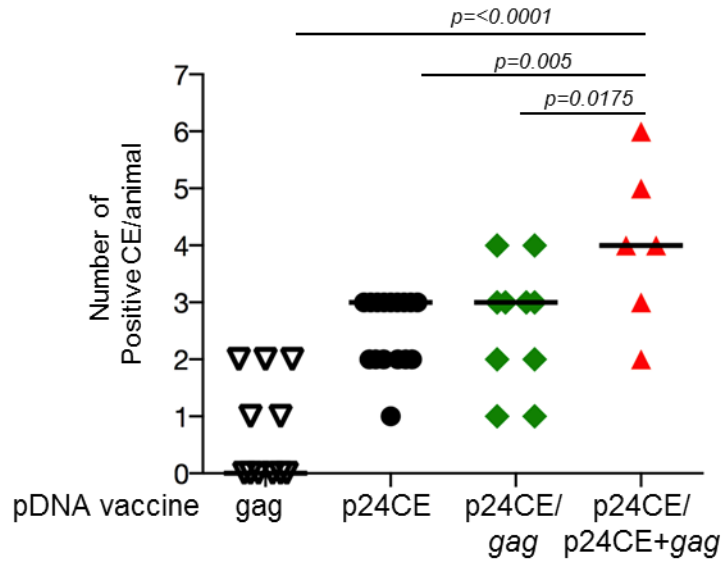



**Figure 4-10 Increased CE recognition induced by the CE/CE+*gag* pDNA vaccine.** Comparison of the breadth of the responses per CE induced by the CE prime/*gag* pDNA prime-boost (green; from studies 4 and 5) and CE prime/ CE+*gag* pDNA prime-boost vaccine (red, study 6). The bars show the percentage of the vaccinated animals recognizing each specific CE.

The breadth of the response, measured by the number of CE recognized per animal, was compared among groups which received different CE based vaccines [CE pDNA only (studies 2 and 3), CE/*gag* pDNA (studies 4 and 5) and CE/CE+*gag* pDNA (study 6) (Figure 4-11). The CE+*gag* pDNA booster vaccination induced responses with significantly increased breadth (2-6 CE per animal), compared to CE or CE/*gag* pDNA vaccines with 1-3 or 1-4 CE per animal (see above studies 4 and 5), with no difference among these groups. All CE based pDNA vaccines induced broader responses than the HIV $p55^{gag}$ pDNA only vaccine, which induced responses to 0-2 CE per animal.




| | | | | |
|---|---|---|---|---|
| Number of vaccinations | 2-6 | 2-3 | 4-5 | 4 |
| Number of vaccinations with p24CE | 0 | 2-3 | 2-3 | 4 |
| Median number of positive CE /animal | 0 | 3 | 3 | 4 |
| Range of CE/animal | 0-2 | 1-3 | 1-4 | 2-6 |
| Number of animals analyzed | 11 | 16 | 10 | 6 |

**Figure 4-11 Increased breadth of the HIV CE-specific responses induced by a CE/CE+*gag* pDNA vaccine regimen.** A comparison of the CE breadth using different vaccine regimens is shown. For details see Table 4-1. The plot shows the number of CE recognized by each macaque immunized with HIV *gag* pDNA only (studies 10, 12, 13), HIV CE pDNA only (studies 2, 3, 6 using the priming data only), CE pDNA prime/*gag* pDNA booster vaccination (studies 4, 5) or CE pDNA prime/CE+*gag* pDNA booster vaccination (study 6). The total number of vaccinations and separately the number of vaccinations including p24CE pDNA are given. The median number of CE recognized, the range of CE responses, and the number of animals analyzed are indicated. P values are from an ANOVA (Dunnett's test). Peptide-stimulated samples were considered positive if the responses were 2-fold higher than that of the unstimulated medium only control and greater than 0.01 after subtracting the medium control value. Samples were acquired on a LSR II or Fortessa flow cytometer (BD Biosciences, San Jose, CA), and the data were analyzed using FlowJo software (Tree Star, Inc., Ashland, OR).

Priming with HIV p24CE pDNA is critical to induce immune responses to subdominant epitopes, and inclusion of the p24CE pDNA together with a plasmid expressing the full-length immunogen (study 6) as a boost is the most effective protocol to induce desirable responses with greatest breadth, magnitude and cytotoxic capability. Importantly, study 6 using HIV vaccine constructs recapitulated the SIV vaccine data (study 9). Hence, this vaccine regimen is proposed for the clinical trial.

### 4.9.4 Immunogenicity of SIV p27CE DNA in macaques

By analogy to the HIV CE immunogens, molecules derived from SIV $p27^{Gag}$ were designed (p27CE1 and p27CE2) and tested in macaques [71]. The findings of the SIV p27CE studies (studies 7-9) recapitulated those of the HIV CE (studies 2-6), each supporting the observation that a priming vaccination with CE pDNA focuses responses to subdominant epitopes, allowing a change of the immunodominance hierarchy and leading to increased breadth and cytotoxicity.

**Study 7 (**Table 4-1**):** Macaques received 3 vaccinations with a combination of p27CE1 and p27CE2 pDNA at 0, 2 and 4 months, with macaque *IL-12* pDNA. Analysis of the vaccine-induced immunity demonstrated that all 14 macaques developed CE-specific cellular responses ranging from 0.03–0.8% of CE-specific IFN-$\gamma^+$ T lymphocytes in blood. All 7 CE were immunogenic (range 1-4 CE/animal; Figure 4-12). This is in contrast to *gag* pDNA vaccinated macaques which induced CE-specific responses in ~50% of animals targeting significantly fewer CE (range 0-3 CE/animal).

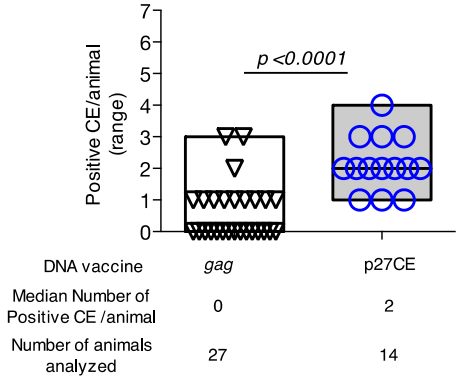



**Figure 4-12 Comparison of breadth of CE-specific T cell responses in p27CE pDNA and *gag* pDNA vaccinated animals.** CE-specific T cell responses were mapped to individual CE. The plot shows the range and median of the CE responses among the two groups. P value is from unpaired t test.

p27CE pDNA vaccination elicited CE-specific T cells with a significant fraction of cytotoxic (IFN-$\gamma^+$, granzyme $B^+$, $CD107a^+$) T cells among both the $CD4^+$ and $CD8^+$ T cell subsets. Thus, these findings mirror the data from HIV p24CE vaccine described in studies 2 and 3.

**Study 8 (**Table 4-1**):** Six of the macaques primed with SIV CE pDNA (from study 7) received a *gag* pDNA booster vaccination given with macaque *IL-12* pDNA. The *gag* pDNA booster vaccination significantly increased the magnitude of the CE-specific responses (p=0.031). These data recapitulate the potent augmentation of HIV p24CE primed T cell responses upon a boost with HIV *gag* pDNA expressing the full-length protein (studies 3 and 4), demonstrating that the immunodominance hierarchy could be altered by CE priming. In addition, the responses after the SIV *gag* pDNA boost were significantly higher (p=0.048) compared to the responses obtained after the p27CE pDNA priming vaccination, similar to observations from the HIV p24CE prime-*gag* DNA boost studies (studies 4 and 5). The *gag* pDNA booster vaccination (study 8) resulted in recognition of 1-4 CE per animal with a similar response breadth as found in the p27CE pDNA only primed animals. Thus, *gag* pDNA booster vaccination increased the magnitude of the CE-specific responses without broadening CE recognition.

Therefore, SIV p27CE pDNA and HIV p24CE pDNA priming vaccinations share the unique feature of effectively inducing immune responses to subdominant Gag epitopes, which is only inefficiently achieved by vaccination with pDNAs expressing full-length SIV Gag or HIV Gag.

**Study 9 (**Table 4-1**):** A second vaccine concept was tested in which macaques (N=6) received a single SIV p27CE pDNA priming vaccination followed by 3 booster vaccinations comprised of a co-delivered mixture of p27CE+*gag* pDNA, plus macaque *IL-12* pDNA. The booster vaccination greatly induced robust CE-specific responses, reaching up to ~1.8% IFN-$\gamma^+$ T cells in blood. No further increase in magnitude of the responses was observed between the 2$^{nd}$ and the 3$^{rd}$ boost, demonstrating that two CE+*gag* pDNA booster vaccinations are sufficient to maximize the magnitude of the primed CE-specific responses using this vaccine regimen.

Both booster vaccines (*gag* pDNA and p27CE+*gag* pDNA) induced overall similar levels of CE-specific T cell responses (studies 8 and 9). However, the p27CE+*gag* pDNA booster vaccination (study 9) significantly expanded the breadth of CE-specific cellular responses (Figure 4-13) to 4-7 CE per animal (Figure 4-14).

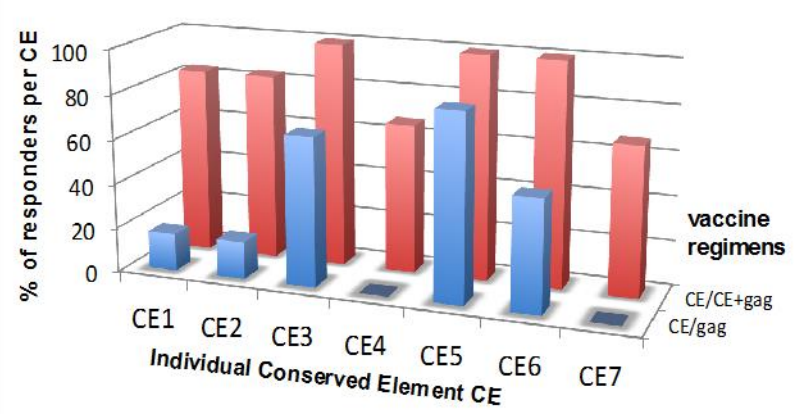



**Figure 4-13 Breadth of CE responses comparing the two prime-boost regimens. Responses to individual CE were mapped in the 2 vaccine groups.** The % responders per CE are plotted. p27CE prime-*gag* pDNA boost (CE/*gag*; N=6; blue bars; front row) from study 8 and p27CE prime-p27CE+*gag* pDNA boost (CE/CE+*gag*; N=6; red bars; back row) from study 9 are compared.

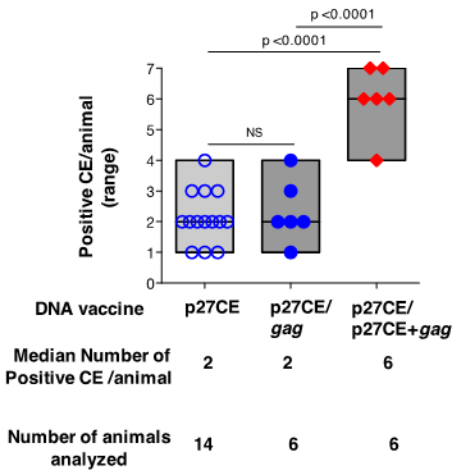



**Figure 4-14 Comparison of breadth of CE-specific T cell responses among the 3 groups of macaques vaccinated with p27CE pDNA based vaccine regimens.** CE-specific T cell responses were mapped to individual CE using macaques described in studies 7-9. The plot shows the range and median of the CE responses among the 3 groups. P values are from ANOVA (Dunnett's test).

Thus, the detailed analysis of the responses to individual CE induced by these two booster vaccination regimens (studies 8 and 9) revealed a fundamental difference in breadth, although the magnitude of CE-specific T cell responses induced by both vaccine regimens is similar.

The frequency of the cytotoxic T cells (granzyme $B^+$) induced in the different CE-based vaccination regimens was also examined. Of note, *gag* pDNA vaccinated macaques which also developed CE-specific responses (N=18) showed significantly (p=0.014) higher frequency of granzyme $B^+$ $p27^{Gag}$-specific T cells than the ~50% of macaques (N=13) which failed to induce CE-specific responses (Figure 4-15, left panel), suggesting that the T cells targeting the CE are functionally more cytotoxic than those targeting the variable regions. This notion is supported by the findings that all protocols in which p27CE pDNA was included as prime induced significantly higher frequency of cytotoxic T cell responses than those induced by the *gag* pDNA vaccination (studies 2-6) (Figure 4-15, right panel).

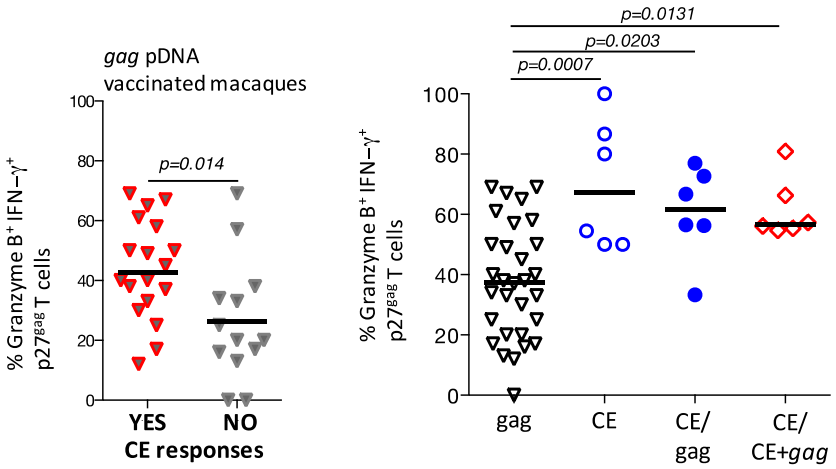



**Figure 4-15 Comparison of cytotoxicity of SIV Gag-specific T cell responses with different vaccine regimens.** The frequency of Gag-specific granzyme $B^+$ IFN-$\gamma^+$ T cell responses was measured in the different vaccine groups. Left panel: Macaques vaccinated with *gag* pDNA were grouped according to their ability to mount T cell responses recognizing CE. P value is from t-test. Right panel: The cytotoxicity of Gag responses is compared among all the *gag* pDNA vaccinated animals and the animals which received a CE based pDNA vaccine (CE only, or CE prime-*gag* pDNA boost or CE prime- p27CE+*gag* pDNA boost) (right panel). The median is indicated. P values are from ANOVA (Dunnett's test).

Overall, priming with CE pDNA and boosting with a mixture of CE and *gag* pDNA is the most efficient protocol to induce CE responses with the broadest coverage, and the subdominant epitopes encoded by the CE immunogen elicit T cell responses of higher functional relevance (more cytotoxic). Data from both the macaques vaccinated with p27CE pDNA and the subset of *gag* DNA vaccinated animals which developed CE-specific responses show a link between CE immunity and CTL responses. Thus, the use of CE pDNA priming vaccine is critical to efficiently induce potent CTL responses to subdominant epitopes.

## 4.10 Clinical studies

### 4.10.1 Clinical studies of p24CE1/2 pDNA

This is a first-in-human study for the p24CE1/2 pDNA vaccine.

### 4.10.2 Clinical studies of p55$^{gag}$ pDNA

DAIDS has developed significant clinical experience with pDNAs that contain *gag* inserts (multiple variants), including the use of intramuscular administration with electroporation to deliver a *gag* pDNA with *IL-12* pDNA. These trials include: HVTN 060, 063, 070, 080 and 087.

### 4.10.3 Clinical studies of HIV-1 DNA vaccines given with GENEVAX® IL-12 pDNA adjuvant IM with Electroporation

**Table 4-2 Summary of clinical studies of HIV-1 DNA vaccines with GENEVAX® IL-12 DNA plasmid and EP in healthy HIV-uninfected adults**

| Study number/ clinicaltrials.gov identifier | Study population | Test article(s) | Trial design / route of delivery/ schedule / N (vaccinee/placebos) |
|---|---|---|---|
| HVTN 080/ NCT00991354 | HIV-uninfected healthy adults | - PENNVAX®-B pDNA GENEVAX® *IL-12* pDNA | Grp 1: 3 mg PV-B alone, IM/EP, month 0, 1, 3<br>N = 10/2<br>Grp 2: 3 mg PV-B + 1 mg *IL-12* pDNA, IM/EP, month 0, 1, 3<br>N = 10/2<br>Grp 3: 3 mg PV-B + 1 mg *IL-12* pDNA, IM/EP, month 0, 1, 3<br>N = 20/4 |
| IAVI study B004/ NCT01496989 | HIV-uninfected healthy adults | - HIV-MAG pDNA + GENEVAX® *IL-12* pDNA<br>- Ad35 GRIN/ENV | Grp 1: 3 mg HIV-MAG pDNA, IM/EP, month 0, 1, 2<br>$2 \times 10^{10}$ vp Ad35 GRIN/ENV, IM, month 6<br>N = 12/3<br>Grp 2: 3 mg HIV-MAG + 0.1 mg *IL-12* pDNA, IM/EP, month 0, 1, 2<br>$2 \times 10^{10}$ vp Ad35 GRIN/ENV, IM, month 6<br>N = 12/3<br>Grp 3: 3 mg HIV-MAG + 1 mg *IL-12* pDNA, IM/EP, month 0, 1, 2<br>$2 \times 10^{10}$ vp Ad35 GRIN/ENV, IM, month 6<br>N = 12/3<br>Grp 4: 3 mg HIV-MAG + 1 mg *IL-12* pDNA, IM/EP, month 0<br>$2 \times 10^{10}$ vp Ad35 GRIN/ENV, IM, month 4<br>N = 12/3<br>Grp 5: $2 \times 10^{10}$ vp Ad35 GRIN/ENV, IM, month 0<br>3 mg HIV-MAG + 1 mg *IL-12* pDNA, IM/EP, month 6<br>N = 12/3 |
| HVTN 087/ NCT01578889 | HIV-uninfected healthy adults | - HIV-MAG pDNA + GENEVAX® *IL-12* pDNA<br>- rVSV HIV *gag* | Grp 1: 3 mg HIV-MAG pDNA alone, IM/EP, month 0, 1, 3<br>$1 \times 10^{8}$ PFU rVSV HIV *gag*, IM, month 6<br>N = 22/3<br>Grp 2: 3 mg HIV-MAG + 0.25 mg *IL-12* pDNA, IM/EP, month 0, 1, 3<br>$1 \times 10^{8}$ PFU rVSV HIV *gag*, IM, month 6<br>N = 22/3<br>Grp 3: 3 mg HIV-MAG + 1 mg *IL-12* pDNA, IM/EP, month 0, 1, 3<br>$1 \times 10^{8}$ PFU rVSV HIV *gag*, IM, month 6<br>N = 22/3<br>Grp 4: 3 mg HIV-MAG + 1.5 mg *IL-12* pDNA, IM/EP, month 0, 1, 3<br>$1 \times 10^{8}$ PFU rVSV HIV *gag*, IM, month 6<br>N = 22/3 |

In HVTN 080, healthy HIV-uninfected volunteers received 3 mg PENNVAX®-B (*gag, pol, env*) with or without 1 mg *IL-12* pDNA by IM injection followed by in vivo EP (IM/EP) (CELLECTRA® EP system) at a single injection site. In this study, adjuvant activity was seen with *IL-12* pDNA. While the difference was not statistically significant, in HVTN 080 the proportion of volunteers making a vaccine-specific CMI response was almost 2-fold greater in the group that received Profectus' GENEVAX® IL-12 pDNA adjuvant given with EP compared to the small group receiving no *IL-12* pDNA (see Figure 4-16, Figure 4-17).

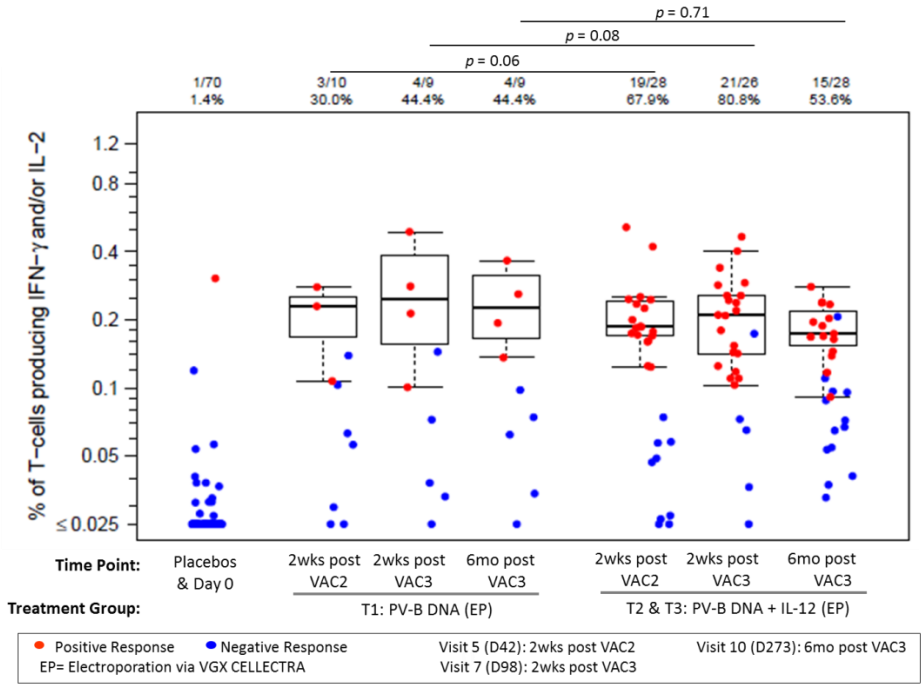



**Figure 4-16 HIV-specific CD4+ T cell ICS responses in HVTN 080**

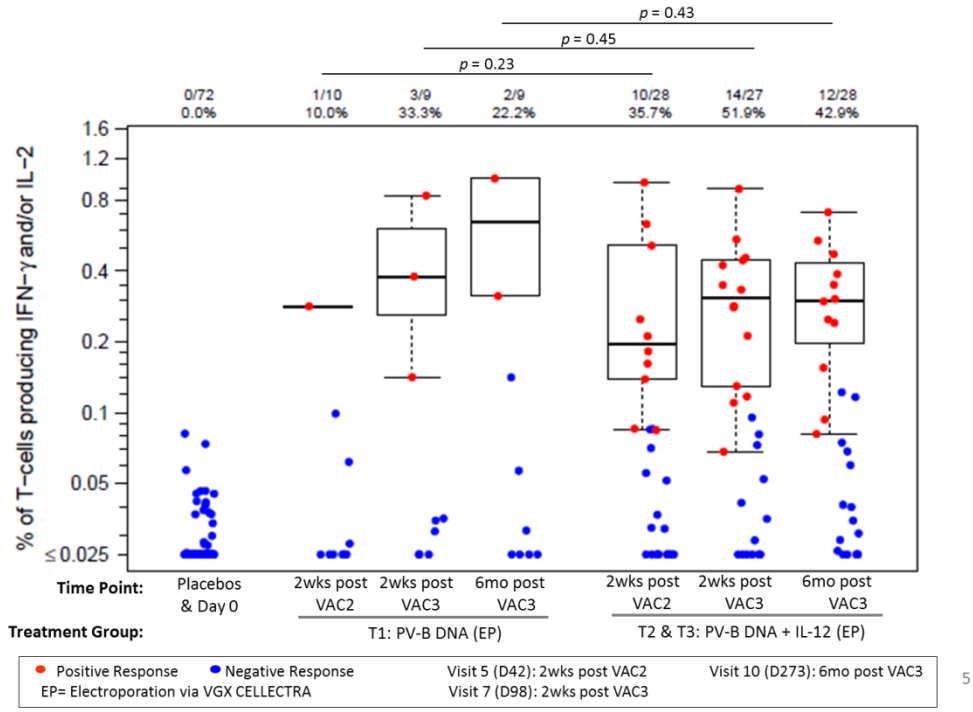



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**Figure 4-17 HIV-specific CD8+ T-cell ICS responses in HVTN 080**

In HVTN 087, several dose levels of *IL-12* pDNA (GENEVAX® IL-12 DNA plasmid) were tested for adjuvant activity, with HIV-MAG (HIV-1 *gag/pol* and HIV-1 *nef/tat/vif, env*) pDNA vaccine delivered IM/EP with the Ichor TDS-IM EP Device. In HVTN 087 the healthy HIV-uninfected volunteers received the HIV-MAG pDNA vaccine given with 0, 0.25, 1 and 1.5 mg *IL-12* pDNA IM/EP divided equally across two injection sites. The HIV-MAG pDNA vaccinations were followed by a VSV HIV *gag* boost. The results of

HVTN 087 with regards to the adjuvant activity of *IL-12* pDNA suggest that the highest dose of *IL-12* pDNA was associated with an increased magnitude of CD8+ T-cell responses following the VSV HIV *gag* boost, compared with group 1, which did not receive *IL-12* pDNA (p = 0.0185, Figure 4-19). The ability to see statistically significant differences between the study arms was limited by the small sample sizes.

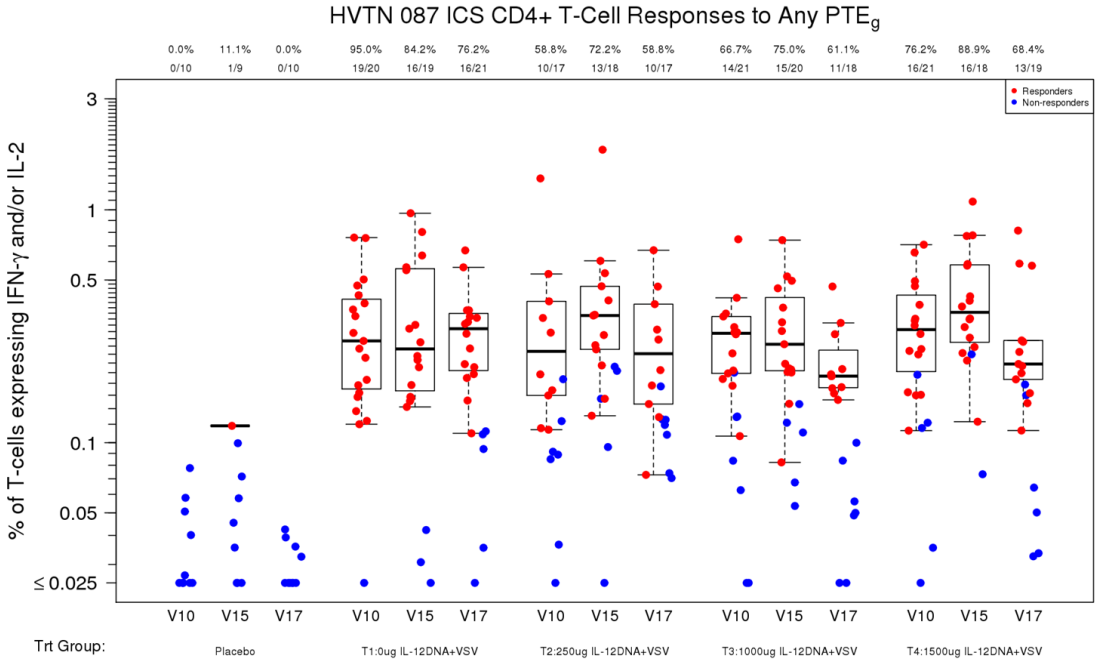


**Figure 4-18 HIV-specific CD4+ T-cell ICS responses in HVTN 087**

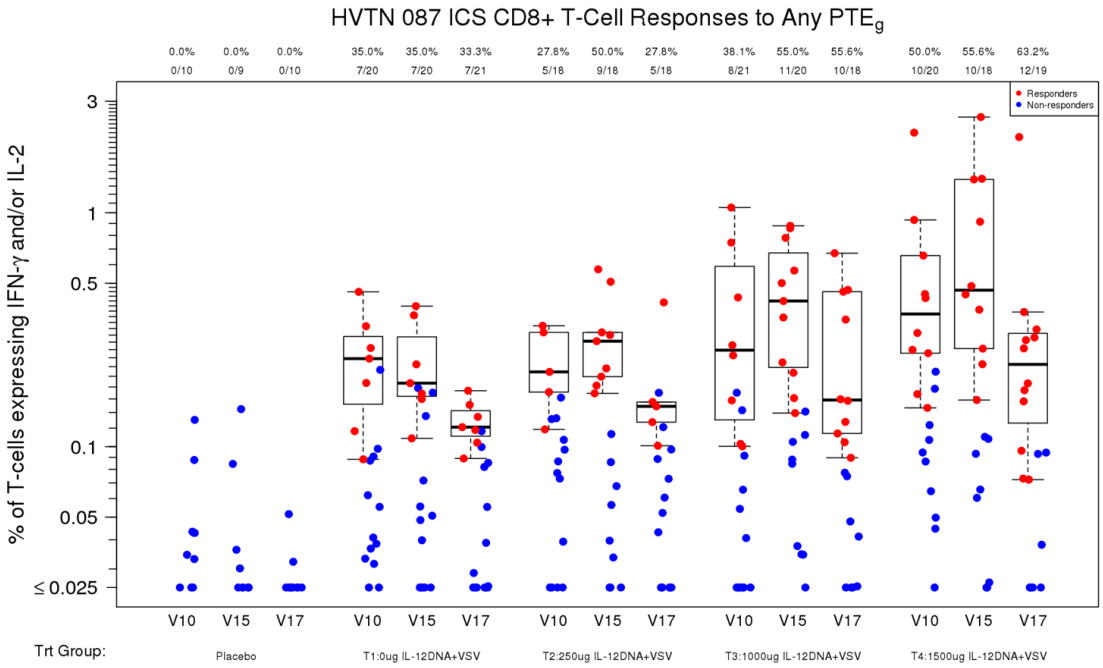


**Figure 4-19 HIV-specific CD8+ T-cell ICS responses in HVTN 087**

However, the highest CD4+ T-cell response rate to DNA vaccination (pre-VSV boost) was seen in group 1, without *IL-12* pDNA (Figure 4-18). Given the ability of IL-12 to shift vaccine-specific immunity toward a Th1 response, *IL-12* pDNA as an adjuvant could contribute to CD8+ cellular immune responses to the p24CE1/2 pDNA vaccine.

IAVI has presented results of its B004 study, which also tested multiple injections of HIV-MAG and GENEVAX® *IL-12*-4532 DNA IM/EP (Ichor TDS-IM EP Device) in healthy volunteers [7]. The study tested the groups shown in Table 4-3. The vaccine regimens tested were adequately safe with no SAEs or AEs that were probably or definitely related to vaccine. The vaccine regimens in the 3 study groups shown were similarly rated with respect to tolerability.

With respect to cellular responses assessed by IFN-γ ELISpot, at baseline there were no responders. After the 3 pDNA priming injections, response rates among vaccinees were 82% (9/11) in Group 1, 64% (7/11) in Group 2, and 42% (5/12) in Group 3. After the Ad35-GRIN/ENV boost, response rates among vaccinees were 73% (8/11) in Group 1, 82% (9/11) in Group 2, and 89% (8/9) in Group 3. Samples from 8 participants in each group were also assessed with 7-color ICS to assess expression of IL-2, TNF-α, and IFN-γ in response to peptides matched to the HIV-MAG vaccine. Groups 1-3 had similar CD4+ and CD8+ T-cell response rates and magnitudes, and the polyfunctionality profiles were similar. An ELISA assay for HIV-specific antibodies to Env A and Env B (matched to the HIV-MAG vaccine) and $p24^{Gag}$ did not detect any HIV-specific antibodies following the DNA primes in any group. In summary, the vaccine regimens tested were immunogenic, but there was no clear indication of enhancement of immunogenicity of HIV-MAG by *IL-12* DNA in this small trial. One shortcoming of this data is that the participants were not all evaluated for CD8+ responses with ICS. It is not clear how the specific participant samples were selected for ICS assay, or how responses would have differed if measured by the HVTN laboratory assays.

**Table 4-3 B004 schema**

| Study arm | N | Month (Week) 0 (0) | Month (Week) 1 (4) | Month (Week) 2 (8) | Month (Week) 6 (24) |
|---|---|---|---|---|---|
| Group 1 | 12 | HIV-MAG | HIV-MAG | HIV-MAG | Ad35-GRIN/ENV |
| | 3 | Placebo | Placebo | Placebo | Placebo |
| Group 2 | 12 | HIV-MAG+ *IL-12* pDNA 0.1 mg | HIV-MAG+ *IL-12* pDNA 0.1 mg | HIV-MAG+ *IL-12* pDNA 0.1 mg | Ad35-GRIN/ENV |
| | 3 | Placebo | Placebo | Placebo | Placebo |
| Group 3 | 12 | HIV-MAG+ *IL-12* pDNA 1 mg | HIV-MAG+ *IL-12* pDNA 1 mg | HIV-MAG+ *IL-12* pDNA 1 mg | Ad35-GRIN/ENV |
| | 3 | Placebo | Placebo | Placebo | Placebo |

Note: HIV-MAG 3 mg delivered IM by EP. GENEVAX® IL-12 DNA plasmid co-administered with HIV-MAG delivered IM by EP. Ad35-GRIN/ENV $2\times10^{10}$ vp delivered IM by standard needle injection.

The HVTN is also evaluating PENNVAX®-GP (Gag, Pol, Env) pDNA Vaccine and *IL-12* plasmid (INO-9012, Inovio Pharmaceuticals, Inc. Plymouth Meeting, Pennsylvania, USA), delivered via intradermal or IM/EP (CELLECTRA® EP system) in HVTN 098 (BB IND #16398, held by DAIDS). In that study, for IM delivery, the dose of PENNVAX®-GP is 8 mg and the dose of the Inovio *IL-12* plasmid is 1 mg, at a single injection site. The study is ongoing. No SAEs related to vaccination have been reported. Injection site pruritus has been reported by 19.1% of participants and presyncope following the vaccination procedure in 5.3%. Another *IL-12* pDNA (pUMVC3-hIL-12-NGVL3, City of Hope Center for Biomedicine and Genetics, Duarte, CA) has been given in doses up to 5.8 mg per administration delivered with EP directly into melanoma tumors, with no dose limiting systemic toxicities noted [91]. Hence, we do not anticipate any significant systemic or local adverse effects related to the use of *IL-12* pDNA IM with EP at the proposed dose.

Taken together, the studies suggest that *IL-12* pDNA delivered with plasmid DNA vaccines with EP may have a dose-dependent adjuvant effect on CD8+ responses. *IL-12* pDNA has been given at doses up to 1 mg at a single injection site with IM/EP and up to 1.5 mg total IM/EP at one visit (2 injection sites). Given the focus of this study on evaluating cellular immune responses, *IL-12* pDNA may provide an advantage to the evaluation of immunogenicity of the p24CE and $p55^{gag}$ pDNA vaccines.

## 4.11 Potential risks of study products and administration

**Table 4-4 Summary of potential risks of study products and administration**

| | |
|---|---|
| Common | • Mild transient cutaneous bleeding, bruising/ecchymosis<br>• Mild to moderate injection site pain, tenderness, pruritus, erythema, or swelling/induration/edema<br>• Malaise/fatigue, myalgia, or headache in the first few days following injection |
| Less common | • Severe injection site pain or tenderness<br>• Transient paresthesia or hypoesthesia in the injected limb<br>• Fever, chills, flu-like syndrome, arthralgia, rash, nausea, or dizziness in the first few days following injection<br>• Vasovagal reaction/lightheadedness/dizziness related to the injection procedure<br>• Transient changes in clinical laboratory values<br>• Injection site hematoma, laceration, bruising/ecchymosis, other transient lesions, itching, or bleeding related to the injection procedure<br>• A vaccine-induced positive HIV antibody test result |
| Uncommon or rare | • Severe localized injection site reaction, such as sterile abscess or secondary bacterial infection<br>• Allergic reaction, including rash, urticaria, angioedema, bronchospasm, or anaphylaxis<br>• Muscle damage at the injection site<br>• Injection needle contact with the bone<br>• Difficulty removing EP device<br>• Syncope |
| Theoretical risks | • Autoimmune disease or cancer<br>• Electrical injury with EP<br>• Disruption of function of implanted electronic medical devices with EP<br>• Exacerbation of cardiac arrhythmias with EP<br>• Effects on a participant's response to an approved HIV vaccine administered in the future<br>• Effects on susceptibility to HIV, if the participant is exposed to HIV<br>• Effects on the course of HIV infection/disease, if the participant is infected with HIV<br>• Effects on the fetus and on pregnancy |

# 5 Objectives and endpoints

## 5.1 Primary objectives and endpoints

*Primary objective 1:*

To evaluate the safety and tolerability of the HIV-1 pDNA vaccines p24CE1/2 and $p55^{gag}$ administered with *IL-12* pDNA by intramuscular injection with electroporation

*Primary endpoints 1:*

- Frequency and severity of local injection/EP site and systemic reactogenicity signs and symptoms
- Frequency of AEs categorized by MedDRA body system, MedDRA preferred term, severity and assessed relationship to study products. Detailed description of all AEs meeting DAIDS criteria for expedited reporting
- The distribution of values of safety laboratory measures at baseline and at follow-up visits post vaccination
- Number of participants with early discontinuation of vaccinations and reason for discontinuation
- Magnitude of local injection/EP site pain as measured by a visual analog scale
- Distribution of responses to questions regarding acceptability of study injection procedures

*Primary objective 2:*

To compare the two pDNA prime/boost strategies with respect to breadth (defined as the number of targeted CEs) of $CD4^+$ and $CD8^+$ T-cell responses to conserved regions of the $p24^{Gag}$ protein

*Primary endpoint 2:*

The number of targeted CEs by $CD4^+$ and $CD8^+$ T-cell responses measured by ICS 2 weeks after the second and fourth vaccinations

## 5.2 Secondary objectives and endpoints

*Secondary objective 1:*

To determine the effect of the different prime/boost regimens on the magnitude and polyfunctionality of T-cell responses induced by the different priming vaccinations

*Secondary endpoint 1:*

Rate, magnitude and polyfunctionality of $CD4^+$ and $CD8^+$ T-cell responses measured by ICS to p24CE and HIV Gag, 2 weeks after the second and fourth vaccinations

*Secondary objective 2:*

To determine the effect of the different prime/boost regimens on the specificity of T-cell responses induced by the different primes

*Secondary endpoint 2:*

Epitope specificity of T-cell responses measured by IFN-γ ELISpot to p24CE and HIV Gag, 2 weeks after the second and fourth vaccinations

*Secondary objective 3:*

To evaluate the humoral immunogenicity of the HIV-1 pDNA vaccines p24CE1/2 and $p55^{gag}$ administered alone, in combination, or sequentially by intramuscular injection with electroporation

*Secondary endpoint 3:*

Rate and magnitude of humoral immune responses to Gag measured 2 weeks after the $4^{th}$ vaccination by binding antibody multiplex assay

*Secondary objective 4:*

To determine the frequency of circulating Tfh in response to each vaccination regimen

*Secondary endpoint 4:*

Rate and magnitude of $CD4^+$ cTfh measured by ICS

## 5.3 Exploratory objectives

*Exploratory objective 1:*

To further evaluate immunogenicity of the different vaccine regimens, additional immunogenicity assays may be performed, including on samples from other time points, based on the HVTN Laboratory Assay Algorithm

*Exploratory objective 2:*

To determine the frequency of plasmablasts in response to each vaccination regimen

*Exploratory objective 3:*

To evaluate the breadth of the T cell receptor repertoire induced after pDNA vaccination by electroporation

*Exploratory objective 4:*

To conduct analyses related to furthering the understanding of HIV, immunology, vaccines, and clinical trial conduct

# 6 Statistical considerations

## 6.1 Accrual and sample size calculations

Recruitment will target enrolling 56 healthy, HIV-uninfected adult participants, aged 18 to 50 years.

To ensure that both men and women will be adequately represented in the trial, the trial will enroll at least approximately 40% of each sex overall. Hence, when approximately 33 participants of one sex are enrolled, recruitment of persons born of that sex will stop.

Since enrollment is concurrent with receiving the first study vaccination, all participants will provide some safety data. However, for immunogenicity analyses, it is possible that data may be missing for various reasons, such as participants terminating from the study early, problems in shipping specimens, low cell viability of processed peripheral blood mononuclear cells (PBMCs) or high background. Immunogenicity data from 17 phase 1 and 2 phase 2a HVTN vaccine trials, which began enrolling after June 2005 (data as of September 2014), indicate that 17% is a reasonable estimate for the rate of missing data at day 182. For this reason, the sample size calculations in Section 6.1.2 account for 4 enrolled participants receiving vaccine having missing data for each study arm at the primary immunogenicity endpoint.

### 6.1.1 Sample size calculations for safety

The goal of the safety evaluation for this study is to identify safety concerns associated with product administration. Sample size calculations for safety are expressed in terms of the ability to detect AEs requiring expedited reporting to DAIDS.

The ability of the study to detect serious adverse events (SAEs) can be expressed by the true event rate above which at least 1 SAE would likely be observed and the true event rate below which no events would likely be observed. Specifically, for each vaccine arm of the study (n =25), there is a 90% chance of observing at least 1 event if the true rate of such an event is 8.8% or more; and there is a 90% chance of observing no events if the true rate is 0.4% or less. As a reference, in HVTN vaccine trials from December 2000 through September 2010, about 4% of participants who received placebos experienced an SAE.

Probabilities of observing 0, 1 or more, and 2 or more events among arms of size 25 are presented in Table 6-1 for a range of possible true adverse event rates. These calculations provide a more complete picture of the sensitivity of this study design to identify potential safety problems with the vaccine.

**Table 6-1 Probability of observing 0 events, 1 or more events, and 2 or more events, among arms of size 25, for different true event rates**

| True event rate (%) | Pr(0/25) | Pr(1+/25) | Pr(2+/25) |
|---|---|---|---|
| 1 | 77.8 | 22.2 | 2.6 |
| 4 | 36 | 64 | 26.4 |
| 5 | 27.7 | 72.3 | 35.8 |
| 10 | 7.2 | 92.8 | 72.9 |
| 20 | 0.4 | 99.6 | 97.3 |
| 30 | <0.1 | >99.9 | 99.8 |
| 40 | <0.1 | >99.9 | >99.9 |

An alternative way of describing the statistical properties of the study design is in terms of the 95% confidence interval for the true rate of an adverse event based on the observed data. Table 6-2 shows the 2-sided 95% confidence intervals for the probability of an event based on a particular observed rate. Calculations are done using the score test method [92]. For a given vaccine arm, if none of the 25 participants receiving a vaccine regimen experience a safety event, the 95% 2-sided upper confidence bound for the true rate of such events in the total vaccinated population is 13.3%.

**Table 6-2 Two-sided 95% confidence intervals based on observing a particular rate of safety endpoints for arms of size 25**

| Observed event rate | 95% CI |
|---|---|
| 0/25 | [0.0, 13.3] |
| 1/25 | [0.2, 19.5] |
| 2/25 | [2.2, 25.0] |

### 6.1.2 Sample size calculations for immunogenicity

For comparing the CD4+ and CD8+ T-cell endpoints between the 2 vaccine regimens, the primary analysis uses head-to-head superiority comparisons. The comparison will be performed separately for each of the primary breadth and secondary magnitude T-cell subset endpoints. No multiplicity adjustments are made for the multiple comparisons given the utility to favor higher power at the expense of a higher accepted false positive rate.

Figure 6-1 below summarizes power available for superiority testing. For these calculations, we assume 17% missing data (n=21 evaluable endpoints from each arm). Sample size calculations for breadth of response are based on data from the HVTN 083 trial and assume a beta-binomial distribution. Sample size calculations for ICS magnitude assume normally distributed magnitudes after natural log transformation with standard deviation = 1. The assumptions for the sample size calculations for ICS magnitude are based on the data from the HVTN 080 trial. Superiority test power is based on the Mann-Whitney U test with 2-sided p-value <0.05 as statistically significant.

If mean CE breadth in the vaccine recipients in Group 1 is 0.2 (0.5) then the study has 80% power to detect superiority in Group 2 if the true CE breadth the 2$^{nd}$ group is at least 1.0 (1.5). A 2.5 fold-change in the vaccine recipients from Group 2 versus Group 1 is required to have 80% power to detect superiority in the T-cell response magnitude.

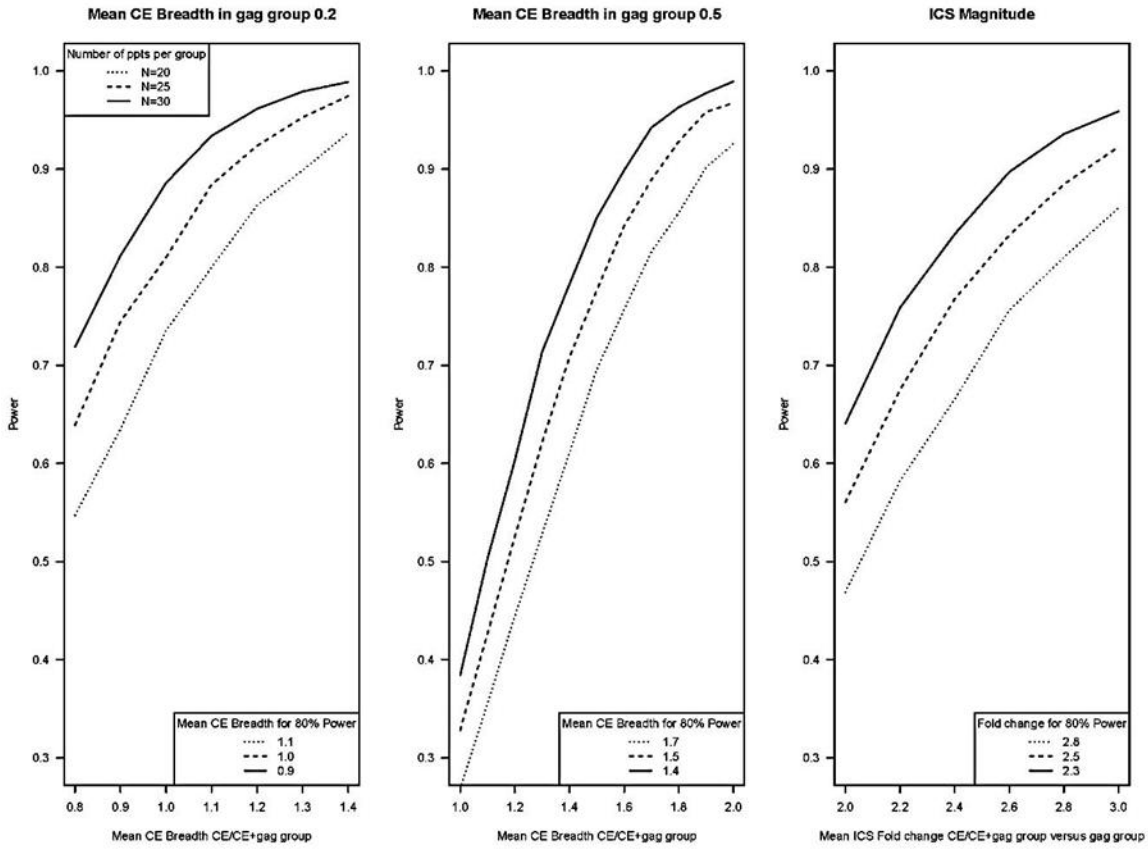


**Figure 6-1 Power to detect superiority in CE Breadth and ICS Magnitude. Power calculations for the breadth of response were run under two different scenarios.** In the first panel power the mean CE Breadth is assumed to be 0.2 for the vaccine recipients in Group 1 while the second panel assumes a mean CE Breadth of 0.5. In the third panel power calculations are shown for the magnitude of response. The x-axis in the first two panels is the CE Breadth for the vaccine recipients in Group 2 and for the third panel it the x-axis is the fold change in ICS response magnitude for vaccine recipients from Group 2 versus Group 1. Power curves are shown for N=20, 25, and 30 vaccine recipients per Group and account for 17% missing immunogenicity data.

## 6.2 Randomization

The randomization sequence will be obtained by computer-generated random numbers and provided to each HVTN CRS through a Web-based randomization system. The randomization will be done in blocks to ensure balance across arms. At each institution, the pharmacist with primary responsibility for dispensing study products is charged with maintaining security of the treatment assignments (except in emergency situations as specified in the HVTN MOP).

## 6.3 Blinding

Participants and site staff (except for site pharmacists) will be blinded as to participant treatment arm assignments (ie, blinded to both group and vaccine/control status). Study product assignments are accessible to those HVTN CRS pharmacists, DAIDS protocol

pharmacists and contract monitors, and SDMC staff who are required to know this information in order to ensure proper trial conduct. Any discussion of study product assignment between pharmacy staff and any other HVTN CRS staff is prohibited. The HVTN SMB members also are unblinded to treatment assignment in order to conduct review of trial safety.

When a participant leaves the trial prior to study completion, the participant will be told he or she must wait until all participants are unblinded to learn his or her treatment assignment.

Emergency unblinding decisions will be made by the site investigator. If time permits, the HVTN PSRT should be consulted before emergency unblinding occurs.

## 6.4 Statistical analyses

This section describes the final study analyses, unblinded as to treatment arm assignment. All data from enrolled participants will be analyzed according to the initial randomization assignment regardless of how many vaccinations they received. In the rare instance that a participant receives the wrong treatment at a specific vaccination time, the Statistical Analysis Plan will address how to analyze the participant's safety data. Analyses are modified intent-to-treat in that individuals who are randomized but not enrolled do not contribute data and hence are excluded. Because of blinding and the brief length of time between randomization and enrollment—typically no more than 4 working days—very few such individuals are expected.

Analyses for primary endpoints will be performed using SAS and R. All other descriptive and inferential statistical analyses will be performed using SAS, StatXact, or R statistical software.

No formal multiple comparison adjustments will be employed for multiple safety endpoints, multiple primary immunogenicity endpoints, or secondary endpoints. However, multiplicity adjustments will be made for certain immunogenicity assays, as discussed below, when the assay endpoint is viewed as a collection of hypotheses (eg, testing multiple peptide pools to determine a positive response).

### 6.4.1 Analysis variables

The analysis variables consist of baseline participant characteristics, safety, and immunogenicity for primary- and secondary-objective analyses.

### 6.4.2 Baseline comparability

Treatment arms will be compared for baseline participant characteristics using descriptive statistics.

### 6.4.3 Safety/tolerability analysis

Since enrollment is concurrent with receiving the first vaccination, all participants will have received at least 1 vaccination and therefore will provide some safety data.

#### 6.4.3.1 Reactogenicity

The number and percentage of participants experiencing each type of reactogenicity sign or symptom will be tabulated by severity and treatment arm and the percentages displayed graphically by arm. For a given sign or symptom, each participant's reactogenicity will be counted once under the maximum severity for all injection visits. In addition, to the individual types of events, the maximum severity of local pain or tenderness, induration or erythema, and of systemic symptoms will be calculated. Kruskal-Wallis tests will be used to test for differences in severity between arms.

#### 6.4.3.2 AEs and SAEs

AEs will be summarized using MedDRA System Organ Class and preferred terms. Tables will show by treatment arm the number and percentage of participants experiencing an AE within a System Organ Class or within preferred term category by severity or by relationship to study product. For the calculations in these tables, a participant with multiple AEs within a category will be counted once under the maximum severity or the strongest recorded causal relationship to study product. Formal statistical testing comparing arms is not planned since interpretation of differences must rely heavily upon clinical judgment.

A listing of SAEs reported to the DAIDS Regulatory Support Center (RSC) Safety Office will provide details of the events including severity, relationship to study product, time between onset and last vaccination, and number of vaccinations received. A separate listing will do the same for AEs of special interest (AESI). AESI for this protocol include but are not limited to autoimmune disorders; a sample list of AESI is provided in Appendix G.

#### 6.4.3.3 Local laboratory values

Box plots of local laboratory values will be generated for baseline values and for values measured during the course of the study by treatment arm and visit. Each box plot will show the first quartile, the median, and the third quartile. Outliers (values outside the box plot) will also be plotted. If appropriate, horizontal lines representing boundaries for abnormal values will be plotted.

For each local laboratory measure, summary statistics will be presented by treatment arm and time point, as well as changes from baseline for post-enrollment values. In addition, the number (percentage) of participants with local laboratory values recorded as meeting Grade 1 AE criteria or above as specified in the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events will be tabulated by treatment arm for each post-vaccination time point. Reportable clinical laboratory abnormalities without an associated clinical diagnosis will also be included in the tabulation of AEs described above.

#### 6.4.3.4 Reasons for vaccination discontinuation and early study termination

The number and percentage of participants who discontinue vaccination and who terminate the study early will be tabulated by reason and treatment arm.

### 6.4.4 Immunogenicity analysis

#### 6.4.4.1 General approach

For the statistical analysis of immunogenicity endpoints, data from enrolled participants will be used according to the initial randomization assignment regardless of how many injections they received. Additional analyses may be performed, limited to participants who received all scheduled injections per protocol. Assay results that are unreliable, from specimens collected outside of the visit window, or from HIV-infected participants postinfection are excluded. Since the exact date of HIV infection is unknown, any assay data from blood draws 4 weeks prior to an infected participant's last seronegative sample and thereafter may be excluded. If an HIV-infected participant does not have a seronegative sample postenrollment, then all data from that participant may be excluded from the analysis.

Discrete categorical assay endpoints (eg, response rates) will be analyzed by tabulating the frequency of positive response for each assay by antigen and treatment arm at each timepoint for which an assessment is performed. Crude response rates will be presented with their corresponding 95% confidence interval estimates calculated using the score test method [92]. Because of the small numbers of control participants in each group, no adjustment will be made to the vaccine arm estimates for the false positive rates in the control arms. Fisher's exact tests will be used to compare the response rates of any 2 vaccine arms, with a significant difference declared if the 2-sided p-value is $\leq 0.05$.

For quantitative assay data (eg, percentage of positive cells from the ICS assay or mean fluorescence intensity from the binding antibody multiplex assay), graphical and tabular summaries of the distributions by antigen, treatment arm, and timepoint will be made. For all primary and secondary immunogenicity endpoints, box plots and plots of estimated reverse cumulative distribution curves will be used for graphical display of all of the study arms. Typically, the results will be shown for each vaccine arm and for the set of control arms pooled into one group.

The differences between treatment groups at 2 weeks post the $2^{nd}$ and $4^{th}$ vaccination timepoints will be tested with a nonparametric Wilcoxon rank sum test if the data are not normally distributed and with a 2-sample t-test if the data appear to be normally distributed.

Some immunologic assays have underlying continuous or count-type readouts that are dichotomized into responder/nonresponder categories (eg, ICS and BAMA). If treatment arm differences for these assays are best summarized by a mixture model, then Lachenbruch's test statistic [93] will be used to evaluate the composite null hypothesis of equal response rates in the 2 arms and equal response distributions among responders in the 2 such arms. This test statistic equals the square of a binomial Z-statistic for comparing the response rates plus the square of a Wilcoxon statistic for comparing the response distributions in the subgroup of responders. A permutation procedure is used to obtain a 2-sided p-value. For estimation, differences in response rates between arms will be estimated using the methods described above, and in the subgroup of positive responders, differences in location parameters between arms will be estimated using the methods described above.

More sophisticated analyses employing repeated measures methodology (eg, linear mixed models or marginal mean models fit by generalized estimating equations) may be utilized

to incorporate immune responses over several timepoints and to test for differences over time. However, inference from such analyses would be limited by the small sample size of this study. All statistical tests will be 2-sided and will be considered statistically significant if $p \leq 0.05$.

Based upon previous HVTN trials, missing 17% of immunogenicity results for a specific assay is common due to study participants terminating from the study early, problems in shipping specimens, or low cell viability of processed peripheral blood mononuclear cells (PBMCs). To achieve unbiased statistical estimation and inferences with standard methods applied in a complete-case manner (only including participants with observed data in the analysis), missing data need to be missing completely at random (MCAR). Following the most commonly used definition, MCAR assumes that the probability of an observation being missing does not depend on any participant characteristics (observed or unobserved). When missing data are minimal (specifically if no more than 20% of participants are missing any values), then standard complete-case methods will be used, because violations of the MCAR assumption will have little impact on the estimates and hypothesis tests.

If a substantial amount of immunogenicity data is missing for an endpoint (at least 1 value missing from more than 20% of participants), then using the methods that require the MCAR assumption may give misleading results. In this situation, analyses of the immunogenicity endpoints at a specific timepoint will be performed using parametric generalized linear models fit by maximum likelihood. These methods provide unbiased estimation and inferences under the parametric modeling assumptions and the assumption that the missing data are missing at random (MAR). MAR assumes that the probability of an observation being missing may depend upon the observed responses and upon observed covariates, but not upon any unobserved factors. Generalized linear models for response rates will use a binomial error distribution and for quantitative endpoints, a normal error distribution. For assessing repeated immunogenicity measurement, linear mixed effects models will be used. If the immunological outcomes are left- and/or right-censored, then the linear mixed effects models of Hughes [94] will be used, because they accommodate the censoring. In addition, secondary analyses of repeated immunogenicity measurements may be done using weighted GEE [95] methods, which are valid under MAR. All of the models described above in this paragraph will include as covariates all available baseline predictors of the missing outcomes.

#### 6.4.4.2 Analysis of $CD4^+$ and $CD8^+$ T-cell response as measured by the ICS assay

The analysis of $CD4^+$ and $CD8^+$ T-cell response rates as measured by the ICS assay will be evaluated and compared as described under the general approach. For each T-cell subset, the positivity call for each peptide pool will include a multiple comparison adjustment for the number of peptide pools used in the assay. In general, the Mixture Models for Single-cell Assays (MIMOSA) statistical framework [96] and/or the Fisher's exact test-based positivity criteria will be used. Details of the positivity criteria will be discussed in the SAP. The magnitude of marginal response will be analyzed as described for quantitative data in the general approach section. For each T-cell subset, graphs will be used to display the background-subtracted magnitudes for each participant by protein, treatment arm and timepoint. Statistical testing comparing the magnitudes will be based on positive responders only. The polyfunctionality of ICS responses will also be analyzed as a secondary endpoint. Besides descriptive plots of the magnitude of polyfunctional responses, the COMPASS (Combinatorial Polyfunctionality analysis of Antigen-Specific T-cell Subsets) statistical framework will also be used to perform joint modelling of

multiple T-cell subsets of different cytokine combinations using the functionality score (FS) and the polyfunctionality score (PFS) to summarize the multi-parameter ICS responses.

#### 6.4.4.3 Analysis of multiplexed immunoassay data

The analysis of response rates and response magnitudes will be evaluated and compared as described under the general approach.

### 6.4.5 Analyses prior to end of scheduled follow-up visits

Any analyses conducted prior to the end of the scheduled follow-up visits should not compromise the integrity of the trial in terms of participant retention or safety or immunogenicity endpoint assessments. In particular, early unblinded analyses by treatment assignment require careful consideration and should be made available on a need to know basis only.

#### 6.4.5.1 Safety

During the course of the trial, unblinded analyses of safety data will be prepared approximately every 4 months during the main study, as defined in Section 3, for review by the SMB. Ad hoc safety reports may also be prepared for SMB review at the request of the HVTN PSRT. The HVTN leadership must approve any other requests for unblinded safety data prior to the end of the scheduled follow-up visits.

#### 6.4.5.2 Immunogenicity

An unblinded statistical analysis by treatment assignment of a primary immunogenicity endpoint may be performed when all participants have completed the corresponding primary immunogenicity visit and data are available for analysis from at least 80% of these participants. Similarly, an unblinded statistical analysis by treatment assignment of a secondary or exploratory immunogenicity endpoint may be performed when all participants have completed the corresponding immunogenicity visit and data are available for analysis from at least 80% of these participants. However, such analyses for a secondary or exploratory immunogenicity endpoint will only take place after at least one of the primary immunogenicity endpoints of the same class (humoral or cell-mediated) or, if no primary endpoint of the same class, at least one of the primary immunogenicity endpoints reaches the aforementioned threshold. The Laboratory Program will review the analysis report prior to distribution to the protocol chairs, DAIDS, vaccine developer, and other key HVTN members and investigators. Distribution of reports will be limited to those with a need to know for the purpose of informing future trial-related decisions. The HVTN leadership must approve any other requests for HVTN immunogenicity analyses prior to the end of the scheduled follow-up visits.

# 7 Selection and withdrawal of participants

Participants will be healthy, HIV uninfected (seronegative) adults who comprehend the purpose of the study and have provided written informed consent. Volunteers will be recruited and screened; those determined to be eligible, based on the inclusion and exclusion criteria, will be enrolled in the study. Final eligibility determination will depend on results of laboratory tests, medical history, physical examinations, and answers to self-administered and/or interview questions.

Investigators should always use good clinical judgment in considering a volunteer's overall fitness for trial participation. Some volunteers may not be appropriate for enrollment even if they meet all inclusion/exclusion criteria. Medical, psychiatric, occupational, or other conditions may make evaluation of safety and/or immunogenicity difficult, and some volunteers may be poor candidates for retention.

The DNA vaccine and adjuvant used in this study will be given with EP. Volunteers may not participate if they have certain metal implants, a surgical or traumatic metal implant in the upper limb and/or shoulder, or a history of cardiac arrhythmias.

Determination of eligibility, taking into account all inclusion and exclusion criteria, must be made within 56 days prior to enrollment unless otherwise noted in Sections 7.1 and 7.2.

## 7.1 Inclusion criteria

**General and Demographic Criteria**

1. **Age** of 18 to 50 years

2. **Access to a participating HVTN CRS** and willingness to be followed for the planned duration of the study

3. Ability and willingness to provide **informed consent**

4. **Assessment of understanding**: volunteer demonstrates understanding of this study; completes a questionnaire prior to first vaccination with verbal demonstration of understanding of all questionnaire items answered incorrectly

5. **Agrees not to enroll in another study** of an investigational research agent prior to completion of last required protocol visit (excludes annual health contact visit)

6. **Good general health** as shown by medical history, physical exam, and screening laboratory tests

**HIV-Related Criteria:**

7. Willingness to receive **HIV test results**

8. Willingness to discuss HIV infection risks and amenable to HIV risk reduction counseling.

9. Assessed by the clinic staff as being at **"low risk" for HIV infection** and committed to maintaining behavior consistent with low risk of HIV exposure through the last required protocol clinic visit.

**Laboratory Inclusion Values**

***Hemogram/CBC***

10. **Hemoglobin** ≥ 11.0 g/dL for volunteers who were born female, ≥ 13.0 g/dL for volunteers who were born male

11. **White blood cell count** = 3,300 to 12,000 cells/$mm^3$

12. **Total lymphocyte count** ≥ 800 cells/$mm^3$

13. **Remaining differential** either within institutional normal range or with site physician approval

14. **Platelets** = 125,000 to 550,000/$mm^3$

***Chemistry***

15. **Chemistry panel**: ALT, AST, and alkaline phosphatase < 1.25 times the institutional upper limit of normal; CPK ≤ 2.0 times the institutional upper limit of normal; creatinine ≤ institutional upper limit of normal.

***Virology***

16. **Negative HIV-1 and -2 blood test**: US volunteers must have a negative FDA-approved enzyme immunoassay (EIA).

17. **Negative Hepatitis B surface antigen (HBsAg)**

18. **Negative anti-Hepatitis C virus antibodies (anti-HCV)**, or negative HCV polymerase chain reaction (PCR) if the anti-HCV is positive

***Urine***

19. **Normal urine**:

    - Negative urine glucose, and
    - Negative or trace urine protein, and
    - Negative or trace urine hemoglobin (if trace hemoglobin is present on dipstick, a microscopic urinalysis with red blood cells levels within institutional normal range).

**Reproductive Status**

20. **Volunteers who were born female**: negative serum or urine beta human chorionic gonadotropin (β-HCG) pregnancy test performed prior to vaccination on the day of initial vaccination. Persons who are NOT of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing.

21. **Reproductive status**: A volunteer who was born female must:

    - Agree to consistently use effective contraception for sexual activity that could lead to pregnancy from at least 21 days prior to enrollment through the last required protocol clinic visit. Effective contraception is defined as using the following methods:
        - Condoms (male or female) with or without a spermicide,
        - Diaphragm or cervical cap with spermicide,
        - Intrauterine device (IUD),
        - Hormonal contraception, or
        - Any other contraceptive method approved by the HVTN PSRT
        - Successful vasectomy in the male partner (considered successful if a volunteer reports that a male partner has [1] documentation of azoospermia by microscopy, or [2] a vasectomy more than 2 years ago with no resultant pregnancy despite sexual activity postvasectomy);
    - Or not be of reproductive potential, such as having reached menopause (no menses for 1 year) or having undergone hysterectomy, bilateral oophorectomy, or tubal ligation;
    - Or be sexually abstinent.

22. **Volunteers who were born female must also agree not to seek pregnancy through alternative methods**, such as artificial insemination or in vitro fertilization until after the last required protocol clinic visit

## 7.2 Exclusion criteria

**General**

1. **Allergy to amide-type local anesthetics** (bupivacaine [Marcaine], lidocaine [Xylocaine], mepivacaine [Polocaine/Carbocaine], etidocaine [Duranest], prilocaine [Citanest, EMLA® cream])

2. **Blood products** received within 120 days before first vaccination

3. **Deltoid skin fold measurement by caliper > 40 mm**

4. **Investigational research agents** received within 30 days before first vaccination

5. **Body mass index (BMI)** ≥ 40; or BMI ≥ 35 with 2 or more of the following: age > 45, systolic blood pressure > 140 mm Hg, diastolic blood pressure > 90 mm Hg, current smoker, known hyperlipidemia

6. **Intent to participate in another study** of an investigational research agent or any other study that requires non-HVTN HIV antibody testing during the planned duration of the study

7. **Pregnant or breastfeeding**

8. **Active duty and reserve US military personnel**

**Vaccines and other Injections**

9. **HIV vaccine(s)** received in a prior HIV vaccine trial. For volunteers who have received control/placebo in an HIV vaccine trial, the HVTN PSRT will determine eligibility on a case-by-case basis.

10. **Non-HIV experimental vaccine(s) received within the last 5 yea**rs in a prior vaccine trial. Exceptions may be made for vaccines that have subsequently undergone licensure by the FDA. For volunteers who have received control/placebo in an experimental vaccine trial, the HVTN PSRT will determine eligibility on a case-by-case basis. For volunteers who have received an experimental vaccine(s) greater than 5 years ago, eligibility for enrollment will be determined by the HVTN PSRT on a case-by-case basis.

11. **Live attenuated vaccines** other than influenza vaccine, received within 30 days before first vaccination or scheduled within 14 days after injection (eg, measles, mumps, and rubella [MMR]; oral polio vaccine [OPV]; varicella; yellow fever)

12. **Influenza vaccine or any vaccines that are not live attenuated vaccines** and were received within 14 days prior to first vaccination (eg, tetanus, pneumococcal, Hepatitis A or B)

13. **Allergy treatment with antigen injections** within 30 days before first vaccination or that are scheduled within 14 days after first vaccination

**Immune System**

14. **Immunosuppressive medications** received within 168 days before first vaccination. (Not exclusionary: [1] corticosteroid nasal spray; [2] inhaled corticosteroids; [3] topical corticosteroids for mild, uncomplicated dermatitis; or [4] a single course of oral/parenteral corticosteroids at doses < 2 mg/kg/day and length of therapy < 11 days with completion at least 30 days prior to enrollment.)

15. **Serious adverse reactions to vaccines or to vaccine components,** including history of anaphylaxis and related symptoms such as hives, respiratory difficulty, angioedema, and/or abdominal pain. (Not excluded from participation: a volunteer who had a nonanaphylactic adverse reaction to pertussis vaccine as a child.)

16. **Immunoglobulin** received within 60 days before first vaccination

17. **Autoimmune disease**

18. **Immunodeficiency**

**Clinically significant medical conditions**

19. **Untreated or incompletely treated syphilis infection**

20. **Clinically significant medical condition**, physical examination findings, clinically significant abnormal laboratory results, or past medical history with clinically significant implications for current health. A clinically significant condition or process includes but is not limited to:

    - A process that would affect the immune response,
    - A process that would require medication that affects the immune response,
    - Any contraindication to repeated injections or blood draws,
    - A condition that requires active medical intervention or monitoring to avert grave danger to the volunteer's health or well-being during the study period,
    - A condition or process for which signs or symptoms could be confused with reactions to vaccine, or
    - Any condition specifically listed among the exclusion criteria below.

21. **Any medical, psychiatric, occupational, or other condition** that, in the judgment of the investigator, would interfere with, or serve as a contraindication to, protocol adherence, assessment of safety or reactogenicity, or a volunteer's ability to give informed consent

22. **Psychiatric condition that precludes compliance with the protocol**. Specifically excluded are persons with psychoses within the past 3 years, ongoing risk for suicide, or history of suicide attempt or gesture within the past 3 years.

23. **Current anti-tuberculosis (TB) prophylaxis or therapy**

24. **Asthma exclusion criteria:**

    **Asthma** other than mild, well-controlled asthma. (Symptoms of asthma severity as defined in the most recent National Asthma Education and Prevention Program (NAEPP) Expert Panel report).

    Exclude a volunteer who:

    - Uses a short-acting rescue inhaler (typically a beta 2 agonist) daily, or
    - Uses moderate/high dose inhaled corticosteroids, or
    - In the past year has either of the following:
        - Greater than 1 exacerbation of symptoms treated with oral/parenteral corticosteroids;

- Needed emergency care, urgent care, hospitalization, or intubation for asthma.

25. **Diabetes mellitus** type 1 or type 2, including cases controlled with diet alone. (Not excluded: history of isolated gestational diabetes.)

26. **Thyroidectomy, or thyroid disease** requiring medication during the last 12 months

27. **Hypertension**:

    - If a person has been found to have elevated blood pressure or hypertension during screening or previously, exclude for blood pressure that is not well controlled. Well-controlled blood pressure is defined as consistently ≤ 140 mm Hg systolic and ≤ 90 mm Hg diastolic, with or without medication, with only isolated, brief instances of higher readings, which must be ≤ 150 mm Hg systolic and ≤ 100 mm Hg diastolic. For these volunteers, blood pressure must be ≤ 140 mm Hg systolic and ≤ 90 mm Hg diastolic at enrollment.

    - If a person has NOT been found to have elevated blood pressure or hypertension during screening or previously, exclude for systolic blood pressure ≥ 150 mm Hg at enrollment or diastolic blood pressure ≥ 100 mm Hg at enrollment.

28. **Bleeding disorder** diagnosed by a doctor (eg, factor deficiency, coagulopathy, or platelet disorder requiring special precautions)

29. **Malignancy** (Not excluded from participation: Volunteer who has had malignancy excised surgically and who, in the investigator's estimation, has a reasonable assurance of sustained cure. or who is unlikely to experience recurrence of malignancy during the period of the study)

30. **Seizure disorder:** History of seizure(s) within past three years. Also exclude if volunteer has used medications in order to prevent or treat seizure(s) at any time within the past 3 years.

31. **Asplenia**: any condition resulting in the absence of a functional spleen

32. History of hereditary **angioedema**, acquired angioedema, or idiopathic angioedema.

33. **Presence of implanted electronic medical device** (eg, cochlear implant, pacemaker, implantable cardioverter defibrillator)

34. **Presence of surgical or traumatic metal implant** at the intended site of administration (including the deltoid muscles and/or overlying skin)

35. **Sinus bradycardia** (defined as < 50 bpm on exam) or a history of cardiac arrhythmia: eg, supraventricular tachycardia, atrial fibrillation, or frequent ectopy.
    NOTE: Sinus arrhythmia is not excluded.

## 7.3 Participant departure from vaccination schedule or withdrawal

This section concerns an individual participant's departure from the vaccination schedule. Pause rules for the trial as a whole are described in Section 11.4.

For information on procedures following EP difficulties, see Sections 9.3.1 and 9.3.2.

### 7.3.1 Delaying vaccinations for a participant

Under certain circumstances, a participant's scheduled vaccination will be delayed. The factors to be considered in such a decision include but are not limited to the following:

- Within 45 days prior to any study injection
  - Receipt of blood products or immunoglobulin
- Within 30 days prior to any study injection
  - Receipt of live attenuated vaccines other than influenza vaccine
  - Receipt of allergy treatment with antigen injections
- Within 14 days prior to any study injection
  - Receipt of influenza vaccine or any vaccines that are not live attenuated vaccines (eg, pneumococcal)
- Pre-vaccination abnormal vital signs or clinical symptoms that may mask assessment of vaccine reaction.

Vaccinations should not be administered outside the visit window period specified in the Study Specific Procedures.

In order to avoid vaccination delays and missed vaccinations, participants who plan to receive licensed vaccines or allergy treatments should be counseled to schedule receipt of these substances, when possible, outside the intervals indicated above. The effects of these substances on safety and immunogenicity assessments and their interactions with study vaccines are unknown. Therefore, if circumstances allow, these substances should also be avoided in the 2 week interval between a study vaccination and completion of the 2 week post vaccination follow-up visit.

### 7.3.2 Participant departure from vaccination schedule

Every effort should be made to follow the vaccination schedule per the protocol. If a participant misses a vaccination and the visit window period for the vaccination has passed, that vaccination cannot be given. The participant should be asked to continue study visits. The participant should resume the vaccination schedule with the next vaccination unless there are circumstances that require further delay or permanent discontinuation of vaccination (see Sections 7.3.1 and 7.3.3).

### 7.3.3 Discontinuing vaccination for a participant

Under certain circumstances, an individual participant's vaccinations will be permanently discontinued. Specific events that will result in stopping a participant's vaccination schedule include:

- Co-enrollment in a study with an investigational research agent (rare exceptions allowing for the continuation of vaccinations may be granted with the unanimous consent of the HVTN PSRT).

- Clinically significant condition (ie, a condition that affects the immune system or for which continued vaccinations and/or blood draws may pose additional risk), including but not limited to the following:
  - Pregnancy (regardless of outcome);
  - Any grade 4 local or systemic reactogenicity symptom, lab abnormality, or AE that is subsequently considered to be related to study products.
  - Any grade 3 lab abnormality or other clinical AE (exception: fever or vomiting and subjective local and systemic symptoms) that is subsequently considered to be related to study products; or
  - Clinically significant type 1 hypersensitivity reaction associated with study vaccination. Consultation with the HVTN PSRT is required prior to subsequent vaccinations following any type 1 hypersensitivity reaction associated with study vaccination; or

- Investigator determination in consultation with Protocol Team leadership (eg, for repeated nonadherence to study staff instructions).

Such participants should be counseled on the importance of continuing with the study and strongly encouraged to participate in follow-up visits and protocol-related procedures per the protocol for the remainder of the trial, unless medically contraindicated.

In addition, vaccinations will be stopped for participants diagnosed with HIV infection. HIV-infected participants will not continue in the trial (see Section 7.3.4).

### 7.3.4 Participant termination from the study

Under certain circumstances, an individual participant may be terminated from participation in this study. Specific events that will result in early termination include:

- Participant refuses further participation,

- Participant relocates and remote follow-up or transfer to another HVTN CRS is not possible,

- HVTN CRS determines that the participant is lost to follow-up,

- Participant becomes HIV infected, or

- Investigator decides, in consultation with Protocol Team leadership, to terminate participation (eg, if participant exhibits inappropriate behavior toward clinic staff).

- Any condition where termination from the study is required by applicable regulations.

# 8 Study product preparation and administration

CRS pharmacists should consult the Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks for standard pharmacy operations. The protocol schema is shown in Table 3-1. See the Investigator's Brochures for further information about study products.

## 8.1 Vaccine regimen

The schedule of vaccination is shown in Section 3 and additional information is given below.

### Group 1

All vaccine regimens including placebo will be administered intramuscularly using the Ichor Medical System intramuscular TriGrid ™ Delivery System (TDS-IM) electroporation (EP) device.

**Treatment 1 (T1):**

p24CE1/2 DNA plasmid 2 mg admixed with GENEVAX® IL-12 DNA plasmid 1 mg administered as 1 mL IM in left deltoid

AND

p24CE1/2 DNA plasmid 2 mg admixed with GENEVAX® IL-12 DNA plasmid 1 mg administered as 1 mL IM in right deltoid at Months 0 and 1.

**THEN**

p24CE1/2 DNA plasmid 1 mg admixed with p55$^{gag}$ DNA plasmid 1 mg and GENEVAX® IL-12 DNA plasmid 1 mg administered as 1 mL IM in left deltoid

AND

p24CE1/2 DNA plasmid 1 mg admixed with p55$^{gag}$ DNA plasmid 1 mg and GENEVAX® IL-12 DNA plasmid 1 mg administered as 1 mL IM in right deltoid at Months 3 and 6.

**Control 1 (C1):**

Placebo for p24CE1/2 + *IL-12* pDNA is Sodium Chloride for Injection, USP 0.9%, administered as 1 mL IM in left deltoid

AND

1 mL IM in right deltoid at Months 0 and 1.

**THEN**

Placebo for p24CE1/2 + p55$^{gag}$ + *IL-12* pDNA is Sodium Chloride for Injection, USP 0.9%, administered as 1 mL IM in left deltoid

AND

1 mL IM in right deltoid at Months 3 and 6.

### Group 2

All vaccine regimens including placebo will be administered intramuscularly using the Ichor Medical System intramuscular TriGrid ™ Delivery System (TDS-IM) electroporation (EP) device.

**Treatment 2 (T2):**

p55$^{gag}$ DNA plasmid 2 mg admixed with GENEVAX® IL-12 DNA plasmid 1 mg administered as 1 mL IM in left deltoid

AND

p55$^{gag}$ DNA plasmid 2 mg admixed with GENEVAX® IL-12 DNA plasmid 1 mg administered as 1 mL IM in right deltoid at Months 0, 1, 3, and 6.

**Control 2 (C2):**

Placebo for p55$^{gag}$ + *IL-12* pDNA is Sodium Chloride for Injection, USP 0.9%, administered as 1 mL IM in left deltoid

AND

1 mL IM in right deltoid at Months 0, 1, 3 and 6.

## 8.2 Study product formulation

#### p24CE1/2 pDNA [Labeled as p24CE1/2 pDNA 4 mg/mL]

p24CE1/2 pDNA is a clear, colorless solution in a 2 mL single use vial. Each 2 mL vial contains 0.7 ± 0.1 mL p24CE1/2 pDNA at a concentration of 4 mg/mL in 30 mM citrate buffer pH 6.5 containing 150 mM NaCl, 0.01% EDTA, and 0.25% bupivacaine-HCl.

It must be stored at -20˚C.

The product is contraindicated in participants with known hypersensitivity to bupivacaine.

**p55$^{gag}$ pDNA [Labeled as p55 Gag pDNA 4 mg/mL]**

The p55$^{gag}$ pDNA is a clear, colorless solution in a 2 mL single use vial. Each 2 mL vial contains 0.7 ± 0.1 mL p55$^{gag}$ pDNA at a concentration of 4 mg/mL in 30 mM citrate buffer pH 6.5 containing 150 mM NaCl, 0.01% EDTA, and 0.25% bupivacaine-HCl.

It must be stored at -20˚C.

The product is contraindicated in participants with known hypersensitivity to bupivacaine.

**GENEVAX® IL-12 DNA Plasmid** (GENEVAX® *IL-12*-4532, WLV-103M, *IL-12* pDNA, *IL-12* plasmid)

GENEVAX® IL-12 DNA plasmid is a clear colorless solution in a 2 mL single use vial. Each 2 mL vial contains 1 ± 0.1 mL of *IL-12* pDNA at a concentration of 2 mg/mL in 30 mM citrate buffer pH 6.5 containing 150 mM NaCl, 0.01% EDTA and 0.25% bupivacaine-HCl.

The product should be stored at 2° to 8°C.

The product is contraindicated in participants with known hypersensitivity to bupivacaine.

## 8.3 Preparation of study products

### 8.3.1 p24CE1/2 + *IL-12* pDNA (Group 1)

Two vials of p24CE1/2 pDNA (4 mg/mL), 2 vials of *IL-12* pDNA (2 mg /mL), and 1 empty sterile vial (5 mL) will be needed to prepare the 2 syringes. Prior to dispensing, the pharmacist will remove 2 vials of p24CE1/2 pDNA from the freezer and allow to thaw at room temperature for 15-30 minutes until no crystals are observed. The pharmacist will remove 2 vials of *IL-12* pDNA from the refrigerator. Gently swirl and invert the vials for at least 10 inversions (do not shake vigorously).

The vials should be visually inspected prior to use. If the vial contains material different from its description above, do not use the vial and contact the protocol pharmacist. Prior to mixing the study products, the pharmacist will also inspect the sterile pouches containing the TriGrid™ Application Cartridges for integrity and verify the Cartridges have not passed expiration. If any tears or gaps in the sterile pouch are noted or if the expiration date has been reached, the Application Cartridge should be placed in quarantine and the protocol pharmacist should be informed. *Note: In the event that the Cartridge Cap has become detached from the Cartridge in the sterile pouch, the Cartridge may still be used if the Cartridge Cap can be replaced without contaminating the patient contact portion of the Cartridge.*

- Using aseptic technique, the pharmacist will withdraw the 0.6 ml from each of the 2 vials of p24CE1/2 pDNA (4 mg/mL) with a 1 mL low void 25 Ga syringe (low dead space syringe; see *HVTN 119 Study Specific Procedures*) and inject them into an empty mixing sterile vial. This mixing vial, now containing 1.2 mL of p24CE1/2 pDNA, will be set aside.

- Using aseptic technique, the pharmacist will then withdraw 0.6 mL from each of the 2 vials containing *IL-12* pDNA (2 mg /mL) with a 1 mL syringe and inject them directly into the mixing vial containing 1.2 mL of p24CE1/2 pDNA. The pharmacist will mix the vial containing 2.4 mL of the mixture of p24CE1/2 pDNA + GENEVAX® IL-12 DNA plasmid. Gently swirl and invert the mixing vial containing the vaccine for at least 10 inversions (do not shake vigorously).

- Using two new 3 mL syringes (BD# 309585, 309657, or Ichor approved) each fitted with a 22 Ga x 1.5" needle (BD# 305156, or Ichor approved), the pharmacist will withdraw 1 mL of the mixed preparation from the mixing vial into each syringe, and affix each syringe with a new 22 Ga x 1.5" needle, and attached into an Application Cartridge as directed in the *HVTN 119 Study Specific Procedures*.

*Note: Use the syringe tabs to insert the syringe into the Application Cartridge. Do not depress the syringe plunger during insertion of the syringe into the Application Cartridge as this will cause the agent to be injected into the Cartridge.*

*Caution: During the syringe loading procedure, do not physically touch the syringe needle or cause it to come in contact with a non-sterile surface. Note that, although the inside of the Application Cartridge is sterile, contact between the syringe needle and the inner housing of the Application Cartridge should be avoided to the extent possible. Contact between the syringe needle and Application Cartridge could result in deformation of the syringe needle and increased discomfort for the subject upon insertion. If contamination and/or deformation of the injection needle is suspected, replace the injection needle with a new BD 22 Ga x 1.5" injection needle (BD model 305156 or Ichor approved). To prevent loss of dosage make sure to withdraw agent from needle before replacing.*

- Each Application Cartridge will have the Participant's ID number written on it using a Sharpie or other similar pen. The cartridge will then be placed back in the pouch that it was removed from and the pouch will be labeled as "HVTN 119 study product or placebo 1 mL."

- The pouch containing the syringe must be labeled with a 4-hour expiration date from the time the vial is removed from the refrigerator/freezer. The label must also contain the words "Administer as soon as possible."

- One of the syringes must also be labeled for administration in RIGHT deltoid and the second syringe must be labeled for administration in the LEFT deltoid.

*Any unused portion of reconstituted vials or expired prefilled syringes is disposed of in accordance with institutional or pharmacy policy.*

### 8.3.2 Placebo for p24CE1/2 + *IL-12* pDNA (Group 1)

One vial containing Sodium Chloride for Injection, USP 0.9% will be needed to prepare the two syringes. The vial should be visually inspected prior to use. The pharmacist will also inspect the sterile pouches containing the TriGrid™ Application Cartridges for integrity and verify the Cartridges have not passed expiration. If any tears or gaps in the sterile pouch are noted or if the expiration date has been reached, the Application Cartridge should be placed in quarantine and the protocol pharmacist should be informed.

*Note: In the event that the Cartridge Cap has become detached from the Cartridge in the sterile pouch, the Cartridge may still be used if the Cartridge Cap can be replaced without contaminating the patient contact portion of the Cartridge.*

- Using aseptic technique, the pharmacist, using two new 3 mL syringes (BD# 309585, 309657, or Ichor approved) each fitted with a 22 Ga x 1.5" needle (BD# 305156 or Ichor approved), will withdraw 1 mL of the Sodium Chloride for Injection, USP 0.9% into each syringe and insert the syringe with an identical new needle attached into the Application Cartridge as directed in the *HVTN 119 Study Specific Procedures*.

*Note: Use the syringe tabs to insert the syringe into the Application Cartridge. Do not depress the syringe plunger during insertion of the syringe into the Application Cartridge as this will cause the agent to be injected into the Cartridge.*

*Caution: During the syringe loading procedure, do not physically touch the syringe needle or cause it to come in contact with a non-sterile surface. Note that, although the inside of the Application Cartridge is sterile, contact between the syringe needle and the inner housing of the Application Cartridge should be avoided to the extent possible. Contact between the syringe needle and Application Cartridge could result in deformation of the syringe needle and increased discomfort for the subject upon insertion. If contamination and/or deformation of the injection needle is suspected, replace the injection needle with a new BD 22 Ga x 1.5" injection needle (BD model 305156 or Ichor approved). To prevent loss of dosage make sure to withdraw agent from needle before replacing.*

- Each Application Cartridge will have the Participant's ID number written on it using a Sharpie or other similar pen. The cartridge will then be placed back in the pouch that it was removed from and the pouch will be labeled as "HVTN 119 study product or placebo 1mL."

- The pouch containing the syringe must be labeled with a four-hour expiration date from the time the vial is removed from the refrigerator. The label must also contain the words "Administer as soon as possible."

- One of the syringes must also be labeled for administration in RIGHT deltoid and the second syringe must be labeled for administration in the LEFT deltoid.

*Any unused portion of reconstituted vials or expired prefilled syringes is disposed of in accordance with institutional or pharmacy policy.*

### 8.3.3 p24CE1/2 + p55$^{gag}$ + *IL-12* pDNA (Group 1)

One vial of p24CE1/2 pDNA (4 mg/mL), 1 vial of p55$^{gag}$ pDNA (4 mg/mL), 2 vials of *IL-12* pDNA (2 mg/mL) and one empty 5 mL sterile vial (for mixing) will be needed to prepare the dose. Prior to dispensing, the pharmacist will remove one vial of p24CE1/2 pDNA and one vial of p55$^{gag}$ pDNA from the freezer and allow to thaw at room temperature for 15 to 30 minutes until no crystals are observed. The pharmacist will remove 2 vials of *IL-12* pDNA from the refrigerator. Gently swirl and invert the vials containing vaccine for at least 10 inversions (do not shake vigorously).

The vials should be visually inspected prior to use. If the vial contains material different from its description above, do not use the vial and contact the protocol pharmacist. Prior to mixing the study products, the pharmacist will also inspect the sterile pouches containing the TriGrid™ Application Cartridges for integrity and verify the Cartridges have not passed expiration. If any tears or gaps in the sterile pouch are noted or if the expiration date has been reached, the Application Cartridge should be placed in quarantine and the protocol pharmacist should be informed. *Note: In the event that the Cartridge Cap has become detached from the Cartridge in the sterile pouch, the Cartridge may still be used if the Cartridge Cap can be replaced without contaminating the patient contact portion of the Cartridge.*

- Using aseptic technique, the pharmacist will withdraw 0.6 ml from the vial containing p24CE1/2 pDNA (4 mg/mL) with a 1 mL low void 25 Ga syringe (low dead space syringe; see *HVTN 119 Study Specific Procedures*) and inject this into the mixing vial. This mixing vial, now containing 0.6 mL of p24CE1/2 pDNA, will be set aside.

- Using aseptic technique, the pharmacist will withdraw 0.6 ml from the vial containing $p55^{gag}$ pDNA (4 mg/mL) with a 1 mL low void 25 Ga syringe (low dead space syringe; see *HVTN 119 Study Specific Procedures*) and inject this into the mixing vial that contains p24CE1/2 pDNA. This mixing vial, now containing 1.2 mL of p24CE1/2 pDNA + $p55^{gag}$ pDNA will be set aside.

- Using aseptic technique, the pharmacist will then withdraw 0.6 mL from each of the 2 vials containing *IL-12* pDNA (2 mg /mL) with a 1 mL syringe and inject them directly into the mixing vial containing 1.2 mL of p24CE1/2 pDNA+ $p55^{gag}$pDNA. The pharmacist will mix the vial containing 2.4 mL of the mixture of p24CE1/2 pDNA + $p55^{gag}$ pDNA +GENEVAX® IL-12 DNA plasmid. Gently swirl and invert the mixing vial containing the vaccine mixture for at least 10 inversions (do not shake vigorously).

- Using two new 3 mL syringes (BD# 309585, 309657, or Ichor approved) each fitted with a 22 Ga x 1.5" needle (BD# 305156 or Ichor approved), the pharmacist will withdraw 1 mL of the mixed preparation from the mixing vial into each syringe, and insert each syringe with an identical new needle attached into an Application Cartridge as directed in the *HVTN 119 Study Specific Procedures*.

*Note: Use the syringe tabs to insert the syringe into the Application Cartridge. Do not depress the syringe plunger during insertion of the syringe into the Application Cartridge as this will cause the agent to be injected into the Cartridge.*

*Caution: During the syringe loading procedure, do not physically touch the syringe needle or cause it to come in contact with a non-sterile surface. Note that, although the inside of the Application Cartridge is sterile, contact between the syringe needle and the inner housing of the Application Cartridge should be avoided to the extent possible. Contact between the syringe needle and Application Cartridge could result in deformation of the syringe needle and increased discomfort for the subject upon insertion. If contamination and/or deformation of the injection needle is suspected, replace the injection needle with a new BD 22 Ga x 1.5" injection needle (BD model 305156 or Ichor approved). To prevent loss of dosage make sure to withdraw agent from needle before replacing.*

- Each Application Cartridge will have the Participant's ID number written on it using a Sharpie or other similar pen. The cartridge will then be placed back in the pouch that it was removed from and the pouch will be labeled as "HVTN 119 study product or placebo 1mL."

- The pouch containing the syringe must be labeled with a four-hour expiration date from the time the vial is removed from the refrigerator. The label must also contain the words "Administer as soon as possible."

- One of the syringes must also be labeled for administration in RIGHT deltoid and the second syringe must be labeled for administration in the LEFT deltoid.

*Any unused portion of reconstituted vials or expired prefilled syringes is disposed of in accordance with institutional or pharmacy policy.*

### 8.3.4 Placebo for p24CE1/2+ p55$^{gag}$ + *IL-12* pDNA (Group 1)

One vial containing Sodium Chloride for Injection, USP 0.9% will be needed to prepare the two syringes. The vial should be visually inspected prior to use. The pharmacist will also inspect the sterile pouch containing the TriGrid™ Application Cartridge for integrity and verify the Cartridge has not passed expiration. If any tears or gaps in the sterile pouch are noted or if the expiration date has been reached, the Application Cartridge should be placed in quarantine and the protocol pharmacist should be informed. *Note: In the event that the Cartridge Cap has become detached from the Cartridge in the sterile pouch, the Cartridge may still be used if the Cartridge Cap can be replaced without contaminating the patient contact portion of the Cartridge.*

- Using aseptic technique, the pharmacist, using two new 3 mL syringes (BD# 309585, 309657, or Ichor approved) each fitted with a 22 Ga x 1.5" needle (BD# 305156 or Ichor approved), will withdraw 1 mL of the Sodium Chloride for Injection, USP 0.9% into each syringe and insert the syringe with an identical new needle attached into the Application Cartridge as directed in the *HVTN 119 Study Specific Procedures*.

*Note: Use the syringe tabs to insert the syringe into the Application Cartridge. Do not depress the syringe plunger during insertion of the syringe into the Application Cartridge as this will cause the agent to be injected into the Cartridge.*

*Caution: During the syringe loading procedure, do not physically touch the syringe needle or cause it to come in contact with a non-sterile surface. Note that, although the inside of the Application Cartridge is sterile, contact between the syringe needle and the inner housing of the Application Cartridge should be avoided to the extent possible. Contact between the syringe needle and Application Cartridge could result in deformation of the syringe needle and increased discomfort for the subject upon insertion. If contamination and/or deformation of the injection needle is suspected, replace the injection needle with a new BD 22 Ga x 1.5" injection needle (BD model 305156 or Ichor approved). To prevent loss of dosage make sure to withdraw agent from needle before replacing.*

- Each Application Cartridge will have the Participant's ID number written on it using a Sharpie or other similar pen. The cartridge will then be placed back in the

pouch that it was removed from and the pouch will be labeled as "HVTN 119 study product or placebo 1 mL."

- The pouch containing the syringe must be labeled with a 4-hour expiration date from the time the vial is removed from the refrigerator/freezer. The label must also contain the words "Administer as soon as possible."

- One of the syringes must also be labeled for administration in RIGHT deltoid and the second syringe must be labeled for administration in the LEFT deltoid.

*Any unused portion of reconstituted vials or expired prefilled syringes is disposed of in accordance with institutional or pharmacy policy.*

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

### 8.3.5 p55$^{gag}$ + *IL-12* pDNA (Group 2)

Two vials of p55$^{gag}$ pDNA (4 mg/mL), 2 vials of *IL-12* pDNA (2 mg /mL), and 1 empty sterile vial (5 mL) will be needed to prepare the 2 syringes. Prior to dispensing, the pharmacist will remove 2 vials of p55$^{gag}$ pDNA from the freezer and allow thaw at room temperature for 15-30 minutes until no crystals are observed. The pharmacist will remove 2 vials of *IL-12* pDNA from the refrigerator. Gently swirl and invert the vials for at least 10 inversions (do not shake vigorously).

The vials should be visually inspected prior to use. If the vial contains material different from its description above, do not use the vial and contact the protocol pharmacist. Prior to mixing the study products, the pharmacist will also inspect the sterile pouches containing the TriGrid™ Application Cartridges for integrity and verify the Cartridges have not passed expiration. If any tears or gaps in the sterile pouch are noted or if the expiration date has been reached, the Application Cartridge should be placed in quarantine and the protocol pharmacist should be informed. *Note: In the event that the Cartridge Cap has become detached from the Cartridge in the sterile pouch, the Cartridge may still be used if the Cartridge Cap can be replaced without contaminating the patient contact portion of the Cartridge.*

- Using aseptic technique, the pharmacist will withdraw 0.6 mL from each of the 2 vials of p55$^{gag}$ pDNA (4 mg/mL) with a 1 mL low void 25 Ga syringe (low dead space syringe; see *HVTN 119 Study Specific Procedures*) and inject this into the mixing vial. This mixing vial, now containing 1.2 mL of p55$^{gag}$pDNA, will be set aside.

- Using aseptic technique, the pharmacist will then withdraw 0.6 mL from each of the 2 vials containing *IL-12* pDNA (2 mg /mL) with a 1 mL syringe and inject them directly into the mixing vial containing 1.2 mL of p55$^{gag}$ pDNA. Now the mixing vial contains 2.4 mL of the mixture of p55$^{gag}$ pDNA + GENEVAX® IL-12 DNA plasmid. The pharmacist will gently swirl and invert the mixing vial containing the vaccine mixture for at least 10 inversions (do not shake vigorously).

- Using two new 3 mL syringes (BD# 309585, 309657, or Ichor approved) each fitted with a 22 Ga x 1.5" needle (BD# 305156, or Ichor approved), the

pharmacist will withdraw 1 mL of the mixed preparation from the mixing vial into each syringe, and insert each syringe with an identical new needle attached into an Application Cartridge as directed in the *HVTN 119 Study Specific Procedures*.

*Note: Use the syringe tabs to insert the syringe into the Application Cartridge. Do not depress the syringe plunger during insertion of the syringe into the Application Cartridge as this will cause the agent to be injected into the Cartridge.*

*Caution: During the syringe loading procedure, do not physically touch the syringe needle or cause it to come in contact with a non-sterile surface. Note that, although the inside of the Application Cartridge is sterile, contact between the syringe needle and the inner housing of the Application Cartridge should be avoided to the extent possible. Contact between the syringe needle and Application Cartridge could result in deformation of the syringe needle and increased discomfort for the subject upon insertion. If contamination and/or deformation of the injection needle is suspected, replace the injection needle with a new BD 22 Ga x 1.5" injection needle (BD model 305156 or Ichor approved). To prevent loss of dosage make sure to withdraw agent from needle before replacing.*

- Each Application Cartridge will have the Participant's ID number written on it using a Sharpie or other similar pen. The cartridge will then be placed back in the pouch that it was removed from and the pouch will be labeled as "HVTN 119 or placebo 1 mL."

- The pouch containing the syringe must be labeled with a 4-hour expiration date from the time the vial is removed from the refrigerator/freezer. The label must also contain the words "Administer as soon as possible."

- One of the syringes must also be labeled for administration in RIGHT deltoid and the second syringe must be labeled for administration in the LEFT deltoid.

*Any unused portion of reconstituted vials or expired prefilled syringes is disposed of in accordance with institutional or pharmacy policy.*

## 8.4 Administration

Any administrator of study product will be blinded to the individual participant's treatment assignment. At sites where registered pharmacists are legally authorized to administer drug, the HVTN CRS may choose to have a blinded HVTN CRS pharmacist administer the vaccinations.

Administration of vaccine or placebo for all groups consists of 2 injections, 1 each in left and right medial deltoids using the Ichor Medical Systems Intramuscular TriGrid Delivery System (TDS-IM) EP device at Months 0, 1, 3 and 6.

The TDS-IM device will be used as directed by Ichor Medical Systems, Inc. (Please refer to the TDS-IM User Manual for further instruction). The TDS-IM has three components: The TDS-IM Pulse Stimulator, the Integrated Applicator (reusable) and the Application Cartridge (single-use). The Application Cartridge is the only patient contact component of the system. Only use Becton Dickinson 3.0 mL sterile syringe (BD# 309585, 309657,

or Ichor approved) and Becton Dickinson 22 Ga x 1.5" injection needle (BD# 305156 or Ichor approved) with the Application Cartridge.

*The used TDS-IM Application Cartridge should be disposed of in accordance with institutional policy in the clinic. It should NOT be returned to pharmacy.*

To accommodate differences in skin thickness, a slidable depth control gauge allows adjustment of injection depth to one of three settings: 12, 17, or 22 millimeters (mm) from the skin surface. The corresponding depth of electrode insertion for these settings is 15, 20, and 25 mm respectively. Prior to applying the EP procedure, the participant's skin to muscle thickness must be assessed for each arm separately. This may be assessed once, at any time prior to the first injection with EP for a participant, and is not required prior to each procedure. However the skin fold thickness should be reassessed for a participant whose body weight has changed by 10% since the previous skin fold thickness assessment. The skin fold thickness for each arm is recorded in the participant study chart. The Application Cartridge penetration depth will be set prior to each procedure according to these measurements.

To assess the thickness of the skin and subcutaneous tissue use your thumb and forefinger to "pinch" the skin and subcutaneous tissue overlying the muscle at the administration site together and measure the fold thickness using skin calipers or other suitable measurement device. Based on this measurement, use the chart below to select the appropriate depth setting on the Application Cartridge for each arm. Record the skin fold thickness for each arm in the participant study chart. The selected depth setting on the Cartridge is indicated by the number of indicator lines visible on Cartridge (see Table 8-1).

**Table 8-1 Selection of device depth setting**

| Range of Measured **skin fold** Thickness [mm] | Device Depth Selection | Number of indicator lines visible |
|---|---|---|
| < 14 mm | A | 3 |
| 14-23 mm | B | 2 |
| 24-40 mm | C | 1 |
| >40 mm | Exclude from protocol | Exclude from protocol |

To ensure intramuscular injection, proper selection of the injection site as well as assessment and setting of the injection depth are important factors in procedure administration. In particular, care should be taken in specific subjects or subject populations with low muscle mass (eg, sarcopenia) to ensure that an administration site with adequate muscle thickness (eg, vastus lateralis) is selected for injection. Specifically, any selected administration site should have sufficient muscle mass to accommodate the 1 inch / 25 mm maximum penetration depth of the device.

## 8.5 Acquisition of study products

p24CE1/2 pDNA and p55$^{gag}$ pDNA will be provided by DAIDS, NIAID, NIH, DHHS.

GENEVAX® IL-12 DNA plasmid will be provided by Profectus BioSciences, Inc.

The TriGrid™ Delivery System and TriGrid™ Application Cartridge (electroporation array), will be provided by Ichor Medical Systems.

The placebo (Sodium Chloride for Injection, USP 0.9%) will not be provided through the protocol but must be purchased by the site.

The syringes and needles used for the preparation and delivery of the study products, including the 3 mL syringes (BD #309585, 309657, or Ichor approved) and Becton Dickinson 22 Ga x1½" needles (BD #305156 or Ichor approved) will not be provided through the protocol and must be purchased by the site.

Once an HVTN CRS is protocol registered, the pharmacist can obtain study products from the NIAID Clinical Research Products Management Center (CRPMC) by following the ordering procedures given in Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks.

## 8.6 Pharmacy records

The HVTN CRS pharmacist is required to maintain complete records of all study products. The pharmacist of record is responsible for maintaining randomization codes and randomization confirmation notices for each participant in a secure manner.

## 8.7 Final disposition of study products

All unused study products must be returned to the CRPMC after the study is completed or terminated unless otherwise instructed by the CRPMC. The procedures and relevant form are included in the Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks.

# 9 Clinical procedures

The schedule of clinical procedures is shown in Appendix F.

## 9.1 Informed consent

Informed consent is the process of working with participants so that they fully understand what will and may happen to them while participating in a research study. The HVTN informed consent form documents that a participant (1) has been informed about the potential risks, benefits, and alternatives to participation, and (2) is willing to participate in an HVTN study. Informed consent encompasses all written or verbal study information HVTN CRS staff provide to the participant, before and during the trial. HVTN CRS staff will obtain informed consent of participants according to HVTN policies and procedures.

The informed consent process continues throughout the study. Key study concepts should be reviewed periodically with the participant and the review should be documented. At each study visit, HVTN CRS staff should consider reviewing the procedures and requirements for that visit and for the remaining visits. Additionally, if any new information is learned that might affect the participants' decisions to stay in the trial, this information will be shared with trial participants. If necessary, participants will be asked to sign revised informed consent forms.

An HVTN CRS may employ recruitment efforts prior to the participant consenting. For example, some HVTN CRSs use a telephone script to prescreen people before they come to the clinic for a full screening visit. Participants must sign a screening or protocol-specific consent before any procedures are performed to determine eligibility. HVTN CRSs must submit recruitment and prescreening materials to their IRB/EC and any applicable RE for human subjects protection review and approval.

Note: As defined in the DAIDS Protocol Registration Manual, an RE is "Any group other than the local IRB/EC responsible for reviewing and/or approving a clinical research protocol and site-specific ICFs [informed consent forms] prior to implementation at a site." CRSs are responsible for knowing the requirements of their applicable REs.

### 9.1.1 Screening consent form

Without a general screening consent, screening for a specific study cannot take place until the site receives protocol registration from the DAIDS RSC Protocol Registration Office.

Some HVTN CRSs have approval from their IRB/EC and any applicable RE to use a general screening consent form that allows screening for an unspecified HIV vaccine trial. In this way, HVTN CRS staff can continually screen potential participants and, when needed, proceed quickly to obtain protocol-specific enrollment consent. Sites conducting general screening or prescreening approved by their IRB/EC and any applicable RE may use the results from this screening to determine eligibility for this protocol, provided the tests are conducted within the time periods specified in the eligibility criteria.

### 9.1.2 Protocol-specific consent forms

The protocol-specific consent forms describe the study products to be used and all aspects of protocol participation, including screening and enrollment procedures. A sample protocol-specific consent form for the main study is located in Appendix A. A separate sample consent form for other uses of specimens is located in Appendix C.

Each HVTN CRS is responsible for developing a protocol-specific consent form(s) for local use, based on the sample protocol-specific consent forms in Appendix A and Appendix C. The consent form(s) must be developed in accordance with requirements of the following:

- CRS's IRB/EC and any applicable REs,
- CRS's institution, and
- Elements of informed consent as described in Title 45, CFR Part 46 and Title 21 CFR, Part 50, and in the International Conference on Harmonisation (ICH) E6, Good Clinical Practice: Consolidated Guidance 4.8.

Study sites are strongly encouraged to have their local CABs review their sites-specific consent forms. This review should include, but should not be limited to, issues of cultural competence, local language considerations, and the level of understandability.

The sample informed consent form includes instructions throughout the document for developing specific content.

Sites should follow the instructions in the Protocol-specific Official Memo distributed along with this protocol regarding when they may begin using their site-specific protocol consent forms.

Regarding protocol registration, sites should follow procedures outlined in the current version of the DAIDS Protocol Registration Manual.

### 9.1.3 Assessment of Understanding

Study staff are responsible for ensuring that participants fully understand the study before enrolling them. This process involves reviewing the informed consent form with the participant, allowing time for the participant to reflect on the procedures and issues presented, and answering all questions completely.

An Assessment of Understanding is used to document the participant's understanding of key concepts in this HIV vaccine trial. The participant must complete the Assessment of Understanding before enrollment. Staff may provide assistance in reading and understanding the questions and responses, if necessary. Participants must verbalize understanding of all questions answered incorrectly. This process and the participant's understanding of the key concepts should be recorded in source documentation at the site.

IRB/EC and any applicable RE may require that a participant has signed either a screening or protocol-specific consent document prior to administering the Assessment of Understanding. The consent process (including the use of the Assessment of

Understanding) should be explained thoroughly to the IRB/EC and any applicable RE, whose recommendations should be followed.

## 9.2 Pre-enrollment procedures

Screening may occur over the course of several contacts/visits, up to and including before vaccination on day 0. All inclusion and exclusion criteria must be assessed within 56 days before enrollment, unless otherwise specified in the eligibility criteria (or below in this section).

After the appropriate informed consent has been obtained and before enrollment, the following procedures are performed:

- Medical history, documented in the case history record;
- Assessment of whether the volunteer is at low risk for HIV infection;
- Complete physical examination, including height, weight, vital signs, and clinical assessments of head, ears, eyes, nose, and throat; neck; lymph nodes; heart; chest; abdomen; extremities; neurological function; and skin;
- Deltoid skin fold measurement (Section 8.4)
- Assessment of concomitant medications the volunteer is taking, including prescription and nonprescription drugs, vitamins, topical products, alternative/complementary medicines (eg, herbal and health food supplements), recreational drugs, vaccinations, and allergy shots;
- Laboratory tests as defined in the inclusion and exclusion criteria, including:
  - Screening HIV test,
  - HBsAg,
  - Anti-HCV antibodies,
  - Syphilis test,
  - CBC with differential and platelets,
  - Chemistry panel (ALT, AST, alkaline phosphatase, creatinine, and CPK),
  - Urine dipstick (as described in Section 9.8). and
  - Urine or serum pregnancy test (participants who were born female)
- Administration of behavioral risk assessment questionnaire;
- Obtaining of volunteer demographics in compliance with the NIH Policy on Reporting Race and Ethnicity Data: Subjects in Clinical Research, Aug. 8, 2001 (available at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-01-053.htmL);

- Counseling on HIV testing and risk reduction, performed in compliance with the US Centers for Disease Control and Prevention (CDC)'s current guidelines or other local guidelines for HIV counseling, testing, and referral as described in Section 9.6; and

- Discussion of pregnancy prevention. A pregnant or breastfeeding person may not be enrolled in this trial. Specific criteria and assessment of contraception and pregnancy status are described in study inclusion criteria. Discussion of pregnancy prevention includes advising a participant who was born female and who reports no current sexual activity that could lead to that participant becoming pregnant to have a plan to begin adequate birth control. This plan would be put to use if, during the study, the participant becomes sexually active in a way that could lead to that participant becoming pregnant.

### 9.2.1 Use of screening results from another HVTN study

If a participant screens for an HVTN study at the same HVTN CRS but then does not join that study, screening results from that effort may be applied to the screening for this protocol, as long as the screening was done under participant consent, the participant has signed a consent form to begin screening for this study, and the tests were conducted within the time periods specified in the eligibility criteria (see Sections 7.1 and 7.2).

## 9.3 Enrollment and vaccination visits

Enrollment is simultaneous with first vaccination. The time interval between randomization and enrollment should not exceed 4 working days. The HVTN CRS registers the participant by scheduling the day 0 visit (enrollment) via the Web-based randomization system, and requests the randomization assignment. Circumstances may require a participant's enrollment visit to be changed. This may exceed the 4-day randomization time limit.

At all vaccination visits, the following procedures are performed before vaccination:

- Abbreviated physical examination, including weight, vital signs, and a symptom-directed evaluation by history and/or appropriate physical exam based on participant self-reported symptoms or complaints;

- Assessment of baseline reactogenicity parameters;

- Assessment of concomitant medications (as described in Section 9.2);

- Assessment of any new or unresolved AEs/intercurrent illnesses; and

- Urine or serum pregnancy test (for participants who were born female). Persons who are NOT of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing.

Following completion of all procedures in the preceding list, and if results indicate that vaccination may proceed, vaccination is prepared and administered (see Sections 8.3 and 8.4).

Administration of all injections during a vaccination visit must be accomplished within 1 calendar day.

Immediately following vaccination, the participant remains in the clinic for observation. Participant pain will be assessed using a Visual Analog Scale (VAS) immediately following EP, then again 5-7 minutes and 25-60 minutes following vaccination (as described in Section 9.9.3). An initial reactogenicity assessment is made at a target of 30 minutes after injection, with an acceptable range of 25-60 minutes. Before leaving the clinic, the participant is given the postvaccination memory tool and is instructed on how to complete it. The site will make arrangements to be in contact with the participant during the reactogenicity period (as described in Section 9.9).

The following procedures will be performed at all vaccination visits. These procedures may be performed prior to or following vaccination:

- Risk reduction counseling (as described in Section 9.6);
- Pregnancy prevention assessment (as described in Section 9.2 and 9.7); and
- Assessment of new or unresolved social impacts (site staff will ask participant about the status of any unresolved social impacts and if s/he has experienced any new social impacts as a result of the trial participation).

Additional procedures will be performed at scheduled visits as specified in Appendix E and Appendix F:

- Administration of the social impact assessment questionnaire (types of impacts assessed involve personal relationships, health insurance, life insurance, educational or employment opportunities, housing, immigration, or travel);
- Administration of a questionnaire that asks the participant about any HIV testing he or she may have received outside of the study. Participants will also be asked whether they believe they received the active vaccine or the control;
- HIV infection assessment including pre-test counseling. A subsequent follow-up contact is conducted to provide post-test counseling and to report results to participant;
- Confirm that participants received HIV test results from previous visit. If not, provide test results and post-test counseling as appropriate; and
- Specimen collection (should be completed prior to vaccination).

### 9.3.1 EP device applied without vaccination

All vaccinations will be given with the Ichor TDS-IM EP device. If the device was applied, but neither electroporation nor study product (vaccine or placebo) was received, and the participant is willing, additional attempts may be made within the vaccination window. For the Month 0 visit, any additional attempts are expected to take place within 4 days of randomization, unless a longer window is approved by the PSRT. For subsequent timepoints, if a participant does not receive study agent (vaccine or placebo,

with or without EP) in at least one arm within the vaccination window, that participant will have missed that vaccination.

At the Month 0 visit, if the participant had the Ichor TDS-IM EP device applied to the participant's arm but the participant was not able to receive any injection of study product at the enrollment visit, the participant is not considered enrolled into the study, and is referred to as a "device-only participant". Refer to the *HVTN 119 Study Specific Procedures* for additional instructions and for the CRFs to be completed. Receipt of study product (ie, any volume of vaccine or placebo, with or without EP) in at least one arm constitutes enrollment.

The CRS staff should contact each device-only participant approximately 14 days after initial application of the device in order to assess any new or unresolved AEs that may have occurred in the interim. This contact does not require a clinic visit, unless medically indicated. As the device-only participant was not enrolled in the trial, no further visits or study procedures are required, except for AE reporting of events associated with the application of the EP device, which should be reported to the SDMC on the appropriate CRF. In addition, AEs requiring expedited reporting should be reported to the DAIDS RSC Safety Office as described in Section 11.2.3.

#### 9.3.2 When a vaccination attempt results in vaccination without EP

If a study product injection (ie, any volume of vaccine or placebo) is given without EP due to human error or device malfunction, this event will be recorded/documented for that arm, and vaccinations should continue as scheduled.

## 9.4 Follow-up visits

The following procedures are performed at all scheduled follow-up visits:

- Risk reduction counseling (as described in Section 9.6);
- Pregnancy prevention assessment (as described in Section 9.2 and 9.7); and
- Assessment of new or unresolved social impacts (site staff will ask participant about the status of any unresolved social impacts and if s/he has experienced any new social impacts as a result of the trial participation);
- Assessment of new or continuing concomitant medications (as described in Section 9.2); and
- Assessment of new or unresolved AEs/intercurrent illnesses.

Additional procedures will be performed at scheduled follow-up visits as specified in Appendix E and Appendix F:

- Administration of an EP acceptability questionnaire;
- Administration of behavioral risk assessment questionnaire;

- Administration of the social impact assessment questionnaire (types of impacts assessed involve personal relationships, health insurance, life insurance, educational or employment opportunities, housing, immigration, or travel);

- Administration of a questionnaire that asks the participant about any HIV testing he or she may have received outside of the study. Participants will also be asked whether they believe they received the active vaccine or the control;

- HIV infection assessment including pre-test counseling. A subsequent follow-up contact is conducted to provide post-test counseling and to report results to participant;

- Confirm that participants received HIV test results from previous visit. If not, provide test results and post-test counseling as appropriate;

- Abbreviated physical examination including weight, vital signs, and a symptom-directed evaluation by history and/or appropriate physical exam based on participant self-reported symptoms or complaints;

- Complete physical examination, including weight, vital signs, and clinical assessments of head, ears, eyes, nose, and throat; neck; lymph nodes; heart; chest; abdomen; extremities; neurological function; and skin;

- Specimen collection;

- Clinical laboratory tests including:
  - CBC with differential,
  - Chemistry panel (see Section 9.2), and
  - Urine dipstick (urinalysis if appropriate; see Section 9.8); and

- Urine or serum pregnancy test (for participants who were born female). Persons who are NOT of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing.

## 9.5 Month 18 health contact

CRS staff will contact study participants at their Month 18 timepoint to collect the information listed below. Clinic visits will only be required if HIV confirmatory testing is necessary (see Section 9.6.1); however, a clinic visit may be arranged for other reasons.

- Confirmation of vital status; if deceased, attempt to learn cause and date of death

- If participant is alive, record any of the following events:
  - New AEs related to study product(s), including:
    - Life threatening adverse experiences;

- Persistent or significant disability/incapacity;
- Hospitalizations and reasons;
- Other important medical events that may jeopardize the participant or may require intervention to prevent 1 of the other outcomes listed above;
- New chronic conditions requiring more than 30 days of medical intervention or medication;
- Other related AEs.

- AESI (Section 11.2.2). A sample list of AESI is provided in Appendix G. AESI are reported regardless of relationship to study product(s);
- New diagnosis of HIV infection; and
- Pregnancies and outcomes, including congenital anomalies/birth defects.

All such events will be recorded and adverse events will be assessed for relationship to study products. A safety monitoring team reviews reports from these contacts quarterly. This monitoring team comprises a DAIDS Medical Officer, Core medical monitor, and a clinical safety specialist (CSS). Other questions may be added by the HVTN 119 Protocol Team for exploratory endpoints.

### 9.5.1 Interim contacts

CRSs may report safety information obtained at a contact other than the annual contact. These contacts are reported as interim visits.

## 9.6 HIV counseling and testing

HIV counseling will be performed in compliance with the CDC's guidelines or other local guidelines for HIV counseling and referral. HIV testing will be performed in accordance with the current HVTN HIV testing algorithm following enrollment.

Participants will be counseled routinely during the trial on the avoidance of HIV infection and on the potential negative social impacts of testing antibody positive due to the vaccine. They will also be counseled on the risks of HIV antibody testing outside of the HVTN CRSs and will be discouraged from doing so during study participation and/or during any period of vaccine-induced positive serology.

Study staff will take particular care to inform study participants of the likelihood of routine HIV testing being offered or performed outside the study CRS at emergency rooms, clinics, and medical offices. Such testing has become more likely due to the CDC's revised guidelines for HIV counseling and testing, as well as policy changes in many countries to make HIV testing more frequent and routine. CRS staff should inform participants of their right to opt out of HIV testing outside the study site. CRS staff should inform study participants if local and/or state policies and regulations permit medical providers to perform HIV testing without first informing patients. If this is the case, then CRS staff should advise study participants that they may decline testing preemptively. CRS staff should also inform participants if positive results must be

reported to local public health authorities. CRS staff should also inform participants of the need to maintain study blinding by getting HIV testing only at the study CRS. CRS staff should provide participants with CRS contact information and should encourage participants to ask medical providers to contact the CRS. The CRS can verify that the participant is in an HIV vaccine clinical trial and should only be tested at the study CRS.

Potential participants identified as being HIV infected during screening are not enrolled. All participants who become HIV infected during the study will be terminated from this study. Potential and enrolled participants identified as HIV infected will be referred for medical treatment, counseling, and management of the HIV infection. These individuals may also be referred to appropriate ongoing clinical trials or observational studies.

### 9.6.1 Distinguishing intercurrent HIV infection from vaccine-induced positive serology

The study product may elicit an antibody response to HIV proteins. Therefore, vaccine-induced positive serology may occur in this study. Several precautionary measures will be taken to distinguish intercurrent HIV infection from vaccine-induced positive serology. These precautionary measures include:

- Participants will have physical examinations at visits specified in Appendix F. Signs or symptoms of an acute HIV infection syndrome, an intercurrent illness consistent with HIV-1 infection, or probable HIV exposure would prompt a diagnostic workup per the HVTN algorithm for Recent Exposure/Acute Infection Testing to determine HIV infection.

- HIV testing will be performed at multiple timepoints throughout the study (see Appendix F). The Laboratory Program (or approved diagnostic laboratory) will follow the HVTN HIV testing algorithm (as described in the HVTN Laboratory Manual of Operations), which is able to distinguish vaccine-induced antibody responses from actual HIV infections.

- All participants can receive HIV-1 diagnostic testing from the site following their last scheduled visit until they are told that they did not receive an HIV vaccine or that they do not have vaccine-induced seropositivity.

- All participants who received vaccine product and who have vaccine-induced positive or indeterminate HIV-1 serology (as measured by the standard anti-HIV antibody screening tests) at or after the study is unblinded will be offered poststudy HIV-1 diagnostic testing (per the HVTN poststudy HIV-1 testing algorithm) periodically and free of charge as medically/socially indicated (approximately every 6 months) unless or until HIV Ab testing is no longer the standard test in clinical settings.

### 9.6.2 VISP registry

Experimental HIV vaccines may induce antibody production to HIV antigens, producing reactive results on commercially available HIV test kits. This is called “vaccine-induced seropositivity” (VISP) (see Section 9.6.1). In order to provide post-study HIV testing to distinguish between VISP and HIV infection, and to mitigate potential social harms resulting from VISP in HIV vaccine recipients who are not infected with HIV, the HVTN has created a VISP registry. Following study unblinding, the registry will allow trained staff to verify that an individual has received an HIV vaccine, and therefore has the

potential for VISP. Information in the VISP registry will not be used for research. Rather, the registry exists to support provision of post-study testing and counseling services to HIV vaccine recipients. The registry contains the names of all study participants, unless they request that their names be removed.

## 9.7 Contraception status

Contraception status is assessed and documented at every scheduled clinic visit for a participant who was born female and who is sexually active in a way that could cause that participant to become pregnant. Prior to enrollment and throughout the study, staff will ask participants to verbally confirm their use of adequate contraceptive methods. A participant who was born female and is sexually active in a way that could cause that participant to become pregnant should be reminded at all scheduled clinic visits of the importance of using contraception and should be referred to specific counseling, information, and advice as needed. (Specific contraception requirements are listed in Section 7.1). This reminder should be documented in the participant's study record.

Self-reported infertility—including having reached menopause (no menses for 1 year) or having undergone hysterectomy, bilateral oophorectomy, or tubal ligation—must be documented in the participant's study record.

## 9.8 Urinalysis

Dipstick testing may be performed in the clinic or the lab, as long as the required elements (glucose, protein, and hemoglobin) are tested. The examination is performed on urine obtained by clean catch.

If the screening dipstick is transiently abnormal due to menses or infection, document this issue in the participant's source documentation. For infection, provide appropriate treatment and/or referral. Following resolution, repeat the dipstick and, if within the eligibility limits specified in the protocol, the participant may be enrolled.

Follow-up urinalysis should be deferred if a participant is menstruating, but should be performed as soon as possible. If a follow-up dipstick is abnormal due to a participant's menstrual period, document in the comment section of the case report form (CRF) and repeat the dipstick once the participant is no longer menstruating. A micro-urinalysis is not required.

## 9.9 Assessments of reactogenicity

For all participants, baseline assessments are performed before and reactogenicity assessments are performed after each vaccination. All reactogenicity symptoms are followed until resolution and graded according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0, November 2014, except as noted in Section 11.2.2.

The reactogenicity assessment period is 7 full days following each vaccination per the assessment schedule shown in Table 9-1. Participants are instructed to record symptoms

using a postvaccination memory tool. Contact between the participant and the site staff should take place at least once between 1-3 days postvaccination, and for events that arise during the period between vaccination and the next scheduled visit. In general, a participant who self-reports any postvaccination reaction greater than mild is seen by a clinician within 48 hours after onset, unless the reaction is improving and/or has completely resolved. Clinic staff will follow new or unresolved reactogenicity symptoms present at day 7 to resolution.

Reactogenicity events are reported using CRFs that correspond to the time of assessment in Table 9-1. Reactogenicity assessments include assessments of systemic and local symptoms, vaccine-related lesions, and lymph nodes. Events not listed on a CRF, or with an onset after the reactogenicity assessment period (day of vaccination and 7 full days after), or those meeting SAE/adverse events requiring expedited reporting to DAIDS criteria, are recorded on an adverse experience log form.

**Table 9-1 Schedule of reactogenicity assessments**

| Day | Time | Performed by |
|---|---|---|
| 0[a] | Baseline: before vaccination | HVTN CRS staff |
| | Early: 25-60 minutes after vaccination | HVTN CRS staff |
| | Between early assessment and 11:59pm day 0 | HVTN CRS staff or participant |
| 1-7[b] | Between 12:00am and 11:59pm on the respective day | HVTN CRS staff or participant |

[a] Day of vaccination

[b] New or unresolved reactogenicity symptoms present on day 7 are followed until resolution

### 9.9.1 Assessment of systemic and local symptoms

Systemic symptoms include increased body temperature, malaise and/or fatigue, myalgia, headache, chills, arthralgia, nausea, and vomiting. Local symptoms include pain and/or tenderness proximal to the injection site. The daily maximum severity reached for each symptom during the assessment period is reported.

Body temperature is measured by oral or infrared thermometry and reported in degrees Celsius. If temperature is measured in Fahrenheit, the conversion to Celsius should be documented in the participant's chart note. A measurement is taken once daily during the assessment period and should be repeated if participant is feeling feverish.

### 9.9.2 Assessment of injection site

Typical injection site reactions are erythema/redness and induration/swelling. The maximum horizontal and maximum vertical measurements for all injection site reactions are recorded.

All injection site reactions are monitored until resolution. Areas greater than 25 $cm^2$ are followed daily; otherwise, the frequency of follow-up is based on clinician judgment.

### 9.9.3 Visual Analog Scale (Pain Scale)

Participant pain will be assessed using a VAS immediately following EP, then again 5-7 minutes and 25-60 minutes following vaccination. A VAS is a horizontal line, 10 cm in length, anchored by word descriptors at each end ("no pain" and "worst pain"). The VAS score is determined by measuring in centimeters from the left hand end of the line to the point that the patient marks, 0 cm being no pain and 10 cm being maximum pain.

### 9.9.4 Assessment of lymph nodes

This assessment is required only when reactogenicity assessments are performed by HVTN CRS staff, not by the participant.

Only the proximally draining lymph nodes are assessed (eg, axillary nodes on the same side of the body for injections given in the deltoid). Lymph nodes are first evaluated for enlargement and tenderness. If they are found to be enlarged, measurements are taken to determine the size (widest diameter) of the enlarged node(s).

## 9.10 Visit windows and missed visits

Visit windows are defined in *HVTN 119 Study Specific Procedures*. For a visit not performed within the window period, a Missed Visit form is completed. If the missed visit is one that required safety assessments or local safety labs, HVTN CRS staff should attempt to bring the participant in for an interim visit as soon as possible.

Procedures performed at an interim visit are usually toxicity/safety assessments (including local safety labs) and HIV testing. With the exception of HIV testing, these procedures are performed only if they were required at the missed visit or if clinically indicated. HIV testing may be performed as deemed appropriate by the study staff. Blood samples for immunogenicity assays are not typically collected at interim visits.

If a missed visit required vaccination, please refer to Section 7.3.2 and Section 7.3.3 for resolution.

## 9.11 Early termination visit

In the event of early participant termination, site staff should consider if the following assessments are appropriate: a final physical examination, clinical laboratory tests (including urine dipstick, CBC with differential, and chemistry panel), pregnancy testing, social impact assessment, and HIV test.

## 9.12 Pregnancy

If a participant becomes pregnant during the course of the study, no more injections of study product will be given, but remaining visits and study procedures should be completed unless medically contraindicated or applicable regulations require termination from the study. In case of required termination, enrollment in an observational study should be offered to the participant. If the participant terminates from the study prior to the pregnancy outcome, the site should make every effort to keep in touch with the participant in order to ascertain the pregnancy outcome. Pregnancies and pregnancy outcomes will be reported.

# 10 Laboratory

## 10.1 HVTN CRS laboratory procedures

The HVTN Site Processing Lab Instructions and study-specific procedures (SSP) provide further guidelines for operational issues concerning the clinical and processing laboratories. The manual includes guidelines for general specimen collection, special considerations for phlebotomy, specimen labeling, whole blood processing, HIV screening/diagnostic testing, and general screening and safety testing.

Tube types for blood collection are specified in Appendix E. For tests performed locally, the local lab may assign appropriate tube types.

In specific situations, the blood collection tubes may be redirected to another laboratory or may require study-specific processing techniques. In these cases, laboratory special instructions will be posted on the protocol-specific section of the HVTN website.

## 10.2 Total blood volume

Required blood volumes per visit are shown in Appendix E. Not shown is any additional blood volume that would be required if a safety lab needs to be repeated, or if a serum pregnancy test needs to be performed. The additional blood volume would likely be minimal. The total blood volume drawn for each participant will not exceed 500 mL in any 56-day (8-week) period.

## 10.3 Primary immunogenicity timepoints

The primary immunogenicity timepoints in this study are at visits 6 (day 42) (ie, 2 weeks after the 2nd vaccination visit) and 11 (day 182) (ie, 2 weeks after the 4th vaccination visit). Endpoint assays for humoral and cellular responses are performed on participants at the primary immunogenicity timepoints and may be performed at baseline. Depending on the number of responders observed, assays for humoral and cellular responses may be performed on participants at other timepoints; the schedule is shown in Appendix E.

## 10.4 Endpoint assays: cellular

### 10.4.1 Flow cytometry

Flow cytometry will be used to examine vaccine-specific $CD4^+$ and $CD8^+$ T-cell responses following stimulation of PBMCs with synthetic HIV peptides that span the proteins encoded by the vaccine construct, including pools covering individual CE regions. ICS parameters will include cytokines such as IFN-γ, IL-2, and TNF-α, and may include other cytokines and phenotypic markers to identify T cells of specific functionality (such as Th2 and Tfh). Markers of cytotoxic potential (eg, granzyme B) must also be included. Data will be reported as percentages of $CD4^+$ or $CD8^+$ T cells

responding to a specific peptide pool. Additional cell surface markers, cytokines, or functional markers may also be analyzed.

### 10.4.2 IFN-γ ELISpot (Epitope Mapping)

Ex vivo HIV-specific T-cells will be assessed by IFN-γ ELISpot for epitope mapping. To this end, PBMCs will be stimulated overnight with synthetic peptide pools that span the proteins encoded by the vaccine constructs, including separate pools containing only CE peptides. Samples with positive responses in this first round of testing will then be tested in a second round using individual peptides comprised in the pools to identify responses to individual epitopes. Data will be reported as the number of spot-forming cells (SFC) per $10^6$ cells recognizing a specific peptide or peptide pool.

## 10.5 Endpoint assays: humoral

### 10.5.1 HIV-1 multiplex antibody assay

IgG antibodies to Clade B Gag and p24CE will be assessed on plasma/serum samples from study participants taken at the primary immunogenicity timepoint and baseline. Specimens from other timepoints as well as other HIV antigens may also be assayed based on the results of the initial assay.

## 10.6 Genotyping

Molecular human leukocyte antigen (HLA) typing may be performed on enrolled participants using cryopreserved PBMC collected at baseline, initially on specimens from participants who demonstrate vaccine-induced T-cell responses at post vaccination timepoints. Other participants (including control recipients) may be HLA-typed to support future studies of immunological interest at the discretion of the HVTN Laboratory Program. Other markers, such as genes associated with immune responses or HIV-1disease progression may also be assessed.

## 10.7 Lab assay algorithm

The Lab Assay Algorithm lists assays to characterize cellular, humoral, and innate immune responses as well as host genetics that may be conducted to determine endpoints in HVTN vaccine trials. The type of assay(s) employed will be dependent on the response obtained by the primary immunogenicity assays at relevant time points. Please note that the Lab Assay Algorithm will be updated periodically to include new assays.

## 10.8 Exploratory studies

Samples may be used for other testing and research related to furthering the understanding of HIV immunology or vaccines. In addition, cryopreserved samples may be used to perform additional assays to support standardization and validation of existing or newly developed methods.

### 10.8.1 T-cell repertoire

Specimens collected at primary immunogenicity timepoints may be compared to those collected at baseline in order to examine evolution of vaccine-induced immune responses by HIV-specific CD4+ T helper cells. Studies that can be performed include detailed T cell receptor alpha beta T cell receptor usage, mapping of epitope-specific responses after transfection of TCR alpha and beta chains into reporter cell lines, and transcriptome analysis of epitope-specific T cells.

## 10.9 Other use of stored specimens

The HVTN stores specimens from all study participants indefinitely, unless a participant requests that specimens be destroyed or if required by IRB/EC, or RE.

Other use of specimens is defined as studies not described in the protocol.

This research may relate to HIV, vaccines, the immune system, and other diseases. This could include limited genetic testing and, potentially, genome-wide studies. This research is done only to the extent authorized in each study site's informed consent form, or as otherwise authorized under applicable law. Other testing on specimens will occur only after review and approval by the HVTN, the IRB/EC of the researcher requesting the specimens, and the CRS's IRBs/ECs if required.

The protocol sample informed consent form is written so that the participant either explicitly allows or does not allow their samples to be used in other research when they sign the form. Participants who initially agree to other use of their samples may rescind their approval once they enter the study; such participants will remain in this study and their samples will only be used for the studies described in this protocol. If a participant decides against allowing other research using his or her samples, or at any time rescinds prior approval for such other use, the study site investigator or designee must notify HVTN Regulatory Affairs in writing. In either case, HVTN Regulatory Affairs directs the HVTN Lab Program not to use samples from these participants for such other uses.

CRSs must notify HVTN Regulatory Affairs if institutional or local governmental requirements pose a conflict with or impose restrictions on other use of specimens.

## 10.10 Biohazard containment

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the CDC and the NIH or other applicable agencies.

All dangerous goods materials, including Biological Substances, Category A or Category B, must be transported according to instructions detailed in the International Air Transport Association Dangerous Goods Regulations.

# 11 Safety monitoring and safety review

## 11.1 Safety monitoring and oversight

### 11.1.1 HVTN 119 PSRT

The HVTN 119 PSRT is composed of the following members:

- DAIDS medical officer representative,
- Protocol chair and cochair,
- Protocol Team leader,
- Core medical monitor, and
- Clinical safety specialist.

The clinician members of HVTN 119 PSRT are responsible for decisions related to participant safety.

The Protocol Team clinic coordinator, clinical data manager, vaccine and device developer representatives, clinical trial manager, and others may also be included in HVTN 119 PSRT meetings.

### 11.1.2 HVTN SMB

The SMB is a multidisciplinary group consisting of biostatisticians, clinicians, and experts in HIV vaccine research that, collectively, has experience in the conduct and monitoring of vaccine trials. Members of the SMB are not directly affiliated with the protocols under review.

The SMB reviews safety data, unblinded as to treatment arm, approximately every 4 months. The reviews consist of evaluation of cumulative reactogenicity events, AE, laboratory safety data, and individual reports of adverse events requiring expedited reporting to DAIDS. To increase the sensitivity for detecting potential safety problems, the SMB will review safety data aggregated across multiple protocols that use the same or similar vaccine candidates. The SMB conducts additional special reviews at the request of the HVTN 119 PSRT.

Study sites will receive SMB summary minutes and are responsible for forwarding them to their IRB/EC and any applicable RE.

### 11.1.3 SDMC roles and responsibilities in safety monitoring

The roles and responsibilities of the SDMC in relation to safety monitoring include:

- Maintaining a central database management system for HVTN clinical data;

- Providing reports of clinical data to appropriate groups such as the HVTN 119 PSRT and HVTN SMB (see Section 11.1.2);

#### 11.1.4 HVTN Core roles and responsibilities in safety monitoring

- Daily monitoring of clinical data for events that meet the safety pause and HVTN 119 PSRT AE review criteria (see Section 11.4);
- Notifying HVTN CRSs and other groups when safety pauses or planned holds are instituted and lifted (see Section 11.4);
- Querying HVTN CRSs for additional information regarding reported clinical data; and
- Providing support to the HVTN 119 PSRT.

## 11.2 Safety reporting

#### 11.2.1 Submission of safety forms to SDMC

Sites must submit all safety forms (eg, reactogenicity, adverse experience, urinalysis, local lab results, concomitant medications) before the end of the next business day after receiving the information. The forms should not be held in anticipation of additional information at a later date. If additional information is received at a later date, the forms should be updated and resubmitted before the end of the next business day after receiving the new information.

#### 11.2.2 AE reporting

An AE is any untoward medical occurrence in a clinical investigation participant administered a study product/procedure(s) and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational study product/procedure(s), whether or not related to the investigational study product/procedure(s). All AEs are graded according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0, November 2014, available on the RSC website at http://rsc.tech-res.com/docs/default-source/safety/daids_ae_grading_table_v2_nov2014.pdf, except:

- Weight loss is required to be reported as an AE only if it is considered to be potentially deleterious to the participant's health (see *HVTN 119 Study Specific Procedures*);
- Injection Site Erythema or Redness and Injection Site Induration or Swelling will not consider interference with usual social and functional activities such that:
  - Grade 1 is: 2.5 to < 5 cm in diameter OR 6.25 to < 25 cm2 surface area;
  - Grade 2 is: ≥ 5 to < 10 cm in diameter OR ≥ 25 to < 100 cm2 surface area;
  - Grade 3 is: ≥ 10 cm in diameter OR ≥ 100 cm2 surface area OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage;

- Grade 4 is: Potentially life-threatening consequences (eg, abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue);

- The grading of Insomnia events will consider the criteria within the Insomnia parameter as well as the general AE functional table such that:
  - Grade 1 is: Mild difficulty falling asleep, staying asleep, or waking up early causing no or minimal interference with usual social and functional activities with intervention not indicated;
  - Grade 2 is: Moderate difficulty falling asleep, staying asleep, or waking up early, causing greater than minimal interference with usual social and functional activities with intervention indicated;
  - Grade 3 is: Severe difficulty falling asleep, staying asleep, or waking up early, causing inability to perform usual social & functional activities with intervention or hospitalization indicated.

If a definition of insomnia falls between 2 grades, the final grading will be selected based on the degree of interference with usual social and functional activities caused by the symptoms.

All AEs are reported to the SDMC on the appropriate CRF. Clinic staff should evaluate every AE to determine if (1) the AE meets the requirements for expedited reporting to DAIDS (Section 11.2.3) and (2) if the AE meets the criteria for a safety pause/prompt AE review (Section 11.4) and (3) if the AE is an AESI. A sample list of AESI is provided as Appendix G.

Sites are expected to notify the CSS of any serious safety concern requiring their attention (see Table 11-1). Telephone numbers and email addresses are found on the protocol home page on the HVTN Members' site (https://members.hvtn.org/protocols/hvtn119). Concerns requiring immediate attention should be communicated by calling the clinical safety phone.

In the case of email notification, the CSS will reply during working hours (US Pacific Time) to confirm that the email has been received and reviewed. If email service is not available, the HVTN CRS should notify the CSS of the event by telephone, then submit CRFs.

In addition, site investigators are required to submit AE information in accordance with IRB/EC and any applicable RE requirements.

### 11.2.3 Expedited reporting of adverse events to DAIDS

Requirements, definitions and methods for expedited reporting of AEs are outlined in Version 2.0 (January 2010) of the *Manual for Expedited Reporting of Adverse Events* to DAIDS (DAIDS EAE Manual), which is available on the RSC website at http://rsc.tech-res.com/clinical-research-sites/safety-reporting. The SAE Reporting Category will be used for this study.

The internet-based DAIDS Adverse Experience Reporting System (DAERS) must be used for expedited AE reporting to DAIDS. In the event of system outages or technical difficulties, expedited AE reports may be submitted via the DAIDS EAE Form. For

questions about DAERS, please contact CRMSsupport@niaid.nih.gov or from within the DAERS application itself.

For questions about expedited AE reporting, please contact the RSC (DAIDSRSCSafetyOffice@tech-res.com).

The study products for which expedited reporting are required are:

- p24CE1/2 pDNA or placebo
- p55$^{gag}$ pDNA or placebo
- *IL-12* pDNA or placebo
- Intramuscular TriGrid Delivery System (TDS-IM) electroporation device

While the participant is in the main study reporting period (See Section 3), the SAE Reporting Category will be used.

If the participant has completed the main study (visit 13, Month 12) and is in the "follow-up health contact" reporting period (see Section 3) the SUSAR Reporting Category will be used (In addition, please note, per Section 9.5, all adverse events that are serious are collected on the month 18 Health Contact Event Log and reported to SCHARP.)

After the participant has completed the month 18 health contact and is off study, sites must report SUSARS if the study site staff becomes aware of the events on a passive basis (eg, from publicly available information)

The NIAID/DAIDS will report all unexpected SAEs related to the study products observed in this clinical trial to the FDA in accordance with 21 CFR 312.32 (IND Safety Reports). However, because safety is a primary study endpoint, the Sponsor Medical Officer will not routinely be unblinded to study treatment assignment when there is an assessment of relatedness of the SAE with the study product(s); and the safety report will be sent to the FDA based on the blinded attribution assessment.

If the PSRT believes unblinding of the site PI to treatment assignment will assist with the clinical management of the SAE, the PSRT will consult the independent HVTN SMB for a recommendation. In the event the HVTN SMB determines that unblinding is indicated, the SMB will inform the site physician of the participant's treatment assignment in such a manner as to maintain the study blind of the PSRT and study team. For additional impact and management of SAEs on the study, refer to Section 11.4.

## 11.3 Safety reviews

### 11.3.1 Initial safety evaluation

Enrollment across all participating HVTN CRSs will be restricted to a maximum of 1 participant per day and restricted to US sites until 10 participants have been enrolled. The HVTN 119 PSRT will review the cumulative safety data including at minimum local and systemic reactogenicity data reported for the first 72 hours postvaccination on each of

these 10 participants, and will determine whether it is safe to proceed with full enrollment.

## 11.4 Safety pause and prompt PSRT AE review

When a trial is placed on safety pause, all enrollment and vaccinations will be held until further notice. The AEs that will lead to a safety pause or prompt HVTN 119 PSRT AE review are summarized in Table 11-1. Vaccinations may be suspended for safety concerns other than those described in the table, or before pause rules are met, if, in the judgment of the HVTN 119 PSRT, participant safety may be threatened. Criteria for an individual participant's departure from the schedule of vaccinations are listed in Section 7.3.

**Table 11-1 AE notification and safety pause/AE review rules**

| Event and relationship to study products | Severity | HVTN CRS action[a] | HVTN Core action |
|---|---|---|---|
| SAE, related | Grade 5 or Grade 4 | Phone immediately, email and submit forms immediately | Immediate pause |
| SAE, not related | Grade 5 | Phone immediately, email and submit forms immediately | Immediate HVTN 119 PSRT notification |
| SAE, related | Grade 3 | Email and submit forms immediately | Prompt HVTN 119 PSRT AE review to consider pause |
| AE[b], related | Grade 4 or 3 | Email and submit forms immediately | Prompt HVTN 119 PSRT AE review to consider pause |

[a] Phone numbers and email addresses are found on the Protocol home page on the HVTN Members' site (https://members.hvtn.org/protocols/hvtn119).

[b] Does not include subjective reactogenicity symptoms (injection site pain, tenderness, fatigue/malaise, myalgia, arthralgia, chills, headache, nausea).

For all safety pauses, HVTN Core notifies the HVTN 119 PSRT, HVTN Regulatory Affairs, DAIDS Pharmaceutical Affairs Branch (PAB), DAIDS Regulatory Affairs Branch (RAB), DAIDS Safety and Pharmacovigilance Team (SPT), and participating HVTN CRSs. When an immediate safety pause is triggered, HVTN Core notifies the SMB.

Once a trial is paused, the HVTN 119 PSRT reviews safety data and decides whether the pause can be lifted or permanent discontinuation of vaccination is appropriate, consulting the SMB if necessary. HVTN Core notifies the participating HVTN CRSs, HVTN Regulatory Affairs, DAIDS PAB, DAIDS RAB, and DAIDS SPT of the decision regarding resumption or discontinuation of study vaccinations. Based on the HVTN 119 PSRT assessment, DAIDS RAB notifies the FDA as needed.

If an immediate HVTN 119 PSRT notification or prompt HVTN 119 PSRT AE review is triggered, HVTN Core notifies the HVTN 119 PSRT as soon as possible during working hours (US Pacific Time)—or, if the information was received during off hours, by the morning of the next work day. If a prompt HVTN 119 PSRT AE review cannot be

completed within 72 hours of notification (excluding weekends and US federal holidays), an automatic safety pause occurs.

The HVTN requires that each CRS submit to its IRB/EC and any applicable RE protocol-related safety information (such as IND safety reports, notification of vaccine holds due to the pause rules, and notification of other unplanned safety pauses). CRSs must also follow all applicable RE reporting requirements.

In addition, all other AEs are reviewed routinely by the HVTN 119 PSRT (see Section 11.5.2).

## 11.5 Review of cumulative safety data

Routine safety review occurs at the start of enrollment and then throughout the study.

Reviews proceed from a standardized set of protocol-specific safety data reports. These reports are produced by the SDMC and include queries to the HVTN CRSs. Events are tracked by internal reports until resolution.

### 11.5.1 Daily review

Blinded daily safety reviews are routinely conducted by HVTN Core for events requiring expedited reporting to DAIDS, and events that meet safety pause criteria or prompt HVTN 119 PSRT AE review criteria.

### 11.5.2 Weekly review

During the injection phase of the trial, the HVTN 119 PSRT reviews clinical safety reports on a weekly basis and conducts calls to review the data as appropriate. After the injections and the final 2-week safety visits are completed, less frequent reporting and safety reviews may be conducted at the discretion of the HVTN 119 PSRT. HVTN Core reviews reports of clinical and laboratory AEs. Events identified during the review that are considered questionable, inconsistent, or unexplained are referred to the HVTN CRS clinic coordinator for verification.

## 11.6 Study termination

This study may be terminated early by the determination of the HVTN 119 PSRT, a pertinent national regulatory authority, NIH, Office for Human Research Protections (OHRP), FDA, or vaccine developer(s). In addition, the conduct of this study at an individual HVTN CRS may be terminated by the determination of the IRB/EC and any applicable RE.

# 12 Protocol conduct

This protocol and all actions and activities connected with it will be conducted in compliance with the principles of GCP (ICHe6), and according to DAIDS and HVTN policies and procedures as specified in the *HVTN Manual of Operations,* DAIDS Clinical Research Policies and Standard Procedures Documents including procedures for the following:

- Protocol registration, activation, and implementation;
- Informed consent, screening, and enrollment;
- Study participant reimbursement;
- Clinical and safety assessments;
- Safety monitoring and reporting;
- Data collection, documentation, transfer, and storage;
- Participant confidentiality;
- Study follow-up and close-out;
- Unblinding of staff and participants;
- Quality control;
- Protocol monitoring and compliance;
- Advocacy and assistance to participants regarding negative social impacts associated with the vaccine trial;
- Risk reduction counseling;
- Specimen collection, processing, and analysis;
- Ancillary studies, and
- Destruction of specimens.

Any policies or procedures that vary from DAIDS and HVTN standards or require additional instructions (eg, instructions for randomization specific to this study) will be described in the *HVTN 119 Study Specific Procedures*.

## 12.1 Social impacts

Participants in this study risk experiencing discrimination or other personal problems, resulting from the study participation itself or from the development of VISP. The HVTN CRS is obliged to provide advocacy for and assistance to participants regarding these negative social impacts associated with the vaccine trial. If HVTN CRS staff have questions regarding ways to assist a participant dealing with a social impact, a designated NIAID or HVTN Core representative can be contacted.

Social harms are tabulated by the SDMC and are subjected to descriptive analysis. The goal is to reduce their incidence and enhance the ability of study staff to mitigate them when possible.

Summary tables of social impact events will be generated weekly, and made available for review by the protocol chairs, protocol team leader, and the designated NIAID representative.

## 12.2 Compliance with NIH guidelines for research involving products containing recombinant or synthetic Nucleic Acid Molecules

Because this study is evaluating products containing recombinant or synthetic DNA, it must comply with regulations set forth in the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (April 2016).* Information about the study must be submitted to site Institutional Biosafety Committees (IBCs) and Institutional Review Boards/Ethics Committees (IRBs/ECs). IBCs and IRBs/ECs must provide a written assessment of whether Recombinant DNA Advisory Committee (RAC) review is warranted. In exceptional cases that meet specific criteria outlined in the *NIH Guidelines Appendix M-I-B*, IBCs and IRBs/ECs assessment can include a request for RAC review. Regardless of the potential for RAC review, the HVTN and DAIDS will register the protocol with the NIH Office of Scientific Policy (OSP). The Protocol Team, jointly with Profectus BioSciences, Inc., will prepare the NIH OSP registration documents.

Investigators at each site are responsible for obtaining IBC approval per NIH Guidelines *section IV-B7-a-(1).* IBC review and approval must be documented by the investigator and submitted as part of DAIDS's initial protocol registration for this trial before participants are enrolled at the site. If this protocol is amended, investigators should follow the requirements of their IBC.

The HVTN and DAIDS will ensure that reporting requirements to NIH OSP, as outlined in *Appendix M-I-C-1.Initiation of the Clinical Investigation*, *Appendix M-I-C-2.Additional Clinical Trial Sites*. *Appendix M-I-C-3.Annual Reports* and *Appendix M-I-C-4.Safety Reporting* are satisfied per the NIH Guidelines.

## 12.3 Emergency communication with study participants

As in all clinical research, this study may generate a need to reach participants quickly to avoid imminent harm, or to report study findings that may otherwise concern their health or welfare.

When such communication is needed, the CRS will request that its IRB/EC and any applicable RE expedite review of the message. If this review cannot be completed in a timeframe consistent with the urgency of the required communication, the site should contact the participant first, and then notify the IRB/EC and any applicable RE of the matter as soon as possible.

# 13 Version history

The Protocol Team may modify the original version of the protocol. Modifications are made to HVTN protocols via clarification memos, letters of amendment, or full protocol amendments.

The version history of, and modifications to, Protocol HVTN 119 are described below.

**Protocol history and modifications**

**Date: February 17, 2017**

*Protocol version:* 1.0
*Protocol modification:*
Original protocol

# 14 Document references (other than literature citations)

Other documents referred to in this protocol, and containing information relevant to the conduct of this study, include:

- Assessment of Understanding. Accessible through the HVTN protocol-specific website.
- Current CDC Guidelines. Revised Recommendations for HIV Testing of Adults, Adolescents, and Pregnant Women in Health-Care Settings. Available at http://www.cdc.gov/mmwr/PDF/rr/rr5514.pdf.
- Division of AIDS (DAIDS) Clinical Research Policies and Standard Procedures Documents. Available at https://www.niaid.nih.gov/research/daids-clinical-research-policies-standard-procedures
- Division of AIDS Protocol Registration Manual. Available at https://www.niaid.nih.gov/sites/default/files/documents/prmanual.pdf
- Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events. Version 2.0, November 2014. Available at http://rsc.tech-res.com/docs/default-source/safety/daids_ae_grading_table_v2_nov2014.pdf
- The Manual for Expedited Reporting of Adverse Events to DAIDS. Version 2.0, January 2010. Available at http://rsc.tech-res.com/clinical-research-sites/safety-reporting/manual
- HVTN Certificate of Confidentiality. Accessible through the HVTN website.
- HVTN 119 Special Instructions. Accessible through the HVTN protocol-specific website.
- HVTN 119 Study Specific Procedures. Accessible through the HVTN protocol-specific website.
- HVTN 119 Site Lab Instructions. Accessible through the HVTN protocol-specific website.
- HVTN Laboratory Manual of Operations. Accessible through the HVTN website.
- HVTN Manual of Operations. Accessible through the HVTN website.
- Dangerous Goods Regulations (updated annually), International Air Transport Association. Available for purchase at http://www.iata.org/publications/dgr/Pages/index.aspx
- Lab assay algorithm

- HVTN algorithm for diagnosis of HIV infections. Part of the HVTN Laboratory Manual of Operations (see above).

- International Conference on Harmonisation (ICH) E6 (R1), Guideline for Good Clinical Practice: Section 4.8, Informed consent of trial subjects. Available at http://www.ich.org/products/guidelines/efficacy/article/efficacy-guidelines.html

- Participants' Bill of Rights and Responsibilities. Accessible through the HVTN website.

- NIH *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*. Available at http://osp.od.nih.gov/sites/default/files/resources/NIH_Guidelines.pdf

- NIH Policy on Reporting Race and Ethnicity Data: Subjects in Clinical Research. Available at http://grants1.nih.gov/grants/guide/notice-files/NOT-OD-01-053.html.

- Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks, July 2008.

- Requirements for Source Documentation in DAIDS Funded and/or Sponsored Clinical Trials. Available at https://www.niaid.nih.gov/research/daids-clinical-site-implementation-operations

- Title 21, Code of Federal Regulations, Part 50. Available at http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=50

- Title 45, Code of Federal Regulations, Part 46. Available at http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.html

See Section 16 for literature cited in the background and statistics sections of this protocol.

# 15 Acronyms and abbreviations

| | |
|---|---|
| AA | amino acid |
| Ab | antibody |
| AE | adverse event |
| AESI | AEs of special interest |
| ALT | alanine aminotransferase |
| ANOVA | analysis of variance |
| AST | aspartate aminotransferase |
| β-HCG | beta human chorionic gonadotropin |
| BMI | body mass index |
| CAB | Community Advisory Board |
| CBC | complete blood count |
| CDC | US Centers for Disease Control and Prevention |
| CE | conserved elements |
| CFR | Code of Federal Regulations |
| CI | confidence intervals |
| CPK | creatine phosphokinase |
| CRF | case report form |
| CRPMC | NIAID Clinical Research Products Management Center |
| CRS* | clinical research site |
| CTL | cytotoxic T lymphocyte |
| DAERS | DAIDS Adverse Experience Reporting System |
| DAIDS | Division of AIDS (US NIH) |
| DHHS | US Department of Health and Human Services |
| DSMB | NIAID Data and Safety Monitoring Board |
| EAE | adverse events requiring expedited reporting to DAIDS |
| EC | Ethics Committee |
| EDTA | ethylenediamine tetraacetic acid |
| EIA | enzyme immunoassay |
| ELISA | enzyme-linked immunosorbent assay |
| ELISpot | enzyme-linked immunospot |
| EP | electroporation |
| FDA | US Food and Drug Administration |
| FHCRC | Fred Hutchinson Cancer Research Center |
| GCP | Good Clinical Practice |
| GEE | generalized estimating equation |
| HBsAg | hepatitis B surface antigen |
| HCV | hepatitis C virus |
| HIPAA | Health Insurance Portability and Accountability Act |
| HIV | human immunodeficiency virus |
| HLA | human leukocyte antigen |

| | |
|---|---|
| HVTN | HIV Vaccine Trials Network |
| IB | Investigator's Brochure |
| IBC | Institutional Biosafety Committee |
| ICH | International Conference on Harmonisation |
| ICS | intracellular cytokine staining |
| IFN-γ | interferon gamma |
| IM | intramuscular |
| IND | Investigational New Drug |
| IRB | Institutional Review Board |
| IUD | intrauterine device |
| MAR | missing at random |
| MCAR | missing completely at random |
| MMR | measles, mumps, and rubella |
| NIAID | National Institute of Allergy and Infectious Diseases (US NIH) |
| NIH | US National Institutes of Health |
| OSP | NIH Office of Scientific Policy |
| OHRP | US Office for Human Research Protections |
| OPV | oral polio vaccine |
| PAB | DAIDS Pharmaceutical Affairs Branch |
| PBMC | peripheral blood mononuclear cell |
| PCR | polymerase chain reaction |
| pDNA | plasmid DNA |
| PI | Principal Investigator |
| PSRT | Protocol Safety Review Team |
| RAB | DAIDS Regulatory Affairs Branch |
| RAC | NIH Recombinant DNA Advisory Committee |
| RE | regulatory entity |
| RSC | DAIDS Regulatory Support Center |
| SAE | serious adverse event |
| SCHARP | Statistical Center for HIV/AIDS Research and Prevention |
| SDMC | statistical and data management center |
| SFC | spot-forming cell |
| SIV | simian immunodeficiency virus |
| SMB | Safety Monitoring Board |
| SPT | DAIDS Safety and Pharmacovigilance Team |
| SV40 | simian virus 40 |
| TB | tuberculosis |
| UW-VSL | University of Washington Virology Specialty Laboratory |
| VISP | Vaccine induced seropositivity |
| VRC | Vaccine Research Center (NIAID) |

* CRSs were formerly referred to as HIV Vaccine Trial Units (HVTUs). Conversion to use of the term CRS is in process, and some HVTN documents may still refer to HVTUs.

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# Appendix A Sample informed consent form

Title: A phase 1 clinical trial to evaluate the safety and immunogenicity of pDNA vaccines expressing HIV M Group $p24^{Gag}$ conserved elements and/or $p55^{Gag}$, administered with *IL-12* pDNA by intramuscular electroporation, in healthy, HIV-uninfected adult participants

HVTN protocol number: HVTN 119

Site: [Insert site name]

Thank you for your interest in our research study. Please read this consent form or ask someone to read it to you. If you decide to join the study, we will ask you to sign or make your mark on this form. We will offer you a copy to keep. We will ask you questions to see if we have explained everything clearly. You can also ask us questions about the study.

Research is not the same as treatment or medical care. The purpose of a research study is to answer scientific questions.

## About the study

The HIV Vaccine Trials Network (HVTN) and [Insert site name] are doing a study to test HIV vaccines. HIV is the virus that causes AIDS.

About 56 people will take part in this study at multiple sites. The researcher in charge of this study at this clinic is [Insert name of site PI]. The US National Institutes of Health (NIH) is paying for the study.

This study is testing HIV vaccines, and another study product called an adjuvant. The study will also test an experimental device, called an electroporation (EP) device, which delivers these products with a small electrical pulse. We will explain these terms in later sections.

1. **We are doing this study to answer several questions.**

   - Are the study products safe to give to people?
   - Are people able to take the study products given with the EP device without becoming too uncomfortable?
   - How do people's immune systems respond to the study products? (Your immune system protects you from disease.)

2. **The study products cannot give you HIV.**

   The study products are not made from actual HIV. It is impossible for the study products to give you HIV. Also, they cannot cause you to give HIV to someone else.

**3. We do not know if the study products will decrease, increase, or not change your risk of becoming infected with HIV if you are exposed to the virus.**

*Sites: Any change to the language in this section requires approval from HVTN Regulatory Affairs.*

Several studies have tested whether HIV vaccines can reduce the risk of getting HIV from another person. In some studies, people who got the vaccine seemed to have the *same* risk of getting HIV as people who did not get the vaccine. In one study, people who got the vaccine seemed to have a *lower* risk of getting HIV than people who did not get the vaccine. In studies with a different vaccine, some people who got the vaccine had a *higher* risk of getting HIV than people who did not get the vaccine.

This study differs from the studies in which people who got the vaccine had a higher or lower risk of getting HIV. The study staff can tell you about the differences.

We do not know whether the vaccines in this study will affect your risk of getting HIV from another person. The risk could be higher, lower, or unchanged. It's very important to avoid exposure to HIV during and after the study. We will tell you how to avoid HIV.

**4. These study products are experimental.**

The study products are called p24CE1/2 vaccine, $p55^{gag}$ vaccine, and *IL-12* pDNA adjuvant. From here on, we will call them "the study vaccines" and "the study adjuvant". These study products are experimental. That means we do not know whether the study products will be safe to use in people, or whether they will work to prevent HIV infection. These study products are used only in research studies.

There is also a placebo in this study. A placebo is a substance that does not contain vaccine. The placebo for the study vaccines is sterile salt water.

The study vaccines are provided by the Division of AIDS (DAIDS). The study adjuvant is provided by Profectus BioSciences, Inc.

The study vaccines are DNA vaccines that contain DNA made in the laboratory. DNA is a natural substance in the body that tells the body to make proteins. Proteins are natural substances that the body uses to build and maintain itself, as well as protect itself against disease. The DNA in the study vaccines tells the body to make proteins, or pieces of proteins, that are found in HIV. Your body's immune system may respond to these proteins by helping your immune cells recognize and respond to infection. Some of these immune cells make antibodies, which fight infection.

So far, experimental DNA vaccines in people have produced mostly weak immune responses. Sometimes vaccines work better when they are combined with another substance that helps to alert the immune system. These substances are called adjuvants. In this study, the study vaccines are always given with the study adjuvant. The study adjuvant is made of DNA that tells the body to make IL-12, a normal protein in the body that helps immune cells work together and makes them multiply.

One of the study vaccines, the p24CE1/2 vaccine, has not been given to people before. It has been tested in mice and monkeys without causing health problems. Even if something looks like it is safe or works in animals, it may not be true for people.

Vaccines very similar to the $p55^{gag}$ study vaccine have been given to about 300 people in 4 different studies. Some of these studies also included the study adjuvant. No serious health problems were found that were related to those study products.

*General risks of vaccines:*

All vaccines can cause fever, chills, rash, aches and pains, nausea, headache, dizziness, and feeling tired. Vaccines can also cause pain, redness, swelling, or itching where you got the injection. Most people can still do their planned activities after getting a vaccine. Rarely, people experience side effects that limit their normal activities or make them go to the doctor.

Rarely, a vaccine can cause an allergic reaction, including a rash, hives, or difficulty breathing. Allergic reactions can be life-threatening. You should tell us if you have ever had a bad reaction to any injection or vaccine.

Very rarely, a vaccine causes an autoimmune disease in a person, or makes an autoimmune disease worse. An autoimmune disease happens when your immune system attacks your own body, instead of attacking an infection.

*Risks of the study products:*

This section lists the side effects of the study products that we know about. There may be others that we don't yet know about, even serious ones. We will tell you if we learn about any new side effects.

The study vaccines and the study adjuvant are made with an anesthetic (a pain reliever) called bupivacaine. Bupivacaine helps DNA get into the muscle. It is similar to the numbing medicine used by dentists. Some people have an allergy to bupivacaine or similar products that are sometimes used by dentists or in anesthetic skin creams. For this reason we will ask if you have ever had an allergic reaction to one of these anesthetics before enrolling you in the study.

Possible risks related to the study vaccines include muscle damage at the site of the injection, the production of antibodies which might react with normal body tissues and cause an autoimmune disease, and insertion of the study product's DNA into the body's DNA.

Since 1995, thousands of people have received experimental DNA vaccines for diseases such as hepatitis, human papilloma virus (HPV, also known as genital warts), and HIV. In these people, the DNA vaccines have not caused serious side effects. We expect the risks of the study vaccines in this study to be similar to those of other DNA vaccines.

In earlier studies of the study adjuvant given at lower doses, there were no severe reactions or serious health problems. We do not know if participants in this study will have similar experiences to those seen in earlier studies.

However, with the study products, there may be new side effects that we don't know about.

**5. The study vaccines and study adjuvant are given using electroporation.**

Besides adjuvants, another way to improve immune responses to DNA vaccines is to use electroporation (EP). EP uses an electric pulse to briefly open tiny pores in the cells. The DNA can enter the cells through these pores. EP has been used for many years in the laboratory to get DNA or other substances into cells. Recently, a study showed that EP increased immune responses to another experimental DNA vaccine.

EP is done in this study using a device called the Intramuscular TriGrid Delivery System. It was developed by Ichor Medical Systems, Inc. From now on we will call it "the EP device." EP in people is an experimental procedure, and the EP device is an experimental device. The EP device is only used in people in research studies. The EP device gives an electrical pulse into the arm muscle where the injection is given.

This electrical pulse is delivered to the muscle through 4 thin needles at the same time as the study products or placebo are given. The EP device has been tested in more than 700 people in other studies with DNA vaccines and placebos. There were no serious side effects.

In other studies, different DNA vaccines and an *IL-12* pDNA adjuvant were given into the muscle with EP to 148 people. However this is the first time these particular study products will be given to people with EP.

*Risks of electroporation (EP):*

EP will cause brief muscle spasms during the procedure. In previous studies using EP, people felt initial pain that ranged from mild to severe. For most people, the pain eased quickly. EP can also cause soreness, a small amount of bleeding, bruising, redness, swelling, itching, or hardness/stiffness in the upper arm where you get the injection. Minor damage to muscle cells is also possible.

Five percent of people (5%) felt lightheaded or faint after an injection with EP, and felt better after lying down. While uncommon, the EP device may cause infection at the part of your body where you got the injection. Rarely, people have reported numbness or a tingling sensation in their arm lasting several minutes. A standard injection with needle and syringe can also have these side effects.

It is possible that the EP device may not work as expected, such as not giving the study products or placebo or the electrical pulse. If this happens, we may ask if we can try again.

In one study, during a person's first injection, the injection needle came in contact with bone, and the EP device was hard to remove, which caused severe pain and stress. A local anesthetic had to be used to ease the removal. This has happened once, in more than 3500 injections using this EP device.

Having the procedure or thinking about it may cause some stress and anxiety. If you feel anxious, please tell us and we will try to help you.

Because EP could possibly interfere with implanted electronic medical devices such as pacemakers or defibrillators or make certain heart problems worse, we will ask whether

you have a history of irregular heartbeat (arrhythmias). We will also make sure you don't have any metal implants in the upper part of your body.

We do not know if EP will change the risks for any of the study products. We do not know all the risks of EP because it has only been used in a limited number of people before this study and not with these study products.

## Joining the study

6. **It is completely up to you whether or not to join the study.**

   Take your time in deciding. If it helps, talk to people you trust, such as your doctor, friends or family. If you decide not to join this study, or if you leave it after you have joined, your other care at this clinic and the benefits or rights you would normally have will not be affected.

   If you join this study, you may not be allowed to join other HIV vaccine or HIV biomedical prevention studies now or in the future. You cannot be in this study while you are in another study where you get a study product. Also during the study, you should not donate blood or tissue.

   If you choose not to join this study, you may be able to join another study.

   *Site: Remove item 7 if you use a separate screening consent that covers these procedures.*

7. **If you want to join the study, we will screen you to see if you are eligible.**

   Screening involves a physical exam, HIV test and health history. A physical exam may include, but is not limited to:

   - Checking your weight, temperature and blood pressure
   - Looking in your mouth and throat
   - Listening to your heart and lungs
   - Feeling your abdomen (stomach and liver)
   - Gently pinching the skin of your upper arm to measure the skin thickness for EP injections

   We will also do blood and urine tests. These tests tell us about some aspects of your health, such as how healthy your kidneys, liver, and immune system are. We will also test you for hepatitis and syphilis. We will ask you about medications you are taking. We will ask you about behaviors that might put you at risk for getting HIV. If you were born female, we will test you for pregnancy.

   We will review the screening results with you. The screening results may show you are not eligible to join the study, even if you want to.

   *(Sites: adapt the following section so it is applicable to the care available at your site)*

8. **If we find that you have a health problem during screening or during the study, we will tell you about the care that we can give here for free.**

   For the care that we cannot give, we will explain how we will help you get care elsewhere. For health problems that are unrelated to the study, we will not pay for care.

9. **If you were born female and could become pregnant, you must agree to use birth control to join this study.**

   *Site: If you want to include Appendix B, Approved birth control methods (for sample informed consent form), in this consent form, paste it below and delete paragraph below.*

   You should not become pregnant during the study because we do not know how the study products could affect the developing baby. You must agree to use effective birth control from 21 days before your first injection until 6 months after your last study injection. We will talk to you about effective birth control methods. They are listed on a handout that we will give to you.

## Being in the study

If you meet the study requirements and want to join, here is what will happen:

10. **You will come to the clinic for scheduled visits about [#] times over [Insert period of time].**

    *Site: Insert number of visits and range of visit lengths. (There is site-specific variation in screening protocols and in the number of possible follow-up visits between protocol-mandated visits.)*

    Visits can last from [#] to [#] hours.

    You may have to come for more visits if you have a lab or health issue.

    We may contact you after the main study ends (for example, to tell you about the study results).

11. **We will give you [Site: Insert compensation] for each study visit you complete.**

    This amount is to cover the costs of [Site: Insert text]

    *Site: Insert any costs to participants (eg, birth control costs for female participants who could become pregnant).*

    Payments you receive for being in the study may be taxable. We may need to ask you for your Social Security number for tax reasons.

    You do not have to pay anything to be in this study.

**12. We will give you either the study products or a placebo.**

Not everyone in this study will get the study products. Some people will get a placebo, a substance that does not contain study vaccine or study adjuvant. We will compare the results from people who got the placebo with results from people who got the study products. In this study, the placebo is sterile salt water.

You have around a 6-in-7 chance of getting the study products. *Site: Modify the randomization metaphor in the next sentence as appropriate to your local culture.* Whether you get the study products or the placebo is completely random, like flipping a coin.

We have no say in whether you get the study products or the placebo. We will not know which one you are getting, and neither will you. Only the pharmacist at this clinic will have this information while the study is going on.

You will have to wait until everyone completes their final study visits to find out whether you got the study products or the placebo. This could be several years. But, if you have a serious medical problem and need to know what you got before the end of the study, we can tell you.

**13. We will give you the study products on a schedule.**

You will be in one of 2 groups. You will get 2 injections of the study products or placebo, one in each upper arm. This will happen 4 times during the study. All injections will be given with EP.

*Site: If a picture version of the injection schedule has been provided in a separate protocol appendix, you may insert it below in place of (or in addition to) the text version or give it as a separate document to volunteers if you believe it will be helpful to them. You are not required to do either.*

| Injection Schedule | | | | |
|---|---|---|---|---|
| **Group** | **First Injection Visit** | **1 month later** | **3 months later** | **6 months later** |
| 1 | p24CE1/2 vaccine<br>+<br>study adjuvant<br><br>**OR**<br><br>placebo | p24CE1/2<br>vaccine<br>+<br>study adjuvant<br><br>**OR**<br><br>placebo | p24CE1/2<br>vaccine<br>+<br>p55gag vaccine<br>+<br>study adjuvant<br><br>**OR**<br><br>placebo | p24CE1/2<br>vaccine<br>+<br>p55gag vaccine<br>+<br>study adjuvant<br><br>**OR**<br><br>placebo |
| 2 | $p55^{gag}$ vaccine<br>+<br>study adjuvant<br><br>**OR**<br><br>placebo | $p55^{gag}$ vaccine<br>+<br>study adjuvant<br><br>**OR**<br><br>placebo | $p55^{gag}$ vaccine<br>+<br>study adjuvant<br><br>**OR**<br><br>placebo | $p55^{gag}$ vaccine<br>+<br>study adjuvant<br><br>**OR**<br><br>placebo |

For all injections, the EP device is pressed against your upper arm firmly. You will feel some pressure against your arm. Once the EP device is set in place, the clinic staff will activate it and you will hear two clicks. At this point the needles are inserted into your arm. For most people, this does not hurt but gives a sensation of pressure. After the needles have been inserted, there will be a 3 to 4 second delay. During this time, the study products or placebo are being injected into your arm. After the injection, a very small amount of electricity is sent to the muscle. The electrical signal will last for about a half second. This procedure will be done once in each upper arm.

You will feel a strong movement in your arm muscle, which is often painful. The level of pain varies from person to person. Some people describe the feeling like a punch in the arm. The intensity of that feeling dies down within a minute or two. On rare occasions, people have reported a mild tingling immediately after EP in their arm or fingers lasting for a few seconds up to several minutes. After EP, your arm may be sore for a few days. We will ask you to rate any pain you feel on a scale.

At each injection visit, you will have to wait in the clinic for about a half hour after your injections to see how you are feeling. Then for that night and for 7 more days, you will need to keep track of how you are feeling and if you have any symptoms. Within 3 days of your injections, we will contact you or ask you to contact us and let us know how you are doing. You should always contact us if you have any issues or concerns after receiving an injection. If you have a problem, we will continue to check on you until it goes away.

**14. In addition to giving you the study products, we will:**

- Do regular HIV testing, as well as counseling on your results and on how to avoid getting HIV;

- Do physical exams;
- Do pregnancy tests if you were born female;
- Ask questions about your health, including medications you may be taking;
- Ask questions about any personal problems or benefits you may have from being in the study;
- Ask questions about your experience with EP; and
- Take urine and blood samples.

When we take blood, the amount will depend on the lab tests we need to do. It will be some amount between 10 mL and 336 mL (2 teaspoons to about 1½ cups). Your body will make new blood to replace the blood we take out.

*Site: You may want to add a sentence to the end of the previous paragraph contextualizing the blood volumes described (eg, "To compare, people who donate blood in the US can give a total of about 500 mL in an 8-week period."). Modify the example for cultural relevance and alter blood volumes as necessary.*

*Site: Insert Appendix [#], Table of procedures (for informed consent form) in this section or distribute it as a separate sheet if it is helpful to your study participants. You are not required to do either.*

We will be looking for side effects. We will review the results of these procedures and tests with you at your next visit, or sooner if necessary. If any of the results are important to your health, we will tell you.

**15. We will counsel you on avoiding HIV infection.**

We will ask you personal questions about your HIV risk factors such as sexual behavior, alcohol, and drug use. We will talk with you about ways to keep your risk of getting HIV low.

**16. We will test your samples for this study.**

We will send your samples (without your name) to labs approved by the HVTN for this study, which are located in the United States. Researchers at these labs will test your samples to see how your immune system responds to the study products. In rare cases, some of your samples may be sent to labs approved by the HVTN in other countries for research related to this study.

Researchers may also do genetic testing related to this study on your samples. Your genes are passed to you from your birth parents. They affect how you look and how your body works. The differences in people's genes can help explain why some people get a disease while others do not. These types of genetic tests involve some of your genes, not all of your genes (your genome). The researchers will study the genes related to the immune system and HIV and those that affect how people get HIV.

If you become HIV infected, the researchers may look at all of the genes of the virus found in your samples. The researchers will use this information to learn more about HIV and the study product(s).

In some cases, researchers may take cells from your samples and grow more of them over time, so that they can continue to contribute to this study.

Tests done on your samples are for research purposes only. The labs will not give the results to you or this clinic, and the results will not become part of your study record.

When your samples are no longer needed for this study, the HVTN will continue to store them.

*Site: Delete next section if using separate consent for use of samples and information in other studies*

**17. When samples are no longer needed for this study, the HVTN wants to keep them for use in other studies by HVTN or other researchers. We will call these "extra samples."**

This section gives you information so you can decide if you want your extra samples and information used in other studies. You will mark your decision at the end of the form. If you have any questions, please ask.

*Do I have to agree?* No. You are free to say yes or no, or to change your mind after you sign this form. At your request, we will destroy all extra samples that we have. Your decision will not affect your being in this study or have any negative consequences here.

*Where are the samples stored?* Extra samples are stored in a secure central place called a repository. Your samples will be stored in the HVTN repository in the United States.

*How long will the samples be stored?* There is no limit on how long your extra samples will be stored. *[Site: insert limits if your regulatory authority imposes them.]*

*Will I be paid for the use of my samples?* No. Also, a researcher may make a new scientific discovery or product based on the use of your samples. If this happens, there is no plan to share any money with you. The researcher is not likely to ever know who you are.

*Will I benefit from allowing my samples to be used in other studies?* Probably not. Results from these other studies are not given to you, this clinic, or your doctor. They are not part of your medical record. The studies are only being done for research purposes.

*Will the HVTN sell my samples and information?* No, but the HVTN may share your samples with other researchers. Once we share your samples and information, we will not be able to get them back.

*How do other researchers get my samples and information?* When a researcher wants to use your samples and/or information, their research plan must be approved by the HVTN. Also, the researcher's institutional review board (IRB) or ethics committee (EC) will review their plan. *[Site: If review by your institution's IRB/EC/RE is also required, insert a sentence stating this.]* IRBs/ECs protect the rights and well-being of people in research.

If the research plan is approved, the HVTN will send your samples to the researcher's location.

*What information is shared with other researchers?* The samples and limited information will be labeled with a code number. Your name will not be part of the information. However, some information that we share may be personal, such as your race, ethnicity, sex, health information from the study, and HIV status. We may share information about the study product you received and how your body responded to the study product.

*What kind of studies might be done with my extra samples and information?* The studies will be related to HIV, vaccines, the immune system and other diseases.

Researchers may also do genetic testing on your samples.

If you become HIV infected, the researchers may look at all of the genes of the virus found in your samples. The researchers will use this information to learn more about HIV and the study product(s).

In some cases, researchers may take cells from your samples and grow more of them over time, so that they can continue to contribute to this study.

If you agree, your samples could also be used for genome wide studies. In these studies, researchers will look at all of your genes (your genome). The researchers compare the genomes of many people, looking for common patterns of genes that could help them understand diseases. The researchers may put the information from the genome-wide studies into a protected database so that other researchers can access it. Usually, no one would be able to look at your genome and link it to you as a person. However, if another database exists that also has information on your genome and your name, someone might be able to compare the databases and identify you. If others found out, it could lead to discrimination or other problems. The risk of this is very small.

*Who will have access to my information in studies using my extra samples?*

People who may see your information are:

- Researchers who use your stored samples and limited information for other research
- Government agencies that fund or monitor the research using your samples or information
- The researcher's Institutional Review Board or Ethics Committee
- The people who work with the researcher

All of these people will do their best to protect your information. The results of any new studies that use your extra samples or information may be published. No publication will use your name or identify you personally.

**18. We will do our best to protect your private information.**

*US sites: Check HIPAA authorization for conflicts with this section.*

Your study records and samples will be kept in a secure location. We will label all of your samples and most of your records with a code number, not your name or other personal information. However, it is possible to identify you, if necessary. We will not share your name with the lab that does the tests on your samples, or with anyone else who does not need to know your name.

*Sites: Any change to the following boxed text requires approval from HVTN Regulatory Affairs.*

We do need to share your name with the HVTN in case you need proof in the future that you participated in an HIV vaccine study. The HVTN will keep your name in a secure file with these items:

- The name of your study
- Your age or date of birth
- Your study ID number
- What study product(s) you received

There are no HIV test results kept in this file. The HVTN will not share any information that could identify you without your agreement. The HVTN will remove your name from the file if you do not want it there.

Clinic staff will have access to your study records. Your records may also be reviewed by groups who watch over this study to see that we are protecting your rights, keeping you safe, and following the study plan. These groups include:

- The US National Institutes of Health and its study monitors,
- The US Food and Drug Administration,
- [Insert name of local IBC],
- [Insert name of local IRB/EC] ,
- [Insert name of local and/or national regulatory authority as appropriate],
- Profectus BioSciences, Inc. and people who work for them,
- Ichor Medical Systems, Inc. and people who work for them,
- The HVTN and people who work for them,
- The HVTN Safety Monitoring Board, and
- The US Office for Human Research Protections.

All reviewers will take steps to keep your records private.

We cannot guarantee absolute privacy. At this clinic, we have to report the following information:

*Site: Include any public health or legal reporting requirements. Bulleted examples should include all appropriate cases (reportable communicable disease, risk of harm to self or others, etc.).*

- [Item 1]
- [Item 2]
- [Item 3]

We have a Certificate of Confidentiality from the US government, to help protect your privacy. With the certificate, we do not have to release information about you to someone who is not connected to the study, such as the courts or police. Sometimes we can't use the certificate. Since the US government funds this research, we cannot withhold information from it. Also, you can still release information about yourself and your study participation to others.

The results of this study may be published. No publication will use your name or identify you personally.

We may share information from the study with other researchers. We will not share your name or information that can identify you.

*Sites: The text below may not be deleted or changed, per FDA requirement.*

A description of this clinical trial will be available on http://www.ClinicalTrials.gov. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

**19. We may stop your injections or take you out of the study at any time. We may do this even if you want to stay in the study and even if you were scheduled for more injections.**

This may happen if:

- you do not follow instructions,
- we think that staying in the study might harm you,
- you get HIV,
- you enroll in a different research study where you get another study product, or
- the study is stopped for any reason.

If we stop your injections, we may ask you to stay in the study to complete other study procedures.

**20. We will stop your injections if you become pregnant during the study.**

We will encourage you to stay in the study if you choose. We will discuss your study options with you.

If you leave the study while you are still pregnant, we will contact you after your due date to ask some questions about your pregnancy and delivery.

**21. If you get infected with HIV during the study, we will help you get care and support.**

You will not be able to stay in this study. We will counsel you about your HIV infection and about telling your partner(s). We will tell you where you can get support and medical care, and if there are other studies you may want to join. *Site: Modify the following sentence as appropriate.* We will not provide or pay for any of your HIV care directly.

## Other Risks

**22. There are other risks to being in this study.**

This section describes the other risks and restrictions we know about. There may also be unknown risks, even serious ones. We will tell you if we learn anything new that may affect your willingness to stay in the study.

*Risks of routine medical procedures:*

In this study, we will do some routine medical procedures. These are taking blood and giving injections. These procedures can cause bruising, pain, fainting, soreness, redness, swelling, itching, a sore, bleeding, and (rarely) muscle damage or infection where you got the injection. Taking blood can cause a low blood cell count (anemia), making you feel tired.

*Personal problems/discrimination/testing HIV antibody positive:*

About 10 to 20% of people who join HVTN studies report personal problems or discrimination because of joining an HIV vaccine study. Family or friends may worry, get upset or angry, or assume that you are infected with HIV or at high risk and treat you unfairly as a result. Rarely, a person has lost a job because the study took too much time away from work, or because their employer thought they had HIV.

The body makes antibodies to fight or prevent infection. Most vaccines cause the body to make antibodies as a way of preventing infection. Your body may make antibodies to HIV because you received HIV study vaccines. The study vaccines may cause you to test positive on some types of HIV antibody tests, even if you are not infected with HIV. This is called vaccine-induced seropositivity (VISP). VISP means that after you get the study vaccines, a routine HIV test done outside this clinic may say you have HIV, even if you don't. For this reason, you should plan to get HIV tests only at this clinic during the study. Our tests can tell the difference between true HIV infection and a positive result that is caused by the study vaccines.

If you have a positive test result caused by the study vaccines at any time, we can arrange free HIV testing for as long as you need it. If this happens, we do not know how long you

will test positive due to the study vaccines. If you receive a positive HIV test result and we determine it is because you have HIV, we will refer you for follow-up care.

It is unlikely, but you could test negative at the end of the study and positive some time later, even though you don't have HIV. This could happen if different HIV tests come into use. We will give you a phone number to call for more information.

*Site: Modify the following paragraph if applicable.*

If someone believes you are infected with HIV even if you are not, you could face discrimination and other problems. For example, in some countries, you could be denied medical or dental care, employment, insurance, a visa, or entry into the military. If you do have a positive HIV antibody test caused by the study vaccines, you will not be able to donate blood or organs. Your family and friends may treat you differently. We will give you a brochure that tells you more about testing HIV positive because of an HIV vaccine, and how you can avoid some of these problems.

If you become pregnant during or after the study and have VISP, we don't know if the antibodies could be passed to your baby. We know that this happens with other vaccines, like tetanus vaccine. These antibodies from the mother are not a danger to the baby, and they go away over time. For most babies antibodies from the mother last for about six months.

You should always tell the delivery staff if you have VISP. However, you may still be tested for HIV using the antibody test when you deliver your baby. If your test is positive and the delivery staff believes you have an HIV infection, your baby may be started on antiretroviral treatment when it is not needed. If this happens, we can arrange for you and the baby to have a test that can tell the difference between true HIV infection and a VISP result. If you or the baby continue to have VISP, we can arrange this testing for free for as long as it is needed.

*Embarrassment/anxiety:*

You may feel embarrassed when we ask about your HIV risks, such as having sex and using drugs. Also, waiting for your HIV test results or other health test results could make you feel anxious. You could feel worried if your test results show that you are infected with HIV. If you feel embarrassed or anxious, please tell us and we will try to help you.

*Risks of disclosure of your personal information:*

We will take several steps to protect your personal information. Although the risk is very low, it is possible that your personal information could be given to someone who should not have it. If that happened, you could face discrimination, stress, and embarrassment. We can tell you more about how we will protect your personal information if you would like it.

*Risks of genetic testing:*

The genetic testing could show you may be at risk for certain diseases. If others found out, it could lead to discrimination or other problems. However, it is almost impossible

for you or others to know your test results from the genetic testing. The results are not part of your study records and are not given to you.

In the very unlikely event that your genetic information becomes linked to your name, a federal law called the Genetic Information Nondiscrimination Act (GINA) helps protect you. GINA keeps health insurance companies and employers from seeing results of genetic testing when deciding about giving you health insurance or offering you work. GINA does not help or protect you against discrimination by companies that sell life, disability or long-term care insurance.

*Unknown risks:*

We do not know if the study vaccines will increase, decrease, or not change your risk of becoming infected with HIV if exposed. If you get infected with HIV, we do not know how the study vaccines might affect your HIV infection or how long it takes to develop AIDS.

We do not know if getting these study vaccines will affect how you respond to any future approved HIV vaccine. It could be that a future HIV vaccine may not work as well for you because you got the study vaccines. Currently, no HIV vaccine has been approved for use.

We do not know how the study vaccines will affect a pregnant participant or a developing baby.

## Benefits

**23. The study may not benefit you.**

We do not know whether getting the study products might benefit you in any way. However, being in the study might still help you in some ways. The counseling that you get as part of the study may help you avoid getting HIV. The lab tests and physical exams that you get while in this study might detect health problems you don't yet know about.

This study may help in the search for a vaccine to prevent HIV. However, if the study products or EP device later become approved and sold, there are no plans to share any money with you.

## Your rights and responsibilities

**24. If you join the study, you have rights and responsibilities.**

You have many rights that we will respect. You also have responsibilities. We list these in the Participant's Bill of Rights and Responsibilities. We will give you a copy of it.

## Leaving the study

**25. Tell us if you decide to leave the study.**

You are free to leave the study at any time and for any reason. Your care at this clinic and your legal rights will not be affected, but it is important for you to let us know.

We will ask you to come back to the clinic one last time for a physical exam, and we may ask to take some blood and urine samples. We will also ask about any personal problems or benefits you have experienced from being in the study. We believe these steps are important to protecting your health, but it is up to you whether to complete them.

## Injuries

*Sites: Do not make changes to the following section without obtaining approval from HVTN Regulatory Affairs at vtn.core.reg@hvtn.org.*

**26. If you get sick or injured during the study, contact us immediately.**

Your health is important to us. *(Sites: adjust the following 2 sentences if applicable to the care available at your site)* We will tell you about the care that we can give here. For the care that we cannot provide, we will explain how we will help you get care elsewhere.

If you become sick or injured in this study, there is a process to decide if it is related to the study products, EP device and/or procedures. If it is, we call it a study-related injury. There are funds to pay for treatment of study-related injuries if certain conditions are met.

The HVTN has limited funds to pay medical costs that it determines are reasonable. There may be other ways that study-related injuries can be funded. We can give you more information, if you would like. *(Sites: insert locale- appropriate medical insurance language in the following sentence)* If the injury is not study related, then you and your health insurance will be responsible for treatment costs.

Some injuries are not physical. For example, you might be harmed emotionally by being in an HIV vaccine study. Or you might lose wages because you cannot go to work. However, there are no funds to pay for these kinds of injuries, even if they are study related.

You may disagree with the decision about whether your injury is study related. If you wish, the HVTN will ask independent experts to review the decision. You always have the right to use the court system if you are not satisfied.

## Health contact visit

**27. After your clinic visits end, we will contact you 18 months after your first injection.**

We will contact you by phone, email, or text message *[Site: Modify mode of contact as appropriate; consult IRB/EC if necessary]* around 18 months after your first injection to ask questions about your health. If you prefer to answer these questions in person, you can come to the clinic to do this.

If we have any concerns about your health, we may need to have more contact with you. You are also welcome to contact us at any time if you have concerns about your health related to being in the study.

If we ask you to come to the clinic, we will give you [Site: Insert compensation amount] for each visit. This amount is to cover the costs of [Site: Insert text].

If someone outside this study clinic told you that you are infected with HIV, we will ask you to come back to the clinic for another HIV test. We will draw about 15 mL (1 tablespoon) of blood. We may ask you to come back more than once for this testing.

Because we will want to contact you once after the main study, please tell us if your contact information changes, if you are moving away, or if you do not want us to contact you anymore.

You can tell us at any time that you don't want us to contact you after the main study. If you do so, you will not lose any benefits or rights you would normally have.

All other information that is discussed earlier in this consent also applies to the 18 month health contact.

## Questions

**28. If you have questions or problems at any time during your participation in this study, use the following important contacts.**

If you have questions about this study, contact
[name and telephone number of the investigator or other study staff].

If you have any symptoms that you think may be related to this study, contact
[name and telephone number of the investigator or other study staff].

If you have questions about your rights as a research participant, or problems or concerns about how you are being treated in this study, contact
[name/title/phone of person on IRB or other appropriate organization].

If you want to leave this study, contact
[name and telephone number of the investigator or other study staff].

## Your permissions and signature

*Site: Delete this section if using a separate consent for use of samples and information in other studies*

**29. In Section 17 of this form, we told you about possible other uses of your extra samples and limited information, outside this study. Please choose only one of the options below and write your initials or make your mark in the box next to it. Whatever you choose, the HVTN keeps track of your decision about how your samples and information can be used.**

☐ I allow my extra samples combined with limited information to be used for other studies related to HIV, vaccines, the immune system, and other diseases. This may include genetic testing and keeping my cells growing over time.

**OR**

☐ I agree to the option above *and* also to allow my extra samples combined with limited information to be used in genome-wide studies.

**OR**

☐ I do not allow my extra samples to be used in any other studies. This includes not allowing genetic testing, growing more of my cells, or genome-wide studies.

**30. If you agree to join this study, you will need to sign or make your mark below. Before you sign or make your mark on this consent form, make sure of the following:**

- You have read this consent form, or someone has read it to you.
- You feel that you understand what the study is about and what will happen to you if you join. You understand what the possible risks and benefits are.
- You have had your questions answered and know that you can ask more.
- You agree to join this study.

You will not be giving up any of your rights by signing this consent form.

| Participant's name (print) | Participant's signature or mark | Date | Time |
|---|---|---|---|
| | | | |

| Clinic staff conducting consent discussion (print) | Clinic staff signature | Date | Time |
|---|---|---|---|
| | | | |

For participants who are unable to read or write, a witness should complete the signature block below:

| Witness's name (print) | Witness's signature | Date | Time |
|---|---|---|---|
| | | | |

*Witness is impartial and was present for the consent process.

# Appendix B Approved birth control methods (for sample informed consent form)

You should not become pregnant during the study because we do not know how the study products could affect the developing baby.

You must agree to use effective birth control from 21 days before your first injection until 6 months after your last study injection.

Effective birth control means using any of the following methods every time you have sex:

- Birth control drugs that prevent pregnancy—given by pills, shots, patches, vaginal rings, or inserts under the skin;
- Male or female condoms, with or without a cream or gel that kills sperm;
- Diaphragm or cervical cap with a cream or gel that kills sperm;
- Intrauterine device (IUD); or
- Any other contraceptive method approved by the researchers.

You do not have to use birth control if:

- You are only having sex with a partner or partners who have had a vasectomy. (We will ask you some questions to confirm that the vasectomy was successful.);
- You have reached menopause, with no menstrual periods for one year;
- You have had a hysterectomy (your uterus removed);
- You have had your ovaries removed;
- You have a tubal ligation (your "tubes tied") or confirmed successful placement of a product that blocks the fallopian tubes;
- You are having sex only with a female partner or partners;
- You only have oral sex; or,
- You are sexually abstinent (no sex at all).

Remember: If you are having sex, you need to use male or female condoms to protect yourself from HIV infection.

If you join the study, we will test you for pregnancy at some visits, including before each study injection.

# Appendix C Sample consent form for use of samples and information in other studies

Title: A phase 1 clinical trial to evaluate the safety and immunogenicity of pDNA vaccines expressing HIV M Group $p24^{Gag}$ conserved elements and/or $p55^{Gag}$, administered with *IL-12* pDNA by intramuscular electroporation, in healthy, HIV-uninfected adult participants

HVTN protocol number: HVTN 119

Site: [Insert site name]

When samples are no longer needed for this study, the HVTN wants to keep them for use in other studies. We will call these "extra samples."

This form gives you information so you can decide if you want your extra samples and information used in other studies. You will mark your decision at the end of the form. If you have any questions, please ask.

**1. Do I have to agree?**

No. You are free to say yes or no, or to change your mind after you sign this form. At your request, we will destroy all extra samples that we have. Your decision will not affect your being in this study or have any negative consequences here.

**2. Where are the samples stored?**

Extra samples are stored in a secure central place called a repository. Your samples will be stored in the HVTN repository in the United States.

**3. How long will the samples be stored?**

There is no limit on how long your extra samples will be stored. *[Site: insert limits if your regulatory authority imposes them.]*

**4. Will I be paid for the use of my samples?**

No. Also, a researcher may make a new scientific discovery or product based on the use of your samples. If this happens, there is no plan to share any money with you. The researcher is not likely to ever know who you are.

**5. Will I benefit from allowing my samples to be used in other studies?**

Probably not. Results from these other studies are not given to you, this clinic, or your doctor. They are not part of your medical record. The studies are only being done for research purposes.

**6. Will the HVTN sell my samples and information?**

No, but the HVTN may share your samples with other researchers. Once we share your samples and information, we will not be able to get them back.

**7. How do other researchers get my samples and information?**

When a researcher wants to use your samples and/or information, their research plan must be approved by the HVTN. Also, the researcher's institutional review board (IRB) or ethics committee (EC) will review their plan. *[Site: If review by your institution's IRB/EC/RE is also required, insert a sentence stating this.]* IRBs/ECs protect the rights and well-being of people in research. If the research plan is approved, the HVTN will send your samples to the researcher's location.

**8. What information is shared with other researchers?**

The samples and limited information will be labeled with a code number. Your name will not be part of the information. However, some information that we share may be personal, such as your race, ethnicity, gender, health information from the study, and HIV status. We may share information about the study product you received and how your body responded to the study product.

**9. What kind of studies might be done with my extra samples and information?**

- The studies will be related to HIV, vaccines, the immune system and other diseases.

Researchers may also do genetic testing related to this study on your samples.

If you become HIV infected, the researchers may look at all of the genes of the virus found in your samples. The researchers will use this information to learn more about HIV and the study product(s).

In some cases, researchers may take cells from your samples and grow more of them over time, so that they can continue to contribute to this study.

If you agree, your samples could also be used for genome wide studies. In these studies, researchers will look at all of your genes (your genome). The researchers compare the genomes of many people, looking for common patterns of genes that could help them understand diseases. The researchers may put the information from the genome-wide studies into a protected database so that other researchers can access it. Usually, no one would be able to look at your genome and link it to you as a person. However, if another database exists that also has information on your genome and your name, someone might be able to compare the databases and identify you. If others found out, it could lead to discrimination or other problems. The risk of this is very small.

**10. What are the risks of genetic testing?**

The genetic testing could show you may be at risk for certain diseases. If others found out, it could lead to discrimination or other problems. However, it is almost impossible for you or others to know your test results from the genetic testing. The results are not part of your study records and are not given to you.

In the very unlikely event that your genetic information becomes linked to your name, a federal law called the Genetic Information Nondiscrimination Act (GINA) helps protect you. GINA keeps health insurance companies and employers from seeing results of genetic testing when deciding about giving you health insurance or offering you work.

GINA does not help or protect you against discrimination by companies that sell life, disability or long-term care insurance.

**11. Who will have access to my information in studies using my extra samples?**

People who may see your information are:

- Researchers who use your stored samples and limited information for other research
- Government agencies that fund or monitor the research using your samples or information
- The researcher's Institutional Review Board or Ethics Committee
- The people who work with the researcher

All of these people will do their best to protect your information. The results of any new studies that use your extra samples or information may be published. No publication will use your name or identify you personally.

## Questions

**12. If you have questions or problems about allowing your samples and information to be used in other studies, use the following important contacts.**

If you have questions about the use of your samples or information or if you want to change your mind about their use, contact
[name and telephone number of the investigator or other study staff].

If you think you may have been harmed because of studies using your samples or information, contact [name and telephone number of the investigator or other study staff].

If you have questions about your rights as a research participant, contact
[name/title/phone of person on IRB or other appropriate organization].

**13. Please choose only one of the options below and write your initials or make your mark in the box next to it. Whatever you choose, the HVTN keeps track of your choice about how your samples and information can be used.**

☐ I allow my extra samples combined with limited information to be used for other studies related to HIV, vaccines, the immune system, and other diseases. This may include genetic testing and keeping my cells growing over time.

**OR**

☐ I agree to the option above and also to allow my extra samples combined with limited information to be used in genome wide studies.

**OR**

☐ I do not allow my extra samples to be used in any other studies. This includes not allowing genetic testing, growing more of my cells, or genome wide studies.

| Participant's name (print) | Participant's signature or mark | Date | Time |
|---|---|---|---|
| | | | |

| Clinic staff conducting consent discussion (print) | Clinic staff signature | Date | Time |
|---|---|---|---|
| | | | |

For participants who are unable to read or write, a witness should complete the signature block below:

| Witness's name (print) | Witness's signature | Date | Time |
|---|---|---|---|
| | | | |

*Witness is impartial and was present for the consent process

# Appendix D Table of procedures (for sample informed consent form)

| Procedure | Screening visit(s) | First injection visit | Time after first injection visit (in months) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0.5 | 1 | 1.25 | 1.5 | 3 | 3.5 | 6 | 6.25 | 6.5 | 9 | 12 | 18[2] |
| Injection | | √ | | √ | | | √ | | √ | | | | | |
| Medical history | √ | | | | | | | | | | | | | |
| Complete physical | √ | | | | | | | | | | | | √ | |
| Brief physical | | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | | |
| Urine test | √ | | √ | | | | | | | | √ | | | |
| Blood drawn | √ | √ | √ | | √ | √ | √ | √ | √ | √ | √ | √ | √ | |
| Pregnancy test (participants born female)[1] | √ | √ | | √ | | | √ | | √ | | | √ | | |
| HIV testing & pretest counseling | √ | | | | | | √ | | √ | | | √ | √ | |
| Risk reduction counseling | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | |
| Interview/questionnaire | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | |
| Health contact | | | | | | | | | | | | | | √ |

Not shown in this table is a time after all study participants have completed their last scheduled visit when you can find out what products you received.

[1]Persons who had a total hysterectomy (removal of the uterus verified by medical records) or removal of both ovaries (verified by medical records), are not required to have a pregnancy test.

[2]Visit at 18 months is a health contact visit.

# Appendix E Laboratory procedures

| | | | | | Tube volume (mL) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *Visit:* | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14**[10] | |
| | | | | *Day:* | Screening visit[3] | D0 | D14 | D28 | D35 | D42 | D84 | D98 | D168 | D175 | D182 | D273 | D364 | D546 | |
| | | | | *Week:* | | W0 | W2 | W4 | W5 | W6 | W12 | W14 | W24 | W25 | W26 | W39 | W52 | W78 | |
| | | | | *Month:* | | M0 | M0.5 | M1 | M1.25 | M1.5 | M3 | M3.5 | M6 | M6.25 | M6.5 | M9 | M12 | M18 | |
| | | | | | | **VAC1** | | **VAC2** | | | **VAC3** | | **VAC4** | | | | | | |
| **Procedure** | **Ship to**[1] | **Assay Location**[2] | **Tube**[4] | **Tube size (vol. capacity)**[4] | | | | | | | | | | | | | | | **Total** |
| BLOOD COLLECTION | | | | | | | | | | | | | | | | | | | |
| Screening or diagnostic assays | | | | | | | | | | | | | | | | | | | |
| Screening HIV test | Local Lab | Local Lab | SST | 5mL | 5 | — | — | — | — | — | — | — | — | — | — | — | — | — | 5 |
| HBsAg/anti-HCV | Local Lab | Local Lab | SST | 5mL | 5 | — | — | — | — | — | — | — | — | — | — | — | — | — | 5 |
| Syphilis | Local Lab | Local Lab | SST | 5mL | 5 | — | — | — | — | — | — | — | — | — | — | — | — | — | 5 |
| HIV diagnostics[9] | UW-VSL | UW-VSL | EDTA | 10mL | — | — | — | — | — | — | 10 | — | 10 | — | — | 10 | 20[9] | — | 50 |
| Safety labs | | | | | | | | | | | | | | | | | | | |
| CBC/ Diff/ platelets | Local lab | Local lab | EDTA | 5mL | 5 | — | 5 | — | — | 5 | — | 5 | — | 5 | — | 5 | — | — | 30 |
| Chemistry panel[5] | Local lab | Local lab | SST | 5mL | 5 | — | 5 | — | — | 5 | — | 5 | — | — | 5 | 5 | — | — | 30 |
| Immunogenicity assays[6] | | | | | | | | | | | | | | | | | | | |
| Host genetics[7] | CSR | HVTN Labs | ACD | 8.5mL | — | 17 | — | — | — | — | — | — | — | — | — | — | — | — | 17 |
| Cellular assays | | | | | | | | | | | | | | | | | | | |
| ICS | CSR | HVTN Labs | ACD | 8.5mL | — | 42.5 | — | — | — | *42.5* | — | — | — | — | *42.5* | — | 68 | — | 195.5 |
| pTfh | CSR | HVTN Labs | ACD | 8.5mL | — | — | — | — | 42.5 | — | — | — | — | 42.5 | — | — | — | — | 85 |
| Epitope mapping | CSR | HVTN Labs | ACD | 8.5mL | — | — | — | — | — | *59.5* | — | — | — | — | *127.5* | — | — | — | 187 |
| T-cell repertoire | CSR | Vanderbilt | ACD | 8.5mL | — | 25.5 | — | — | — | — | — | 25.5 | — | — | 25.5 | — | 25.5 | — | 102 |
| Humoral assays | | | | | | | | | | | | | | | | | | | |
| Binding Ab | CSR | HVTN Labs | SST | 8.5mL | — | 8.5 | — | — | — | 8.5 | — | — | — | — | *8.5* | — | 8.5 | — | 34 |
| Specimen storage | | | | | | | | | | | | | | | | | | | |
| PBMC | CSR | | ACD | 8.5mL | — | 42.5 | — | — | 25.5 | 68 | — | 85 | — | 25.5 | 85 | — | 85 | — | 416.5 |
| Plasma | CSR | | ACD | 8.5mL | — | z | — | — | z | z | — | z | — | z | z | — | z | — | 0 |
| Serum | CSR | | SST | 8.5mL | — | 17 | — | — | 8.5 | 17 | — | 17 | — | 8.5 | 17 | — | 17 | — | 102 |
| **Visit total** | | | | | **25** | **153** | **10** | **0** | **76.5** | **205.5** | **10** | **137.5** | **10** | **81.5** | **311** | **20** | **224** | **0** | **1264** |
| **56-Day total** | | | | | **25** | **178** | **188** | **188** | **264.5** | **470** | **292** | **353** | **10** | **91.5** | **402.5** | **20** | **224** | **0** | |
| URINE COLLECTION | | | | | | | | | | | | | | | | | | | |
| Urine dipstick[11] | Local lab | Local lab | | | X | — | X | — | — | — | — | — | — | — | X | — | — | — | |
| Pregnancy test[8] | Local lab | Local lab | | | X | X | — | X | — | — | X | — | X | — | — | X | — | — | |

[1]CSR = central specimen repository; UW-VSL = University of Washington Virology Specialty Laboratory (Seattle, Washington, USA).

[2]HVTN Laboratories include: Fred Hutchinson Cancer Research Center (Seattle, Washington, USA); Duke University Medical Center (Durham, North Carolina, USA). Non-HVTN laboratory: Vanderbilt (Nashville, Tennessee, USA).

[3]Screening may occur over the course of several contacts/visits up to and including day 0 prior to vaccination.

[4]Local labs may assign appropriate alternative tube types for locally performed tests.

[5]Chemistry panels are defined in Section 9.2 (pre-enrollment) and Section 9.4 (postenrollment).

[6]Immunogenicity assays will be performed at M0 (for binding Ab assay), and M1.5 and M6.5. Based on the number of responders observed at these timepoints, lab assays may be performed on participants for humoral and cellular responses at other time points.

[7]Genotyping may be performed on enrolled participants using cryopreserved PBMC collected at baseline, initially in participants who demonstrate vaccine-induced T-cell responses at postvaccination timepoints.

[8]For a participant born female, pregnancy test must be performed on the day of vaccination with negative results received prior to vaccination. Persons who are NOT of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing.

[9]At an early termination visit for a withdrawn or terminated participant (see Section 9.11), blood should be drawn for HIV diagnostic testing, as shown for visit 13 above.

[10]For information concerning the Month 18 health contact, see Section 9.5. Clinic visits are not required except that any participant reporting a diagnosis of HIV infection from testing outside the HVTN will be asked to come to the clinic to collect specimens for HIV testing with HVTN HIV diagnostic algorithms.

[11]And microscopy if needed.

z= Up to 10 x 1mL aliquots of ACD plasma will be harvested for storage during PBMC processing; no separate blood draw is needed.

# Appendix F Procedures at HVTN CRS

| Visit: | 01[1] | 02 | 03 | 04 | 05 | 06 | 07 | 08 | 09 | 10 | 11 | 12 | 13 | 14[8] | Post |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day: | | D0 | D14 | D28 | D35 | D42 | D84 | D98 | D168 | D175 | D182 | D273 | D364 | D546 | |
| Month: | | M0 | M0.5 | M1 | M1.25 | M1.5 | M3 | M3.5 | M6 | M6.25 | M6.5 | M9 | M12 | M18 | |
| Procedure | Scr. | VAC1 | | VAC2 | | | VAC3 | | VAC4 | | | | | | |
| **Study procedures[2]** | | | | | | | | | | | | | | | |
| Signed screening consent (if used) | X | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Assessment of understanding | X | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Signed protocol consent | X | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Medical history | X | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Complete physical exam | X | — | — | — | — | — | — | — | — | — | — | — | X | — | — |
| Abbreviated physical exam | — | X | X | X | X | X | X | X | X | X | X | X | — | — | — |
| Risk reduction counseling | X | X | X | X | X | X | X | X | X | X | X | X | X | — | — |
| Pregnancy prevention assessment[3] | X | X | X | X | X | X | X | X | X | X | X | X | X | — | — |
| Behavioral risk assessment | X | — | — | — | — | — | — | — | — | X | — | — | X | — | — |
| Confirm eligibility, obtain demographics, randomize | X | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Social impact assessment | — | X | X | X | X | X | X | X | X | X | X | X | X | — | — |
| Social impact assessment questionnaire | — | — | — | — | — | — | X | — | X | — | — | — | X | — | — |
| Outside testing and belief questionnaire | — | — | — | — | — | — | — | — | X | — | — | — | X | — | — |
| Concomitant medications | X | X | X | X | X | X | X | X | X | X | X | X | X | — | — |
| Intercurrent illness/adverse experience | — | X | X | X | X | X | X | X | X | X | X | X | X | — | — |
| HIV infection assessment[4] | X | — | — | — | — | — | X | — | X | — | — | X | X | — | — |
| Confirm HIV test results provided to participant | — | X | — | — | — | — | — | X | — | X | — | — | X | X | — |
| Visual Analog Pain Scale (Pain Assessment) | — | X | — | X | — | — | X | — | X | — | — | — | — | — | — |
| Acceptability questionnaire | — | — | X | — | X | — | — | X | — | X | — | — | — | — | — |
| **Local lab assessment** | | | | | | | | | | | | | | | |
| Urine dipstick | X | — | X | — | — | — | — | — | — | — | X | — | — | — | — |
| Pregnancy (urine or serum HCG)[5] | X | X | — | X | — | — | X | — | X | — | — | X | — | — | — |
| CBC, differential | X | — | X | — | — | X | — | X | — | X | — | X | — | — | — |
| Chemistry panel (see Section 9.2) | X | — | X | — | — | X | — | X | — | — | X | X | — | — | — |
| Syphilis, Hepatitis B, Hepatitis C | X | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| **Vaccination procedures** | | | | | | | | | | | | | | | |
| Vaccination[6] | — | X | — | X | — | — | X | — | X | — | — | — | — | — | — |
| Reactogenicity assessments[7] | — | X | — | X | — | — | X | — | X | — | — | — | — | — | — |

| Visit: | **01[1]** | **02** | **03** | **04** | **05** | **06** | **07** | **08** | **09** | **10** | **11** | **12** | **13** | **14[8]** | **Post** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Day: | | D0 | D14 | D28 | D35 | D42 | D84 | D98 | D168 | D175 | D182 | D273 | D364 | D546 | |
| Month: | | M0 | M0.5 | M1 | M1.25 | M1.5 | M3 | M3.5 | M6 | M6.25 | M6.5 | M9 | M12 | M18 | |
| Procedure | Scr. | VAC1 | | VAC2 | | | VAC3 | | VAC4 | | | | | | |
| **Poststudy** | | | | | | | | | | | | | | | |
| Unblind participant | — | — | — | — | — | — | — | — | — | — | — | — | — | — | X |

[1] Screening may occur over the course of several contacts/visits up to and including day 0 prior to vaccination.
[2] For specimen collection requirements see Appendix E.
[3] Pregnancy prevention assessment is required only for participants who were born female and are capable of becoming pregnant.
[4] Includes pre-test counseling. A subsequent follow-up contact is conducted to provide post-test counseling and to report results to participant.
[5] For a participant who was born female, pregnancy test must be performed on the day of vaccination prior to vaccination. Pregnancy test to determine initial eligibility may be performed at screening, but must also be done on Day 0 prior to first vaccination. Persons who are NOT of reproductive potential due to having undergone total hysterectomy or bilateral oophorectomy (verified by medical records) are not required to undergo pregnancy testing. Serum pregnancy test may be used to confirm the results of, or substitute for, a urine pregnancy test.
[6] Blood draws required at vaccination visits must be performed prior to administration of study product, however it is not necessary to have results prior to administration, except for results of a serum pregnancy test, if indicated. Lab tests may be drawn within the 3 days prior to vaccination.
[7] Reactogenicity assessments performed daily for at least7 days postvaccination (see Section 9.9).
[8] For information concerning the Visit 14 health contact, see Section 9.5. Clinic visits are not required, except that any participant reporting a diagnosis of HIV infection will be asked to come to the clinic so that HIV status can be confirmed.

# Appendix G Adverse events of special interest

AEs of special interest (AESI) for this protocol include but are not limited to autoimmune disorders; representative examples of AESI are listed below. Updates to AESI will be provided as an appendix to the *HVTN 119 Study Specific Procedures*.

| Neuroinflammatory disorders | Musculoskeletal disorders | Skin disorders |
|---|---|---|
| • Cranial nerve disorders, including paralyses/paresis (eg Bell's palsy)<br>• Optic neuritis<br>• Multiple sclerosis<br>• Transverse myelitis<br>• Guillain-Barré syndrome, including Miller Fisher syndrome and other variants<br>• Acute disseminated encephalomyelitis, including site specific variants: eg non-infectious encephalitis, encephalomyelitis, myelitis, myeloradiculoneuritis<br>• Myasthenia gravis, including Lambert-Eaton myasthenic syndrome<br>• Immune-mediated peripheral neuropathies and plexopathies, (including chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy and polyneuropathies associated with monoclonal gammopathy).<br>• Narcolepsy | • Systemic lupus erythematosus and associated conditions<br>• Systemic scleroderma (Systemic sclerosis), including diffuse systemic form and CREST syndrome<br>• Idiopathic inflammatory myopathies, including dermatomyositis<br>• Polymyositis<br>• Antisynthetase syndrome<br>• Rheumatoid arthritis, and associated conditions including juvenile chronic arthritis and Still's disease<br>• Polymyalgia rheumatica<br>• Spondyloarthritis, including ankylosing spondylitis, reactive arthritis (Reiter's Syndrome) and undifferentiated spondyloarthritis<br>• Psoriatic arthropathy<br>• Relapsing polychondritis<br>• Mixed connective tissue disorder | • Psoriasis<br>• Vitiligo<br>• Erythema nodosum<br>• Autoimmune bullous skin diseases (including pemphigus, pemphigoid and dermatitis herpetiformis)<br>• Alopecia areata<br>• Lichen planus<br>• Sweet's syndrome<br>• Localized Scleroderma (Morphea)<br>• Cutaneous lupus erythematosus<br>**Metabolic disorders**<br>• Addison's disease<br>• Autoimmune thyroiditis (including Hashimoto thyroiditis)<br>• Diabetes mellitus type I<br>• Grave's or Basedow's disease |
| **Vasculitides** | **Blood disorders** | **Others** |
| • Large vessels vasculitis including: giant cell arteritis such as Takayasu's arteritis and temporal arteritis.<br>• Medium sized and/or small vessels vasculitis including: polyarteritis nodosa, Kawasaki's disease, microscopic polyangiitis, Wegener's granulomatosis, Churg-Strauss syndrome (allergic granulomatous angiitis), Buerger's disease (thromboangiitis obliterans), necrotizing vasculitis and anti-neutrophil cytoplasmic antibody (ANCA) positive vasculitis (type unspecified), Henoch-Schonlein purpura, Behcet's syndrome, leukocytoclastic vasculitis. | • Autoimmune hemolytic anemia<br>• Autoimmune thrombocytopenia<br>• Antiphospholipid syndrome<br>• Pernicious anemia<br>• Autoimmune aplastic anemia<br>• Autoimmune neutropenia<br>• Autoimmune pancytopenia<br>**Gastrointestinal disorders**<br>• Celiac disease<br>• Crohn's disease<br>• Ulcerative colitis<br>• Ulcerative proctitis<br>**Liver disorders**<br>• Autoimmune cholangitis<br>• Autoimmune hepatitis<br>• Primary biliary cirrhosis<br>• Primary sclerosing cholangitis | • Autoimmune glomerulonephritis (including IgA nephropathy, glomerulonephritis rapidly progressive, membranous glomerulonephritis, membranoproliferative glomerulonephritis, and mesangioproliferative glomerulonephritis)<br>• Ocular autoimmune diseases (including autoimmune uveitis and autoimmune retinopathy)<br>• Autoimmune myocarditis/cardiomyopathy<br>• Sarcoidosis<br>• Stevens-Johnson syndrome<br>• Sjögren's syndrome<br>• Idiopathic pulmonary fibrosis<br>• Goodpasture syndrome<br>• Raynaud's phenomenon |