PROTOCOL

HVTN 098

A phase 1 clinical trial to evaluate the safety and immunogenicity of PENNVAX®-GP (*gag*, *pol*, *env*) DNA vaccine and *IL-12* plasmid, delivered via intradermal or intramuscular electroporation in healthy, HIV–uninfected adult participants

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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 PRODUCTS PROVIDED BY

Inovio Pharmaceuticals, Inc.
Plymouth Meeting, Pennsylvania, USA

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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 recognizes the importance of institutional review and values the role of in country Institutional Review Boards (IRBs) and Ethics Committees (ECs) 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 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 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 (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 subject 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 098 Protocol Safety Review Team (PSRT). In addition, the HVTN Safety Monitoring Board (SMB) or a Data and Safety Monitoring Board (DSMB) 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 a Confidentiality Agreement 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 PENNVAX®-GP (gag, pol, env) DNA vaccine and IL-12 plasmid, delivered via intradermal or intramuscular electroporation in healthy, HIV–uninfected adult participants

Primary objective

To evaluate the safety and tolerability of PENNVAX®-GP, an HIV-1 *env* A, *env* C, *gag*, *pol* plasmid deoxyribonucleic acid (DNA) vaccine, and interleukin 12 (*IL-12*) DNA, given by intradermal (ID) or intramuscular (IM) injection with electroporation (EP), in healthy HIV-uninfected adult volunteers

Study products and routes of administration

- **Vaccine: PENNVAX®-GP:** PENNVAX®-GP (10 mg/mL) is an admixture of SynCon® INO-6112 (*env* A/ *env* C) with SynCon® INO-6145(*Mpol* / *gag*). SynCon® INO-6112 consists of 2 plasmids encoding for synthetic consensus clade A and C HIV-1 envelope protein. SynCon® INO-6145 consists of 2 plasmids containing synthetic HIV-1 multiclade (A, B, C and D) consensus *pol*, and *gag*. Specific doses for each component are indicated in Table 3-1 below for each study group.

- **Adjuvant: *IL-12* DNA: *IL-12* DNA (**INO-9012) (10 mg/mL) consists of a single plasmid containing a dual promoter system for expression of both the IL-12 p35 and p40 genes necessary for production of the active heterodimeric IL-12 protein.

- **Placebo:** Sterile Water for Injection, USP

- **Electroporation device:** The CELLECTRA® Adaptive Constant Current Electroporation Device is a portable, battery-powered medical device designed to facilitate the introduction of DNA into the muscle or skin through EP. The CELLECTRA® 3P EP system will be used for intradermal delivery. The CELLECTRA® 5P EP system will be used for intramuscular delivery.

Table 3-1 Schema

				PENNVAX®-GP					Adjuvant
Group	N		Vol per injection	env A	env C	gag	pol	Total PENNVAX®-GP	Total IL-12 DNA
1	5	*ID*	0.1 mL	0.1 mg	0.1 mg	0.2 mg	0.2 mg	0.6 mg	0.2 mg
	1	*ID*	0.1 mL	Placebo	Placebo	Placebo	Placebo	Placebo	Placebo
2	20	*ID**	0.1 mL (2 sites)	0.6 mg	0.6 mg	0.2 mg	0.2 mg	1.6 mg	0 mg
	2	*ID**	0.1 mL (2 sites)	Placebo	Placebo	Placebo	Placebo	Placebo	Placebo
3	30	*ID**	0.1 mL (2 sites)	0.6 mg	0.6 mg	0.2 mg	0.2 mg	1.6 mg	0.4 mg
	3	*ID**	0.1 mL (2 sites)	Placebo	Placebo	Placebo	Placebo	Placebo	Placebo
4	30	*IM*	1 mL	3 mg	3 mg	1 mg	1 mg	8 mg	1 mg
	3	*IM*	1 mL	Placebo	Placebo	Placebo	Placebo	Placebo	Placebo
Total 94 (85 vaccine/9 placebo)									

Notes

*Groups 2 and 3 receive the doses of PENNVAX®-GP and *IL-12* DNA indicated divided over 2 injection sites on the arm(s). Each of 2 injection sites will receive 0.1 mL ID.

Participants in all groups are scheduled to receive injections at four timepoints: day 0, months 1, 3, and 6.

All injections are given with EP. ID injections are given into the skin overlying the deltoid, with EP using the CELLECTRA® 3P EP system. IM injections are given into the deltoid using the CELLECTRA® 5P EP System.

Enrollment begins with Group 1. Enrollment in Group 1 will be restricted to one person per day across all sites. Enrollment will then be held until all available safety and reactogenicity data reported through day 14 for participants in Group 1 are reviewed by the HVTN 098 PSRT. If the data are acceptable, Groups 2, 3 and 4 will open to enrollment simultaneously. For Groups 2, 3, and 4, enrollment will be restricted to one person per day across all participating HVTN CRSs for the first 15 participants (5 participants in each group). Enrollment will then be held until all available safety and reactogenicity data from day 14 from these 15 participants will be reviewed. If the data are acceptable, Groups 2, 3 and 4 will reopen to enrollment.

If ID administration of the study products in Group 1, 2, or 3 results in significant local reactogenicity and/or injection site AEs related to the route of delivery, but no other significant safety issues are identified that would be expected to occur with IM administration, the HVTN 098 PSRT may opt to proceed with enrollment for Group 4 only.

Participants

Healthy, HIV–uninfected volunteers aged 18 to 55 years: 85 vaccinees, 9 placebo recipients

Design

Multicenter, randomized, placebo-controlled, double-blind trial

Duration per participant

18 months of scheduled clinic visits

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

- **PENNVAX®-GP**: Inovio Pharmaceuticals, Inc. (Plymouth Meeting, Pennsylvania, USA)
- ***IL-12* DNA**: Inovio Pharmaceuticals, Inc. (Plymouth Meeting, Pennsylvania, USA)

Electroporator provider

- **CELLECTRA® EP System**: Inovio Pharmaceuticals, Inc. (Plymouth Meeting, Pennsylvania, 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)

HVTN Statistical and Data Management Center (SDMC), FHCRC (Seattle, Washington, USA)

HIV diagnostic laboratory

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

Endpoint assay laboratories

- FHCRC/University of Washington (Seattle, Washington, USA)

- Duke Human Vaccine Institute, Duke University Medical Center (Durham, North Carolina, USA)
- Duke Neutralizing Antibody Assay Laboratory, Duke University Medical Center (Durham, North Carolina, USA)
- Duke Antibody-Dependent Cellular Cytotoxicity Assay Laboratory, Duke University Medical Center (Durham, North Carolina, USA)
- Vanderbilt University (Nashville, Tennessee, USA)

Study sites

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

Safety monitoring

HVTN 098 PSRT; HVTN SMB

3.1 Protocol Team

Protocol leadership

Chair	Srilatha Edupuganti Emory University 404-712-1434 sedupug@emory.edu	*Statistician*	Xuesong Yu SCHARP, FHCRC 206-667-7008 xyu@scharp.org
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Other contributors to the original protocol

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	Niranjan Y. Sardesai Inovio, Inc.	*Clinical trials manager*	Marianne Hansen HVTN Core, FHCRC
	Jessica Lee Inovio, Inc.		
	Mark Bagarazzi Inovio, Inc.	*Project manager*	Huguette Redinger SCHARP, FHCRC
Laboratory Program representative	Eva Chung HVTN Laboratory Program, FHCRC	*DAIDS Project Officer*	Jeff Pullen DAIDS, NIAID
Regulatory affairs	Renee Rivers HVTN Core, FHCRC	*Protocol development coordinator*	Ramey Fair HVTN Core, FHCRC
Clinic coordinator	Vickie Grimes Emory University	*Community engagement unit representative*	Gail Broder HVTN Core, FHCRC
Community Advisory Board (CAB) members	Brian Green University of Pennsylvania CAB	*Technical editor*	Adi Ferrara HVTN Core, FHCRC
	Justin Wooley University of Illinois - Chicago CAB	*Technical editor*	Erik Schwab HVTN Core, FHCRC
Community educator/recruiter	Marcus Bolton Emory University		

4 Background

4.1 Rationale for trial concept

DNA-based immunization has a number of advantages that make it an attractive vaccine platform [4]. First, it has an improved safety profile compared to live attenuated viruses, which may revert back to their virulent form or spread to unintended individuals. DNA vaccines are simple and relatively inexpensive to construct, are readily produced in large quantities, and are generally stable for long periods of time. If DNA vaccination proves to be efficacious, production and delivery to individuals in low and middle income countries may be more economically and logistically feasible than with other types of vaccines. Taken together, these advantages make DNA-based immunizations a desirable vaccine modality for HIV.

Early immunogenicity studies using DNA vaccines in numerous clinical trials related to infectious diseases did not live up to expectations. More recently, several groups have been developing methods to improve the immune responses of DNA vaccines, such as EP, use of cytokine adjuvants, genetic optimization and use of highly concentrated DNA formulations, multiple RNA optimizations and the addition of improved leader sequences. Inovio Pharmaceuticals has been very active in this area, employing all these strategies, and has particularly focused its work on improving the transfection of DNA using *in vivo* EP. This physical process exposes the target tissue to a brief electric field pulse that induces temporary and reversible pores in the cell membrane and has been shown to be an efficient way to introduce DNA into cells [5,6]. This technology has been used for more than three decades by molecular biologists for cell transfection. To date, however, EP remains experimental in humans; it has not been licensed by the FDA for clinical use. More recently, clinical applications of EP have been tested in treatment of cancer and gene therapy [7-9].

For HIV DNA vaccines, the hypothesis is that EP will increase the uptake and processing of plasmid DNA (DNA), generating significantly increased immunogenicity. Recent studies have demonstrated the ability of EP to augment HIV-specific cellular immune responses in mice [10], and simian immunodeficiency virus (SIV)-specific immune responses in macaques [11]. Luckay *et al.* found that IM EP of SIV DNA vaccines increased the potency of these vaccines 50-200 fold [11]. There are various means of EP (i.e., constant current vs. constant voltage) and testing these strategies in mice and pigs has shown that a constant current device may be the most efficient at generating immune responses [12]. Studies in macaques found that EP of SIV DNA+*IL-12* plasmids yielded 10-fold higher responses than DNA without EP [13], and that this immune response was boosted with additional vaccinations. The magnitude of achievable responses is similar to that seen with natural SIV infection, on the order of 10,000 spot forming units (SFU)/million peripheral blood mononuclear cells (PBMC) in an enzyme-linked immunospot (ELISpot) assay. These responses are also polyfunctional, as defined by their ability to generate interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α) and IL-2. Following successful proof-of-concept studies in animals, Inovio has optimized both pulse pattern and voltage to increase transfection efficiency. The early exciting results in human clinical trials with HIV (particularly data from the recently completed HVTN 080 clinical protocol) and other vaccine candidates merit consideration for further HIV DNA vaccine trials using EP.

Preclinical studies showed that the immunogenicity of DNA vaccines can be substantially increased by the use of cytokine adjuvants [14-22]. Coadministration of cytokine plasmids with HIV DNA vaccines has been studied in rodents by the Weiner Laboratory [23]. These studies demonstrated a dramatic increase in specific cytotoxic T-lymphocyte (CTL) activity when a *gag*/*pol* plasmid or an *env* plasmid was coadministered with an *IL-12* plasmid, as compared with results in animals receiving *env* or *gag*/*pol* plasmids alone. The molecular adjuvant activity of several Th1 cytokines (GM-CSF, IL-2, IL-12, IL-15, and IL-18) was then evaluated in mice in a subsequent study by the Weiner Laboratory [17]. This study revealed that the *IL-12* plasmid was the best driver of major histocompatibility complex (MHC)-restricted CD8+ CTL activity. Codelivery of *IL-12* DNA and HIV DNA vaccines was also evaluated by the Weiner Laboratory in macaques and chimpanzees with enhanced responses to DNA immunogens with *IL-12* plasmid injections [24-26]. Wyeth also demonstrated a substantial enhancement of cellular immune responses with *IL-12* DNA and *gag* DNA administration compared with *gag* DNA alone in macaques. *IL-12* is a p70 disulfide-linked heterodimer composed of two separately encoded subunits, a heavy chain of 40 kDa and a 35-kDa light chain [27-29]. Although p35 gene transcripts are rather ubiquitous, p40 transcripts are exclusive of cells producing biologically active IL-12, which include monocytes, macrophages, dendritic cells, polymorphonuclear leukocytes, and B cells [29]. The functional consequence of coadministered DNA+*IL-12* is an improved ability of HIV-specific CD8+ T cells to proliferate in culture in response to antigenic stimulation [13].

Results of earlier studies with DNA vaccination with *IL-12* DNA as a molecular adjuvant (but without EP mediated delivery) in clinical trials of HIV vaccines did not replicate the results of using *IL-12* plasmid in nonhuman primates [30]. HVTN 060 (DAIDS-ES ID 10057) tested the immunogenicity of a truncated (p37) *gag* DNA vaccine (1.5 mg) with and without *IL-12* DNA adjuvant (GENEVAX® *IL-12*, up to 1.5 mg). HVTN 063 (DAIDS-ES ID 10058) tested the immunogenicity of p37 *gag* DNA vaccine (1.5 mg) with and without an IL-15 DNA adjuvant (up to 1.5 mg), followed in some participants by *gag* DNA vaccine with *IL-12* DNA (1.5mg each). In both trials, which gave injections IM without EP, the combination of the *gag* DNA vaccine and *IL-12* DNA at these doses were well tolerated. However, few vaccine-induced T-cell responses were detected by IFN-γ ELISpot to consensus *gag* peptides in either protocol.

4.1.1 PENNVAX®-B and the effects of electroporation

PENNVAX®-B, a multigene HIV-1 DNA immunogen (clade B *gag*, *pol*, *env* plasmids) was studied in HVTN 070 (BB-IND 13449), alone or adjuvanted with plasmid *IL-12* (GENEVAX® *IL-12*) which had been highly optimized for gene expression. HVTN 070 enrolled 120 participants and 100 of them (83%) received all four vaccinations. After 4 vaccinations with DNA by IM injection (without EP), 38.5% (10/26) of subjects developed an HIV-specific CD4+ T-cell response and 7.4% (2/27) had a detectable CD8+ T-cell response. These results were not improved by the addition of plasmid *IL-12* (40.7% [11/27] subjects had CD4+ responses; 3.6% [1/28] individuals had CD8+ responses). Thus, it is possible that DNA-based strategies, such as using full length *gag*, additional HIV genes, higher doses of plasmids, and optimizing the plasmids for higher levels of gene expression, may only have a limited impact on the immunogenicity of DNA vaccines. The reasons behind the striking differences in immunogenicity between animals and humans are unclear, but may be the poor uptake of DNA into cells, and the limited amount of protein that is expressed.

In contrast, the results with EP have been quite different. In the HVTN 080 protocol, PENNVAX®-B or placebo were delivered via IM injection with the CELLECTRA® EP device in 48 healthy human volunteers. The regimen consisted of 1 mg of each plasmid (clade B *gag*, *pol*, *env*) with or without a plasmid encoding the GENEVAX® *IL-12* adjuvant. Immunizations were given at months 0, 1 and 3. Vaccinations with EP were well tolerated with only two participants discontinuing vaccination due to injection site pain, and all but one of the participants responding that they were willing to undergo EP for a new vaccine against a serious disease for which they were at risk. In the group that received the 4 plasmid combination including *IL-12* adjuvant, CD4+ responses were detected in 67% and 81% of volunteers after 2 and 3 immunizations, respectively. CD8+ responses were observed in 36% after 2 immunizations and 52% of volunteers after 3 immunizations. Overall, 89% of volunteers produced either a CD4+ or CD8+ T-cell response with 11/27 subjects producing both. Furthermore, these T cell results compare favorably with responses observed by HVTN using other prime-boost approaches (see Table 4-1). Thus, this study establishes that a highly engineered DNA vaccine delivered using EP intramuscularly can induce robust T-cell response in humans [31].

Table 4-1 Summary of select HIV vaccine trials including DNA conducted by the HVTN

Protocol	Vaccines	Sequence	Schedule	%CD4 response*	%CD8 response
HVTN 073-SAAVI	DNA, MVA-C boost	D, D, D, M, M	Day 0, 28, 56, 112, 140	70	33
HVTN 205-GeoVax	DNA, MVA-B boost	D, D, M, M	Day 0, 56, 112, 140	66	22
HVTN 080-PENNVAX®-B	DNA/IL+12/EP	D, D, D	Day 0, 28, 84	81	52

*measured following last vaccination
MVA = modified vaccinia Ankara

In HVTN 080, overall immune responses were most often detected against HIV Pol(CD4+ response 69.2%, 18/26; CD8+ response 48.1%, 13/27) compared to HIV Gag(CD4+ response 65.4%, 17/26; CD8+ response 7.4%, 2/27) and HIV Env(CD4+ response 0%, 0/26; CD8+ response 14.8%, 4/27). In contrast to other DNA vaccination studies, Gag responses were frequent but very few individuals responded to HIV Env peptides. Binding antibody response to Env measured using the CON S antigen were also minimal and neutralizing antibody (nAb) responses to Tier 1 isolates were low. The reason for the lack of Env-specific immune responses in this study is not clear. PENNVAX®-B contained a synthetic consensus clade B *env* construct that was designed to generate a T-cell response. In addition, the 1 mg dose of *env* DNA was lower than in some other trials of HIV DNA vaccines, such as HVTN 204, which used 2 mg total *env* DNA per injection with Biojector. There was some concern that the peptide set used for evaluation of immune responses was not identically matched to the epitopes encoded by the plasmid DNA. However, additional assays using a set of peptides matched for the vaccine *env* plasmid did not significantly improve the number of Env responders. It is possible that Env priming was present but not detectable without a boost [32]. Finally, significant boosting of the immune response was repeatedly demonstrated in nonhuman primate (NHP) studies following a fourth vaccination, while the HVTN 080 protocol included only 3 total vaccinations.

Two clinical trials, HVTN 087 and IAVI B004, evaluated a different DNA vaccine (HIV-MAG, Profectus Biosciences, Inc., Tarrytown, NY) with or without GENEVAX® *IL-12* given IM with EP, and confirmed that EP could elicit T cell responses in the majority of volunteers (see sections 4.8.3.5 and 4.8.3.6). In HVTN 087, after 3 vaccinations, 77% (48 out of 62) and 40% (25 of 62) of vaccinated individuals mounted a CD4+ T-cell or CD8+ T-cell vaccine-specific ICS response, respectively. These responses included HIV Env-specific CD4+ responses in 69% (43/62) of participants. In IAVI B004, HIV-MAG given with EP elicited IFNγ ELISpot responses in 21/34 (62%) of participants. These study results are preliminary, but have confirmed that DNA vaccination with GENEVAX® *IL-12* DNA given by EP is immunogenic, and that Env responses may be elicited with a multiantigen DNA vaccine.

4.1.2 Intradermal electroporation

The use of *in vivo* EP has focused on IM delivery due to the durability of gene expression achievable from this tissue. Skin is an attractive target tissue for delivering DNA vaccines for multiple reasons: skin is the largest organ of the human body and readily accessible; it is highly immunocompetent, harboring Langerhans and other antigen-presenting cells, so it is capable of developing a broad immune response to antigens; it offers the possibility of reducing invasiveness by requiring less penetration and avoiding stimulation of muscle tissue.

While Inovio's current IM delivery technologies are well tolerated, the company is also advancing device development to achieve various desirable attributes. Its ID EP device penetrates to no more than 3 mm, compared to IM devices that penetrate up to 18 mm. Inovio's aim is to make EP delivery amenable to mass prophylactic vaccination by decreasing dose levels, increasing tolerability of the vaccination, and increasing the breadth of viable vaccine targets.

As indicated in Section 4.8.4, a phase 1 clinical trial of a novel DNA vaccine for influenza has shown that individuals reported lower pain scores with ID injection with EP compared to IM injection with EP. A preclinical study featuring a head-to-head comparison of an ID and IM-delivered 8-component smallpox vaccine in a NHP challenge model showed that the monkeys vaccinated with ID EP mounted stronger antibody responses and protection against a virus challenge compared to those vaccinated via IM EP. Even more significant, the antibody titers produced by ID EP vaccination in the monkey model were comparable to those produced by the currently stock-piled but no longer manufactured Dryvax™ smallpox vaccine in the same monkey model [33].

This trial will build on the promising CD4+ and CD8+ immune responses demonstrated with DNA vaccines delivered with EP in HVTN 080. The proposed trial has the potential to demonstrate improved T-cell responses and also elicit antibody responses to multi-envelope antigens (*env* A and C). Furthermore, this study will compare the immunogenicity of HIV immunogens delivered using the more tolerable ID delivery route. Finally, this study will provide support to product development decisions for evaluating antigens, cytokine adjuvants, dose, and route for potential phase 2 studies with these antigens.

To date, HIV DNA vaccines by themselves have not generated robust antibody responses without a vector or protein boost. The magnitude of antibody responses elicited by the proposed regimens would be expected to further increase with protein boost injections, as suggested by the guinea pig studies (Inovio Pharmaceuticals, unpublished results). If a

suitable protein boost becomes available, the study protocol can be amended to incorporate protein boost injections for consenting participants.

4.2 PENNVAX®- GP

The DNA vaccine in this protocol, PENNVAX®-GP (*env* A and C plasmids, consensus HIV-1 clade A, B, C and D *gag* and *pol* plasmids), was developed using SynCon® technology with the goal of increasing the magnitude and breadth of the immune response by increasing the cumulative coverage of potential T-cell epitopes (PTE) relative to clade B alone. This process involves synthetically deriving consensus genes and optimizing the DNA inserts at the genetic level to give them high expression capability in human cells. Briefly, to develop *gag* and *pol* multiclade consensus immunogens, HIV-1 clade A, B, C and D *gag* and *pol* consensus sequences were constructed by generating intraclade consensus sequences of *gag* and *pol* genes for each clade and then generating a single interclade consensus sequence from the four intraclade consensus sequences, thus avoiding bias towards heavily sequenced clades. Several additional modifications were introduced after generating multiclade *gag*, *pol* and clade A and C consensus *env* sequences. Specifically, the HIV *env* V1 and V2 regions were shortened based on the early transmitter sequences and the cytoplasmic tail was truncated to prevent envelope recycling [34]. A constitutive transport element was added to the *gag* and *pol* sequences. Three mutations were introduced into HIV *pol* to deactivate the protease, reverse transcriptase and RNase H. Additionally, the integrase protein was removed. An efficient IgE leader sequence was added to all DNA antigen sequences to improve expression. The resulting optimized HIV DNA immunogens were codon- and RNA-optimized, synthesized, and cloned into the pVAX1 expression vector to create optimized expression constructs. The vector contains the human cytomegalovirus (hCMV) immediate-early promoter for high-level expression in a wide range of mammalian cells, the bovine growth hormone (BGH) polyadenylation signal for efficient transcription termination and polyadenylation of mRNA, and a kanamycin resistance gene for selection in *E. coli*. The plasmids have been tested for expression *in vitro* and for immunogenicity in mice and guinea pigs. The current vaccine plasmids have been manufactured by VGXI, Inc. (The Woodlands, TX). The *env* A and C plasmids, and consensus *gag* plasmid are currently being evaluated in the clinical trial RV262 by US Military HIV Research Program (MHRP) (Section 4.8.1); however cell banks are newly manufactured for this study and the bulk plasmid product has been formulated in sterile water and at a higher concentration. The consensus *pol* plasmid has not previously been tested in humans.

The differences between the *gag* and *pol* in HVTN 070 and HVTN 080 and the *gag* and *pol* used in the current study are:

- The *gag* and *pol* in this study are multiclade consensus immunogens, while the previous *gag* and *pol* were based on a single clade B viral isolate, HXB2. Therefore, the *gag* and *pol* to be used in this study have the potential to induce broader immune responses.

- The *gag* and *pol* in this study are more codon-optimized for human expression than the previous plasmids.

- While the integrase of the previous *pol* was present with several mutations made to active site residues to knock out integrase activity, in the current *pol* construct

the integrase domain was deleted to further mitigate any residual theoretical risk associated with the theoretical potential for integration. Thus, the current *pol* expressed protein sequence is shorter in length than the *pol* used in the HVTN 070 and HVTN 080 protocols by about 200 amino acids and may provide a more effective immune target.

PENNVAX®-GP is formulated by combining two biologic products, INO-6112 and INO-6145, prior to administration. SynCon® INO-6112 consists of 2 plasmids encoding clade A and C HIV-1 envelope protein (pGX1001 and pGX1002, respectively). SynCon® INO-6145 contains 2 plasmids for synthetic multi-clade pol (pGX1004), and gag (pGX1005).

4.3 *IL-12* DNA adjuvant

The cytokine adjuvant consists of a single plasmid, pGX6001, containing a dual promoter system for expression of both the IL-12 p35 and p40 genes necessary for production of the active heterodimeric IL-12 protein. The p35 subunit is under the control of the hCMV promoter/enhancer and the simian virus 40 (SV40) polyadenylation signal whereas the p40 subunit is under the control of the simian cytomegalovirus promoter and the BGH polyadenylation signal. *IL-12* plasmid (INO-9012) is manufactured by VGXI (The Woodlands, Texas). It is formulated in sterile water, and has not been tested in humans previously.

A different product, *IL-12* plasmid formulated with bupivacaine (0.25%) (GENEVAX® *IL-12*-4532 and GENEVAX® *IL-12*-6285), was used previously in HVTN 060, HVTN 070 and HVTN 080 clinical studies. Those plasmids were manufactured by DSM Biologics, Groningen, Netherlands for Wyeth Vaccines Research, now Pfizer, Inc.

4.4 Trial design rationale

The primary objective of the trial is to evaluate the safety and tolerability of the PENNVAX®-GP HIV-1 DNA vaccine, with *IL-12* DNA, delivered with EP by ID and IM injection.

An earlier study, HVTN 080, has provided important initial safety and immunogenicity data on the use of EP with a similar HIV-1 DNA vaccine and an *IL-12* DNA given IM. This study, HVTN 098, will evaluate a different DNA vaccine, as well as a different *IL-12* DNA product, and test ID injections to determine whether the ID route might afford better tolerability and immunogenicity, particularly for induction of antibody responses.

4.4.1 Dose (amount and number)

Group 1 is a small pilot safety group to test the study products given ID with EP for the first time. As *IL-12* DNA has not been given ID with EP to healthy individuals previously, the effects of the adjuvant on local skin reactions to vaccination is not known. Taking a cautious approach, Group 1 tests a low dose of PENNVAX®-GP with *IL-12* DNA given ID with EP, in one injection site. Env (A and C), Gag, and Pol are given at 0.2 mg each. The study also provides for a limited initial pace of enrollment for this group to monitor carefully for adverse events (AEs) related to vaccination including injection site skin changes, and the effects of any visible lesions on the willingness of participants to continue receiving injections with EP. The size of this group (5 to receive

vaccine, 1 to receive placebo) limits the immunogenicity information that will be available for the products at these doses.

If Group 1 vaccinations are found to be safe and well-tolerated after a safety review, then Groups 2, 3 and 4 will be enrolled. Groups 2 and 3 will give ID vaccinations with EP, one group without *IL-12* DNA and the other group with *IL-12* DNA. Groups 2 and 3 deliver the vaccine at two EP injection sites for each timepoint, a progression from the single injection site tested in Group 1. The purpose of this study design is to determine whether the ID route can be more comfortable and tolerable for participants, equally safe, and have similar or better immunogenicity compared to IM with EP.

Group 3 will also evaluate the effects of *IL-12* DNA on safety and immunogenicity of the ID vaccinations, compared to Group 2. Two clinical trials in addition to HVTN 080, HVTN 087 and IAVI B004, evaluated a different DNA vaccine (HIV-MAG, Profectus Biosciences, Inc., Tarrytown, NY) given IM with EP, and preliminary results have found increased CD8+ T cell response rates by ICS with GENEVAX® *IL-12* DNA adjuvant, relative to DNA vaccine given alone, however, CD4+ response rates were decreased (see sections 4.8.3.5 and 4.8.3.6). Therefore it continues to be important to test a comparator group without *IL-12* DNA, especially since the adjuvant is being given by the ID route for the first time. Groups 2, 3, and 4 test total doses of *env* that are increased 6-fold relative to *gag* or *pol*. This will assess whether increasing the relative dose of *env* plasmid, and having 2 clades of Env represented, will result in *env*-specific responses more commensurate with the *gag* and *pol* responses previously seen in HVTN 080.

Group 4 gives IM vaccinations with EP, with *IL-12* DNA. The rationale for Group 4 is primarily to confirm and expand on the results of HVTN 080 by testing the same *gag*, *pol* and *IL-12* doses (1 mg dose of each plasmid) that were given in that study, in which the *IL-12* product increased the frequency of CD4+ and CD8+ T cell responses. The study products will be given IM with EP to provide a comparison with the same products given ID with EP in Group 3, and to results from HVTN 080 in which participants received 3 mg PENNVAX®-B and 1 mg GENEVAX® *IL-12* DNA IM with EP. The total dose of PENNVAX®-GP in Group 3 is 8 mg per injection. The 6-fold higher dose of Env DNA compared to HVTN 080 is intended to evaluate if the much higher dose will improve the frequency of cellular and humoral responses against Env.

Prior trials have tested vaccines IM with EP similar to the current vaccine at doses of 3-6 mg. PENNVAX®-B has been tested IM+EP at 3 mg and PENNVAX®-G at 4 mg. In addition, clinical data of IM DNA vaccination with the CELLECTRA® EP device are available for total DNA doses of up to 6 mg. Inovio has completed a phase 1 dose-escalation trial with a therapeutic HPV DNA vaccine, VGX-3100, containing the identical plasmid backbone as each of the vaccine components in PENNVAX®-GP , delivered via IM injection followed by EP (BB-IND-13683). The highest dose administered was 6 mg/vaccination in a volume of 1 mL. A total of 16 patients received at least one 6 mg dose of VGX-3100 (3 patients received 3 doses, 3 patients received 4 doses and 10 patients received one 6 mg dose).

In addition, Inovio is currently conducting a randomized, blinded, placebo-controlled study of VGX-3100 delivered via IM injection followed by EP. A total of 167 patients have been enrolled in the phase 2 trial and 157 have completed the 3 dose regimen. Based on the most recent blinded data review, no serious adverse events related to the vaccine were reported following three 6 mg doses of DNA.

Another study was conducted in both the US and Korea with dose escalation of a DNA-based influenza vaccine (VGX-3400X) via IM injection followed by EP. Across the US and South Korea, 20 patients received at least one dose of 6 mg. The vaccine regimen was well tolerated with no serious adverse events related to the vaccine and there was no dose related toxicity reported from these repeated 6 mg DNA doses. Based on these studies, the 8 mg dose of PENNVAX®-GP is expected to have an acceptable safety profile.

In all groups, a series of 4 vaccinations are given, compared to 3 vaccinations in HVTN 080, to determine whether the frequency or magnitude of either cellular or humoral immune responses may be increased with a fourth vaccination.

In the study, information will be collected on device performance and any difficulties that clinical research site (CRS) staff may have with using the EP device, especially when these issues result in a missed vaccination or missed electroporation. Feedback on device performance may assist the developer in the design of next-generation electroporation devices.

4.4.2 Schedule

The schedule of injections was chosen to be comparable to the schedule of injections at Months 0, 1, and 3 used in HVTN 080 with similar products given IM with EP. An additional vaccination at Month 6 has been added to test whether an additional administration may further increase immune responses.

4.4.3 Potential for future protein boost

If a suitable protein boost becomes available, the study protocol may be amended to incorporate protein boost injections for consenting participants.

4.4.4 Choice of placebo

The placebo will be Sterile Water for Injection, USP. This has been used as the placebo in the clinical trial for Inovio's investigational DNA vaccine for influenza and has been well-tolerated in IM with EP and ID with EP administrations.

The primary reason to include placebos in HVTN 098 is to blind participants and clinical staff to the receipt of active product or placebo by individual subjects to avoid bias in safety reporting. The DNA vaccine is diluted in sterile water and the final study product for injection is hypotonic. Therefore, the study team chose to use sterile water rather than sterile saline for the placebo group to minimize the possibility of unintended unblinding, and to more closely match the study product. This is a new factor being introduced in this protocol in terms of evaluation of local reactogenicity, as other HVTN protocols with EP have used sterile saline as the placebo.

4.4.5 Body measurements and immunogenicity

In this study, we plan to measure both body mass index (BMI) and waist circumference (WC) to explore whether these anthropometric measurements might have a relationship to the frequency and/or magnitude of vaccine-elicited immune responses. As indicated in Section 4.8.3.4, in HVTN 080, participants who developed T cell responses after receiving PV+IL12 IM+EP had lower median BMI than non-responders (p=0.04 at both

time points). BMI did not affect response magnitudes. It is interesting to note that the median BMI (25.4) of the non-responders was less than 30 and technically not in the obese range. It is possible, however, that some people could fall into the category of "normal-weight obesity" [35], highlighting the need for a refined measurement of body fat distribution. As such, BMI remains an insensitive measure overall since it does not take into consideration the distribution of body fat or the lean muscle mass. Increased BMI is associated with increased risk for infectious diseases (2009 H1N1 influenza and others), a decreased response to vaccinations such as Hepatitis B and tetanus toxoid in children, and a more rapid decline in HAI titers and lower CD8 T cells in response to influenza vaccines in adults [36-38].

WC is highly correlated with abdominal fat which is associated with cardiovascular disease, mortality, and other obesity-related complications [39,40]. Increased disease risk is noted when the WC is > 40 inches in men and >35 inches in women in overweight (BMI of > 25) and obese (BMI of > 30) individuals but also in persons of normal range BMI. WC is best measured (as per NIH guidelines) at the superior border of the iliac crest with a measuring tape and can be incorporated into the vital signs portion of the physical exam [41].

4.5 Plans for future product development and testing

This study will provide support to product development decisions for evaluating antigens, cytokine adjuvants, dose, and route for potential phase 2 studies with these antigens given with EP. Ideally this trial would set a new standard for immune responses to a HIV DNA-only regimen and set the stage to explore boosting with other products that may further optimize cellular and antibody responses.

4.6 Preclinical safety studies

Table 4-2 Summary of preclinical safety studies

Study number	Product	Type of study	Animal	N	Dose groups	Route	Schedule
1195-07910	PENNVAX®-B	Toxicity	NZW rabbit	10m, 10f	(see below)	IM	Study Day 1, 15, 29, 43, 57
1195-07911	PENNVAX®-B	Biodistribution	NZW rabbit	15m, 15f	(see below)	IM	Study Day 1

NZW = New Zealand White
m = male
f = female

The above table, Table 4-2, lists the most relevant preclinical safety studies, however, there have been four other independent IND-enabling good laboratory practice (GLP) toxicology and biodistribution studies in New Zealand White (NZW) rabbits with DNA plasmids (delivered with *in vivo* EP) sharing the identical plasmid backbone as the aforementioned *gag*, *pol*, *env* A and *env* C plasmids, differing only in the expressed antigen.

4.6.1 Toxicology and biodistribution of PENNVAX®-B in NZW rabbits

A GLP preclinical study titled: "PENNVAX®-B DNA Plasmids Vaccine and Adjuvant *IL-12* DNA Plasmid: An 85-Day Multiple Vaccine Repeat Intramuscular Dose Toxicity Study in the New Zealand White Rabbit" (Study No. 1195-07910) was performed at Bridge Laboratories (Gaithersburg, MD). The study evaluated the potential toxicity of PENNVAX®-B (PV-B) vaccine plus GENEVAX® *IL-12*-4532 in the NZW rabbit when administered IM by EP on study days (sd) 1, 15, 29, 43, and 57. Persistence, reversibility, or delayed onsets of any effects were recorded during a 28-day no-treatment recovery period. The study consisted of 4 groups of rabbits (10 per sex per group) for a total of 80 animals. Dosing was performed IM with a constant dose volume of 1.3 mL with either placebo (sterile saline) with EP (Group 1), PV-B vaccine and *IL-12*-4532 adjuvant with EP (Group 2), PV-B vaccine plus placebo with EP (Group 3), or the placebo without EP (Group 4) (see Table 4-3). Five rabbits per sex per group were designated for terminal sacrifice on sd 58 and the remaining animals designated for recovery sacrifice on sd 85. Evaluation included mortality, clinical and cageside observations, dermal Draize observations, body weight data, food consumption, ophthalmology, clinical pathology, immunology, gross pathology, absolute and relative organ weight data, and histopathology.

Table 4-3 Toxicology study no. 1195-07910 design

Group	Treatment	Dose Volume (mL)	Route
1	Placebo + EP	1.3	IM
2	PV-B 3 mg+ *IL-12* 1mg + EP	1.3	IM
3	PV-B 3 mg + Placebo + EP	1.3	IM
4	Placebo	1.3	IM

Treatment with PV-B alone or in combination with adjuvant *IL-12*-4532 with EP had no effect on mortality with the exception of one female animal where the cause of death is unknown. For males, the incidence and severity of injection site tissue (Draize) changes were comparable in the EP-treated (Groups 1-3) animals; but of higher incidence when compared to non-EP Group 4 (placebo alone) animals. For females, the incidence and severity of injection site tissue (Draize) changes was greater in the Group 2 (PV-B + *IL-12* with EP) animals followed by the remaining EP-treated groups (Groups 1 and 3, respectively). However the severity of the tissue changes was also observed in isolation on sd 30 for the non-EP (Group 4) females, which otherwise showed minimal or unremarkable tissue changes. All Draize observations had resolved by sd 71.

Elevated creatine kinase values were noted in Groups 1-3 (EP treatment groups) and were thought to be due to the transient muscle degeneration and trauma associated with EP. Treatment with PV-B DNA with EP (Group 3) or in combination with adjuvant *IL-12* DNA with EP (Group 2) resulted in neutrophilia up to sd 58 and this is thought to be the result of inflammation as a secondary effect due to vaccination with the test article and/or adjuvant, respectively.

The IM injection of PV-B alone or in combination with adjuvant *IL-12* DNA plasmid with a series of five treatments followed by a 28 day recovery period was generally well-tolerated in male and female NZW rabbits. Treatment related effects were noted in the injection site tissues and in the spleen. A secondary effect, due to treatment with the test article and/or adjuvant with EP, was the inflammation-induced elevation of neutrophils and fibrinogen. Of these findings, only the histopathologic observations in the spleen persisted, but were lower in incidence suggesting reversibility. The observed effects were not considered adverse or biologically significant and there was no delayed effect.

4.6.1.1 Biodistribution and Integration study

A GLP study entitled "PENNVAX®-B DNA Plasmids Vaccine plus Adjuvant *IL-12*: A Single Intramuscular Dose Biodistribution and Integration Study During a 90-Day Study Period in the New Zealand White Rabbit" (Study No. 1195-07911) was performed at Bridge Laboratories (Gaithersburg, MD). The dose for the study was selected to deliver up to the maximum expected dose to be delivered into humans.

Fifteen rabbits/sex/group were assigned to the study, for a total of 60 animals. On sd 1, the animals received 2 separate 0.62 mL IM injections (contralateral limbs) with EP to achieve a dose volume of 1.24 mL of sterile saline or PV-B vaccine plus *IL-12* adjuvant (see Table 4-4). Total DNA/1.24 mL consisted of 1 mg of the 3 encoded genes plus 1 mg *IL-12* DNA. Each animal was anesthetized (propofol and isoflurane) during dose administration and each injection administered at a clipped/marked site. Five rabbits/sex/group were euthanized on sd 9, 60, and 90 and tissues collected to evaluate the biodistribution of PV-B plus *IL-12* adjuvant. On sd 90, samples were collected to determine the integration of plasmids in genomic DNA.

Table 4-4 Biodistribution/integration study no. 1195-07911 design

Group	Treatment	Day of Dose	Dose Volume (mL)
1	Placebo[a] + EP	sd1	1.24
2	PV-B 3 mg+ *IL-12* 1mg + EP	sd1	1.24[b]

[a] Sterile saline
[b] Total DNA/1.24 mL consisting of 1 mg of the three encoded genes plus 1 mg *IL-12* DNA

There were no effects noted on mortality, clinical observations, body weight data, food consumption, or individual gross necropsy. At 90 days after IM administration of vector to the NZW rabbits, except for the brain of animal 10048 at sd 9, there was no evidence for the distribution or persistence of PV-B vaccine and *IL-12* adjuvant in tissues of treated animals other than in the skin and muscle at the injection sites. There was no evidence of amplification of PV-B DNA plasmids within the genomic DNA.

Other than a transient reduction in food consumption, body weight and body weight changes during the initial interval of sd 1 - 8, a single IM injection of PV-B DNA plasmid vaccine plus *IL-12* DNA plasmid adjuvant via EP to NZW rabbits was generally well-tolerated.

4.7 Preclinical immunogenicity study

Table 4-5 Preclinical immunogenicity study

Product	Animal	N	Dose groups	Route	Schedule	Assay
HIV MGag, MPol	Macaque	4f	0.5 mg MGag, 0.5 mg MPol	IM+EP	3x 4wks	ELISpot, Flow Cytometry
HIV MGag, MPol, *IL-12*	Macaque	4f	0.5 mg MGag, 0.5 mg MPol, 0.3 mg *IL-12*	IM+EP	3x 4wks	ELISpot, Flow Cytometry
HIV *env* clade A and C	Macaque	5	1.5 mg *env* A, 1.5 mg *env* C	IM+EP	4x 6 wks	ELISA

As shown in Table 4-5, two groups of Rhesus macaques (n=4) were immunized intramuscularly using EP via the CELLECTRA® device with 0.5mg MGag (HIV) and 0.5 mg MPol (HIV) plasmids (multiclade *gag* and *pol* constructs which are the same plasmids proposed for use in the current protocol, furthermore have demonstrated immunogenicity in the RV-262 protocol, as summarized in Section 4.8.1) 3 times, 4 weeks apart, with or without the addition of 0.3mg of the macaque *IL-12* DNA adjuvant. IFN-γ ELISpot was used as a primary readout for Th1 biased immune responses in PBMCs of vaccinated animals. Analysis of the data revealed that animals vaccinated with MGag and MPol exhibited roughly 1000 SFU/million PBMC after 2 immunizations, while the group receiving the *IL-12* DNA adjuvant exhibited close to 3000 SFU, suggesting that inclusion of the *IL-12* DNA adjuvant led to a dose-sparing effect. Three immunizations with the MGag and MPol constructs resulted in a mean group IFN-γ ELISpot average of just over 4000 SFU per 10^6 PBMC, whereas the animals receiving *IL-12* adjuvant with the vaccine exhibited greater than 4500 SFU per 10^6 PBMC. Following this analysis, all animals were allowed to rest for 3 months, at which point mesenteric lymph nodes were harvested and CD8-mediated HIV-specific cytotoxicity was assayed. PBMCs isolated from the mesenteric lymph nodes were put through a single round of *in vitro* stimulation with HIV Gag and Pol peptides (Day 6) or left unstimulated (Day 0). Cytotoxic activity was measured using a flow-cytometric based assay that detects the presence of active granzyme B that has been delivered to target cells in an antigen-specific fashion. Without prior stimulation, neither group showed robust killing activity at Day 0 (1.59% for the Gag/Pol group and 2.3% for the *IL-12* DNA group). However, after a single round of *in vitro* stimulation, the Gag/Pol group exhibited 19.8% killing activity while the group receiving the *IL-12* DNA showed a statistically significant increase to 27.5%. Taken together these data indicate that the HIV plasmids MGag and MPol are immunogenic when administered intramuscularly using the CELLECTRA® device and that the addition of the *IL-12* DNA further increases immune responses to these antigens both in the form of IFN-γ production from PBMCs and in the form of cytotoxicity from CD8+ T cells taken from the mesenteric lymph nodes.

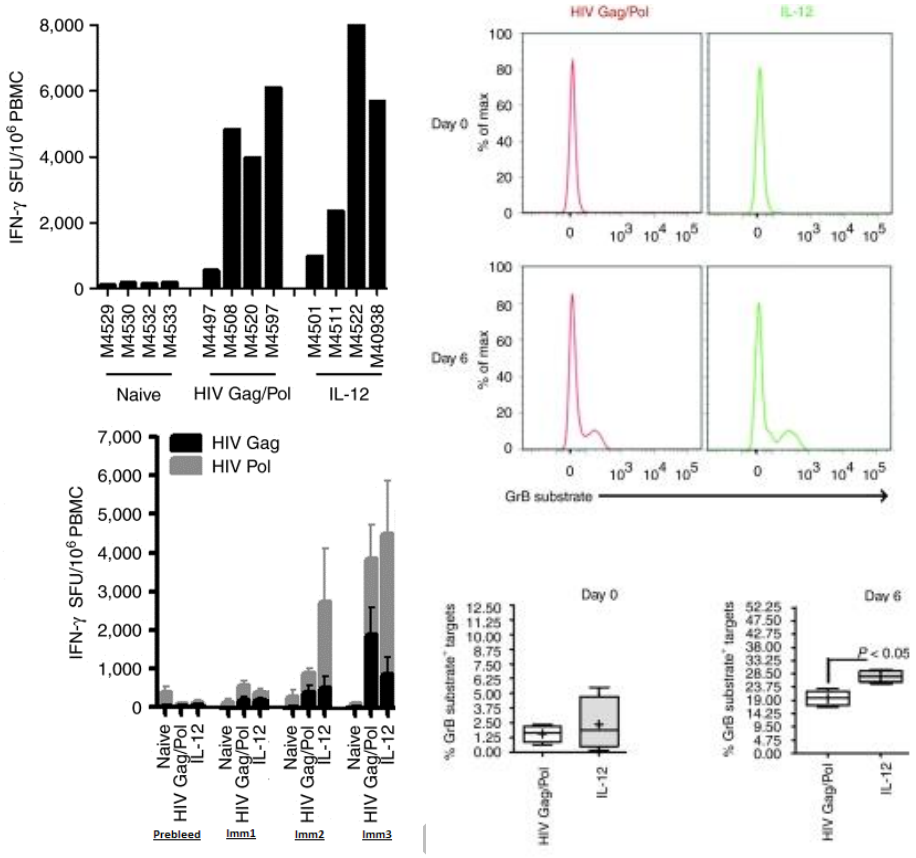

Figure 4-1 IFN-γ ELISpot and cytotoxicity assays from HIV vaccines with and without *IL-12* DNA. IFN-γ ELISpot assays performed on PBMCs isolated 2 weeks after the final vaccination indicate that IM immunization with the MGag and MPol constructs using the CELLECTRA® device drives robust HIV gag and HIV pol IFN-γ responses after a third vaccination, with or without *IL-12*DNA. Furthermore, cytotoxicity assays performed 3 months after the final vaccination using cells isolated from mesenteric lymph nodes indicate that both vaccines were able to induce HIV specific killing activity, with the vaccine containing the IL-12 adjuvant contributing additional killing activity that achieved statistical significance when compared to vaccination with antigen alone.

The *env* C construct has been shown to be immunogenic in previous published studies, demonstrating both antibody and cellular responses in mice [34] and in NHP studies [42] when delivered using *in vivo* EP. In a follow on study, vaccinating nonhuman primates with 1.5 mg each of the *env* A and C constructs induced binding antibody titers that were maintained throughout the 4 vaccination regimen (shown in Figure 4-2). These data indicate that the *env* A and C constructs are able to induce both humoral and cellular immunity in animal models. Furthermore, these vaccines have been shown to be immunogenic in RV-262 clinical trial (results are presented in Section 4.8.1).

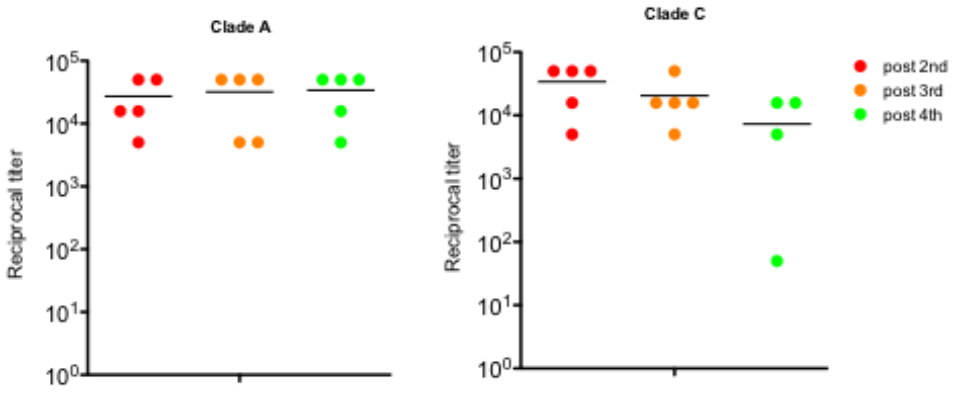

Figure 4-2 Non-human primates were vaccinated with *env* A and C constructs using IM injection followed by EP. Binding antibody titers were measured by enzyme-linked immunosorbent assay (ELISA) following second (red, post 2nd), third (orange, post 3rd) and fourth (green, post 4th) vaccinations. Proteins used to measure binding were consensus clade A and consensus clade C *env* proteins (Immune Therapies Co.)

4.8 Clinical studies

4.8.1 Clinical studies of PENNVAX®-G

RV262 is being conducted in 2 parts. Part A is an open-label safety and immunogenicity study conducted at the US MHRP in Rockville, MD in 13 HIV-uninfected adults (12 total subjects initially projected). Part B is a randomized placebo-controlled study in Kenya, Tanzania and Uganda which enrolled 87 HIV-uninfected adults (80 total subjects initially projected). A phase 1, randomized trial (RV262) is currently being performed by the US MHRP with a regimen consisting of two priming vaccinations with PENNVAX®-G DNA (PVG -consensus *env* clade A, C, D, 0.7 mg each; consensus *gag*, 2 mg) delivered either with Biojector (BJ) or electroporation (EP) at Month 0 and 1, followed by 2 boosts at Month 3 and 6 with recombinant poxvirus (MVA-CMDR: *env* A/E and *gag/pol* A/E, 10^8 plaque forming units (PFU) with safety and immunogenicity as the primary endpoints. The *gag* and clade A and C *env* constructs used in the RV 262 study derive from the same original plasmids (and therefore represent the same sequences) as those to be used in the HVTN 098 trial with the exception that the constructs for the HVTN 098 study are newly manufactured and have been formulated at a higher concentration. Additionally, while the primary seed stock of plasmids for the RV-262 and HVTN 098 derive from the same source (Weiner lab), new master and working cell banks were created for the plasmids being used in the HVTN 098 trial. The cell banks were expanded and plasmids manufactured under clinical good manufacturing practices at VGXI, Inc. (The Woodlands, TX) to yield high concentration bulk material and formulated material to support the high dose and ID administration proposed in HVTN 098. The RV-262 material was manufactured by Althea, Inc. and is not suitable to address the specific hypotheses outlined in this protocol – namely ID administration, and high concentration IM delivery.

The open label Part A (N=13, US) was followed by the double-blinded Part B (N=87, East Africa). Part B was randomized to BJ or EP and to placebo or PENNVAX™-G DNA/ MVA-CMDR (1:4). For 11 Part A and 16 Part B subjects, IFN-γ ELISPOT

responses were measured to vaccine env, gag, and pol inserts at baseline, 2 weeks post second DNA injection and 2 weeks post second MVA-CMDR injection. For 11 Part A subjects, ELISA binding antibody (bAb) endpoint titers and neutralizing antibody (TZMbl and PBMC) responses were measured at baseline, 1 and 2 weeks post second MVA, and at month 12. Part B remains blinded.

To date, the DNA prime and MVA boost injections have been safe and generally well tolerated. In the 100 enrolled participants (76 males, 24 females) with a mean age of 29.02 years, there were 197 DNA/placebo injections given across 4 clinical sites; 98 (49.7%) by CELLECTRA® and 99 (50.3%) by Biojector®. Three volunteers did not receive the second DNA/placebo injection due to relocation. There was no difference in mean age ($p=0.7904$) or gender ($p=0.196$) between the two groups. Immediately post injection (<5 min), 38.4% of the CELLECTRA® group and 2.1% of the Biojector® group reported moderate pain in the arm ($p<0.0001$). Severe pain was reported by 3.0% in CELLECTRA® but by none in Biojector. Visible muscle contraction was seen after electroporation in all injections except for one. At 45 minutes post injection, mild pain was reported by 61.2% in CELLECTRA® and by 27.3% in Biojector® ($p<0.0001$). Moderate pain was reported by 4.1% in the CELLECTRA® group only. Mild tenderness was also higher in CELLECTRA® (49.0%) compared to Biojector® (12.1%) ($p<0.0001$). There was no significant difference between the two groups in reported local swelling ($p=0.067$), warmth ($p=0.243$) and pruritus ($p=0.994$) [unpublished data]. The Biojector® needleless device was better tolerated than the CELLECTRA® electroporation device in terms of local pain and tenderness. However, the two devices showed no difference in tolerability in terms of local swelling, warmth and pruritus, and retention for repeat vaccinations.

All related AEs were mild or moderate. There have been no related SAEs, deaths or life-threatening AEs in this study. One subject had an elevated CPK of 4561 U/L, (grade 4 by DAIDS AE severity tables) 12 days after receiving the first DNA vaccination via the Biojector® device after 5 hours of strenuous exercise the day prior. The subject experienced mild myalgia after exercise. The following day, CPK decreased to 2637 U/L (DAIDS grade 3). The lab abnormality was not serious per ICH criteria and was assessed as not related to study product. Repeat CPK and AST testing showed that the lab values returned to normal. The same volunteer had another asymptomatic grade 4 CPK elevation (9950 U/L) which occurred one day after strenuous exercise but prior to receiving his final MVA boost on the same day. The elevated CPK returned to normal within 2 days (CPK 501 U/L) and was assessed to be unrelated to the study vaccine. One participant died during the study's follow up period. The subject was enrolled in Group III (PV-G or placebo by BJ followed by MVA-CMDR or placebo) and had successfully completed the period of active study visits, through 6 months after final vaccination. Five days after being discharged from the study, during the passive study follow-up period, he was admitted to the hospital with "acute abdominal catastrophe", collapsed and died enroute to surgery. The event was assessed as not related to the study product by the investigator.

IFN-γ ELISPOT responses post second DNA vaccination were not detected. After the second MVA, responses to the MVA-CMDR env were most frequent, with 7/14 BJ and 5/13 EP responders. 6/6 BJ and 4/5 EP recipients developed p24 bAb responses peaking two weeks post 2nd MVA, with responses persisting at 6 months in 5/6 BJ and 3/5 EP recipients. All 11 subjects developed bAb to gp120 (CM234), however responses waned in the majority by month 12. Neutralizing antibodies were detected only in the PBMC assay using a CRF01_AE CM235 infectious molecular clone (6/6 BJ and 3/5 EP).

A similar pattern was noted for the intracellular cytokine staining (ICS) assays. For Part A participants, responses to the DNA vaccines were not seen until after the boost injections, except in 1 participant who received DNA vaccine by BJ who had a CD4+ response to Gag and 1 participant who had a CD8+ response to Env. After the first MVA boost all participants (11/11) in both BJ and EP groups had a CD4+ response to MVA-CMDR-matched Env, and 6/11 (2 EP. 4 BJ) had a CD4+ response to Gag. The magnitude of the responses to DNA ranged from 0.06-0.19% HIV specific CD4+ T cells for the EP group and 0.06-0.58% HIV specific CD4+ T cells for the BJ group. In addition, 5/11 (2 EP, 3 BJ) had a CD8+ response to Env, and 2/11 (both BJ) had a CD8+ response to Gag. Response rates decreased overall after the second MVA vaccination, as well as the magnitude of responses.

Preliminary data on the 11/13 US participants who completed the study (there were 2 study unrelated withdrawals) demonstrate this PVG/MVA-CMDR regimen to be safe with no significant difference between DNA delivery methods. To date, the DNA prime and modified vaccinia Ankara (MVA) boost have been well tolerated. There were no related serious adverse events (SAEs), no pregnancies, and no HIV infections in this group. The early results from this very small initial study will be augmented by data generated from the vaccinations in additional subjects in Part B. Significantly all vaccinees (11/11) mounted an Env specific response following DNA prime MVA boost vaccinations, whereas in the phase 1 trial of MVA-CMDR alone, only 5/10 participants who received 10^8 PFU per vaccination responded to Env after 2 injections at months 0 and 1, as detected by both ICS and ELISpot assays [43]. This difference suggests the effectiveness of the PENNVAX®-G Env constructs in priming the immune response. It is important to note that the RV-262 study did not use *IL-12* DNA adjuvant.

4.8.2 Clinical studies of the proposed product combination

While individual components *env* A, *env* C, *gag*, and *IL-12* as well as a longer length analog of *pol* (95% identity) have been tested with IM administration and EP in previous clinical trials, there have been no previous clinical studies using this specific product combination and vaccine regimen with EP-mediated delivery.

4.8.3 Clinical studies of DNA vaccines with *IL-12* DNA adjuvant, with and without IM electroporation

While the human *IL-12* DNA formulation to be used in the current study has not been given to humans previously, *IL-12* DNA has been tested for adjuvant activity in a number of clinical studies conducted in healthy adult volunteers (HVTN 060, HVTN 063, and HVTN 070; and delivered with IM EP in HVTN 080, HVTN 087, and IAVI B004).

4.8.3.1 Phase 1 study of prototype HIV-1 *gag* p37-expressing DNA construct in combination with human *IL-12* DNA delivered by standard IM injection in HIV-negative adults (HVTN 060)

The HVTN has conducted a phase 1 trial, HVTN 060 (BB#12367, DAIDS-ES ID 10057), which evaluated the safety and immunogenicity of a prototype HIV-1 *gag* p37-expressing DNA vaccine (GENEVAX® *gag*-2962) alone or in combination with human *IL-12* DNA (GENEVAX® *IL-12*-6285 or -4532). Participants included 132 volunteers in the US and 12 Part B volunteers in Thailand. See Table 4-6.

Table 4-6 Trial schema for HVTN 060

		Vaccination schedule in months (days)						
		Dose (mcg)		Priming			Boosting	
Groups	N	gag	IL-12	0 (0)	1 (28)	3 (84)	6 (168)	9 (273)
Part A								
1	10	1500	—	*gag* DNA	*gag* DNA	*gag* DNA	—	—
	2	—	—	placebo	placebo	placebo	—	—
2	10	1500	100	*gag* DNA + *IL-12* DNA	*gag* DNA + *IL-12* DNA	*gag* DNA + *IL-12* DNA	—	—
	2	—	—	placebo	placebo	placebo	—	—
3	10	1500	500	*gag* DNA + *IL-12* DNA	*gag* DNA + *IL-12* DNA	*gag* DNA + *IL-12* DNA	—	—
	2	—	—	placebo	placebo	placebo	—	—
4	10	1500	1500	*gag* DNA + *IL-12* DNA	*gag* DNA + *IL-12* DNA	*gag* DNA + *IL-12* DNA	—	—
	2	—	—	placebo	placebo	placebo	—	—
Part B								
5	30	1500	—	*gag* DNA	*gag* DNA	*gag* DNA	*gag* DNA	*gag* DNA
	6	—	—	placebo	placebo	placebo	placebo	placebo
6	30	1500	1500	*gag* DNA + *IL-12* DNA	*gag* DNA + *IL-12* DNA	*gag* DNA + *IL-12* DNA	*gag* DNA + *IL-12* DNA	*gag* DNA + *IL-12* DNA
	6	—	—	placebo	placebo	placebo	placebo	placebo
7	20	1500	1500	*gag* DNA + *IL-12* DNA	*gag* DNA + *IL-12* DNA	*gag* DNA + *IL-12* DNA	—	—
	4	—	—	placebo	placebo	placebo	placebo	placebo
Total	48 (Part A) + 96 (Part B) = 144							

There were no AEs among vaccinated participants that were considered probably or definitely related to vaccination. The vaccine and cytokine adjuvant did not have any apparent effects on hematologic parameters (complete blood count), CD4 counts, or serum chemistries compared to placebo. There were no fatalities reported during the trial, no life-threatening AEs, and no serious adverse events (SAEs).

An assay for anti-*IL-12* antibody was performed, to determine whether administration of *IL-12* DNA might have elicited antibody that might affect native IL-12. Evaluated samples were blinded to group assignment and to assignment to the vaccine or placebo arm. One person had a positive result (32.3 neutralizing units per milliliter (NU/mL); a positive response was >30 NU/mL) for antibody to IL-12 at Day 14 that was not present at baseline, Day 42, or Day 98.

Immunogenicity was assessed by IFN-γ ELISpot after third and fourth vaccinations, using peptide pools representing clade B consensus *gag*. Unblinded results from Group 1 and Group 5 (*gag* DNA alone) pooled show a 2/33 (6.1%) IFN-γ ELISpot response rate 2 weeks after the third injection; both participants were in Group 5. Two weeks after the fourth injection 2/23 participants (8.7%) in Group 5 also had positive ELISpot responses; 1 of those had responded earlier. There were no responses in Group 2 (*gag* and *IL-12* DNA 100 mcg). In Group 3, 4/9 (44.4%) participants responded after 3 vaccinations with *gag* DNA and *IL-12* DNA 500 mcg (95% confidence interval 18.9%-73.3%). In Groups 4, 6, and 7, no responses were seen after 3 vaccinations of *gag* with *IL-12* DNA 1500 mcg among 49 samples assayed. In addition, 24 samples from Group 6 were tested after the fourth injection, and 11 samples were tested after the fifth injection, and no responses were detected at those timepoints. The range of background adjusted spot counts among the positive samples from Groups 3 and 5 was 55-141 spot-forming cells (SFC)/10^6 PBMC.

Serological tests for binding antibodies to p55 *gag* were assessed by a validated ELISA using single serum dilutions (1/100), at baseline, 2 weeks after third vaccination, and after fifth vaccination for Part B participants. No samples were positive for binding antibodies to p55 *gag*.

In summary, local and systemic reactions to vaccination with the HIV-1 *gag* p37 DNA vaccine GENEVAX® *gag*-2962) and *IL-12* DNA (GENEVAX® *IL-12*-6285 and GENEVAX® *IL-12*-4532) were mild to moderate in the HVTN 060 phase 1 trial. No pattern of systemic AEs emerged during the study, and no SAEs related to the study products were observed. The DNA vaccine and the *IL-12* DNA cytokine adjuvant have been well-tolerated, with an acceptable safety profile in this trial. The DNA vaccine regimen delivered by standard IM injection was very minimally immunogenic.

4.8.3.2 Phase 1 study of prototype HIV-1 *gag* p37-expressing DNA construct in combination with human IL-15 DNA or *IL-12* DNA delivered by standard IM injection in HIV-negative adults (HVTN 063)

HVTN 063 (BB IND# 12439, DAIDS-ES ID 10058) tested the safety and immunogenicity of a prototype HIV-1 *gag* (p37) DNA vaccine (GENEVAX® *gag*-2962) alone or with plasmids encoding *IL-15* (GENEVAX® *IL-15*-1696), and *IL-12* (GENEVAX® *IL-12*-4532) in healthy, HIV-1–uninfected adults. This was the first-time-in-humans evaluation for GENEVAX® *IL-15*-1696 as a molecular adjuvant.

In this study, participants received 1500 mcg HIV *gag* p37 DNA vaccine with *IL-15* DNA at 0, 100, 500, or 1500 mcg, or placebo. The study had two parts: Part A, a dose escalation study of *IL-15* DNA, and Part B, a regimen selection study, of *gag* DNA with *IL-15* DNA, given up to 5 times, or as 3 injections subsequently boosted by 2 injections of *gag* DNA with *IL-12* DNA. See Table 4-7.

Table 4-7 Trial schema for HVTN 063

Study arm	N	*gag* DNA dose (mcg)	*IL-15* DNA dose (mcg)	*IL-12* DNA dose (mcg)	Vaccination schedule in months (days) 0 (0)	1 (28)	3 (84)	Booster schedule in months (days) 6 (168)	9 (273)
Part A									
1	10	1500	—	—	*gag* DNA	*gag* DNA	*gag* DNA	—	—
	2	—	—	—	placebo	Placebo	placebo	—	—
2	10	1500	100	—	*gag* DNA + *IL-15* DNA	*gag* DNA + *IL-15* DNA	*gag* DNA + *IL-15* DNA	—	—
	2	—	—	—	placebo	Placebo	placebo	—	—
3	10	1500	500	—	*gag* DNA + *IL-15* DNA	*gag* DNA + *IL-15* DNA	*gag* DNA + *IL-15* DNA	—	—
	2	—	—	—	placebo	Placebo	placebo	—	—
4	10	1500	1500	—	*gag* DNA + *IL-15* DNA	*gag* DNA + *IL-15* DNA	*gag* DNA + *IL-15* DNA	—	—
	2	—	—	—	placebo	Placebo	placebo	—	—
Pause for safety evaluation									
Part B									
5	30	1500	1500	—	*gag* DNA + *IL-15* DNA	*gag* DNA + *IL-15* DNA	*gag* DNA + *IL-15* DNA	*gag* DNA + *IL-15* DNA	*gag* DNA + *IL-15* DNA
	6	—	—	—	placebo	placebo	placebo	placebo	placebo
6	0	—	—	—	—	—	—	—	—
	0	—	—	—	—	—	—	—	—
7	30	1500	1500	1500	*gag* DNA + *IL-15* DNA	*gag* DNA + *IL-15* DNA	*gag* DNA + *IL-15* DNA	*gag* DNA + *IL-12* DNA	*gag* DNA + *IL-12* DNA
	6	—	—	—	placebo	placebo	placebo	placebo	placebo
Total: A (48) + B (72) = 120									

In all, 100 people received the *gag* DNA vaccine, 56 men and 44 women: 10 received *gag* DNA vaccine alone with no adjuvant, 64 received vaccine with *IL-15* DNA adjuvant, and 26 received vaccine with each of the two adjuvants (*IL-15* DNA and *IL-12* DNA). Twenty people received the placebo (Sodium Chloride for Injection USP, 0.9%).

The vaccinations were well tolerated. Injection site pain and/or tenderness, and systemic symptoms such as malaise/fatigue, chills, nausea, myalgia, arthralgia or headache, were mild or absent in most participants. There were no severe reactions related to vaccine. There were no fatalities reported during the trial.

AEs that were reported as "definitely related" or "probably related" to vaccination were mild in severity: 1 event each of injection site pruritus treatment group 1(T1), injection site swelling (T1), injection site pain (T7), injection site papule (T7), pyrexia (T7), and injection site hematoma (T5).

An assay for *IL-15* nAb was performed, to determine whether administration of *IL-15* DNA might have elicited antibody that might affect native IL-15. For the 65 participants tested from Part B groups 5 and 7, an assay for *IL-12* nAb was also done. Evaluated samples were blinded to group assignment and to assignment to the vaccine or placebo arm. There were no positive results in 38 Part A vaccinees and 8 controls tested at baseline and after the third vaccination. From Part B, 2/26 Group 7 vaccinees, 0/28 Group 5 vaccinees, and 0/11 controls had positive results for *IL-15* nAb at day 273, after 4 vaccinations. A positive response was defined as a result > 11 NU/mL. The Group 7

participants had responses of 11.5 and 17.7 NU/mL, respectively. No Part B participant had a positive result for *IL-12* nAb, defined as a result > 30 NU/mL.

Immunogenicity was assessed by validated IFN-γ ELISpot 2 weeks after third and fourth vaccinations, using cryopreserved PBMC stimulated overnight with synthetic peptide pools representing clade B consensus *gag*. The sums of background-corrected responses to *gag* ConB from the 3 responders from treatment group 5 were: 195, 185 and 105 SFC/10^6 PBMC. The sum of background-corrected responses to *gag* ConB from the single responder in treatment group 7 was 113 SFC /10^6 PBMC. One participant in a placebo group from Part A had a positive response at baseline and after 3 injections of placebo. Due to the low frequency of responses after the third and fourth vaccinations, samples after the fifth vaccination were not assessed.

Serological tests for binding antibodies to p55 *gag* were assessed by a validated ELISA using single serum dilutions (1/100), at baseline, 2 weeks after third vaccination for Part A participants (Groups 1-4: 37 vaccinees and 8 controls tested), and after fifth vaccination for Part B participants (Groups 5 and 7: 56 vaccinees and 12 controls tested). No samples were positive for vaccine-induced binding antibodies to p55 *gag*. One participant in Group 7 had a positive result at baseline and after fifth vaccination.

In summary, local and systemic reactions to vaccination with the Gag DNA vaccine GENEVAX® Gag-2962) and *IL-15* DNA (GENEVAX® *IL-15*-1696), and *IL-12* DNA (GENEVAX® *IL-12*-4532) have been mild to moderate in the HVTN 063 phase 1 trial. No pattern of systemic AEs related to vaccination emerged during the study, and no SAEs related to the study vaccines were observed. The vaccines have been well-tolerated, with an acceptable safety profile in this trial.

4.8.3.3 Phase 1 study of PENNVAX®-B DNA construct expressing HIV *gag*, *pol* and *env* in combination with human *IL-15* DNA or *IL-12* DNA delivered by standard IM injection in HIV-negative adults (HVTN 070)

The HVTN 070 clinical trial (BB IND# 13449, DAIDS-ES ID 10490) was designed to evaluate the safety and immunogenicity of PV-B (*gag*, *pol*, *env*) alone, with *IL-12* DNA (GENEVAX®-*IL-12*-4532), or with a dose escalation of *IL-15* DNA (pIL15EAM) (Table 4-8). PV-B and *IL-15* DNA were provided by David Weiner, University of Pennsylvania School of Medicine (Philadelphia, PA, USA). The *IL-12* DNA was provided by Profectus Biosciences, (Tarrytown, NY, USA). HVTN 070 was initiated in October, 2007. Enrollment was completed in January, 2009, and all vaccinations were completed by August, 2009.

Table 4-8 Trial schema for HVTN 070

		Dose			Vaccination schedule in months (days)			
Study arm	**Number**	**PV-B**	***IL-15* DNA**	***IL-12* DNA**	**0 (0)**	**1 (28)**	**3 (84)**	**6 (168)**
Group 1	10	6 mg	0.8 mg	—	PV-B + *IL-15*	PV-B + *IL-15*	PV-B + *IL-15*	PV-B + *IL-15*
	2	—	—	—	Control	Control	Control	Control
Group 2	30	6 mg	—	—	PV-B	PV-B	PV-B	PV-B
	6	—	—	—	Control	Control	Control	Control
Group 3	30	6 mg	—	1.5 mg	PV-B + *IL-12*	PV-B + *IL-12*	PV-B + *IL-12*	PV-B + *IL-12*
	6	—	—	—	Control	Control	Control	Control
Group 4	30	6 mg	2 mg	—	PV-B + *IL-15*	PV-B + *IL-15*	PV-B + *IL-15*	PV-B + *IL-15*
	6	—	—	—	Control	Control	Control	Control
Total	120 (100 vaccine + 20 control)							

PV-B = PENNVAX®-B

Thirteen participants discontinued the vaccination series early.

Six participants were discontinued from vaccination by the HVTN 070 PSRT for clinical reasons or AEs. All were vaccine recipients:

- One person in Group 1 was discontinued from vaccinations after the second vaccination, due to Grade 2 neutropenia, probably not related to vaccination.

- One person in Group 2 was discontinued from vaccinations after the third vaccination, due to the subject's relapse into drug abuse involving intranasal heroin, and an associated Grade 3 weight loss, which were not related to vaccination.

- One person in Group 4 was discontinued from vaccinations after the second vaccination, for symptoms occurring 10 minutes after vaccination, including globus sensation, bilateral hand paresthesias, nausea, and throat tingling, which were considered probably not related to study vaccine. Although symptoms were mild and self-limited and the site clinician suspected the symptoms were due to pre-existing gastroesophageal reflux disease and anxiety, the site clinician and the PSRT were concerned that the symptoms could possibly represent an allergic reaction and opted not to revaccinate the participant.

- One participant in Group 4 was discontinued for active cocaine use.

- One participant in Group 2 was discontinued for moderate atypical lateral epicondylitis, considered not related to vaccination.

- One participant in Group 2 was discontinued for cervical radiculopathy with an exacerbation of peripheral sensorimotor neuropathy (a pre-existing condition that was undisclosed at study entry) after 1 vaccination. This was considered to be possibly related to vaccination by the site investigator, and was also reported as a serious adverse event (SAE).

Thirteen participants discontinued the study early: 1 was unable to adhere to the visit schedule, 5 relocated, 6 were unable to be contacted, and 1 was no longer available.

No participant reported severe reactogenicity symptoms. The maximum severity of reactogenicity symptoms can be compared in Figure 4-3 to the same assessments in HVTN 080, which gave the same products with EP.

There were no statistically significant differences observed for any of the reactogenicity signs or symptoms between the 4 vaccine arms. Combining vaccine arms and comparing to placebos, the vaccine arms had more pain ($p=0.02$), tenderness ($p < 0.0001$), and pain and/or tenderness ($p=.0008$). There were no differences for erythema, induration or systemic symptoms.

Four SAEs have been reported for this study: the exacerbation of peripheral sensorimotor neuropathy mentioned above, possibly related, which led to the participant's discontinuation of vaccination in Group 2; a death in Group 2 which occurred 6 months after vaccination, which was considered probably not related to vaccination; a spontaneous abortion, probably not related to PV+*IL12* vaccination 38 days earlier; and a hospitalization of a participant in Group 3 with fever, flank pain, and abdominal lymphadenopathy which was considered probably not related to vaccination.

One adverse event (AE) was assessed by site investigators as probably or definitely related to vaccination with PV-B alone-- injection site pruritus. One AE was probably or definitely related to vaccination with PV-B with *IL-12* DNA-- injection site hematoma.

Flow cytometry was used to examine HIV-1–specific CD4+ and CD8+ T-cell responses using a validated ICS assay for IL-2 and/or IFN-γ. PBMC are stimulated with synthetic HIV-1 peptides that span the proteins encoded by the vaccine construct. The method used is based on PTE. The primary cellular immunogenicity endpoints for HVTN 070 are responses at Days 98 and 182 (ie Visits 7 and 9), corresponding to 2 weeks following the third and fourth vaccinations.

Responses were primarily for CD4+ T-cells, ranging from 19.2% - 28.0% among vaccinees at Day 98 and from 28.6% - 40.7% at Day 182. Responses to *pol* (26/33) and *gag* (20/33) were more frequent than responses to *env* (10/33). CD8+ T-cell response rates were < 8% for all treatment arms at both Days 98 and 182. These are summarized in Figure 4-4 for comparison with results from HVTN 080 which gave the same products with EP.

Neutralizing antibody titers against HIV-1 strains MN and SF162.LS were observed in low titers in a few vaccine and placebo recipients. The false positive rate from controls was 5/36 = 14% for MN and 1/36 = 3% for SF162.LS. A small number of titers in vaccine groups were slightly higher than the false positives but overall the nAb responses were weak. For the MN isolate at Day 98, vaccinees who received PV-B with *IL-15* 2 mg had the highest response rate, 25.9% (7/27), followed by participants receiving PV-B with *IL-12* DNA with 20.0% (6/30), and PV-B alone, 3.7% (1/27). MN response rates were lower at Day 182. No one in the group that received PV-B with *IL-15* DNA 0.8 mg responded at either timepoint. There were no responses in the vaccine arms to SF162.LS, at either timepoint.

Binding antibodies to consensus B Env and Gag were assessed by a validated ELISA using single serum dilutions (1/20). There was one low level responder (out of 94 vaccinees assessed) who received PV-B + *IL-12* DNA.

In summary, local and systemic reactions have been mild to moderate in the HVTN 070 phase 1 trial. Thirty participants have received PV-B 6 mg with *IL-12* DNA (GENEVAX®-*IL-12*-4532) 1.5 mg, of whom 27 have received 4 injections. Also, an additional 70 participants have received PV-B 6 mg with or without *IL-15* DNA (pIL15EAM), of whom 56 have received 4 injections. No severe reactions or other safety concerns related to the study products have been observed. Overall, the study products have been well-tolerated, with an acceptable safety profile to date. The T-cell responses to PV-B were more frequent than previously seen in HVTN 060 and HVTN 063 with the Wyeth p37 *gag* DNA vaccine, and were CD4+. Responses were more frequent to Gag and Pol antigens relative to Env.

4.8.3.4 A phase 1 clinical trial to evaluate the safety and immunogenicity of PENNVAX®-B (*gag*, *pol*, *env*) vaccine, with or without *IL-12* DNA plasmid, delivered via EP in healthy, HIV-1–uninfected adult participants (HVTN 080)

IL-12 DNA plasmid was evaluated with PV-B (*gag*, *pol*, *env*) vaccine in HIV-uninfected healthy adults in HVTN 080 (Table 4-9). Both study products were delivered using the Inovio CELLECTRA® EP device.

The trial schema is given below.

Table 4-9 Trial schema for HVTN 080

			Vaccination schedule in months (days)		
Study arm	**N**	**Dose via EP**	**0 (0)**	**1 (28)**	**3 (84)**
Group 1	10	3 mg PV-B	PV-B	PV-B	PV-B
	2		Placebo	Placebo	Placebo
Group 2	10	3 mg PV-B + 1 mg *IL-12* DNA	PV-B+ *IL-12* DNA	PV-B+ *IL-12* DNA	PV-B+ *IL-12* DNA
	2		Placebo	Placebo	Placebo
Group 3	20	3 mg PV-B + 1 mg *IL-12* DNA	PV-B+ *IL-12* DNA	PV-B+ *IL-12* DNA	PV-B+ *IL-12* DNA
	4		Placebo	Placebo	Placebo
Total		40 + 8 = 48			

PV-B = PENNVAX®-B

The first participant was enrolled November 9, 2009. Injections for the study were completed August 26, 2010.

As one measure of tolerability, participants were asked to mark along a 10 cm line (0 cm = no pain to 10 cm = worst pain) to indicate their perceived severity of discomfort related to EP at 3 timepoints: immediately after injection with EP, at 5 minutes, and at 30 minutes. All participants experienced some pain immediately following each vaccination (median VAS, 5.0–5.4 across vaccinations; range 0.4–9.0), with no statistically significant differences between arms. VAS scores decreased at 5 minutes (median, 0.7–

0.9; range, 0–6.9) and 25 minutes (median, 0.5–1.0; range, 0–6.0). PV-B recipients recorded higher VAS scores than PV-B plus *IL-12* DNA recipients 5 and 25 minutes after each vaccination ($P < .001$ at 5 minutes, and $P<.04$ at 25 minutes). Controls had more pain than PV-B plus *IL-12* DNA recipients 5 minutes after the second and third vaccinations (P = .02 for both comparisons). One possibility for the decreased pain associated with the PV-B plus *IL-12* DNA is that this was due to the increased total dose of bupivacaine given with the addition of *IL-12* DNA. Both the vaccine and adjuvant preparations were formulated in 0.25% bupivacaine.

No SAEs or safety concerns related to vaccination have been identified for this study. One AE reported by the group that received PV-B vaccine given with EP that was considered related to the study product was injection site induration. The AEs that were considered related to PV-B with *IL-12* DNA given by EP were: injection site pain (2 events), injection site reaction, presyncope, and device malfunction.

Three participants discontinued vaccinations early. One participant was discontinued from vaccination due to the discovery of an ongoing pre-existing condition, not previously known to the site, of bony disease of the spine. The participant presented with pain and numbness in the right leg which eventually led to an AE report of moderate exacerbation of pre-existing spinal stenosis L1-2, which was ongoing at the end of study participation. Two participants refused to receive additional vaccinations, as they considered the process or the reactogenicity symptoms too painful.

Reactogenicity refers to solicited injection site or systemic symptoms within the first 3 days after vaccination. The maximum severity of reactogenicity symptoms reported by participants, occurring within 3 days of vaccination, are summarized in Figure 4-3 on the right in comparison to results from HVTN 070, left. There were no severe reactogenicity symptoms.

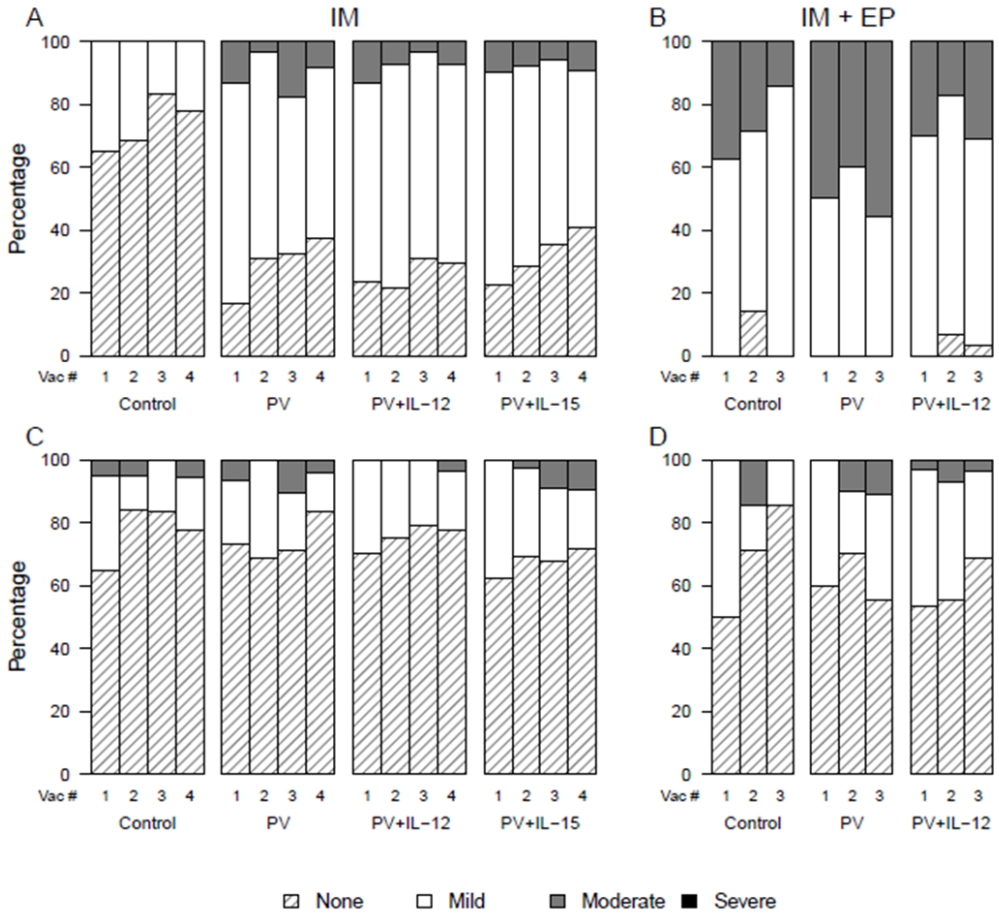

Figure 4-3 Maximum severity of reactogenicity symptoms after vaccination with control or PV-B with *IL-12* DNA or IL-15 DNA, and with or without EP. (A) Severity of injection site symptoms (pain/tenderness) in HVTN 070 (B) Severity of injection site symptoms in HVTN 080 (C) Severity of systemic symptoms (malaise and/or fatigue, myalgia, headache, nausea, vomiting, chills, arthralgia) in HVTN 070 (D) Severity of systemic symptoms in HVTN 080.

For HVTN 080 there were no statistically significant differences for any reactogenicity signs/symptoms between PV-B alone compared to PV-B + *IL-12* DNA or for the vaccine arms compared to placebos.

Immune responses from this trial have been summarized in Section 4.1.1.

EP in this study showed a significant dose-sparing effect. In HVTN 080, people were vaccinated with cumulative doses of 9 mg PV-B and 3 mg *IL-12* DNA, compared to HVTN 070 in which people received up to 24 mg PV-B in the course of the study and yet had lower response rates. Although a small group of 10 subjects was evaluated with DNA delivered by EP without *IL-12*, this was primarily a safety arm and was not large enough for a formal comparison. Four of 9 evaluable individuals in this arm developed CD4+ immune responses, and 3/9 developed CD8+ T-cell responses. While these response rates appear lower than with DNA+*IL12* delivered via EP, these differences were not statistically significant. No significant differences were observed in the magnitude of responses between arms with or without *IL-12* DNA or between like-arms with or without EP.

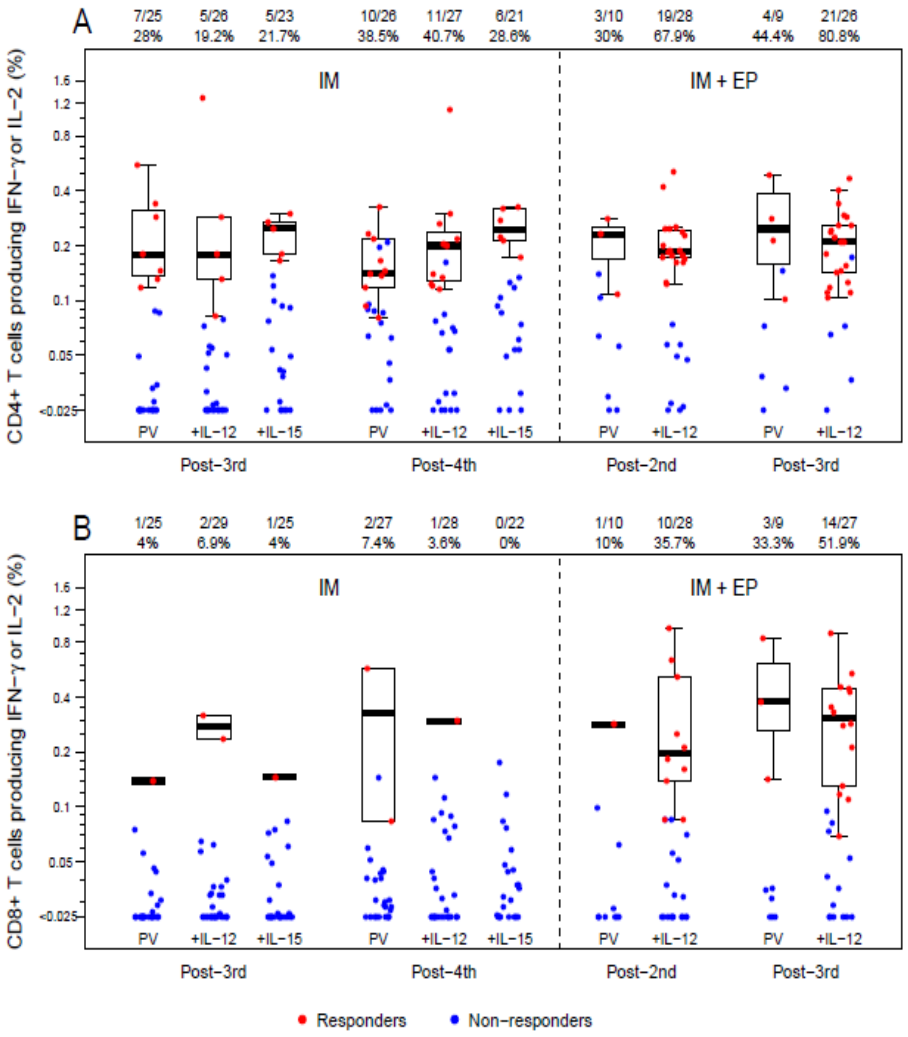

Figure 4-4 HVTN 070 and HVTN 080 ICS responses against ANY global PTE peptides (Env, Gag, or Pol), comparing standard IM injection (HVTN 070) to IM injection with EP and lower doses of product (HVTN 080). (A) CD4+ T cells (B) CD8+ T cells.

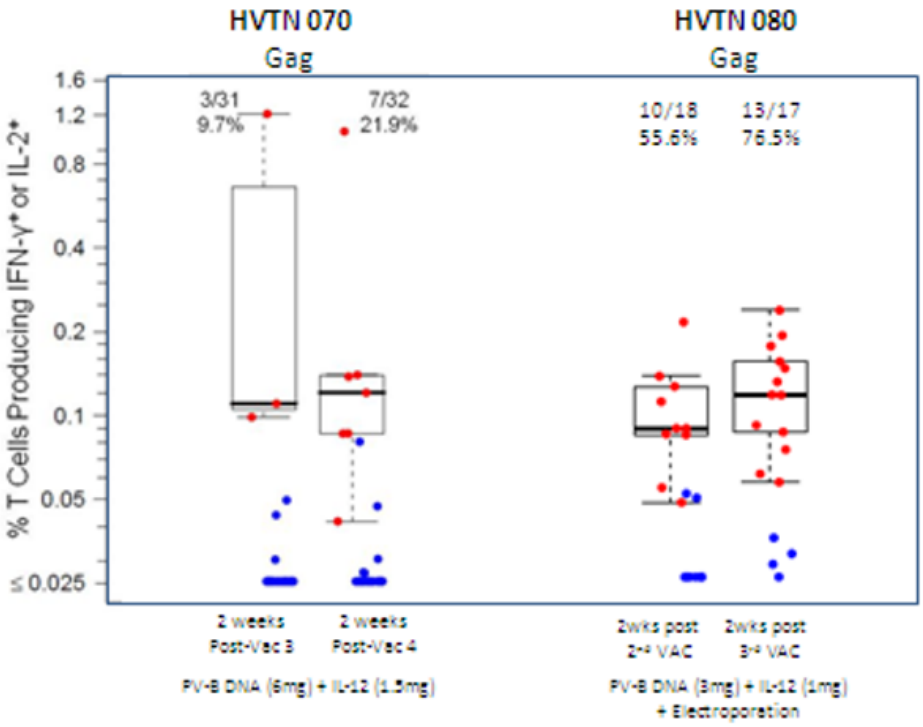

Figure 4-5 HVTN 070 and HVTN 080 ICS responses against Gag global PTE peptides. The timepoint following the third vaccination is most comparable (left graph for HVTN 070, 9.7%, and right graph for HVTN 080, 76.5%).

Interestingly, IM+EP PV+*IL12* CD4+ responders after the 2nd vaccination had a significantly lower BMI than nonresponders (median BMI 23.3 vs 25.4; p=.04), however after the 3rd vaccination, the majority of subjects had a CD4+ response, and this difference was not significant. In contrast, for CD8+ T cell responses the BMI of responders was lower than that of non-responders after 2 or 3 vaccinations (p=0.04 at both time points). Among responders, there were no significant correlations between BMI and response magnitudes. We found no relationship between gender, and either CD4+ or CD8+ response with IM+EP administration.

Neutralizing antibodies against HIV-1 strains Bal.26, MN.3, MW965.26, NPO3.13 and SF162.LS were measured. Low level (titer < 20) positive responses to the MW965.26 isolate were observed in 10 participants. For Group 1 receiving PV-B alone the response rate was 60.0% at Day 42 and 20.0% at Day 98. For the combined Groups 2 and 3 receiving PV-B and *IL-12* DNA, the response rate was 20.0% at Day 42 and 30.0% at Day 98. One placebo recipient had a positive response for MW965.26 at Day 98. Four participants (2 from Group 1 and 2 from Group 2) had a positive response at both timepoints. No responses were observed against Bal.26, MN.3, NPO3.13, and SF162.LS viruses.

IL-12 neutralization activity was measured in six placebo-treated and thirty volunteers randomized to the two arms receiving the PENNVAX®-B vaccine with *IL-12* DNA plasmid. IL-12 neutralization was measured by in vitro stimulation of natural killer cells with participant sera with subsequent measurement of secreted IFN-γ in the culture supernatant using a sandwich ELISA. The lower limit of quantitation for this assay was determined to be 8 NU/mL and titers equal to or less than 8 NU/mL were considered negative. Assays on clinical samples resulted in such high background in prevaccination

(baseline) serum samples that quantities up to 30 NU/mL were considered negative; however this response appears to be independent of the vaccination.

Data were available for analysis at baseline (visit 2) for all 36 participants. Data were available from day 98 for 27 vaccinees and 6 placebo participants. The 3 vaccinees without a day 98 specimen had data from day 42 (n=2) or day 273 (n=1).

No placebo-treated subjects showed any positive responses. Two subjects tested positive pre-vaccination (39 NU/mL and 31 NU/mL) and one of these subjects also tested positive post-vaccination (49 NU/mL). No other sera tested positive for IL-12 neutralization.

These results indicate that subjects receiving *IL-12* DNA plasmid and the PENNVAX®-B vaccine using in vivo electroporation did not develop any IL-12 neutralization activity.

In summary, PV-B and *IL-12* DNA can be safely administered with IM injection and EP using the Inovio CELLECTRA® EP system. IM vaccination with EP is adequately tolerated by participants although it is associated with a moderate amount of transient discomfort. EP significantly enhances the cellular immunogenicity of DNA. EP technology has the potential to change the prospects for DNA vaccines.

4.8.3.5 A phase 1 trial to evaluate the safety, tolerability, and immunogenicity of an *IL-12* pDNA enhanced HIV-1 multiantigen pDNA vaccine delivered intramuscularly with electroporation, with an HIV-1 rVSV vaccine boost, in healthy HIV-uninfected adult participants

HVTN 087 is another study which tested GENEVAX® *IL-12*-4532 DNA with an HIV DNA vaccine, HIV-MAG (Profectus Biosciences, Inc, Tarrytown, NY) using IM injection with EP. The schema for HVTN 087 is shown in (Table 4-10).

Table 4-10 Trial schema for HVTN 087

Study arm	N	Dose *IL-12* pDNA	Month 0	Month 1	Month 3	Month 6
Group 1	22	0 mcg	HIV-MAG*	HIV-MAG	HIV-MAG	VSV HIV *gag***
	3	—	placebo	placebo	placebo	placebo
Group 2	22	250 mcg	HIV-MAG + *IL-12* DNA	HIV-MAG + *IL-12* DNA	HIV-MAG + *IL-12* DNA	VSV HIV *gag*
	3	—	placebo	placebo	placebo	placebo
Group 3	22	1000 mcg	HIV-MAG + *IL-12* DNA	HIV-MAG + *IL-12* DNA	HIV-MAG + *IL-12* DNA	VSV HIV *gag*
	3	—	placebo	placebo	placebo	placebo
Group 4	22	1500 mcg	HIV-MAG + *IL-12* DNA	HIV-MAG + *IL-12* DNA	HIV-MAG + *IL-12* DNA	VSV HIV *gag*
	3	—	placebo	placebo	placebo	placebo
Total: 100 (88 vaccine / 12 placebo)						

*Dose of HIV-MAG was 3 mg throughout the trial. When given with adjuvant, HIV-MAG and IL-12 DNA are admixed into the same syringe.

**The dose of VSV HIV *gag* throughout the trial was 3.4×10^7 PFU.

The HIV-MAG vaccine consists of 2 plasmid DNA expression vectors, HIV-1 *gag*/*pol* and HIV-1 *nef/tat/vif, env*. In HVTN 087, HIV-MAG vaccine with or without GENEVAX® *IL-12* DNA was safe and generally well tolerated when given via EP. Preliminary immunogenicity data are available. After 3 vaccinations of the HIV-MAG DNA vaccine and GENEVAX® *IL-12* DNA, (Figure 4-6; v10), in a per protocol analysis, 77% (48 out of 62) and 40% (25 of 62) of vaccinated individuals mounted a CD4+ T-cell or CD8+ T-cell vaccine-specific ICS response, respectively. In HVTN 087, the Group 3

regimen with the same DNA doses given in HVTN 080 (3 mg HIV-MAG given with 1 mg of *IL-12* DNA IM+EP) was immunogenic, with CD4+ T cell responses seen in 69% of vaccinated participants (per protocol analysis), and a CD8+ T cell responses in 50%, after 3 doses.

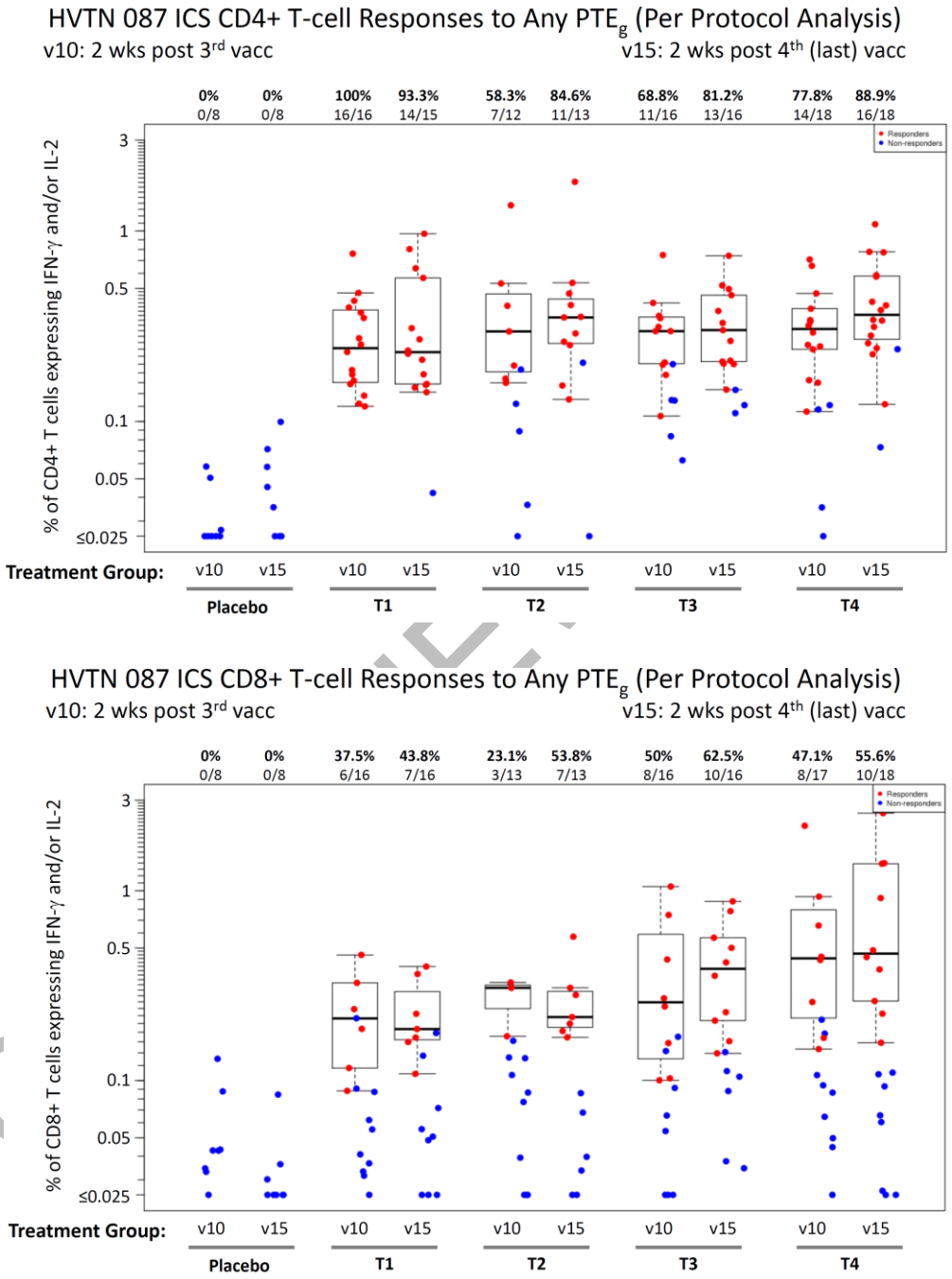

Figure 4-6 HIV-specific ICS responses in HVTN 087, after 3 DNA+EP vaccinations (visit 10) and after VSV HIV gag boost (visit 15).

The results of HVTN 087 indicate that the highest dose of *IL-12* pDNA (T4) was associated with an increased magnitude of CD8+ T-cell responses after VSV HIV *gag* boost (visit 15) compared with the magnitude of responses from participants in Group 1

(T1) who did not receive *IL-12* pDNA (p = 0.02, Figure 4-6, lower panel). The ability to see significant dose effects between the study arms was limited by the small sample sizes.

In summary, HIV-MAG with *IL-12* DNA IM+EP was immunogenic, with similar CD4+ and CD8+ T cell response rates as in HVTN 080, and with a significantly increased magnitude of CD8+ T cell responses at the highest dose of *IL-12* DNA after VSV boosting, compared to vaccination without the cytokine adjuvant.

4.8.3.6 Phase I Safety and Immunogenicity of Electroporated HIV DNA +/-Interleukin 12 and Ad35-GRIN/ENV in Healthy HIV-seronegative African Volunteers

IAVI has presented results of its B004 study, which also tested multiple injections of HIV-MAG and GENEVAX® *IL-12*-4532 DNA IM+EP in healthy volunteers.[44]

The study tested the following groups:

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 DNA 100 mcg	HIV-MAG+ IL-12 DNA 100 mcg	HIV-MAG +IL-12 DNA 100 mcg	Ad35-GRIN/ENV
	3	Placebo	Placebo	Placebo	Placebo
Group 3	12	HIV-MAG+ IL-12 DNA 1000 mcg	HIV-MAG+ IL-12 DNA 1000 mcg	HIV-MAG+ IL-12 DNA 1000 mcg	Ad35-GRIN/ENV
	3	Placebo	Placebo	Placebo	Placebo

Notes:

HIV-MAG 3 mg delivered IM by in vivo electroporation. GENEVAX® IL-12 co-administered with HIV-MAG delivered IM by in vivo electroporation. Ad35-GRIN/ENV $2x10^{10}$vp delivered IM by standard needle injection.

The investigators report that 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 IFNγ ELISpot, at baseline there were no responders. After the 3 DNA 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, TNFα, and IFNγ 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.

4.8.4 Clinical studies of ID electroporation

In an effort to improve tolerability of the procedure of injection with EP procedure, Inovio has also focused efforts on development of its ID delivery system which uses less electrical energy and shallower injection depth than the IM delivery system. While the results from the HVTN 080 study using the IM approach are clearly encouraging, the ID procedure represents a more tolerable approach that does not sacrifice immunogenicity in preclinical models. Inovio is currently testing a novel DNA vaccine for influenza where individuals were primed using an IM injection with EP (study FLU001) followed by boosting with an ID injection with lower energy EP in a phase 1 clinical study (FLU002). Patients were asked to rate their pain on a modified VAS (0 being "no pain" and 10 being "worst pain imaginable"). Figure 4-7 demonstrates that the same subjects rated the ID procedure to be much more tolerable than the IM procedure. VAS scores immediately after the procedure (0 minutes) were clearly lower and the initial pain of the procedure resolved much more quickly (5 and 10 minute postprocedure). This simple, efficient and tolerable method of delivery has great potential for DNA vaccination.

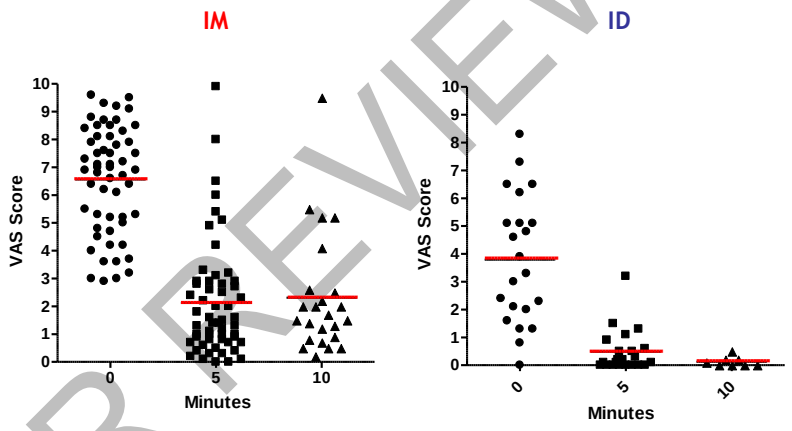

Figure 4-7 VAS scores Inovio influenza vaccination with EP, IM versus ID

Recent trials at Inovio using DNA vaccines and ID EP:

1. FLU002: A 2-dose series of a prophylactic H5 HA influenza DNA plasmid (INO-3401) was delivered via ID injection with the CELLECTRA®-3P device (ID+EP), to healthy volunteers previously immunized with an H5 combination (HA, NA, M2e-NP) influenza plasmid vaccine (VGX-3400X) administered IM (1 mL) followed by EP with CELLECTRA®-5P in the FLU001 study.

    - 22 subjects received at least 1 dose (0.9 mg DNA / dose)

    - 17 subjects received the Month 3 vaccination (second ID boost)

- No study product related Grade 3 or 4 laboratory abnormalities, AEs, SAEs or injection site reactions have been reported.

- Injection site pain was reported by 45% of participants and redness was reported by 55%. All injection site reactions resolved without sequelae. Other injection site AEs reported were: nodule (1), haematoma (1), oedema (1), pruritus (6) and injection site reaction (visible needle marks at injection site).

2. FLU101: A phase 1 clinical trial (BB-IND 14682) to investigate various dosing regimens of a prophylactic H1 and H5 influenza DNA plasmid vaccine (INO-3510) delivered by ID+EP with CELLECTRA®-3P

    - Of the 116 enrolled, twenty subjects discontinued early including 15 who were lost to follow up and 5 who discontinued for reasons unrelated to the study or vaccination.

    - Doses of 0.9 mg DNA were given per injection site; except for one study arm (Arm D), in which 10 participants received 0.3 mg DNA per injection. 71 participants in Arms A-G received 3 doses. In Arm H, 10 participants received 2 doses. Arm I was a comparator arm of seasonal influenza vaccine given by needle and syringe. 9 subjects in Arm J received 1.8 mg DNA, given as 2 doses of 0.9 mg each at 3 vaccination visits (6 doses total).

    - There were no deaths or life-threatening AEs reported. No grade 3 or 4 injection site reactions were reported and all injection site reactions resolved without sequelae.

    - There was one grade 3 adverse event that was considered "probably related" to study product. A grade 3 fever (103.2ºF) was reported by one subject one day following 3rd vaccination/EP. The event was assessed by the investigator as "probably related" to study product. The site confirmed there was no other underlying condition that contributed to the event and that the subject did not seek medical attention, no medications were taken, and the event resolved the following day. The subject did not report any injection site pain, tenderness, or erythema in either arm. Injection site swelling was reported as 0.7 x 0.8 cm in the left arm and 0.7 x 0.9 cm in the right arm, which resolved the following week. The subject reported one additional adverse event (injection site pruritus (right arm)) during the above noted event. This event was assessed as a grade 1, "definitely related" event lasting 75 days.

    Table 4-11 shows the summary of post-vaccination pain reported within 4 weeks following each administration as assessed by the Investigator by severity for FLU-101.

Table 4-11 Summary of post-vaccination pain by severity for FLU-101

Greatest Severity After Each Vaccination	Subject Count (%) Vaccination 1 (n = 116), 2 (n = 95), 3 (n = 80)		
	Mild Grade 1	Moderate Grade 2	Severe Grade 3
Pain			
- Post Vaccination 1	3 (3)	0 (0)	0 (0)
- Post Vaccination 2	2 (2)	0 (0)	0 (0)
- Post Vaccination 3	3 (4)	0 (0)	0 (0)

Source: Table 14.3.1.4, Summary of Post-Vaccination Reactions (Worst Reaction Reported within 4 Weeks After Each Vaccination), run date 21 May 2014.An event occurring more than once in the same subject is counted once at the highest grade for that reporting period

In these studies, as previously observed with other ID vaccinations, skin changes have been noted at the two vaccination sites (volar aspect of the forearm and the skin above the deltoid muscle) in 63/138 subjects (45.7%). Vaccine dose did not appear to affect the frequency of skin changes. These first appear as erythematous papules with small eschars at the sites where EP electrodes have entered the skin, and heal with occasional postinflammatory hypo- or hyperpigmentation or scar. The skin changes appear to be more prominent in darker-skinned individuals and have been observed to persist up to 9 months after the initial vaccination.

3. FLUPRIME: An investigator-initiated study to evaluate ID+EP administration of Inovio's INO-3605 and INO-3609 H1 influenza DNA vaccines alone or in a prime-boost regimen with licensed inactivated influenza vaccine in healthy Canadian adults over the age of 65 years.

   - The study enrolled 50 participants into 3 treatment groups; 2 active arms that received 2-4 doses of DNA vaccine with 1-2 booster(s) of seasonal influenza vaccine and one placebo arm who received 2 doses of sterile water for injection and 2 doses of seasonal influenza vaccine.

   - As of October 22, 2013, all ID EP procedures (146: 20 using placebo, 126 with DNA) have been completed. Forty participants (80%) reported at least one AE. Most events were judged to be mild (63/117, 54%, 15% of these related) or moderate (49/117, 42%, 9% of these related) by the investigator. Twenty-nine (25%) AEs were assessed as possibly or definitely related to the study product.

   - There have been no grade 3 or 4 injection site reactions or grade 3 or 4 related AEs reported. There were two deaths, two life-threatening AEs and two non-life-

threatening SAEs reported. All were considered unrelated to study drug. There were no injection site AEs related to ID EP reported in the study.

4.9 Potential risks of study products and administration

Table 4-12 Summary of potential risks of study products and administration

Common	• Mild to moderate injection site pain, tenderness, pruritus, erythema, or swelling/induration/edema • Malaise/fatigue, myalgia, or headache in the first few days following injection • A vaccine-induced positive HIV antibody test result • Visible lesion(s) at the injection site, such as erythematous papules with eschar, hypopigmentation, hyperpigmentation, or scar
Less common	• Severe injection site pain or tenderness • Fever, chills, flu-like syndrome, arthralgia, rash, nausea, or dizziness in the first few days following injection • Vasovagal reaction/lightheadedness/dizziness related to the injection procedure • Transient changes in clinical laboratory values • Injection site hematoma, bruising/ecchymosis, laceration, other transient lesions, or bleeding related to the injection procedure
Uncommon or rare	• Severe localized injection site reaction, such as sterile abscess or secondary bacterial infection • Allergic reaction, including rash, urticaria, angioedema, bronchospasm, or anaphylaxis
Unknown frequency or theoretical risks	• Muscle damage at the injection site • Autoimmune disease or cancer • Electrical injury with EP • Disruption of function of implanted electronic medical devices with EP • Exacerbation of cardiac arrhythmia with EP • Effects on a participant's response to an approved HIV vaccine administered in the future • Effects on susceptibility to HIV, if the participant is exposed to HIV • Effects on the course of HIV infection/disease, if the participant is infected with HIV • Effects on the fetus and on pregnancy

5 Objectives and endpoints

5.1 Primary objectives and endpoints

Primary objective:

To evaluate the safety and tolerability of PENNVAX®-GP, an HIV-1 *env* A, *env* C, *gag*, *pol* plasmid DNA vaccine, and human *IL-12* DNA, given by intradermal or intramuscular injection with electroporation, in healthy HIV-uninfected adult volunteers

Primary endpoints:

- Frequency and severity of reactogenicity signs and symptoms
- Magnitude of local injection/EP site pain as measured by a VAS.
- 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: white blood cells, neutrophils, lymphocytes, hemoglobin, alkaline phosphatase, platelets, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, and creatine phosphokinase (CPK) at baseline and at follow-up visits postvaccination.
- Number of participants with early discontinuation of vaccinations and reason for discontinuation.
- Distribution of responses to questions regarding acceptability of study injection procedures.

5.2 Secondary objectives and endpoints

Secondary objective:

To characterize and rank the immunogenicity of 3 vaccine regimens: PENNVAX®-GP with and without *IL-12* DNA, given by intradermal injection with electroporation, and PENNVAX®-GP with *IL-12* given by intramuscular injection with electroporation

Secondary endpoints:

- Response rate of CD4+ T-cell responses measured by flow cytometry, to HIV-1-specific peptide pools representing *gag*, *pol*, *env* following the third and fourth vaccinations

- Response rate of CD8+ T-cell responses measured by flow cytometry, to HIV-1-specific peptide pools representing *gag*, *pol*, *env* following the third and fourth vaccinations

- Frequency and magnitude of HIV-1specific binding antibody (Ab) responses as assessed by multiplex assay following the third and fourth vaccinations

- Neutralizing antibody magnitude and breadth against tier 1 and, if applicable, tier 2 HIV-1 isolates as assessed by area under the magnitude-breadth curves following the third and fourth vaccinations

- B-cell response rate and magnitude measured by B-cell ELISpot to quantify Env-specific antibody producing B cells following the third and fourth vaccinations

5.3 Exploratory objectives and endpoints

Exploratory objective 1:

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

Exploratory endpoint 1:

T-cell receptor beta chain sequence diversity of HIV-specific T cells

Exploratory objective 2:

To further characterize B cell immune responses

Exploratory endpoint 2:

Titers of antibody-dependent cellular cytotoxicity-mediating antibodies following the third and fourth vaccinations

Exploratory objective 3:

To describe and document the range of injection site skin changes that can be expected 2 weeks and at later timepoints after intradermal or intramuscular injection with electroporation

Exploratory endpoint 3:

Findings (including skin lesion descriptions, measurements) from post-reactogenicity injection site assessments and photography

Exploratory objective 4:

To evaluate for a relationship between anthropometric measurements (BMI and waist circumference) and vaccine-elicited immune responses

Exploratory endpoint 4:

To determine the relationship between BMI and waist circumference and the magnitude and frequency of CD4+ T-cell, CD8+ T-cell, B-cell and antibody responses

Exploratory objective 5:

To collect information on CELLECTRA® EP system performance

Exploratory endpoint 5:

Frequency and types of device issues or clinician / operator error, and frequency of missed vaccinations related to these events

6 Statistical considerations

6.1 Accrual and sample size calculations

Recruitment will target up to 94 (85 vaccinees and 9 placebos) healthy, HIV-uninfected adult participants. Enrollment will begin with Group 1. If the safety data are acceptable, Groups 2,3, and 4 will open to enrollment simultaneously.

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 PBMCs, or high background. Immunogenicity data from nine phase 1 and one phase 2a HVTN vaccine trials, which began enrolling after June 2005 (data as of June 2011), indicate that 10% is a reasonable estimate for the rate of missing data. For this reason, the sample size calculations in Section 6.1.2 account for 10% of enrolled participants having missing data for 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 (see Section 11).

The ability of the study to detect SAEs (see Section 11) 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 the vaccine arm in Group 1 (n = 5), there is a 90% chance of observing at least 1 event if the true rate of such an event is 37% or more; and there is a 90% chance of observing no events if the true rate is 2% or less. Group 2 (n = 20), there is a 90% chance of observing at least 1 event if the true rate of such an event is 10.9% or more; and there is a 90% chance of observing no events if the true rate is 0.5% or less. For each vaccine arm in Groups 3 and 4 (n =30) , there is a 90% chance of observing at least 1 event if the true rate of such an event is 7.4% or more; and there is a 90% chance of observing no events if the true rate is 0.3% or less. As a reference, in HVTN vaccine trials from December 2000 through December 2012, 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 5, 20 and 30 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 5, 20 and 30, for different true event rates

True event rate (%)	Pr(0/30)	Pr(1+/30)	Pr(2+/30)	Pr(0/20)	Pr(1+/20)	Pr(2+/20)
1	74.0	26.0	3.6	81.8	18.2	1.7
3	40.1	59.9	22.7	54.4	45.6	12
5	21.5	78.5	44.6	35.8	64.2	26.4
7	11.3	88.7	63.1	23.4	76.6	41.3
9	5.9	94.1	76.6	15.2	84.8	54.8
10	4.2	95.8	81.6	12.2	87.8	60.8
20	0.1	99.9	98.9	1.2	98.8	93.1
30	<0.1	>99.9	>99.9	0.1	99.9	99.2
40	<0.1	>99.9	>99.9	<0.1	>99.9	99.9
True event rate (%)	Pr(0/5)	Pr(1+/5)	Pr(2+/5)			
20	32.8	67.2	26.3			
40	7.8	92.2	66.3			
60	1	99	91.3			
80	<0.1	>99.9	99.3			

An alternative way of describing the statistical properties of the study design is in terms of the 95% confidence interval (CI) for the true rate of an adverse event based on the observed data. Table 6-2 shows the 2-sided 95% CIs for the probability of an event based on a particular observed rate. Calculations are done using the score test method [45]. If none of the 20 participants receiving PENNVAX®-GP without IL-12 ID experience a safety event, the 95% 2-sided upper confidence bound for the true rate of such events in the vaccinated population is 16.1%, and if none of the 30 participants receiving PENNVAX®-GP with IL-12 by ID or IM experience a safety event, the 95% 2-sided upper confidence bound for the true rate of such events in the vaccinated population is 11.4%.

Table 6-2 Two-sided 95% CIs based on observing a particular rate of safety endpoints for arms of size 5, 20 and 30

Observed event rate	CI (%)
0/5	[0.0, 43.4]
1/5	[3.6, 62.4]
2/5	[11.8, 76.9]
0/20	[0, 16.1]
1/20	[0.9, 23.6]
2/20	[2.8, 30.1]
0/30	[0.0, 11.4]
1/30	[0.6, 16.7]
2/30	[1.8, 21.3]

6.1.2 Sample size calculations for immunogenicity

6.1.2.1 Comparison of immune response rates among vaccine arms

The main goals of this trial regarding immunogenicity outcomes involve a preliminary estimation of response rates based on data from the B cell and T cell assays among vaccinees. No adjustment for multiple comparisons will be made for the use of multiple assays. The precision with which the true response rate can be estimated from the observed data depends on the true underlying response rate and the sample size. Two-sided 95% CIs for the response rate based on observing a particular rate of responses in the vaccinees is shown in Table 6-3. Calculations are done using the score test method [45]. The table indicates n=18 and 27, assuming a 10% loss of data. The sample size for Group 1 is not designed for a good estimation of response rates, for example, if the observed response rate is 60%, two-sided 95% CI for the true response rate is between 23.1% and 88.2% for n=5.

Table 6-3 Two-sided 95% CIs for the true response rate based on observing a particular rate of responses in the vaccinees (n = 18, 27)

No. of responses	Observed response rate (%)	CI
1/18	5.6	[1, 25.8]
3/18	16.7	[5.8, 39.2]
5/18	27.8	[12.5, 50.9]
7/18	38.9	[20.3, 61.4]
9/18	50	[29, 71]
11/18	61.1	[38.6, 79.7]
13/18	72.2	[49.1, 87.5]
15/18	83.3	[60.8, 94.2]
17/18	94.4	[74.2, 99]
1/27	3.7	[0.7, 18.3]
3/27	11.1	[3.9, 28.1]
5/27	18.5	[8.2, 36.7]]
7/27	25.9	[13.2, 44.7]
9/27	33.3	[18.6, 52.2]
11/27	40.7	[24.5, 59.3]
13/27	48.1	[30.7, 66.0]
15/27	55.6	[37.3, 72.4]
17/27	63.0	[44.2, 78.5]
19/27	70.4	[51.5, 84.1]
21/27	77.8	[59.2, 89.4]
23/27	85.2	[67.5, 94.1]

A formal comparison of immune response rates between Group 1 and Groups 2, 3, 4 will not be conducted due to the limited sample size in Group 1. As shown in Table 6-4, there is limited power for a formal comparison of immunogenicity response rates between Group 2 of size n=20 and Groups 3 and 4 of size n = 30. For either 80% or 90% power, the sizes of differences that the trial is powered to detect are fairly large. These calculations use a Fisher's exact 2-sided test with a Type I error rate of 0.05.

Table 6-4 Power for comparison of response rates between 2 arms of size 27 and between n1=18 and n2=27 with 2-sided statistical tests

	Minimum true response rate in Arm 2 in order to detect a difference	
True response rate Arm 1 (%)	80% power	90% power
n1=18	n2=27	
10	53	60
20	66	72
30	76	82
40	85	90
50	92	96
n1=27	n2=27	
10	47	52
20	60	66
30	72	77
40	80	85
50	88	92

An alternative to formal comparisons of arms is to rank the arms by their response rates. For Groups 2, 3 and 4, we can assess the reliability of this study to select the best arm with respect to the magnitude of response rates. Ranking will be performed separately for each of these assays. Selection of the best regimen will depend upon scientific judgment as to the pattern of rankings and magnitude of differences observed. Table 6-5 shows various true response rates for which this study will correctly select the arm with the highest response rate with 0.8 or 0.9 probabilities. Each line in the table shows the results based on 40,000 simulated datasets of response rates for 3 arms of size 18, 27 and 27 generated using different binomial probabilities, with the best response probability used to generate data for one arm and the second best response probability used to generate data for the other arms. The top panel is for the case that the best arm has sample size 27 and the other 2 arms have 18 and 27. The bottom panel is for the case that the best arm has sample size 18 and the other 2 arms have 27. If the difference in response between the best and second best arms is smaller than the assumed difference, the chance of correctly selecting the arm with the true highest response will be less than 80% (90%).

Table 6-5 True immunogenicity response rates for which the regimen with the highest response probability will be correctly selected with 0.8 (0.9) probability among 3 arms of size 18, 27 and 27

Second best response probability	Best response probability	Difference
	Best arm n=27	
10%	22%(28%)	12%(18%)
20%	35%(41%)	15%(21%)
30%	47%(53%)	17%(23%)
40%	57%(63%)	17%(23%)
50%	67%(73%)	17%(23%)
60%	77%(82%)	17%(22%)
70%	85%(89%)	15%(19%)
80%	93%(96%)	13%(16%)
90%	99%(100%)	9%(10%)

	Best arm n=18	
10%	23%(29%)	13%(19%)
20%	36%(42%)	16%(22%)
30%	47%(53%)	17%(23%)
40%	58%(64%)	18%(24%)
50%	67%(73%)	17%(23%)
60%	76%(81%)	16%(21%)
70%	85%(89%)	15%(19%)
80%	92%(95%)	12%(15%)
90%	98%(100%)	8%(10%)

6.1.2.2 Comparison of neutralizing response among vaccine arms

Secondary endpoints also include the area under the magnitude-breadth curve (AUC-MB) endpoint, calculated for both the tier 1 isolates and for the tier 2 isolates. For Groups 2, 3 and 4, we can assess the reliability of this study to select the best arm with respect to the AUC-MB. Table 6-6 shows various true AUC-MB means for which this study will correctly select the arm with the highest AUC-MB mean with 0.8 or 0.9 probabilities. Each line in the table shows the results based on 40,000 simulated datasets of response means for 3 arms of size 18, 27 and 27 generated using different normal distributions, with the best (highest) response mean used to generate data for one arm and the second best (lower) response mean used to generate data for the other arms. The same standard deviation (SD) (0.204) is used for all the arms. The standard deviation of AUC-MB is estimated based on the neutralization data from 90 recipients of the AIDSVAX bivalent subtype B vaccine in the North America/Netherlands VaxGen efficacy trial (Gilbert *et al.*, unpublished data). For these 90 vaccine recipients, the sample average of the AUC-MB to a panel of 12 tier 2 subtype B isolates was 1.04 and the sample SD was 0.204. The top panel is for the case that the best arm has sample size 27 and the other 2 arms have 18 and 27. The bottom panel is for the case that the best arm has sample size 18 and the other 2 arms have 27. If the difference in response means between the best and second best arms is smaller than the assumed difference, the chance of correctly selecting the arm with the true highest response will be less than 80% (90%).

Table 6-6 True AUC-MB mean responses for which the regimen with the best/highest neutralization AUC-MB will be correctly selected with 0.8 (0.9) probability for 3 arms

Second best response mean	Best response mean	Difference
	Best arm n=27	
1.04	1.11(1.14)	0.07(0.1)
1.08	1.15(1.18)	0.07(0.1)
1.12	1.19(1.22)	0.07(0.1)
1.16	1.23(1.26)	0.07(0.1)
1.2	1.27(1.3)	0.07(0.1)
	Best arm n=18	
1.04	1.12(1.14)	0.08(0.1)
1.08	1.16(1.18)	0.08(0.1)
1.12	1.2(1.22)	0.08(0.1)
1.16	1.24(1.26)	0.08(0.1)
1.2	1.28(1.3)	0.08(0.1)

6.2 Randomization

The randomization sequence will be obtained by computer-generated random numbers and provided to each HVTN CRS through the SDMC's 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.

6.3 Blinding

Participants and site staff (except for site pharmacists) will be blinded as to participant treatment arm assignments (eg, vaccine or control) but not to group. 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 098 PSRT should be consulted before emergency unblinding occurs.

6.4 Statistical analysis

This section describes the final study analysis, 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. The analysis is a modified intent-to-treat analysis 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 characteristic, 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 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

Boxplots 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 boxplot will show the first quartile, the median, and the third quartile. Outliers (values outside the boxplot) 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 timepoint, as well as changes from baseline for postenrollment 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 DAIDS AE Grading Table (see Section 9.9) will be tabulated by treatment arm for each postvaccination timepoint. Reportable clinical laboratory abnormalities without an associated clinical diagnosis will also be included in the tabulation of AEs described above.

6.4.3.4 Injection/EP site pain assessment

The magnitude of injection/EP site pain, as assessed by a VAS, will be summarized statistically and graphically by the pain assessment time intervals (0, 5-7, and 25-60 minutes) after each vaccination, by vaccination timepoint and by treatment arm.

6.4.3.5 Acceptability

The number and percentage of responses to the acceptability questions will be tabulated by vaccination timepoint, overall and by treatment arm.

6.4.3.6 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 are excluded. Since the exact date of HIV infection is unknown, any assay data from blood draws 4 weeks, or less, 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% CI estimates calculated using the score test method [45]. 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 ≤ 0.05.

In addition to response rate estimates for each timepoint, the probability of observing at least 1 positive response by a given timepoint and the probability of observing more than 1 positive response by a given timepoint will be estimated, with corresponding CIs, for each vaccine arm using maximum likelihood-based methods [46].

For quantitative assay data (eg, magnitude of HIV-1 *env*-specific binding Ab responses, percentage of positive cells from ICS assay), graphical and tabular summaries of the distributions by antigen, treatment arm, and timepoint will be made. The difference between arms at a specific timepoint 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. An appropriate data transformation (eg, $\log_{10}$ transformation) may be applied to better satisfy assumptions of symmetry and homoscedasticity (constant variance).

More sophisticated analyses employing repeated measures methodology (for example, repeated measures analysis of variance or 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 AIDS Vaccine Evaluation Group and HVTN trials, missing 10% 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 PBMCs. To achieve unbiased statistical estimation and inferences with nonparametric tests and generalized linear models fit by generalized estimating equation (GEE) methods, missing data need to be missing completely at random (MCAR). MCAR assumes that the probability of an observation being missing does not depend upon the observed responses or upon any unobserved covariates but may depend upon covariates included in the model (eg, missing more among whites than nonwhites). When missing data are minimal (specifically if no more than 20% of participants are missing any values), then nonparametric tests and GEE methods will be used, because violations of the MCAR assumption will have little impact on the estimates and hypothesis tests. These models will include as covariates all available baseline predictors of the missing outcomes.

If a substantial amount of immunogenicity data are missing (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 [47] will be used, because they accommodate the censoring. In addition, secondary analyses of repeated immunogenicity measurements may be done using weighted GEE [48] methods, which are valid under MAR. All of the models described above will include as covariates all available baseline predictors of the missing outcomes.

6.4.4.2 Analyses of neutralization magnitude-breadth curves

Tier 1 screen of vaccine regimens versus placebo

The AUC-MB to the tier 1 isolates will be computed for each participant with evaluable neutralization data, as described in [49]. Dunnett's procedure will be applied with 2-sided alpha = 0.05 to determine which of the vaccine groups have a significantly higher mean AUC-MB than that of the pooled placebo groups, as described in [50]. This procedure will be applied to construct 95% CIs about the differences in mean AUC-MB for each vaccine regimen versus the pooled placebo groups (vaccine – placebo), which simultaneously have at least 95% coverage probability. The rule for a vaccine regimen passing the tier 1 screen is that the lower confidence limit about the mean difference is above zero. The vaccine regimens passing the tier 1 screen will be advanced to tier 2 evaluation, and regimens failing the tier 1 screen are not planned to undergo evaluation for neutralization of the tier 2 isolates.

Tier 2 screen of vaccine regimens versus placebo

For the set of vaccine regimens passing the tier 1 screen, the same Dunnett's procedure as described above, using the AUC-MB endpoint for the tier 2 isolates, will be used to determine the set of vaccine regimens that pass the tier 2 screen.

Select the best vaccine regimen among those passing the tier 2 screen

The vaccine regimens that passed the tier 2 screen will be ranked by the estimated mean of the AUC-MB curves. The vaccine regimen with the highest estimated mean will be selected as the best regimen.

6.4.4.3 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 using the discrete Bonferroni adjustment. The magnitude of 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, with a box plot of data from positive responders superimposed on the individual data values. Statistical testing comparing the magnitudes will be based on positive responders only.

6.4.4.4 Analysis of HIV-specific binding responses to the vaccine regimens

Frequency and magnitude of HIV-1-specific antibodies will be compared among the randomized vaccine arms using the approach for qualitative and continuous assay variables described in Section 6.4.4.1 above

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 for review by the SMB. Ad hoc safety reports may also be prepared for SMB review at the request of the HVTN 098 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, cell-mediated) reaches the aforementioned threshold. The HVTN 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. Any analyses conducted prior to the end of the study should not compromise the integrity of the trial in terms of participant retention or safety or immunogenicity endpoint assessments.

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.

As the DNA vaccine and adjuvant used in this study will be given by EP, volunteers may not participate if they have certain metal implants, a surgical or traumatic metal implant in the upper limb and or torso, 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 55 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

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/Complete blood count (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; creatinine ≤ institutional upper limit of normal; CPK ≤ 2.0 times the institutional upper limit of normal.

Virology

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

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

18. **Negative anti-Hepatitis C virus antibodies (anti-HCV)**, or negative HCV polymerase chain reaction 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 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. **A volunteer who was born female must:**

    - Agree to consistently use effective contraception (see Appendix B) 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 any of the following methods:
        - Condoms (male or female) with or without a spermicide,
        - Diaphragm or cervical cap with spermicide,
        - Intrauterine device ,
        - Hormonal contraception,
        - Any other contraceptive method approved by the HVTN 098 PSRT, or
        - 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. **Blood products** received within 120 days before first vaccination
2. **Investigational research agents** received within 30 days before first vaccination
3. **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
4. **Intent to participate in another study** of an investigational research agent during the planned duration of the HVTN 098 study
5. **Pregnant or breastfeeding**

6. **Subcutaneous contraceptive device**

Vaccines and other Injections

7. **HIV vaccine(s)** received in a prior HIV vaccine trial. For volunteers who have received control/placebo in an HIV vaccine trial, the HVTN 098 PSRT will determine eligibility on a case-by-case basis.

8. **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 098 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 098 PSRT on a case-by-case basis.

9. **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)

10. **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)

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

Immune System

12. **Immunosuppressive medications** received within 168 days before first vaccination. (Not excluded: [1] corticosteroid nasal spray; [2] low-dose 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.

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

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

15. **Autoimmune disease**

16. **Immunodeficiency**

Clinically significant medical conditions

17. History or presence of **keloid scar formation or hypertrophic scar**

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

19. **Presence of surgical or traumatic metal implant** in the upper arm and/or upper torso

20. **History of cardiac arrhythmia** (eg, supraventricular tachycardia, atrial fibrillation, or frequent ectopy)

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

22. **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.

23. **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. For example:

    - Tattoo overlying the injection site
    - Skin conditions at the injection site.

24. **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.

25. **Current anti-tuberculosis prophylaxis or therapy**

26. **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 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.

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

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

29. **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.

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

31. **Malignancy** (Not excluded: 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)

32. **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.

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

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

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.

7.3.1 EP device applied without vaccination

For Group 4 participants, at the Month 0 visit, if the IM CELLECTRA® EP needle array is inserted into a participant's arm but for any reason the participant does not receive the study injection, then this device-only participant is not considered to be enrolled in the study. Clinic procedures and safety reporting requirements for device-only participants are specified in Section 9.3.1. Refer to the HVTN 098 Study Specific Procedures for additional instructions.

At other visits if the IM CELLECTRA® EP needle array is inserted into a participant's arm but for any reason the participant does not receive the study injection within the specified window period, then the participant will have missed the vaccination.

If the IM EP device is applied, but no electroporation and no study agent (vaccine or placebo) was received, and the participant is willing, additional attempts may be made within the vaccination window. For the Month 0 visit, this is expected to be within 4 days of randomization, unless a longer window is approved by the PSRT.

7.3.2 Vaccination without EP

If a study product injection is given without EP (eg, EP unsuccessful, EP error, or EP refused), this event will be recorded/documented for that arm, and vaccinations should continue as scheduled. This applies to participants in all groups. See Section 9.3.2.

7.3.3 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)
  - Receipt of systemic corticosteroids—more than 2 mg/kg for 5 days.
- Prevaccination 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 HVTN 098 Study Specific Procedures.

In order to avoid vaccination delays and missed vaccinations, participants who plan to receive licensed vaccines, 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 postvaccination follow-up visit.

7.3.4 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 7.3.5).

7.3.5 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 098 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 vaccination;
  - 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 vaccination; or
  - Clinically significant type 1 hypersensitivity reaction associated with study vaccination. Consultation with the HVTN 098 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 non-adherence 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 or termination from the study is required by applicable regulations.

In addition, vaccinations will be stopped for participants diagnosed with HIV infection. HIV-infected participants will not continue in the trial (see Sections 7.3.6 and 9.6.1).

7.3.6 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 (TO BE ADMINISTERED AS 1 INJECTION)

Treatment 1 (T1):

PENNVAX®-GP 0.6 mg admixed with *IL-12* DNA 0.2 mg to be administered as 0.1 mL ID (intradermal) over either deltoid at months 0, 1, 3, and 6 using the CELLECTRA® 3P EP system

[Note: PENNVAX®-GP will require the admixing of Env A / Env C (vial labeled as SynCon® INO-6112) with Mpol / Gag (vial labeled as SynCon® INO-6145)]

Placebo 1 (P1):

Placebo for PENNVAX®-GP / *IL-12* DNA (labeled as Sterile Water for Injection, USP) to be administered as 0.1 mL ID (intradermal) over either deltoid at months 0, 1, 3, and 6 using the CELLECTRA® 3P EP system

Group 2 (TO BE ADMINISTERED AS 2 INJECTIONS)

Treatment 2 (T2):

PENNVAX®-GP 0.8 mg to be administered as 0.1 mL ID (intradermal) over LEFT deltoid (unless medically contraindicated) at months 0, 1, 3, and 6 using the CELLECTRA® 3P EP system

AND

PENNVAX®-GP 0.8 mg to be administered as 0.1 mL ID (intradermal) over RIGHT deltoid (unless medically contraindicated) at months 0, 1, 3, and 6 using the CELLECTRA® 3P EP system

[Note: PENNVAX®-GP will require the admixing of Env A / Env C (vial labeled as SynCon® INO-6112) with Mpol / Gag (vial labeled as SynCon® INO-6145)]

Placebo 2 (P2):

Placebo for PENNVAX®-GP (labeled as Sterile Water for Injection, USP) to be administered as 0.1 mL ID (intradermal) over LEFT deltoid (unless medically contraindicated) at months 0, 1, 3, and 6 using the CELLECTRA® 3P EP system

AND

Placebo for PENNVAX®-GP (labeled as Sterile Water for Injection, USP) to be administered as 0.1 mL ID (intradermal) over RIGHT deltoid (unless medically contraindicated) at months 0, 1, 3, and 6 using the CELLECTRA® 3P EP system

Group 3 (TO BE ADMINISTERED AS 2 INJECTIONS)

Treatment 3 (T3):

PENNVAX®-GP 0.8 mg admixed with *IL-12* DNA 0.2 mg to be administered as 0.1 mL ID (intradermal) over LEFT deltoid (unless medically contraindicated) at months 0, 1, 3, and 6 using the CELLECTRA® 3P EP system

AND

PENNVAX®-GP 0.8 mg admixed with *IL-12* DNA 0.2 mg to be administered as 0.1 mL ID (intradermal) over RIGHT deltoid (unless medically contraindicated) at months 0, 1, 3, and 6 using the CELLECTRA® 3P EP system

[Note: PENNVAX®-GP will require the admixing of Env A / Env C (vial labeled as SynCon® INO-6112) with Mpol / Gag (vial labeled as SynCon® INO-6145)]

Placebo 3 (P3):

Placebo for PENNVAX®-GP / *IL-12* DNA (labeled as Sterile Water for Injection, USP) to be administered as 0.1 mL ID (intradermal) over LEFT deltoid (unless medically contraindicated) at months 0, 1, 3, and 6 using the CELLECTRA® 3P EP system

AND

Placebo for PENNVAX®-GP / *IL-12* DNA (labeled as Sterile Water for Injection, USP) to be administered as 0.1 mL ID (intradermal) over RIGHT deltoid (unless medically contraindicated) at months 0, 1, 3, and 6 using the CELLECTRA® 3P EP system

Group 4 (TO BE ADMINISTERED AS 1 INJECTION)

Treatment 4 (T4):

PENNVAX®-GP 8 mg admixed with *IL-12* DNA 1 mg to be administered as 1 mL IM in either deltoid at months 0, 1, 3, and 6 using the CELLECTRA® 5P EP system

[Note: PENNVAX®-GP will require the admixing of Env A / Env C (vial labeled as SynCon® INO-6112) with Mpol / Gag (vial labeled as SynCon® INO-6145)]

Placebo 4 (P4):

Placebo for PENNVAX®-GP / *IL-12* DNA (labeled as Sterile Water for Injection, USP) to be administered as 1 mL IM in either deltoid at months 0, 1, 3, and 6 using the CELLECTRA® 5P EP system

8.2 Study product formulation

The study products are described in further detail in the Investigator's Brochure.

PENNVAX®-GP (prepared by pharmacy admixing the appropriate contents of the two study products below)

Env A / Env C (labeled as SynCon® INO-6112)

The Env A / Env C component of the PENNVAX®-GP vaccine will be provided in a sterile, single-use vial containing 1.2 mL ± 0.2 mL (10 mg/mL) of product. The product should be stored frozen (at or below -15°C) in a -20 freezer.

Mpol / Gag (labeled as SynCon® INO-6145)

The Mpol / Gag component of the PENNVAX®-GP vaccine will be provided in a sterile, single-use vial containing 0.6 mL ± 0.2 mL (10 mg/mL) of product. The product should be stored frozen (at or below -15°C) in a -20 freezer.

IL-12 DNA (labeled as INO-9012)

Each vial contains 0.5 mL ± 0.1 mL of INO-9012 *IL-12* DNA at a concentration of 10 mg/mL. The product should be stored frozen (at or below -15°C) in a -20 freezer.

Diluent

Sterile Water for Injection, USP (preservative-free sterile water for injection) will be used by the pharmacist during preparation of the injection(s). The vials must be stored as directed by the manufacturer of the product.

Placebo for all Study Products

Sterile Water for Injection, USP will be used as the Placebo for PENNVAX®-GP and PENNVAX®-GP / IL-12 DNA vaccinations. These vials contain no bacteriostat, antimicrobial agent, or added buffer. The vials must be stored as directed by the manufacturer of the product.

8.3 Preparation of study products

For All Groups

On the day of administration, for frozen product(s), the pharmacist will allow all of the vials to thaw at room temperature in an upright position.

Group 1 (T1) - PENNVAX®-GP 0.6 mg admixed with IL-12 DNA 0.2 mg to be administered over either deltoid

One vial of SynCon® INO-6112, one vial of SynCon® INO-6145, one vial of INO-9012, and one vial of Sterile Water for Injection, USP will be needed to prepare the one syringe needed for the ID injections in Group 1 (T1).

Once the contents of the vials are thawed, the pharmacist, using aseptic technique, will withdraw 0.15 mL from the vial SynCon® INO-6112 (Env A/ Env C) with a 0.3 mL or 0.5 mL syringe and inject this into an empty sterile vial. This "mixing" vial, now containing 0.15 mL of Env A/ Env C, will be set aside.

The pharmacist, using a new 0.5 mL or 1 mL syringe and still using aseptic technique will withdraw 0.3 mL from the vial SynCon® INO-6145 (Mpol / Gag) and add the 0.3 mL to the "mixing" vial containing 0.15 mL of Env A/ Env C. Once again, this "mixing" vial, now containing 0.45 mL, will be set aside.

Using a new 0.3 mL or 0.5 mL syringe, the pharmacist will withdraw 0.15 mL of INO-9012 (IL-12) and add the 0.15 mL to the "mixing" vial containing the 0.45 mL of PENNVAX®-GP (Env A/ Env C/Mpol/Gag).

The pharmacist will then, using a new 0.3 mL or 0.5 mL syringe, withdraw 0.15 mL of Sterile Water for Injection and add this 0.15 mL to the "mixing" vial containing PENNVAX®-GP / IL-12 DNA. The contents of the vial should be gently swirled. The final volume of this "mixing" vial should now be 0.75 mL of PENNVAX®-GP 4.5 mg / IL-12 DNA 1.5 mg.

Using aseptic technique, the pharmacist will use a 1 cc syringe or smaller (e.g. BD tuberculin syringe) to withdraw 0.1 mL from the "mixing" vial containing PENNVAX®-GP 4.5 mg / IL-12 DNA 1.5 mg. Prior to dispensing, the syringe should be labeled by the pharmacist as *PENNVAX®-GP 0.6 mg / IL-12 0.2 mg or Placebo* and *Administer ID over deltoid.*

The syringe MUST be labeled with the date and time the products were removed from storage as well as the words *ADMINISTER AS SOON AS POSSIBLE*. Keep the syringe at controlled room temperature until administration.

Any unused portion of entered vials, mixing vials, and expired pre-filled syringes should be disposed of in accordance with institutional or pharmacy policy.

Group 2 (T2) - PENNVAX®-GP 0.8 mg to be administered over LEFT deltoid and PENNVAX®-GP 0.8 mg to be administered over RIGHT deltoid for a total dose of PENNVAX®-GP 1.6 mg

One vial of SynCon®INO-6112, one vial of SynCon® INO-6145, and one vial of Sterile Water for Injection, USP are used to prepare the two syringes needed for the ID injections in Group 2 (T2).

Once the contents of the vials are thawed, the pharmacist, using aseptic technique, will withdraw 0.6 mL from the vial SynCon® INO-6112 (Env A/ Env C) with a 1 mL syringe and inject this into an empty sterile vial. This "mixing" vial, now containing 0.6 mL of Env A/ Env C, will be set aside.

The pharmacist, using a new 0.5 mL or 1 mL syringe and still using aseptic technique, will withdraw 0.2 mL from the vial SynCon®INO-6145 (Mpol / Gag) and add the 0.2 mL to the "mixing" vial containing 0.6 mL of Env A/ Env C. Once again, this "mixing" vial, now containing 0.8 mL, will be set aside.

Using a new 0.5 mL or 1 mL syringe, the pharmacist will withdraw 0.2 mL of Sterile Water for Injection, USP and add the 0.2 mL to the "mixing" vial containing the 0.8 mL of PENNVAX®-GP (Env A/ Env C/Mpol/Gag). The contents of the vial should be gently swirled. The final volume of this vial should now be 1 mL of PENNVAX®-GP 8 mg.

Using aseptic technique, the pharmacist will use a 1 cc syringe or smaller (e.g. BD tuberculin syringe) to withdraw 0.1 mL from the "mixing" vial containing PENNVAX®-GP 8 mg. The pharmacist will repeat this process a second time. Prior to dispensing, each syringe should be labeled by the pharmacist as *PENNVAX®-GP 0.8 mg or Placebo* and *Administer ID over LEFT deltoid* (one syringe) / and *Administer ID over RIGHT deltoid* (second syringe).

Each syringe MUST be labeled with the date and time the products were removed from storage as well as the words *ADMINISTER AS SOON AS POSSIBLE*. Keep the syringes at controlled room temperature until administration.

Any unused portion of entered vials, mixing vials, and expired pre-filled syringes should be disposed of in accordance with institutional or pharmacy policy.

Group 3 (T3) - PENNVAX®-GP 0.8 mg admixed with IL-12 DNA 0.2 mg to be administered over LEFT deltoid and PENNVAX®-GP 0.8 mg admixed with IL-12 DNA 0.2 mg to be administered over RIGHT deltoid for a total dose of PENNVAX®-GP 1.6 mg / IL-12 DNA 0.4 mg

One vial of SynCon®INO-6112, one vial of SynCon® INO-6145, and one vial of INO-9012 are used to prepare the two syringes needed for the ID injections in Group 2 (T2).

Once the contents of the vials are thawed, the pharmacist, using aseptic technique, will withdraw 0.6 mL from the vial SynCon® INO-6112 (Env A/ Env C) with a 1 mL syringe and inject this into an empty sterile vial. This "mixing" vial, now containing 0.6 mL of Env A/ Env C, will be set aside.

The pharmacist, using a new 0.5 mL or 1 mL syringe and still using aseptic technique, will withdraw 0.2 mL from the vial SynCon®INO-6145 (Mpol / Gag) and add the 0.2 mL to the "mixing" vial containing 0.6 mL of Env A/ Env C. Once again, this "mixing" vial, now containing 0.8 mL, will be set aside.

Using a new 0.5 mL or 1 mL syringe, the pharmacist will withdraw 0.2 mL of INO-9012 (IL-12) and add the 0.2 mL to the "mixing" vial containing the 0.8 mL of PENNVAX®-GP (Env A/ Env C/Mpol/Gag). The contents of the vial should be gently swirled. The final volume of this vial should now be 1 mL of PENNVAX®-GP 8 mg / IL-12 2 mg.

Using aseptic technique, the pharmacist will use a 1 cc syringe or smaller (e.g. BD tuberculin syringe) to withdraw 0.1 mL from the "mixing" vial containing PENNVAX®-GP 8 mg / IL-12 2 mg. The pharmacist will repeat this process a second time. Prior to dispensing, each syringe should be labeled by the pharmacist as *PENNVAX®-GP 0.8 mg /*

IL-12 0.2 mg or Placebo and *Administer ID over LEFT deltoid* (one syringe) / and *Administer ID over RIGHT deltoid* (second syringe).

Each syringe MUST be labeled with the date and time the products were removed from storage as well as the words *ADMINISTER AS SOON AS POSSIBLE*. Keep the syringes at controlled room temperature until administration.

Any unused portion of entered vials, mixing vials, and expired pre-filled syringes should be disposed of in accordance with institutional or pharmacy policy.

Group 4 (T4) - PENNVAX®-GP 8 mg admixed with IL-12 DNA 1 mg to be administered in either deltoid

One vial of SynCon® INO-6112, one vial of SynCon® INO-6145, one vial of INO-9012, and one vial of Sterile Water for Injection, USP will be needed to prepare the one syringe needed for the IM injections in Group 3 (T3).

Once the contents of the vials are thawed, the pharmacist, using aseptic technique, will withdraw 0.9 mL from the vial SynCon® INO-6112 (Env A/ Env C) with a 1 or 3 mL syringe and inject this into an empty sterile vial. This "mixing" vial, now containing 0.9 mL of Env A/ Env C, will be set aside.

The pharmacist, using a new 0.5 mL or 1 mL syringe and still using aseptic technique, will withdraw 0.3 mL from the vial SynCon® INO-6145 (Mpol / Gag) and add the 0.3 mL to the "mixing" vial containing 0.9 mL of Env A/ Env C. Once again, this "mixing" vial, now containing 1.2 mL, will be set aside.

Using a new 0.3 mL or 0.5 mL syringe, the pharmacist will withdraw 0.15 mL of INO-9012 (IL-12) and add the 0.15 mL to the "mixing" vial containing the 1.2 mL of PENNVAX®-GP (Env A/ Env C/Mpol/Gag). Once again, this "mixing" vial, now containing 1.35 mL, will be set aside.

Using aseptic technique, the pharmacist will use a new 0.3 mL or 0.5 mL syringe, to withdraw 0.15 mL of Sterile Water for Injection and add this 0.15 mL to the "mixing" vial containing PENNVAX®-GP / IL-12. The contents of the vial should be gently swirled. The final volume of this vial should now be 1.5 mL of PENNVAX®-GP 12 mg / IL-12 1.5 mg.

Using a 3 cc Luer-lock syringe (e.g. BD syringe), the pharmacist will withdraw 1 mL from the vial containing PENNVAX®-GP 12 mg / IL-12 1.5 mg. Prior to dispensing, the syringe should be labeled by the pharmacist as *PENNVAX®-GP 8 mg / IL-12 1mg or Placebo* and *Administer IM in deltoid*.

The syringe MUST be labeled with the date and time the products were removed from storage as well as the words *ADMINISTER AS SOON AS POSSIBLE*. Keep the syringe at controlled room temperature until administration.

Any unused portion of entered vials, mixing vials, and expired pre-filled syringes should be disposed of in accordance with institutional or pharmacy policy.

Group 1 (P1) - Placebo for PENNVAX®-GP / IL-12 DNA to be administered over either deltoid

One vial of Sterile Water for Injection, USP will be needed to prepare the one syringe needed for the ID injections in Group 1 (P1).

The pharmacist, using aseptic technique, will withdraw 0.1 mL from the vial containing Sterile Water for Injection, USP using a 1 cc syringe or smaller (e.g. BD tuberculin syringe). The syringe should be labeled by the pharmacist as *PENNVAX®-GP 0.6 mg / IL-12 0.2 mg or Placebo* and *Administer ID over deltoid*.

The syringe MUST be labeled with the date and time the product was removed from storage as well as the words *ADMINISTER AS SOON AS POSSIBLE*. Keep the syringe at controlled room temperature until administration.

Any unused portion of entered vials and expired pre-filled syringes should be disposed of in accordance with institutional or pharmacy policy.

Group 2 (P2) - Placebo for PENNVAX®-GP to be administered over LEFT deltoid and Placebo for PENNVAX®-GP to be administered over RIGHT deltoid

One vial of Sterile Water for Injection, USP will be needed to prepare the two syringes needed for the ID injections in Group 2 (P2).

The pharmacist, using aseptic technique, will withdraw 0.1 mL from the vial containing Sterile Water for Injection, USP using a 1 cc syringe or smaller (e.g. BD tuberculin syringe). This process will then be repeated a second time. Each syringe should be labeled by the pharmacist as *PENNVAX®-GP 0.8 mg or Placebo* and *Administer ID over LEFT deltoid* (one syringe) / and *Administer ID over RIGHT deltoid* (second syringe).

Each syringe MUST be labeled with the date and time the product was removed from storage as well as the words *ADMINISTER AS SOON AS POSSIBLE*. Keep the syringes at controlled room temperature until administration.

Any unused portion of entered vials and expired pre-filled syringes should be disposed of in accordance with institutional or pharmacy policy.

Group 3 (P3) - Placebo for PENNVAX®-GP / IL-12 DNA to be administered over LEFT deltoid and Placebo for PENNVAX®-GP / IL-12 DNA to be administered over RIGHT deltoid

One vial of Sterile Water for Injection, USP will be needed to prepare the two syringes needed for the ID injections in Group 3 (P3).

The pharmacist, using aseptic technique, will withdraw 0.1 mL from the vial containing Sterile Water for Injection, USP using a 1 cc syringe or smaller (e.g. BD tuberculin syringe). This process will then be repeated a second time. Each syringe should be labeled by the pharmacist as *PENNVAX®-GP 0.8 mg / IL-12 0.2 mg or Placebo* and *Administer ID over LEFT deltoid* (one syringe) / and *Administer ID over RIGHT deltoid* (second syringe).

Each syringe MUST be labeled with the date and time the product was removed from storage as well as the words *ADMINISTER AS SOON AS POSSIBLE*. Keep the syringes at controlled room temperature until administration.

Any unused portion of entered vials and expired pre-filled syringes should be disposed of in accordance with institutional or pharmacy policy.

Group 4 (P4) - Placebo for PENNVAX®-GP / IL-12 DNA to be administered in either deltoid

One vial of Sterile Water for Injection, USP will be needed to prepare the one syringe needed for the IM injections in Group 4 (P4).

The pharmacist, using aseptic technique, will withdraw 1 mL from the vial containing Sterile Water for Injection, USP using a 3 cc Luer-lock syringe (e.g. BD syringe). The syringe should be labeled by the pharmacist as *PENNVAX®-GP 8 mg / IL-12 1mg or Placebo* and *Administer IM in deltoid*.

The syringe MUST be labeled with the date and time the product was removed from storage as well as the words *ADMINISTER AS SOON AS POSSIBLE*. Keep the syringe at controlled room temperature until administration.

Any unused portion of entered vials and expired pre-filled syringes should be disposed of in accordance with institutional or pharmacy policy.

8.4 Administration

Groups 1, 2, and 3 - Intradermal (ID) injections

Groups 1, 2, and 3 (Vaccine or Placebo) will be administered ID using the CELLECTRA® 3P EP system. The outside and inside console will contain yellow color-coded labels as well as the wording "CELLECTRA® INTRADERMAL 3P-ID". In addition, the CELLECTRA® Applicator cord will be flagged with the same yellow color-coded label and wording.

The CELLECTRA® Adaptive Constant Current Electroporation Device will be used as directed by Inovio Pharmaceuticals, Inc. Note: This EP device will have a different password than the device used for Group 4. (Refer to the CELLECTRA® User Manual for further instructions.) As with all vaccinations, the injection site is disinfected and the area is allowed to dry completely. The ID injections are to be given as a volume of 0.1 mL using a standard intradermal injection needle (e.g. 25-gauge needle with a length of 5/8 inch). The ID injections will be administered in the skin overlying the deltoid area of the arm. The needle will be inserted into the skin at a 5 to 15 degree angle to the skin and bevel side up until the bevel is seen to be fully under the skin. The syringe contents will be injected to form a small bleb. The clinician will then continue the EP treatment as directed in the User Manual.

If two ID injections are required (Groups 2 and 3), then one injection should be completed (including electroporation) prior to administering the second one.

For Groups 2 and 3 if an injection is administered over the deltoid contralateral to that specified on the syringe label due to a medical contraindication, the appropriate staff should document this clearly. Under this circumstance, this is NOT a protocol violation. Two injections administered into the same arm should be at least 2 cm apart.

The CELLECTRA® Applicator will be used with a disposable plastic sheath to prevent contamination. The used CELLECTRA® Sterile Disposable Array and the used sheaths should be disposed of in accordance with institutional policy in the clinic. They should NOT be returned to pharmacy. The CELLECTRA® 3P Applicator should be cleaned with Clorox wipes (or equivalent) after each vaccination and stored for subsequent vaccinations in a secure location.

When preparing a dose in a syringe and administering the dose, consideration should be given to the volume of solution in the needle before and after the dose is administered. Particularly, if the needle used to withdraw the product is replaced prior to vaccine administration, consideration should be given to conserving the full dose of product. The pharmacy and clinic staff members are encouraged to work together to administer the dose specified in the protocol.

Group 4 - Intramuscular (IM) injections

Group 4 (Vaccine or Placebo) will be administered IM as a 1 mL injection using the CELLECTRA® 5P EP system. The outside and inside console will contain blue color-coded labels as well as the wording "CELLECTRA INTRAMUSCULAR 5P-IM". In addition, the CELLECTRA® Applicator cord and the arrays will be flagged with the same blue color-coded label and wording.

The CELLECTRA® Adaptive Constant Current Electroporation Device will be used as directed by Inovio Pharmaceuticals, Inc. Note: This EP device will have a different password than the device used for Groups 1, 2, and 3. (Refer to the CELLECTRA® User Manual for further instructions.) As with all vaccinations, the injection site is disinfected and the area is allowed to dry completely. The syringe (3 mL syringe with 21 G 2" needle) containing the vaccine/placebo will be inserted into the injection port of the array. **The clinician MUST pull up on the plunger to ensure the needle is not in a blood vessel**. The vaccine/placebo is then injected into the deltoid, and the needle and the syringe which had contained the vaccine/placebo is removed from the deltoid and discarded. The clinician will then continue the EP treatment as directed in the User Manual.

Each subject will be assigned a unique CELLECTRA® 5P Applicator, which should be cleaned with Clorox wipes (or equivalent) after each vaccination and stored in a secure location for subsequent vaccinations in the same subject. The used CELLECTRA® Sterile Disposable Array should be disposed of in accordance with institutional policy in the clinic. They should NOT be returned to pharmacy.

When preparing a dose in a syringe and administering the dose, consideration should be given to the volume of solution in the needle before and after the dose is administered. Particularly, if the needle used to withdraw the product is replaced prior to vaccine administration, consideration should be given to conserving the full dose of product. The pharmacy and clinic staff members are encouraged to work together to administer the dose specified in the protocol.

8.5 Acquisition of study products

All study products will be provided by Inovio Pharmaceuticals.

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 ensuring that participants 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 IRB/EC and any applicable Regulatory Entity (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 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 Protocol Registration Office's Regulatory Support Center (RSC).

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 form

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 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 for local use, based on the sample protocol-specific consent forms in Appendix A and Appendix C. The consent form must be developed in accordance with requirements of the following:

- CRS's IRB/EC,
- CRS's institution and any applicable REs, 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 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 poststudy 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 poststudy 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.

9.1.4 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;
- 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 (record the complete generic name for all medications);
- Laboratory tests as defined in the inclusion and exclusion criteria, including:
  - Screening HIV test,
  - HBsAg,
  - Anti-HCV,
  - Syphilis test,
  - CBC with differential and platelets,
  - Chemistry panel (ALT, AST, alkaline phosphatase, creatinine, and CPK),

  - Urine dipstick (as described in Section 9.8),
  - 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;

- Photograph injection sites (as described in Section 9.10);

- 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 VAS immediately following EP, then again 5-7 minutes and 25-60 minutes following vaccination. 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 symptom log and is instructed on how to complete it. The site will make arrangements to obtain daily reports of reactogenicity events from 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).

The following procedure will be performed at all vaccination visits. This procedure will be performed following vaccination:

- Visual Analog Scale (VAS) (pain assessment) (as described in Table 9-1)

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

- Waist circumference

- 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 behavioral risk assessment questionnaire;

- 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 placebo;

- 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)

If the CELLECTRA® EP device is applied to the participant's arm but vaccination with study product cannot be completed within the specified visit window period, then the participant will have missed that vaccination (see Section 7.3.1). The participant will not need to complete the postvaccination symptom log or report reactogenicity events during the reactogenicity period. Normal safety reporting requirements apply.

9.3.1 Procedures for participants with IM EP device contact who were unable to receive a study injection (Device-only participants)

If the participant was assigned to Group 3 and the CELLECTRA® 5P EP System needle array was inserted into the participant's arm but the participant was not able to receive any injection of study product at that visit, the participant is not considered enrolled into the study. Refer to the HVTN 098 Study Specific Procedures for additional instructions and for the CRFs to be completed.

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 and adverse events requiring expedited reporting to DAIDS (EAE) reporting of events associated with the application of the EP device, which should be reported to the SDMC on the appropriate case report form (CRF). In addition, AEs requiring expedited reporting should be reported to the DAIDS RCC Safety Office as described in Section 11.2.3.

9.3.2 Procedures for participants who received a study injection but were unable to receive EP (Vaccination-only participants)

If the participant was vaccinated but without EP (eg, EP unsuccessful, EP error, or EP refused), the participant remains enrolled. Other procedures specified for the visit (with the exceptions of pain assessment using the VAS scale) should be completed and data collected should be reported to the SDMC using the appropriate CRFs (see Section 11.2.2). 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);

- 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);

- Assessment of new or unresolved AEs/intercurrent illnesses;

- Injection site assessment as described in Section 9.10; and

- Photograph injection sites (Optional)

Additional procedures will be performed at scheduled follow-up visits as specified in 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 placebo;

- Administration of behavioral risk assessment questionnaire;

- Administration of acceptability questionnaire;

- HIV infection assessment including pretest 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;

- Waist circumference;

- Specimen collection;

- Clinical laboratory tests including:
  - CBC with differential and platelet count,
  - 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) or if an unresolved injection site change is reported, which should be assessed and photographed in person; 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 the participant's responses to the following:

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

    - Assessment of unresolved injection site skin changes (if present, arrange clinic visit for post-reactogenicity injection site assessment and photography of injection site(s)); and

    - Assessment of new or unresolved AEs/intercurrent illnesses:

        - 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;

        - AESI (refer to Section 11.2.2. A sample list of AESI is provided in Appendix G);

        - 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 098 Protocol Team for exploratory endpoints.

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 E). The Laboratory Program (or approved diagnostic laboratory) will follow the HVTN HIV testing algorithm (as described in the HVTN Site Lab Reference Manual), 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). Participants who report a new diagnosis of HIV-1 infection at the Month 18 health contact will be asked to undergo confirmatory HIV-1 diagnostic testing (per the HVTN poststudy HIV-1 testing algorithm).

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 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 (DAIDS AE Grading Table), Version 2.0 [November 2014].

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 symptom log daily during the assessment period. Clinic staff will follow new or unresolved reactogenicity symptoms present at day 7 to resolution. Participants are instructed to contact the clinic 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.

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 skin changes, 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 and VAS assessments

Day	Time	Performed by
0[a]	Reactogenicity baseline: before vaccination	HVTN CRS staff
	VAS immediate: post vaccination	HVTN CRS staff
	VAS 5-7 minutes: post vaccination	HVTN CRS staff
	VAS 25-60 minutes: post vaccination	HVTN CRS staff
	Reactogenicity early 25-60 minutes: post vaccination	HVTN CRS staff
1	Reactogenicity between 12:00 AM and 11:59 PM day 1	HVTN CRS staff or participant
2	Reactogenicity between 12:00 AM and 11:59 PM day 2	HVTN CRS staff or participant
3[b]	Reactogenicity between 12:00 AM and 11:59 PM day 3	HVTN CRS staff or participant
4-7[b]	Reactogenicity between 12:00 AM and 11:59 PM days 4-7	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 Reactogenicity assessment of injection site

Typical injection site reactions are erythema and induration/swelling/edema. 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 Injection site assessment and photography

To document the appearance of injection sites over time, the area to be injected will be assessed prior to receipt of an injection, and at subsequent scheduled visits. The purpose of this post-reactogenicity assessment is to evaluate the appearance of the injection sites after time has allowed for healing. A description including type and size of any skin changes related to vaccination that are not described in Section 9.9.2 will be recorded.

If the participant agrees, the area(s) to be injected will be photographed, prior to receipt of an injection, and at all subsequent scheduled visits. Photography of injection site(s) at interim visits and at the time of the 18 month health contact is strongly recommended if the participant has any unresolved injection site symptoms or concerns.

9.11 Visit windows and missed visits

Visit windows are defined in HVTN 098 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 7.3.4 and Section 7.3.5 for resolution.

9.12 Early termination visit

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

9.13 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.

10 Laboratory

10.1 HVTN CRS laboratory procedures

The HVTN Site Lab Reference Manual provides 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 7 (day 98) and 9 (day 182) (ie 2 weeks after the third and fourth vaccination visits). 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 global PTE peptides or synthetic HIV peptide equivalents that span the proteins encoded by the vaccine construct. Parameters assessed by ICS will include cytokines such as IFN-γ, IL-2, and TNF-α, and may include other cytokines to identify T cells of specific functionality (such as Th2 and Th17). Markers of cytotoxic potential (Granzyme B, perforin and CD57) may also be included. Data will be reported as percentages of CD4+ or CD8+ T cells responding to a specific

peptide pool among CD4+ or CD8+ T cell subsets. Additional cell surface markers, cytokines, or functional markers may also be analyzed.

10.4.2 B-cell ELISpot

B-cell ELISpot assays may be conducted on PBMCs to enumerate B cells that secrete HIV-specific antibodies. The total number of B cells secreting IgG and/or IgA will be compared to the number of B cells secreting HIV-specific antibodies using Env proteins as antigens. Responses will be reported as the percentage of antigen-specific B cells among total antibody-secreting cells.

10.5 Endpoint assays: humoral

10.5.1 HIV-1 multiplex antibody assay

Total binding IgG (IgG1, IgG2, IgG3, IgG4) and IgA antibodies to (Consensus *env* A, *env* C, and *gag*) will be assessed on plasma/serum samples from study participants taken at the primary immunogenicity timepoints and baseline. Specimens from other timepoints as well as other HIV antigens may also be assayed based on the results of the initial assay.

10.5.2 Neutralizing antibody assay

HIV-1–specific neutralizing antibody assays will be performed on serum samples from all study participants taken at the primary immunogenicity timepoints. Specimens from the baseline and other timepoints may also be analyzed at the discretion of the HVTN Laboratory Program, which may be contingent on the results of the primary immunogenicity timepoints. Tier 1 assays will test neutralization of HIV-1 strains represented in the highly neutralization-sensitive tier 1 viruses. The tier 2 assays will test neutralization of a panel of heterologous primary isolates. [51]

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 postvaccination timepoints. Other participants (including placebo 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 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.

10.7.1 T-cell repertoire

Changes to the T cell repertoire will be assessed by deep sequencing techniques. Specimens collected at primary immunogenicity timepoints will be compared to those collected at baseline in order to examine evolution of vaccine-induced immune responses by HIV-specific CD4+ T helper cells.

10.7.2 Antibody-dependent cellular cytotoxicity (ADCC) assay

As an exploratory analysis, the induction of antibodies capable of mediating ADCC may be investigated. The ADCC assay will use either gp120-coated cells or HIV-1 infected cells as targets (eg, CEM.NK.CCR5 cells) and either cryopreserved PBMC or a suitable cell line as effector cells. Percent specific ADCC activity in plasma will be based on either degranulation or lysis, and will be quantified by either flow cytometry or luminescence, respectively. If reactivity is detected against viruses of the same subtype as the vaccine strain, the breadth of responses will also be evaluated.

10.8 Other use of stored specimens

The HVTN aims not only to test vaccine candidates but also to continue to explore the correlates of immunity to HIV. In order to do so, the HVTN intends to store blood samples from participants. These samples will be used for other testing and research related to furthering the understanding of virology, immunology, or vaccinology to the extent authorized in each study site's informed consent form, or as otherwise authorized under applicable law. Other testing on specimens will only occur, at a minimum, after review and approval by the HVTN and the IRB/EC and any applicable Regulatory bodies of the researcher requesting the specimens.

The protocol sample informed consent form is written so that the participant either explicitly allows or does not allow sample storage for other research when he or she signs the form. Participants who initially agree to other use of their samples may rescind their approval once they enter the study; such participants will still remain in this study. 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 assures the samples from theses participants are not used for such other uses.

Study sites must notify HVTN Regulatory Affairs if applicable requirements pose a conflict with or impose restrictions on the use of stored specimens.

10.9 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 098 PSRT

The HVTN 098 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 098 PSRT are responsible for decisions related to participant safety.

The Protocol Team clinic coordinator, project manager, vaccine developer representative, clinical trial manager, and others may also be included in HVTN 098 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, AEs, 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 098 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 098 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 098 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 098 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, and 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 refaxed 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 (DAIDS AE Grading Table), Version 2.0 [November 2014], available on the RSC website at http://rsc.tech-res.com/safetyandpharmacovigilance/gradingtables.aspx, except that weight loss of less than 9% loss in body weight from baseline is not required to be reported as an adverse event.

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 can be found in the Key Contacts under Safety Reporting and Clinical Monitoring on the HVTN 098 home page on the HVTN Members' site (https://members.hvtn.org/protocols/hvtn098).

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/safetyandpharmacovigilance/. The SAE Reporting Category will be used for this study.

The internet-based DAIDS Adverse Event 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 DAIDS-ESSupport@niaid.nih.gov or from within the DAERS application itself.

Sites where DAERS has not been implemented will submit expedited AE reports by documenting the information on the current DAIDS EAE Form. This form is available on the RSC website: http://rsc.tech-res.com/safetyandpharmacovigilance/. For questions about expedited AE reporting, please contact the RSC (DAIDSRSCSafetyOffice@tech-res.com).

The study products for which expedited reporting are required are:

- PENNVAX®-GP or placebo
- *IL-12* DNA (INO-9012) or placebo
- CELLECTRA® 3P EP System
- CELLECTRA® 5P EP System

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

After the protocol-defined AE reporting period for the study, unless otherwise noted, only Suspected, Unexpected Serious Adverse Reactions as defined in Version 2.0 of the DAIDS EAE Manual must be reported to DAIDS, if the study staff become aware of the events.

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 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 principal investigator 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 begins with Group 1. Enrollment in Group 1 will be restricted to one person per day across all sites. Enrollment will then be held until all available safety and reactogenicity data reported through day 14 for participants in Group 1 are reviewed by the HVTN 098 PSRT. If the data are acceptable, Groups 2, 3, and 4 will open for enrollment simultaneously. For Groups 2, 3, and 4, enrollment will be restricted to one person per day across all participating HVTN CRSs for the first 15 participants (5 participants in each group). Enrollment will then be held until all available safety and reactogenicity data from day 14 from these 15 participants will be reviewed. If the data are acceptable, Groups 2, 3, and 4 will reopen to enrollment.

If ID administration of the study products in Group 1, 2 or 3 results in significant local reactogenicity and/or injection site AEs related to the route of delivery, but no other significant safety issues are identified that would be expected to also occur with IM administration, the HVTN 098 PSRT may opt to proceed with enrollment for Group 4 only.

11.4 Safety pause and prompt PSRT AE review

When a trial is placed on safety pause, all enrollment and vaccination with the product related to the event that triggered the pause will be held until further notice. The AEs that will lead to a safety pause or prompt HVTN 098 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 098 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	HVTN Core action
SAE, related	Grade 5 or Grade 4	Phone immediately, email and fax forms immediately[a]	Immediate pause
SAE, not related	Grade 5	Phone immediately, email and fax forms immediately	Immediate HVTN 098 PSRT notification
SAE, related	Grade 3	Email and fax forms immediately	Prompt HVTN 098 PSRT AE review to consider pause
AE[b], related	Grade 4 or 3	Email and fax forms immediately	Prompt HVTN 098 PSRT AE review to consider pause

[a] Phone numbers and email addresses can be found in the Key Contacts under Safety Reporting and Clinical Monitoring on the HVTN 098 home page on the HVTN Members' site (https://members.hvtn.org/protocols/hvtn098).

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

For all safety pauses, the HVTN 098 PSRT, HVTN Regulatory Affairs, DAIDS Pharmaceutical Affairs Branch (PAB), DAIDS Regulatory Affairs Branch (RAB), DAIDS Safety and Pharmacovigilance Team (SPT), and participating HVTN CRSs will be notified by HVTN Core. When an immediate safety pause is triggered, the HVTN SMB is also notified.

Once a trial is paused, the HVTN 098 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 098 PSRT assessment, DAIDS RAB notifies the FDA as needed.

If an immediate HVTN 098 PSRT notification or prompt HVTN 098 PSRT AE review is triggered, HVTN Core notifies the HVTN 098 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 098 PSRT AE review cannot be completed within 72 hours of notification (excluding weekends and US federal holidays), an automatic safety pause occurs.

Each HVTN CRS is responsible for submitting 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, etc.), as required.

In addition, all other AEs are reviewed routinely by the HVTN 098 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 098 PSRT AE review criteria.

11.5.2 Weekly review

During the injection phase of the trial, the HVTN 098 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 098 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 098 PSRT, HVTN SMB, FDA, NIH, Office for Human Research Protections, or vaccine developer. 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; and
- Specimen collection, processing, and analysis.

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 098 *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 DNA

Because this study is evaluating products containing recombinant DNA, it must comply with regulations set forth in the NIH's *Guidelines for Research Involving Recombinant DNA Molecules.* Information about the study must be submitted to site Institutional Biosafety Committees (IBC) and must be approved before participants are enrolled at the site. Investigators at each site are responsible for obtaining IBC approval and periodic review of the research per NIH guidelines *section IV-B07-b-(6)* and *section IV-B-2-b.* IBC review and approval must be documented by the investigator and submitted as part of initial protocol registration for this trial. If this protocol is amended, investigators should follow the requirements of their IBC.

The NIH guidelines also require that human gene transfer trials conducted at or sponsored by institutions that receive NIH funds must be submitted to the NIH Office of Biotechnology Activities for review by the Recombinant DNA Advisory Committee (RAC). The NIH guidelines create exceptions to RAC review, but the HVTN 098 Protocol Team determined that the exceptions did not apply. Therefore, the Protocol Team, jointly with Inovio Pharmaceuticals, Inc., submitted the application with the study concept proposal for RAC review and responded to RAC comments. The application followed the guidance provided in the NIH Guidelines.

The HVTN and DAIDS will ensure that reporting requirements to RAC, as outlined in *Appendix M-I-C-1.Initiation of the Clinical Investigation*, *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 098 are described below.

Protocol history and modifications

Date: February 18, 2015

Protocol version: 1.0

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 http://www3.niaid.nih.gov/research/resources/DAIDSClinRsrch/
- Division of AIDS Protocol Registration Manual. Available at http://www.niaid.nih.gov/LabsAndResources/resources/DAIDSClinRsrch/Documents/prmanual.pdf
- U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0. [November 2014]. Available from: http://rsc.tech-res.com/safetyandpharmacovigilance/gradingtables.aspx
- The Manual for Expedited Reporting of Adverse Events to DAIDS. Version 2.0, January 2010. Available at http://rsc.tech-res.com/safetyandpharmacovigilance
- HVTN Certificate of Confidentiality. Accessible through the HVTN website.
- HVTN 098 Special Instructions. Accessible through the HVTN protocol-specific website.
- HVTN 098 Study Specific Procedures. Accessible through the HVTN protocol-specific website.
- HVTN Site Lab Reference Manual. 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/ps/publications/dgr/Pages/index.aspx.
- HVTN algorithm for diagnosis of HIV infections. Part of the HVTN Site Lab Reference Manual (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/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6_R1/Step4/E6_R1__Guideline.pdf

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

- NIH Guidelines for Research Involving Recombinant DNA Molecules. Available at http://oba.od.nih.gov/rdna/nih_guidelines_oba.html.

- 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://phacs.nichdclinicalstudies.org/publicDocs/DAIDS_SourceDocPolicy.pdf

- Title 21, Code of Federal Regulations, Part 50. Available at http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&sid=2e2429c70115b7df5635f222901ae8f7&rgn=div5&view=text&node=21:1.0.1.1.19&idno=21

- Title 45, Code of Federal Regulations, Part 46. Available at http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&sid=2e2429c70115b7df5635f222901ae8f7&rgn=div5&view=text&node=45:1.0.1.1.25&idno=45

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

15 Acronyms and abbreviations

Ab	antibody
ADCC	antibody-dependent cellular cytotoxicity
AE	adverse event
AESI	AEs of special interest
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BGH	bovine growth hormone
BMI	body mass index
CAB	Community Advisory Board
CBC	complete blood count
CDC	US Centers for Disease Control and Prevention
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 Event Reporting System
DAIDS	Division of AIDS (US NIH)
DNA	Deoxyribonucleic acid
DHHS	US Department of Health and Human Services
EAE	adverse events requiring expedited reporting to DAIDS
EC	Ethics Committee
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
GLP	good laboratory practice
HBsAg	hepatitis B surface antigen
hCMV	human cytomegalovirus
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HPV	human papillomarvirus
HVTN	HIV Vaccine Trials Network
IBC	Institutional Biosafety Committee
ICH	International Conference on Harmonisation

ICS	intracellular cytokine staining
ID	intradermal
IFN-γ	interferon gamma
IL-12	Interleukin 12
IM	intramuscular
IND	Investigational New Drug
IRB	Institutional Review Board
IUD	intrauterine device
MAR	missing at random
MCAR	missing completely at random
MHRP	Military HIV Research Program
MMR	measles, mumps, and rubella
MVA	modified vaccinia Ankara
nAb	neutralizing antibody
NHP	nonhuman primate
NIAID	National Institute of Allergy and Infectious Diseases (US NIH)
NIH	US National Institutes of Health
NU/mL	neutralizing units per milliliter
NZW	New Zealand White
OPV	oral polio vaccine
PAB	DAIDS Pharmaceutical Affairs Branch
PBMC	peripheral blood mononuclear cell
PFU	plaque forming units
PSRT	Protocol Safety Review Team
PTE	potential T-cell epitope
PV-B	PENNVAX®-B
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
sd	study day
SD	standard deviation
SDMC	statistical and data management center
SFC	spot-forming cell
SFU	spot-forming unit
SIV	simian immunodeficiency virus
SMB	Safety Monitoring Board
SPT	DAIDS Safety and Pharmacovigilance Team
UW-VSL	University of Washington Virology Specialty Laboratory
VAS	visual analog scale
VISP	Vaccine induced seropositivity

vs	versus
WC	waist circumference

* 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.

16 Literature cited

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Appendix A Sample informed consent form

Title: A phase 1 clinical trial to evaluate the safety and immunogenicity of PENNVAX®-GP (*gag*, *pol*, *env*) DNA vaccine and *IL-12* plasmid, delivered via intradermal or intramuscular electroporation in healthy, HIV–uninfected adult participants
HVTN protocol number: HVTN 098
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 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 an HIV vaccine. HIV is the virus that causes AIDS.

Up to 94 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 a vaccine, another study product called an adjuvant, and an experimental procedure called electroporation (EP). We will define these terms in a later section.

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 with EP without becoming too uncomfortable?
   - How do people's immune systems respond to the study products? (Your immune system protects you from disease.)
   - Do the study products have different effects if they are given into the skin or into the muscle with EP?

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

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

3. We do not know if the study vaccine will decrease, increase, or not change your chance 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 another study, some men who got the vaccine had a *higher* risk of getting HIV than men 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 vaccine 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 PENNVAX®-GP vaccine and interleukin-12 (IL-12) DNA adjuvant. From here on, we will call them "the study vaccine" and "the study adjuvant." The 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 products are used only in research studies.

The study products are provided by Inovio Pharmaceuticals, Inc.

The study vaccine is a DNA vaccine which contains 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 vaccine tells the body to make a few 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, some people will be given just the study vaccine, and some people will be given the study vaccine and the study adjuvant together. This study adjuvant is made of DNA that will tell your body to make IL-12, a normal protein in the body that helps immune cells work together and makes them multiply.

These study products have not been given together in people before. Some monkeys have received different parts of the study vaccine and the study adjuvant, but not all the study products together. There were no serious health problems seen in these monkeys. Even if something looks like it is safe or works in animals, it may not be true for people.

Other studies have tested very similar DNA vaccines in at least 140 people. Some of those studies also tested a similar IL-12 DNA adjuvant in at least 60 people. No serious health problems were found that were related to those study products.

General risks of vaccines:

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.

All vaccines can cause fever, chills, rash, aches and pains, nausea, headache, dizziness, and feeling tired. 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.

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:

We do not know all the risks of the study vaccine because it has not been given to people before. Possible risks related to DNA 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 vaccine DNA into the body's DNA. We think the risk of these things happening is low. Scientists also think it may be possible that a vaccine could cause cancer, but we have never seen this happen with any experimental HIV vaccine. There could be unknown side effects. More than 1000 people have been given DNA vaccines being tested against HIV and none of these things has happened so far. Thousands of people have received other experimental DNA vaccines. In those people, the DNA vaccines have not caused serious health problems. We expect the risks of the DNA vaccine in this study to be similar to those of other DNA vaccines.

In earlier studies of an adjuvant similar to the study adjuvant, 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.

5. The study vaccine 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 CELLECTRA® EP System. It was developed by Inovio Pharmaceuticals, 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 skin or arm muscle where the injection is given.

The EP device has been used with injections into the skin in at least 127 people. It has been used with injections into the upper arm muscle in at least 188 people. In another study, a similar DNA vaccine and an IL-12 DNA adjuvant were given into the muscle with EP to 30 people. However this is the first time these study products will be given together into the skin or muscle with EP.

Risks of electroporation (EP):

EP will cause brief muscle contractions 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, bruising, redness, swelling, itching, or hardness/stiffness in the upper arm where you got the injection. If the injection and EP are given to the skin, the needles may leave marks, such as red bumps and scabs. Later the marks may heal but might leave light or dark spots or small scars. In some people these marks have lasted 9 months or more. These marks tend to be more noticeable on darker skin. We do not know how the skin at the injection site will change in appearance after EP with these study products because they have not been given with EP before.

In a previous study of the EP device in healthy volunteers, some people said that they felt only a little discomfort while others said it was very painful, even after the injections were over. However, only 3 out of 48 volunteers discontinued the study because of pain and discomfort.

If the injection and EP are given into the muscle, minor damage to muscle cells is also possible. On rare occasions, the device may cause infection at the part of your body where you got the injection.

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.

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 prevention studies now or in the future. 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 decide 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)

   We will also do blood and urine tests. These tests tell us about some key aspects of your health, such as how healthy your kidneys, liver, and immune system are. We will also test you for these diseases: Hepatitis B, Hepatitis C, 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, and offer you counseling and referral if you need medical care. We will not pay for this medical care. The screening results may show you are not eligible to join the study, even if you want to. You cannot be in this study while you are in another study where you receive a study product.

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

   Site: List approved birth control methods here if you do not want to hand out the separate Approved Birth Control Methods sheet.

   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 three weeks 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. *Site: Delete the preceding sentence if you list approved birth control methods in this consent form.* If you join the study, we will test you for pregnancy at some visits, including before each study injection.

Being in the study

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

9. **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).

10. 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. This happens if we pay you more than $600 between January 1 and December 31 of the same year. The clinic staff 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.

11. We will give you either the study vaccine or a placebo.

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

The first 6 people in the study will have a 5 in 6 chance of receiving the study vaccine. Other people in the study will have a 10 in 11 chance of receiving the study vaccine.

Site: Modify the randomization metaphor in the below paragraph as appropriate to your local culture.

Whether you get the study vaccine or the placebo is completely random, like flipping a coin.

The reason we are testing the study vaccine is because we do not know whether it is safe and whether it works. That means we do not know whether it is better to get the vaccine or to get the placebo. In either case, you need to take steps to protect yourself from HIV infection.

The clinic staff has no say in whether you get the study vaccine or the placebo. They will not know which one you are getting, and neither will you. Only the pharmacist at your site will have this information while the study is going on, and he or she will keep it a secret.

You will have to wait until everyone completes their final study visits to find out whether you got the study vaccine 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.

12. We will give you the study products on a schedule.

You will be in one of 4 groups. We will tell you which group you are in. Each group tests a different dose of the study vaccine and adjuvant. The first group will get the lowest

doses of study vaccine and adjuvant. We will look at how people's bodies react to the lowest dose before enrolling the other groups at higher doses.

You will get injections at 4 study visits. The first 6 people in the study will be in Group 1. They will get injections into the skin of one upper arm. Other people in the study will be assigned to Group 2, Group 3, or Group 4. If you are assigned to Group 2 or 3, you will get injections into the skin of both upper arms. Both arms will be used each time unless there is a reason one arm cannot get injections. In that case, 2 injections may be given into one arm. If you are assigned to Group 4, you will get injections into the muscle of only one upper arm. The groups test different doses of the vaccine, as shown in the table. People in Group 2 do not receive the study adjuvant.

We cannot give injections in exactly the same place twice. Depending on your group, you will get injections in 4 or 8 different places in your upper arms.

Injection Schedule				
Groups	First Injection Visit (given with EP)	1 Month Later (given with EP)	3 Months Later (given with EP)	6 Months Later (given with EP)
Group 1 (between the layers of the skin of one upper arm)	**Low dose** study vaccine + study adjuvant or placebo	**Low dose** study vaccine + study adjuvant or placebo	**Low dose** study vaccine + study adjuvant or placebo	**Low dose** study vaccine + study adjuvant or placebo
Group 2 (between the layers of the skin of both upper arms)	**Medium dose** study vaccine or placebo	**Medium dose** study vaccine or placebo	**Medium dose** study vaccine or placebo	**Medium dose** study vaccine or placebo
Group 3 (between the layers of the skin of both upper arms)	**Medium dose** study vaccine + study adjuvant or placebo	**Medium dose** study vaccine + study adjuvant or placebo	**Medium dose** study vaccine + study adjuvant or placebo	**Medium dose** study vaccine + study adjuvant or placebo
Group 4 (into muscle of one upper arm)	**High dose** study vaccine + study adjuvant or placebo	**High dose** study vaccine + study adjuvant or placebo	**High dose** study vaccine + study adjuvant or placebo	**High dose** study vaccine + study adjuvant or placebo

For injections between the layers of the skin, the injection will be given on the upper arm with a needle and syringe. Then the EP device will be pressed firmly against your upper arm where you just received the injection. It will insert 3 very short needles into your skin. The study staff will activate the device and a very small amount of electricity will be sent in 4 short electrical pulses from the needles into your arm. Each of these pulses will last less than one second. Your arm will move because of the electrical pulses.

For injections into the muscle, the EP device will be pressed firmly against your upper arm. It will insert 5 needles into your arm. While the EP device is in your arm, the study products will be injected into your arm muscle with a needle and syringe. The study staff will activate the device and a very small amount of electricity will be sent in 3 short electrical pulses from the needles into your arm. Each of these pulses will last less than one second. Your arm will move because of the electrical pulses.

These injection procedures will take less than one minute. During the procedure and right after, you will feel some pain or discomfort. The intensity of that feeling lessens or may go away in a couple of minutes. After that, your arm may be sore for a day or two. We will ask you to rate the level of pain you feel on a scale.

You will have to wait in the clinic for about a half hour after each injection to see if there are any problems. Then for that night and for seven more days, you will need to write down how you are feeling and if you have any symptoms. Contact the clinic staff 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.

13. 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;
- Perform physical exams;
- Measure your waist;
- Take blood and urine samples;
- Do pregnancy tests if you were born female;
- Ask questions about your health, including medications you may be taking;
- Ask personal questions about your HIV risk, including sexual behavior and drug use;
- Ask questions about any personal problems or benefits you may have from being in the study;
- Photograph your injection site(s) (optional);
- Ask questions about your EP experience; and
- Have you rate your pain on a scale.

When we take blood, the amount will depend on the lab tests we need to do. It will be some amount between 18.5 mL and 220 mL (1 tablespoon to 1 cup). 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: Paste table of procedures in this section or distribute it as a separate sheet if it is helpful to your study participants.

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. We will also offer you counseling and referral for needed care.

14. We would like to take photographs of the injection sites on your arm(s).

The pictures will not include your face. You can refuse to have your picture taken at any time.

It is common for a mark to be left on the skin after an injection is given, with or without EP. The EP devices have 3 or 5 needles and may leave several marks on the skin. We are interested in what these marks will look like, or if you have a mark at all. We will take pictures of each injection site even if there is no mark. The pictures will show us how the injection sites heal and will help us make decisions about the EP study and devices.

15. We will counsel you on avoiding HIV infection.

We will ask you personal questions about your HIV risk factors such as sexual behavior and drug use. We will talk with you about ways to keep your risk of getting HIV low. Some topics we may discuss include:

- What you think may cause risky behavior for you.
- Methods to avoid getting HIV.

These may include not having sex, using condoms, or behavior changes, such as cutting down on alcohol. We will talk with you about which methods of HIV prevention may be right for you.

16. We will test your samples for reactions to the study products.

We will be looking for side effects. If any of the results are important to your health, we will tell you.

We will send your samples (without your name) to a lab to see how your immune system responds to the study products. The researchers may:

- Take cells from your samples and grow more of them. We may grow more of your cells over time, so that they can continue to contribute to this study.
- Do limited genetic testing. 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. Limited genetic testing involves only some of your genes, not all of your genes (your genome). The researchers will not look at all of your genes, only the genes related to the immune system and diseases.

These tests are for research purposes only. The lab will not give the results to you or this clinic, and the results will not become part of your study record.

17. After your clinic visits end, we will contact you around 6 months later.

We will contact you by phone or email *[Site: Modify mode of contact as appropriate]* after your study visits 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, please tell us if your address or phone number changes, if you are moving away, or if you do not want us to contact you anymore.

Delete next section if using separate Other Use of Specimen consent

18. When we take samples from you for this study, we take extra samples in case we have to repeat tests. 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 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. *[Site: insert specific information if your regulatory authority requires it.]* The central repositories for the HVTN are located in the United States and South Africa.

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 needed for your medical care. 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: insert review by your institution's IRB/EC, if applicable.]* IRBs/ECs protect the rights and well-being of people in research. The HVTN keeps track of your decision about how your samples and information can be used.

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.

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. The researchers may:

- Take cells from your samples and grow more of them. This means the researchers may keep your cells growing over time.
- Do limited genetic testing, which involves only looking at some of your genes, not all of your genes.

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.

19. 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.

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],
- Inovio Pharmaceuticals, Inc. and people who work for them,
- The HVTN and people who work for them,

- The HVTN Safety Monitoring Board or the National Institute of Allergy and Infectious Diseases Data and 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]

US sites: Include the following boxed text. You can remove the box.

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.
When the study is done, we may share the information from the study with others so they can see it and use it. We will not share any information that will let someone identify you.

20. 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 additional injections.

This may happen if:

- you do not follow instructions,

- you are in Group 4 and the EP device signals that it cannot give the electrical pulse and you have not yet received any study products,

- the researcher thinks that staying in the study might harm you,

- you get HIV,

- you enroll in a different research study where you receive 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.

21. If you become pregnant during the study, we will continue with some procedures but not injections.

We will do this for as long as it is safe for you and your developing baby.

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.

22. 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 about 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.

Risks

23. There are other risks to being in this study.

This section describes the 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 the needle was inserted. 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 an HIV study vaccine. The study vaccine may cause you to test positive on some types of HIV tests, even if you are not infected with HIV. This is called vaccine-induced seropositivity (VISP). VISP means that after you get the study vaccine, 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 vaccine.

If you receive a positive test result caused by the study vaccine at any time, we can provide you with 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 vaccine. 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, 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 vaccine, 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.

Site: Delete the following paragraph if local HIV testing of newborns is done via nucleic acid test.

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.

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 (HIV) 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 the baby continues to have VISP, we can do this testing for free for as long as it is needed. For most babies antibodies from the mother last for about six months.

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.

U.S. Sites, include the following paragraph 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 vaccine 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 vaccine might affect your HIV infection or how long it takes to develop AIDS.

We do not know if getting this study vaccine 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 vaccine. Currently, no HIV vaccine has been approved for use.

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

Benefits

24. 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 later become approved and sold, there are no plans to share any money with you.

Your rights and responsibilities

25. 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 Bill of Rights and Responsibilities. We will give you a copy of it.

Leaving the study

26. 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, and pictures of your injection sites. 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

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

Your health is important to us. We will help you get the medical care you need.

If someone gets sick or injured in an HVTN study, the HVTN decides whether the injury is probably related to the study products and/or procedures. If the HVTN decides it was more likely due to the study products or procedures than any other cause, then the HVTN and/or Inovio Pharmaceuticals, Inc. will use their funds to pay for treatment. If the HVTN decides otherwise, then you and your health insurance *(Sites: insert locale-appropriate medical insurance language in the preceding paragraph)* would be responsible for treatment costs. You may disagree with the decision the HVTN makes about your injuries. At your request the HVTN will ask experts who are not connected with the HVTN to review its decision.

In this study, Inovio Pharmaceuticals, Inc. will pay the cost of medical expenses that arise from injuries caused by the study products, including the EP device.

For injuries caused by study procedures other than EP, the HVTN has limited funds to cover the cost of medical treatment.

No matter what, you still have the right to use the court system to address payment for your injuries if you are not satisfied.

Some injuries are not physical. For example, someone might be harmed psychologically or emotionally by being in an HIV vaccine study. Or they might lose wages from injuries because they could not go to work. No funds have been set aside to pay for nonphysical injuries, even if they are related to participation in the study.

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 18 of this form, we told you about possible other uses of your extra samples and limited information, outside this study. Please write your initials or make your mark in the box next to the option you choose.

☐ I allow my extra samples combined with limited information to be used for other studies related to HIV, the immune system, and other diseases. This may include limited 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 limited genetic testing, growing more of my cells, or genome wide studies.

30. If you agree to join this study, you will need to sign below. Before you sign 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.

If you were born female and are sexually active in a way that could lead you to get pregnant, you must agree to use effective birth control, from 3 weeks 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, or vaginal rings;
- 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.

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 PENNVAX®-GP (gag, pol, env) DNA vaccine and IL-12 plasmid, delivered via intradermal or intramuscular electroporation in healthy, HIV–uninfected adult participants

HVTN protocol number: HVTN 098

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. *[Site: insert specific information if your regulatory authority requires it.]* The central repositories for the HVTN are located in the United States and South Africa.

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 needed for your medical care. They are not part of your medical record.

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: insert review by your institution's IRB/EC, if applicable.]* IRBs/ECs protect the rights and well-being of people in research. The HVTN keeps track of your decision about how your samples and information can be used.

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. The researchers may:

- Take cells from your samples and grow more of them. This means the researchers may keep your cells growing over time.
- Do limited genetic testing, which involves only looking at some of your genes, not all of your genes.

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.

U.S. Sites, include the following paragraph

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 write your initials or make your mark in the box next to the option you choose.

☐ I allow my extra samples combined with limited information to be used for other studies related to HIV, the immune system and other diseases. This may include limited 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 limited 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)

				Visits (number, months, and time after injection)								
	Screening	**1st injection**	2 weeks after 1st injection	**2nd injection** (Month 1)	2 weeks after 2nd injection	**3rd injection** (Month 3)	2 weeks after 3rd injection	**4th injection** (Month 6)	2 weeks after 4th injection	Month 9	Month 12	Month 18**
Procedure	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12
Injection and electroporation		√		√		√		√				
Medical history	√											
Complete physical	√										√	
Brief physical, injection site check		√	√	√	√	√	√	√	√	√		
Waist measurement		√					√		√		√	
Urine test	√		√				√					
Blood drawn	√	√	√		√		√		√	√	√	
Pregnancy test (participants born female)*	√	√		√		√		√		√		
HIV testing and pretest counseling	√						√		√	√	√	
Risk reduction counseling	√	√	√	√	√	√	√	√	√	√	√	
Interview/questionnaire	√	√	√	√	√	√	√	√	√	√	√	√
Pain assessment		√		√		√		√				
Photograph injection site (optional)		√	√	√	√	√	√	√	√	√	√	
Health contact**												√

*People who had a complete hysterectomy (removal of the uterus), or removal of both ovaries (verified by medical records) are not required to have a pregnancy test.

**Clinic visit is not required. However, if someone outside the study told you that you are infected with HIV we will ask you to come back to the clinic for another HIV test.

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.

Appendix E Laboratory procedures

					Visit:	1	2	3	4	5	6	7	8	9	10	11	12*	
					Day:	Screening visit[4]	D0	D14	D28	D42	D84	D98	D168	D182	D273	D364	D547	
					Month:		M0	M0.5	M1	M1.5	M3	M3.5	M6	M6.5	M9	M12	M18	
							VAC1		VAC2		VAC3		VAC4					
Description	**Ship to[1]**	**Assay location[2,3]**	**Tube[5]**	**Tube size (vol capacity)**			DNA		DNA		DNA		DNA					**Total**
BLOOD COLLECTION																		
Screening or diagnostic assays																		
Screening HIV test	Local lab	Local lab	SST	5mL		5	-	-	-	-	-	-	-	-	-	-	-	**5**
HBsAg/anti-HCV/Syphilis	Local lab	Local lab	SST	10mL		10	-	-	-	-	-	-	-	-	-	-	-	**10**
HIV diagnostic algorithm[10]	UW-VSL	UW-VSL	EDTA	10mL		-	-	-	-	-	-	10	-	10	10	20	-	**50**
Safety labs																		
CBC/ Diff/ platelets	Local lab	Local lab	EDTA	5mL		5	-	5	-	5	-	5	-	5	5	-	-	**30**
Chemistry Panel[6]	Local lab	Local lab	SST	5mL		5	-	5	-	5	-	5	-	5	5	-	-	**30**
Immunogenicity assays[7]																		
HLA Typing[8]	CSR	FHCRC	ACD	8.5mL		-	17	-	-	-	-	-	-	-	-	-		**17**
Cellular Assays																		
ICS	CSR	FHCRC	ACD	8.5mL		-	59.5	-	-	-	-	***59.5***	-	***59.5***	-	59.5	-	**238**
B-cell ELISpot	CSR	FHCRC	ACD	8.5mL		-	17	-	-	-	-	***17***	-	***17***	-	17	-	**68**
T cell repertoire	CSR	Vanderbilt	ACD	8.5mL		-	25.5	-	-	-	-	***25.5***	-	***25.5***	-	25.5	-	**102**
Humoral Assays																		
HIV-1 multiplex ab assay	CSR	Duke-DHVI	SST	8.5ml		-	8.5	-	-	-	-	***8.5***	-	***8.5***	-	8.5	-	**34**
HIV neut ab assay	CSR	Duke-NAB	SST	8.5ml		-	8.5	-	-	-	-	***8.5***	-	***8.5***	-	8.5	-	**34**
ADCC	CSR	Duke-ADCC	SST	-		-	y	-	-	-	-	***y***	-	***y***	-	y	-	**0**
Storage																		
Serum storage	CSR	—	SST	5mL or 8.5mL		-	13.5	8.5	-	8.5	-	13.5	-	13.5	-	13.5	-	**71**
Plasma storage	CSR	—	ACD	---		-	z	-	-	-	-	z	-	z	-	z	-	**0**
PBMC storage	CSR	—	ACD	8.5mL		-	42.5	-	-	59.5	-	42.5	-	42.5	-	42.5	-	**229.5**
Maximum Total						**25**	**192**	**18.5**	**0**	**78**	**0**	**195**	**0**	**195**	**20**	**195**	**0**	**918.5**
Maximum 56-Day Total						**25**	**217**	**235.5**	**235.5**	**313.5**	**78**	**273**	**0**	**195**	**20**	**195**	**0**	
URINE COLLECTION																		
Urinalysis	Local lab	Local lab				X	-	X	-	-	-	X	-	-	-	-	-	
Pregnancy Test[9]	Local lab	Local lab				X	X	-	X	-	X	-	X	-	X	-	-	

*For information concerning the Month 18 health contact see Section 9.5. Clinic visits are not required, except that any participant reporting diagnosis of HIV infection will be asked to come to the clinic so that HIV status can be confirmed.

y = 8.5mL of SST blood collected for the HIV nAb assay will also cover specimen needs for the ADCC/ADCVI; no separate blood draw is needed

z = up to 5mL of plasma extracted from ACD blood processed for PBMCs at the site processing lab; no separate blood draw is needed

[1] CSR = central specimen repository

[2] HVTN Laboratory Program includes laboratories at UW-VSL, Duke-DHVI, Duke-NAB, Duke-ADCC, and FHCRC. UW-VSL = University of Washington Virology Specialty Laboratory (Seattle, Washington, USA); Duke-DHVI = Duke Human Vaccine Institute, Duke University Medical Center (Durham, North Carolina, USA); Duke-NAB = Duke Neutralizing Antibody Assay Laboratory, Duke University Medical Center (Durham, North Carolina, USA); Duke-ADCC = Duke Antibody-Dependent Cellular Cytotoxicity Assay Laboratory, Duke University Medical Center (Durham, North Carolina, USA); FHCRC = Fred Hutchinson Cancer Research Center (Seattle, Washington, USA)

[3] Non-HVTN laboratories: Vanderbilt. Vanderbilt = Vanderbilt University (Nashville, Tennessee, USA)

[4] Screening may occur over the course of several contacts/visits up to and including day 0 prior to vaccination.

[5] Local labs may assign appropriate alternative tube types for locally performed tests.

[6] Chemistry panels are defined in Section 9.2 (pre-enrollment) and Section 9.4 (postenrollment).

[7] Immunogenicity assays will be performed at M0 (for binding Ab assay) M3.5 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 timepoints

[8] 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.

[9] Pregnancy test may be performed on blood specimens. 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.

[10] At an early termination visit for a withdrawn or terminated participant (see Section 9.12), blood should be drawn for HIV diagnostic testing, as shown for visit 11 above.

Appendix F Procedures at HVTN CRS

Visit:	01[a]	02	03	04	05	06	07	08	09	10	11	12	Post
Day:		D0	D14	D28	D42	D84	D98	D168	D182	D273	D364	D547	
Month:		M0	M0.5	M1	M1.5	M3	M3.5	M6	M6.5	M9	M12	M18[b]	
Procedure	Scr.	VAC1		VAC2		VAC3		VAC4					
Study procedures[c]													
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	—	—	—
Waist circumference measurement	—	X	—	—	—	—	X	—	X	—	X	—	—
Risk reduction counseling	X	X	X	X	X	X	X	X	X	X	X	—	—
Pregnancy prevention assessment[d]	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	—	—
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	—
Intercurrent illness/adverse experience	—	X	X	X	X	X	X	X	X	X	X	X	—
HIV infection assessment[e]	X	—		—	—	—	X		X	X	X	—	—
Confirm HIV test results provided to participant	—	X	—	—	—	—	—	X		X	X	X	—
Visual Analog Scale (pain assessment)	—	X	—	X	—	X	—	X	—	—	—	—	—
Acceptability questionnaires	—	—	X	—	X	—	X	—	X	—	X	—	—
Health contact	—	—	—	—	—	—	—	—	—	—	—	X	—
Local lab assessment												—	
Screening HIV test	X	—	—	—	—	—	—	—	—	—	—	—	—
Urine dipstick	X	—	X	—	—	—	X	—	—	—	—	—	—
Pregnancy (urine or serum HCG)[f]	X	X	—	X	—	X	—	X	—	X	—	—	—
CBC, differential, platelets	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[g]	—	X	—	X	—	X	—	X	—	—	—	—	—
Reactogenicity assessments[h]	—	X	—	X	—	X	—	X	—	—	—	—	—
Post-reactogenicity injection site assessment and photography[i]		X	X	X	X	X	X	X	X	X	X	X[j]	—
Poststudy													
Unblind participant	—	—	—	—	—	—	—	—	—	—	—	—	X

[a] Screening may occur over the course of several contacts/visits up to and including day 0 prior to vaccination.

[b] 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 will be asked to come to the clinic so that HIV status can be confirmed.

[c] For specimen collection requirements, see Appendix E.

[d] Pregnancy prevention assessment occurs only with participants who were born female and are capable of becoming pregnant.

[e] Includes pretest counseling. A subsequent follow-up contact is conducted to provide post-test counseling and to report results to participant.

[f] For a participant who was born female, pregnancy test must be performed on the day of vaccination prior to vaccination. Pregnancy test to determine eligibility may be performed at screening or 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 tests may be used to confirm the results of, or substitute for, a urine pregnancy test.

[g] 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. Lab tests may be drawn within the 3 days prior to vaccination.

[h] Reactogenicity assessments performed daily for at least 7 days postvaccination (see Section 9.9).

[i] Photograph of injection site(s) are to be taken prevaccination and at all subsequent scheduled visits if participant agrees.

[j] Assessment of unresolved injection site skin changes and photography of injection site(s)

Appendix G Adverse Events of Special Interest (AESI)

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 098 Study Specific Procedures*.

Neuroinflammatory disorders

Optic neuritis
Multiple sclerosis
Demyelinating disease
Transverse myelitis
Guillain-Barré syndrome
Myasthenia gravis
Encephalitis
Neuritis
Bell's palsy

Musculoskeletal disorders

Systemic lupus erythematous
Cutaneous lupus
Sjogren's syndrome
Scleroderma, dermatomyositis
Polymyositis
Rheumatoid arthritis
Juvenile rheumatoid arthritis
Polymyalgia rheumatica
Reactive arthritis
Psoriatic arthropathy
Ankylosing spondylitis
Spondyloarthropathy

Gastrointestinal disorders

Crohn's disease
Ulcerative colitis
Celiac disease

Metabolic diseases

Autoimmune thyroiditis
Grave's or Basedow's disease
Hashimoto thyroiditis
Insulin-dependent diabetes mellitus [IDDM]
Addison's disease

Skin disorders

Psoriasis
Vitiligo
Raynaud's phenomenon
Erythema nodosum
Autoimmune bullous skin diseases

Others

Autoimmune hemolytic anemia
Idiopathic thrombocytopenic purpura
Antiphospholipid syndrome
Temporal arteritis
Behcet's syndrome
Pernicious anemia
Autoimmune hepatitis
Primary biliary cirrhosis
Primary sclerosing cholangitis
Autoimmune glomerulonephritis
Autoimmune uveitis
Autoimmune cardiomyopathy
Sarcoidosis
Stevens-Johnson syndrome
Vasculitides