HIV VACCINE
TRIALS NETWORK

PROTOCOL

HVTN 115

A phase 1 clinical trial to evaluate the safety and immunogenicity of EnvSeq-1 Envs adjuvanted with GLA-SE, administered alone or with DNA Mosaic-Tre *env*, in healthy, HIV-uninfected adult participants

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CLINICAL TRIAL SPONSORED BY

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

2 IRB/EC review considerations

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

2.1 Minimized risks to participants

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

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

2.2 Reasonable risk/benefit balance

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

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

2.3 Equitable participant selection

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

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

2.4 Appropriate informed consent

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

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

2.5 Adequate safety monitoring

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

This protocol has extensive safety monitoring in place (see Section 11). Safety is monitored daily by HVTN Core and routinely by the HVTN 115 Protocol Safety Review Team (PSRT). In addition, the HVTN Safety Monitoring Board (SMB) periodically reviews study data.

2.6 Protect privacy/confidentiality

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

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

3 Overview

Title

A phase 1 clinical trial to evaluate the safety and immunogenicity of EnvSeq-1 Envs adjuvanted with GLA-SE, administered alone or with DNA Mosaic-Tre env, in healthy, HIV-uninfected adult participants

Primary objectives Part A

- To evaluate the safety and tolerability of different doses of CH505TF Env gp120 in HIV-uninfected healthy adults
- To evaluate binding antibody responses elicited by different doses of the CH505TF gp120 vaccine

Primary objectives Part B

- To evaluate the safety and tolerability of EnvSeq-1 vaccines administered with or without DNA Mosaic-Tre *env* in HIV-uninfected healthy adults
- To evaluate and compare vaccine-induced humoral responses elicited by EnvSeq-1 vaccines administered in sequential and additive protein immunizations, with and without DNA

Study products and routes of administration

- CH505TF HIV gp120 transmitted/founder with GLA-SE SE (glucopyranosyl lipid adjuvant-stable emulsion (synthetic lipid A derivative mixed with squalene oil)
- CH505w53 HIV gp120 with GLA-SE
- CH505w78 HIV gp120 with GLA-SE
- CH505w100 HIV gp120 with GLA-SE
- (EnvSeq-1 refers to the 4 component gp120 vaccine including CH505TF, w53, w78, and w100 HIV gp120s)
- DNA Mosaic-Tre *env*: trivalent vaccine composed of mosaic HV13284, HV13285 and HV13286 plasmids that are designed for global HIV-1 T-cell response coverage. All express gp160 Env protein.
- Placebo: Sodium Chloride for Injection, 0.9% USP
- All administrations will be intramuscular (IM) injections

Table 3-1 Schema

Study arm	N	Month 0 (Day 0)	Month 2 (Day 56)	Month 4 (Day 112)	Month 8 (Day 224)	Month 12 (Day 364)
Part A						
Group 1	12	20 mcg CH505TF	20 mcg CH505TF	20 mcg CH505TF	20 mcg CH505TF	20 mcg CH505TF
Group 2	12	100 mcg CH505TF	100 mcg CH505TF	100 mcg CH505TF	100 mcg CH505TF	100 mcg CH505TF
Group 3	12	400 mcg CH505TF	400 mcg CH505TF	400 mcg CH505TF	400 mcg CH505TF	400 mcg CH505TF
Group 4	6	placebo	placebo	placebo	Placebo	placebo
Total Part A	42 (36/6)					

Study arm	N	Month 0 (Day 0)	Month 2 (Day 56)	Month 4 (Day 112)	Month 8 (Day 224)	Month 12 (Day 364)	Month 16 (Day 485)
Part B							
Group 5	20	TBD mcg CH505TF + placebo	TBD mcg CH505w53 + placebo	TBD mcg CH505w78 + placebo	TBD mcg CH505w100 + placebo	TBD mcg CH505w100 + placebo	TBD mcg CH505w100 + placebo
Group 6	20	TBD mcg CH505TF + 4 mg DNA Mosaic-Tre	TBD mcg CH505w53 + 4 mg DNA Mosaic-Tre	TBD mcg CH505w78 + 4 mg DNA Mosaic-Tre	TBD mcg CH505w100 + 4 mg DNA Mosaic-Tre	TBD mcg CH505w100 + 4 mg DNA Mosaic-Tre	TBD mcg CH505w100 + 4 mg DNA Mosaic-Tre
Group 7	20	TBD mcg CH505TF + placebo	TBD mcg CH505TF, w53 + placebo	TBD mcg CH505TF, w53, w78 + placebo	TBD mcg CH505w53, w78, w100 + placebo	TBD mcg CH505w78, w100 + placebo	TBD mcg CH505w100 + placebo
Group 8	20	TBD mcg CH505TF + 4 mg DNA Mosaic-Tre	TBD mcg CH505TF, w53 + 4 mg DNA Mosaic-Tre	TBD mcg CH505TF, w53, w78 + 4 mg DNA Mosaic-Tre	TBD mcg CH505w53, w78, w100 + 4 mg DNA Mosaic-Tre	TBD mcg CH505w78, w100 + 4 mg DNA Mosaic-Tre	TBD mcg CH505w100 + 4 mg DNA Mosaic-Tre
Group 9	10	placebo + placebo	placebo + placebo	placebo + placebo	placebo + placebo	placebo + placebo	placebo + placebo
Total Part B	90 (80/10)						
Total Part A + Part B	132 (116/16)						

Safety data and immune responses after Month 4 in Part A will inform the protein dose for Part B.

GLA-SE will be admixed with all proteins. The total dose of GLA-SE will be 10 mcg at all timepoints. The total volume for protein plus adjuvant for injection is 1 mL, mixed 1:1 by volume.

If the dose of each protein in Groups 5 and 6 is 20 mcg, the total dose of each protein in Groups 7 and 8 will also be 20 mcg. If the dose of each protein in Groups 5 and 6 is 100 mcg or 400 mcg, the dose of each protein in Groups 7 and 8 will be 100 mcg.

All injections will be administered IM in the thigh. Part A injections will consist of 1 mL injections administered by needle and syringe. Part B injections will consist of 1 mL of protein or placebo administered by needle and syringe and 1 mL of DNA or placebo administered by Biojector 2000® Needle-Free Injection Management System™ (Biojector 2000®) into the same thigh. When necessary, the total protein volume will be supplemented with Sodium Chloride for Injection, 0.9% USP, at the time of mixing to make the total volume of injection 1 mL.

Participants

132 healthy, HIV-1–uninfected volunteers aged 18 to 50 years; In **Part A**, 36 vaccinees, 6 placebo recipients; in **Part B**, 80 vaccinees, 10 placebo recipients

Design

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

Duration per participant

Part A: 18 months of scheduled clinic visits (main study) followed by a health contact at month 24

Part B: 22 months of scheduled clinic visits (main study) followed by a health contact at month 28

Estimated total study duration

48 months (includes enrollment, planned safety and immunogenicity holds, in-clinic follow-up, and health contact)

Investigational New Drug (IND) sponsor

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

Study product providers

- EnvSeq-1 (CH505TF, w53, w78, and w100) HIV gp120s: DAIDS, NIAID, NIH, DHHS (Bethesda, Maryland, USA)
- DNA Mosaic-Tre *env* trivalent vaccine: DAIDS, NIAID, NIH, DHHS (Bethesda, Maryland, USA)
- GLA-SE adjuvant: Infectious Disease Research Institute (IDRI) (Seattle, Washington, USA)

Core operations

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

Statistical and data management center (SDMC)

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

HIV diagnostic laboratory

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

Endpoint assay laboratories

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

Study sites

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

Safety monitoring

HVTN 115 PSRT; HVTN Safety Monitoring Board (SMB)

3.1 Protocol Team

Protocol leadership

Chair	Magda Sobieszczyk Columbia University 212-305-7185 mes52@cumc.columbia.edu	*Statistician*	Paul Edlefsen SCHARP, FHCRC 206-667-4086 pedlefsen@gmail.com
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Vaccine developer representative	Barton Haynes Duke University	*Clinical trials manager*	Charlie Gregor HVTN Core, FHCRC
	Zachary Sagawa IDRI	*Project manager*	Andrew Sigman SCHARP, FHCRC
Laboratory Program representative	Georgia Tomaras Duke University	*SCHARP Protocol operations manager*	Gina Escamilla SCHARP, FHCRC
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Community Advisory Board (CAB) members	Ramil Sapinoro Rochester CAB	*Community engagement unit representative*	Jim Maynard HVTN Core, FHCRC
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4 Background

4.1 Rationale for trial concept

One of the major obstacles to developing an efficacious preventive HIV-1 vaccine is the challenge of inducing broadly neutralizing antibodies (bnAbs) against the virus. There are several reasons why eliciting bnAbs has been challenging and these include the conformational structure of the viral envelope, molecular mimicry of host antigens by conserved epitopes which may lead to the suppression of potentially useful antibody responses, and the high level of somatic mutations in the variable domains and the requirement for complex maturation pathways [4-6]. It has been shown that approximately 15% of HIV-1 – infected individuals develop high levels of very bnAbs that are detected 2-4 years after infection. To date, all bnAbs have 1 or more of these unusual antibody traits: high levels of somatic mutation, autoreactivity with host antigens, and long heavy chain third complementarity determining regions (HCDR3s)—all traits that are controlled or modified by host immunoregulatory mechanisms. Thus, the hypothesis has been put forth that typical vaccinations of single invariant immunogen primes and boosts will not suffice to be able to induce bnAbs; rather, it will take sequential immunizations with different Env immunogens, perhaps over a prolonged period of time, to mimic the bnAb induction that occurs in chronically infected individuals [7].

The B-cell lineage immunogen design tested in this trial is a process to give the bnAb lineages that are typically subdominant in infected patients a survival advantage wherein sequential Env immunogens are chosen that have high affinities for the B cell receptors of the unmutated common ancestor (UCA) or germline gene of the bnAb clonal lineage (see Figure 4-1) [8]. Envs for immunization can either be selected for binding or derived from the evolutionary pathways of Envs that actually give rise to bnAbs *in vivo*. Liao and colleagues, using serum samples of HIV-infected individuals collected over time under the CHAVI-ID program, recently described the co-evolution of HIV-1 and a CD4-binding site (CD4bs) bnAb from the time of seroconversion to the development of plasma bnAb induction, thereby presenting an opportunity to map out the pathways that lead to generation of this type of CD4bs bnAb (see Figure 4-2) [9].

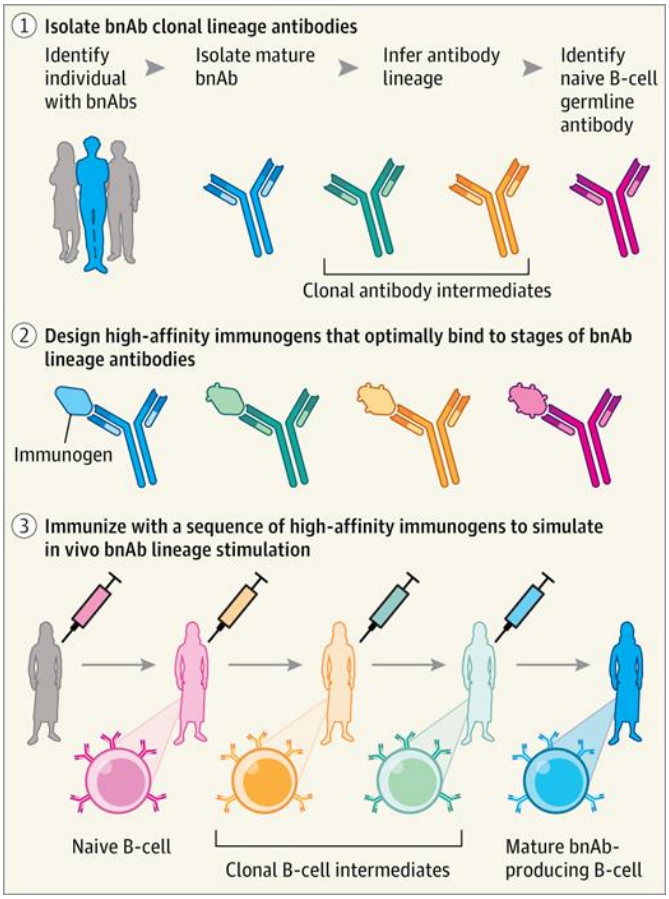

Figure 4-1. Steps of a B-cell-lineage-based approach to vaccine design

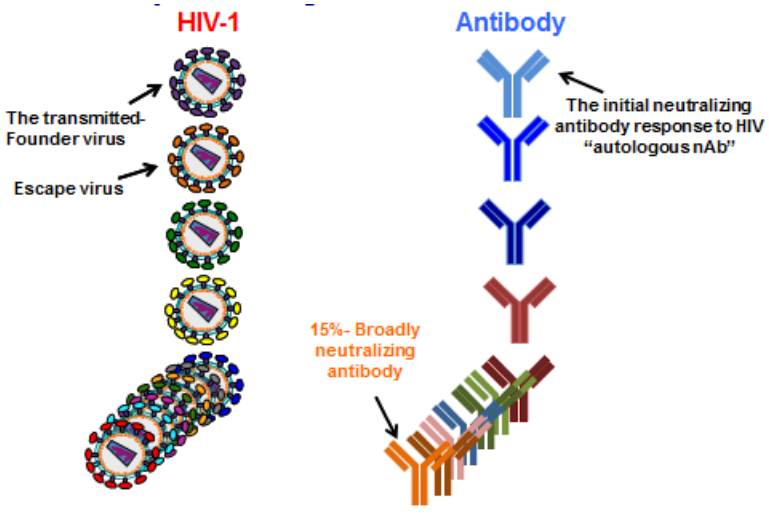

Figure 4-2. Schema of co-evolution of HIV-1 and broadly neutralizing antibodies in HIV-1–infected individuals. Most HIV-I infected individuals make either no or low levels of plasma broadly neutralizing antibodies, while ~15% of HIV-1–infected individuals will eventually make high levels of very broadly neutralizing plasma antibodies. The goal of this protocol is attempt to recreate this process by sequential Env vaccination.

This study demonstrated that the single transmitted/founder virus was able to bind to the bnAb UCA, and identified a series of evolved envelope proteins of the founder virus that were likely stimulators of the bnAb lineage. Analysis of the frequency of the B cell immunoglobulin gene variable-diversity-joining gene segment (VDJ) recombinations that occurred in this B cell lineage (the V_H4-59, V_λ3-1, CH103 CD4bs bnAb lineage) through collaborative studies of Bart Haynes and Tom Kepler (Boston University) has revealed that we can expect similar germline precursors in most vaccinated individuals. Specifically, the analysis found these precursors in 9/9 individuals tested by next generation sequencing. Thus, this work presents the HVTN with an opportunity to vaccinate with naturally derived viral envelopes that could drive the desired B-cell responses and induce the development of broad and potent neutralizing antibodies. While the human antibody repertoire is diverse, it has been found that only a few types of B-cell lineages can lead to bnAb development, and that these lineages are similar across a number of individuals [10,11]. Thus, it is feasible that use of Envs from 1 individual will generalize to others.

The approach in this study to address the challenge of eliciting bnAbs involves recreating the Env immunogens that induced a CD4bs bnAb clonal lineage in an HIV-infected individual, by making sequential recombinant Envs from that individual and using these Envs for vaccination. The B-cell lineage vaccine strategy thus includes designing immunogens based on unmutated ancestors as well as intermediate ancestors of known bnAb lineages. A candidate vaccine could use transmitted/founder virus envelopes to, at first, stimulate the beginning stages of a bnAb lineage, and subsequently boost with evolved Env variants to recapitulate the high level of somatic mutation needed for affinity maturation and bnAb activity. The goal of such a strategy is to selectively drive desired bnAb pathways.

4.1.1 B-cell lineage immunogen design

Over the past 5 years the HIV vaccine development field has realized that immunization with a single HIV envelope protein is not going to be successful for induction of broadly neutralizing antibodies [4]. Moreover, the biology of bnAbs has also become considerably clearer, with the evidence for a role of the host immune tolerance control mechanisms in limiting the induction of bnAbs (reviewed in [4,12]). While the role of the structure of the Env immunogens is undoubtedly important, ie, the Env must contain sufficiently native bnAb epitopes to bind in nM affinities to the unmutated common ancestor (naïve B cell receptors) of bnAb lineages [7,13], it is as yet unknown whether a native trimer is needed for this purpose or if a highly antigenic Env subunit will suffice. Studies in mice in basic B cell biology have demonstrated, however, that what is important for B cell survival in the germinal center (GC) is the affinity of the immunogen for the GC B cell receptor [14,15].

Thus, the Haynes group has proposed the concept of B cell lineage immunogen design, whereby lineages of bnAbs are elucidated, and Envs are chosen for sequential immunizations based on optimized affinity of Env immunogens for B-cell receptor (BCR) at sequential steps of the affinity maturation pathway of bnAb lineages (see Figure 4-1) [7]. While Envs have been designed for reacting with UCAs of heterologous bnAb lineages [13,16], in order to take the guessing out of Env selection, Haynes et al. have taken the approach of defining in select HIV-infected individuals, who make bnAbs, the natural sequence of Envs that in that individual induced the bnAb lineage. Thus, from the HIV-infected African individual CH505, both sequential Envs and bnAbs were isolated over time and the co-evolution of the CH103 CD4 binding site bnAb lineage was mapped

(see Figure 4-2)[9]. The Haynes group first screened thirty CH505 Env mutants, and of this group 4 candidates were found that reacted with the UCA, intermediate antibodies, and mature antibodies of the CH103 bnAb lineage. The proteins that optimally reacted with each step of the CH103 lineage were the transmitted/founder (TF) Env, the week 53.16 Env, the week 78.33 Env, and the week 100.B6 Env. In surface plasmon resonance (SPR) assays, the TF Env gp120 reacted with the UCA of the CH103 lineage with a K_D of ~200 nM.

4.1.2 Rationale for this study

This proposed study represents an important step to learning how to induce bnAbs in a vaccine recipient, by using a strategy of immunization to attempt to recreate the natural evolution of Env that promotes CD4 binding site-specific antibody evolution. This is the first time this concept will be studied in humans, and it will be undertaken in a stepwise approach. The vaccine is called the CH505 sequential envelope vaccine or the EnvSeq-1 vaccine. It is made up of 4 Env gp120 components, the transmitted/founder (TF) gp120 Env (CH505TF), the CH505 week 53 gp120 Env (CH505w53), the week 78 gp120 Env (CH505w78) and the week 100 gp120 Env (CH505w100). Gp120 forms of Env were chosen because a) the efficiency of immunization is related to binding affinity to B cell receptors of responding B cells [14,15], b) the gp120 forms bound with nM affinity to the unmutated ancestor antibody of the bnAb lineage and as well bound with nM affinities to other bnAb lineage intermediates, and c) it was desirable to leave out gp41 so as not to divert the immune response to non-neutralizing gp41 determinants.

The first step in the clinical trial is a dose-ranging study that will evaluate the induced antibodies to determine if the early stages of bnAb lineage can be stimulated with TF virus envelope (Part A of this proposal). This will be followed by a second part (Part B) that will assess boosting of CH505TF gp120 with evolved Env variants (CH505w53, w78, and w100) in order to attempt to recreate the Env diversity observed during *in vivo* affinity maturation in the CH505 individual. The second part of the study will also incorporate administration of a DNA with mosaic *env* insert together with the CH505 EnvSeq-1 variants. The rationale for this strategy is to provide T-cell help from heterologous Envs using the mosaic Envs designed to overcome virus diversity and to provide “universal CD4 T-cell help” (see Section 4.1.3 on Rationale for DNA Mosaic-Tre *env* as a universal helper immunogen) [17-19]. Our overall aim is to trigger the desired CD4 binding site bnAb lineage. We hypothesize that this can be accomplished in a clinical study, given the nonhuman primate (NHP) data (see Section 4.8 on Preclinical immunogenicity studies) demonstrating that it is possible to initiate the CH103-like CD4 binding site lineage in rhesus macaques.

The overarching aims of the proposed trial are to determine the safety and immunogenicity of CH505 EnvSeq-1 vaccine administered in combination with the GLA-SE adjuvant. The specific aim of Part A of the study is to make a rational choice for the optimal dose of gp120 Env protein that will be used in the other groups. The dose-ranging study of this vaccine product with GLA-SE in NHP (NHP 106; see Section 4.8.1 on Preclinical immunogenicity studies) demonstrated that after the second injection there was clearly a hierarchy of doses with respect to inducing Env binding as well as neutralizing antibodies and thus, the first part of this study will be a critical first step leading to the selection of a protein dose in humans and to Part B of the trial. The other important aspect of Part A is to evaluate the immunogenicity of CH505TF gp120 and compare to responses induced with the different combinations of the 4 Env mutant proteins in order to assess whether Env mutants, and not just CH505TF gp120 alone, are

needed to induce the desired lineage. The second part of the study will allow us to focus in on the essence of the concept of how to induce the desired bnAb pathways. And thus, the aims of the second part of the strategy (Part B) will be to assess the ability of the CH505 gp120 EnvSeq-1 mutant variants to induce CD4-binding site bnAb lineages. The EnvSeq-1 proteins will be administered either by combining the proteins with an additive approach with overlapping exposure to antigens (mimicking the coexistence of antigens in the environment in which they evolved naturally) and in a sequential immunization approach with each of the proteins administered separately in the order in which they evolved. An important secondary objective will be to explore the repertoire of B cell responses and isolate clones of the desired bnAb lineage.

Exploratory objectives proposed for this study will investigate the correlation between B-cell responses to the gp120 Env variants and the diversity of responses to the antigens present in gut flora. The role of the gut microbiome on the maturation of the immune system and the pathogenesis of chronic HIV infection has been well described [20-22] and there are emerging data about the role of gut microbiome in shaping the initial response to HIV. It has been shown, for example, that the initial antibody response to HIV infection is directed against gp41 and does not control early viremia [23]. Previous work demonstrated that immunization with DNA prime, rAd5 boost HIV-1 vaccine regimen selectively recruited a dominant gp41 memory B-cell response that was cross reactive with intestinal commensal-bacterial antigens [24]. Specific composition of the microbiota is thus an important factor influencing the host immune system and individual differences in the human microbiome can modulate vaccine-induced responses. This proposal will explore further how immune responses to this vaccine correlate with gut flora responses and whether the composition of gut microbiota in a vaccine recipient selects for cross-reactive responses similar to what has been previously noted.

4.1.3 Rationale for DNA Mosaic-Tre *env* as a universal helper immunogen

It is known that considerable T-cell help is required for the amount of affinity maturation needed to drive bnAbs and that those individuals chronically infected with HIV who spontaneously make bnAbs have higher levels of blood CD4 T cells that are phenotypically similar to T follicular helper T cells (Tfh) [25]. Previous work in non-human primates suggested that including a DNA vaccine with the protein can increase the quantity and quality of the induced antibody response compared to immunization with protein alone [26,27]. Furthermore co-administration of DNA and protein in the same limb elicited higher systemic binding and neutralizing antibodies compared to DNA alone or DNA prime followed by an antibody boost [26]. Pavlakis and colleagues illustrated the potential immunologic advantages of co-administration of protein with DNA in non-human primate studies whereby the animals that received DNA vaccine (either alone or co-administered with a protein) had higher levels of vaccine-induced T-cell responses. Moreover, the group that received DNA concurrently with protein had higher binding Ab responses than DNA or protein alone [28,29]. Pissani, et al. also showed in rabbits that DNA and protein co-administration increased the magnitude, avidity, and neutralizing potency compared to administration of protein alone. In addition, their study indicated that antibody responses using a mismatched DNA and protein elicited comparable or higher responses compared to matched vaccines [27]. These data support inclusion of a DNA vaccine in the regimen to assess and compare binding antibody responses and differential binding induced by immunization with and without the DNA. We hypothesize that the combined administration of DNA and protein will elicit stronger humoral and cellular responses than the protein alone.

Previous work demonstrated that immunization with a DNA prime/rAd5 boost HIV-1 vaccine regimen induced a non-neutralizing dominant Env gp41-reactive Ab response directed to non-neutralizing epitopes [24]. Although the DNA Mosaic-Tre vaccine in this protocol contains gp41 epitopes, a couple of key characteristics distinguish it from the DNA vaccine made by the Vaccine Research Center (VRC) which may result in a different quality of responses: 1) the mosaic gp160 DNA (DNA Mosaic-Tre) vaccine has been optimized for T cell diversity and CD4 (as well as CD8) epitopes; and 2) it is expected that this DNA gp160s product will form more native Env trimers compared to the VRC gp140 CFI misfolded proteins used in the DNA/rAd5 regimen. Importantly, current design of Part B will permit comparison of the immune responses induced by the protein + DNA Mosaic-Tre (Groups 6 and 8) vs. protein alone (Groups 5 and 7) to evaluate the effect of gp41 and whether or not co-administration of DNA results in diverting the immune response to non-neutralizing gp41 determinants.

Therefore, in this study we will evaluate and compare the immunogenicity of the CH505 gp120 proteins in sequential and additive immunization regimens administered with or without the DNA Mosaic-Tre *env* vaccine.

4.1.4 Rationale for GLA-SE adjuvant

The EnvSeq-1 proteins will be formulated in the GLA-SE adjuvant that has been used extensively in human phase 1 trials for influenza and leishmaniasis vaccines [30,31]. The 10 mcg dose of GLA-SE to be used in this clinical trial has been evaluated in 48 participants in 2 other clinical trials, 1 for a leishmaniasis vaccine candidate and 1 for a schistosomiasis vaccine candidate (ClinicalTrials.gov identifiers NCT02071758 and NCT01154049). And importantly, when administered with CH505TF gp120 in guinea pigs and NHP studies, the GLA-SE adjuvant has been shown to be a potent adjuvant in induction of binding and neutralizing antibodies [B. Haynes, unpublished]. In the guinea pig study, GLA-SE adjuvant was necessary for optimal immune responses to gp120 at all doses (Figure 4-3). The 10 mcg dose in this clinical trial was selected based upon the preclinical record of safety and immunogenicity of the CH505 gp120 + GLA-SE vaccine in guinea pigs and rhesus macaques, and the established safety profile of the GLA-SE adjuvant in human clinical trials.

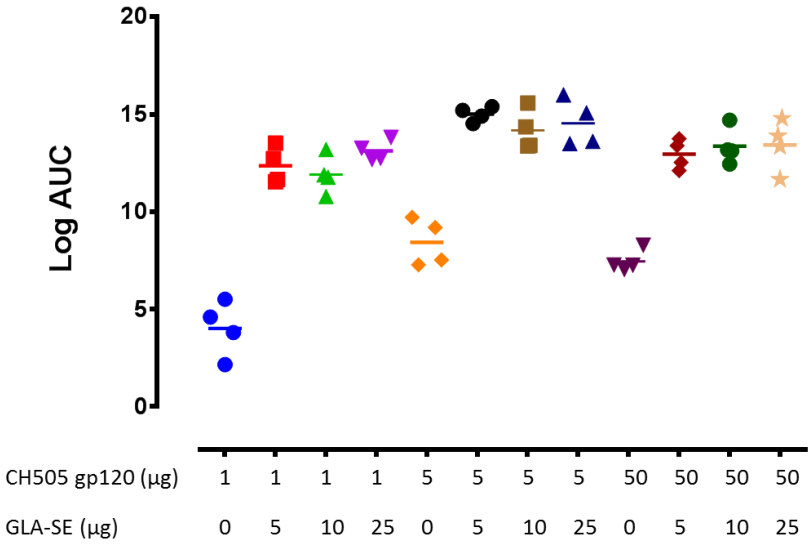

Figure 4-3. A dose study of both GLA-SE (0 to 25 mcg per immunization) and 1-50 mcg per immunization of CH505 gp120 IM was performed with 4 guinea pigs per group. Data show that for each dose of gp120, the response to the unadjuvanted protein was lower than the adjuvanted. Thus, the adjuvant is required for optimal response to gp120.

4.2 CH505 Sequential Env (EnvSeq-1)

The 4 individual EnvSeq-1 vaccine Envs used in this study are called CH505TF, CH505w53, CH505w78, and CH505w100 and were chosen based on their binding affinity in assays such as shown in Figure 4-4, which demonstrates the binding of the CH103 lineage versus a panel of recombinantly expressed sequential Envs.

	Binding of CH103 lineage antibodies to the autologous CH0505 Env, EC50, ug/ml												
Antigen	UCA	IA8	IA7	IA6	IA5	IA4	IA3	IA2	IA1	CH105	CH103	CH104	CH106
CH0505 TFD8gp120	2.116	1.277	0.412	0.534	0.254	0.133	0.177	0.147	0.179	0.194	0.095	0.138	0.086
CH0505 TF gp140C	4.714	1.931	0.402	0.536	0.203	0.164	0.132	0.111	0.122	0.130	0.072	0.180	0.073
CH505.s.03.D8.gp120	23.184	1.870	>100	0.525	0.236	0.266	0.126	0.189	0.218	0.192	0.078	0.218	0.097
CH505.s.03.gp140C	2.732	0.849	5.439	0.299	0.223	0.770	0.107	0.083	0.093	0.095	0.059	0.087	0.058
CH505.08.D11gp120	>100	3.116	0.973	1.837	1.504	>100	0.473	0.746	0.633	0.387	0.452	0.613	0.271
CH505.08.gp140C	>100	1.745	>100	2.245	0.578	0.324	0.401	0.307	0.487	0.560	0.335	0.483	0.266
CH505.30.e6 gp140	NB	NB	2.212	>100	0.597	>100	0.320	0.286	0.281	0.296	0.158	0.274	0.181
H0505.w30.23 D8 gp120	NB	NB	NB	>100	>100	NB	0.360	0.248	0.356	0.399	0.123	0.402	0.217
CH0505.w30.23gp140C	NB	NB	>100	7.029	0.196	6.689	0.132	0.108	0.127	0.125	0.067	0.110	0.081
CH505 W53.e16.D8 gp120	NB	NB	NB	NB	>100	NB	0.171	0.092	0.126	0.162	0.038	0.101	0.074
CH505.w53.e16.gp140C	NB	NB	NB	NB	>100	NB	0.141	0.081	0.103	0.132	0.046	0.090	0.069
CH505.w78.1.D8.gp120	NB	NB	NB	NB	NB	NB	0.779	0.359	0.498	0.774	0.067	0.449	0.652
CH505.w78.1.gp140C	>100	NB	NB	NB	NB	NB	0.528	0.263	0.339	0.480	0.110	0.344	0.589
CH505.w78.7.D8.gp120	NB	NB	NB	NB	NB	NB	0.233	0.119	0.164	0.198	0.047	0.211	0.240
cH505.w78.7.gp140C	NB	NB	NB	NB	NB	NB	0.179	0.284	0.151	0.167	0.055	0.140	0.210
CH505.w78.16.D8gp140	NB	NB	NB	NB	NB	NB	2.689	0.217	0.292	0.507	0.071	0.302	0.248
CH505.w78.16.gp140C	NB	NB	NB	NB	NB	NB	1.220	0.276	0.336	0.492	0.089	0.349	0.193
CH505.w78.25.D8.gp120	NB	NB	NB	NB	NB	NB	1.332	0.499	0.767	1.179	0.125	0.655	0.480
CH505.w78.25.gp140C	NB	NB	NB	NB	NB	NB	0.515	0.405	0.317	0.374	0.102	0.266	0.197
CH505.w78.33.D8.gp120	NB	NB	NB	NB	NB	NB	0.086	0.090	0.096	0.125	0.041	0.121	0.088
CH505.w78.33.gp140C	NB	NB	NB	NB	NB	NB	0.100	0.053	0.062	0.085	0.036	0.058	0.047
CH505.w78.38.D8.gp120	NB	NB	NB	NB	>100	NB	>100	0.709	0.867	1.182	0.219	0.797	0.589
CH505.w78.38.gpc140C	NB	NB	NB	NB	>100	NB	>100	>100	>100	>100	0.724	>100	0.716
CH505_w100.A4.D8.gp120	NB	NB	NB	NB	>100	NB	0.238	0.074	0.097	0.149	0.028	0.164	0.074
CH505.w100.A4.gp140c	NB	NB	NB	NB	>100	NB	0.789	1.164	0.326	0.491	0.122	0.422	0.248
CH505.w100.B6.D8.gp120	NB	NB	NB	NB	NB	NB	0.101	0.024	0.035	0.068	0.583	0.031	0.046
CH505.w100.B6.gp140C	NB	NB	NB	NB	NB	NB	0.026	0.015	0.016	0.023	0.110	0.016	0.018

Figure 4-4. Binding of CH103 lineage antibodies to autologous CH505 Envs. Choice of transmitted/founder and week 53, 78, and 100 CH505 Envs by binding EC50 levels to recombinant sequential CH505 Envs. Data are in EC50 binding levels in ELISA. (Red shading highlights the selected antigens and the antibodies they bind to.)

The EnvSeq-1 immunogen is composed of 4 recombinant protein Env gp120s, each with an N-terminal deletion to facilitate production by decreasing protein dimer formation and increasing production yield [32]. The individual components of the immunogen are the transmitted/founder CH505 Env (called CH505TF), the week 53 CH505 Env (called CH505w53), the week 78 CH505 Env (called CH505w78), and the week 100 CH505 Env (called CH505w100). They are all derived from the African clade C transmitted founder virus CH505 as described by Liao and colleagues [9].

4.3 DNA Mosaic-Tre *env* vaccine

This is a trivalent mosaic vaccine, Mosaic-Tre *env*, composed of mosaic HV13284, HV13285 and HV13286 *env*s that optimize global coverage. The individual Env components are termed TCM3.1 (plasmid name HV13284), TCM3.2 (plasmid name HV13285) and TCM3.3 (plasmid name HV13286). The mosaic immunogens are designed by *in silico* homologous recombinations to result in 3 *env* genes that a) have natural joining sites for CD4 and CD8 T cell epitopes, and b) provide the most extensive coverage of all the isolates possible in the Los Alamos HIV Sequence Database. Figure 4-5 shows the comparison of the coverage predicted for the Mosaic-Tre *env* versus the coverage with group M consensus CON-S *env* and a wild-type *env* B.1059. Figure 4-6 shows Mosaic-Tre *env* coverage for 9-mer coverage (CD8 cells) and 12-mer coverage (CD4 cells). The Mosaic-Tre *env*, optimized for 9-mers, is composed of 3 mosaic gp160s with transmembrane and cytoplasmic domain regions, and are encoded in the VRC8400 plasmid.

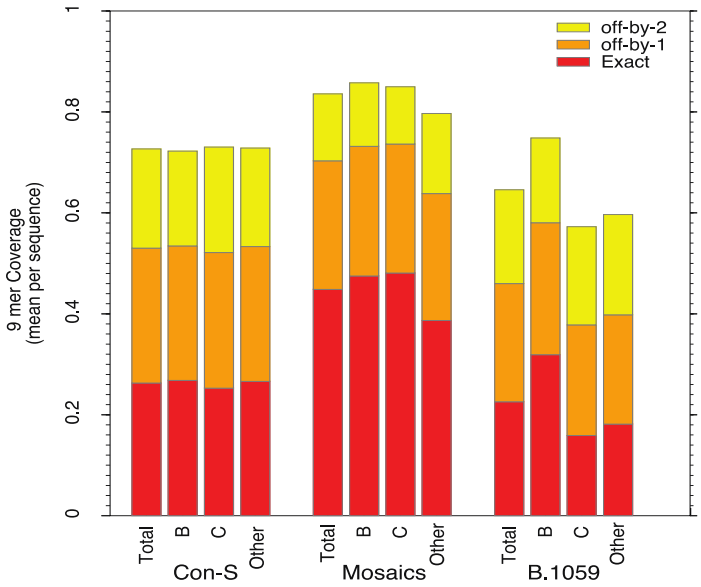

Figure 4-5. Average coverage of 9-mers in the Los Alamos database by different HIV-1 Env vaccine antigens used in this study. The red bar indicates the fraction of 9-mers that were perfectly matched by a 9-mer included in the vaccine, the orange adds on the frequency of 9-mers with an 8 of 9 match, and the yellow a match of 7 of 9. This plot is alignment independent, based on splintering all M group proteins, 1 sequence per person, into all possible 9-mers, attending to their frequencies, and then calculating the frequency of matches and near matches in the full data base to each vaccine antigen or protein cocktail. Each vaccine is summarized 4 ways. The "total" represents all sequences in the database alignment at the time these sequences were designed (2008). The "B" is just the subset that are B clade, "C" the subset that are C clade, and "Other" are the remaining M group sequences that are not B or C clade (all other clades and recombinants). Mosaics were optimized such that they maximized the red bar on the left, the exact matches in full, or total, dataset, but they provide excellent coverage of B clade, C clade, and other sequences. The best single natural TF virus, B.1059, was also selected to maximize the number of exact matches in the full database, given the constraints of selecting form among TF viruses and using a natural sequence. The single best natural is of course a B Env, as B clade dominates the data base, and thus the B clade has the best coverage for B.1059, with C and other having markedly reduced coverage. Con S, as expected, provides much more even coverage for all clades.

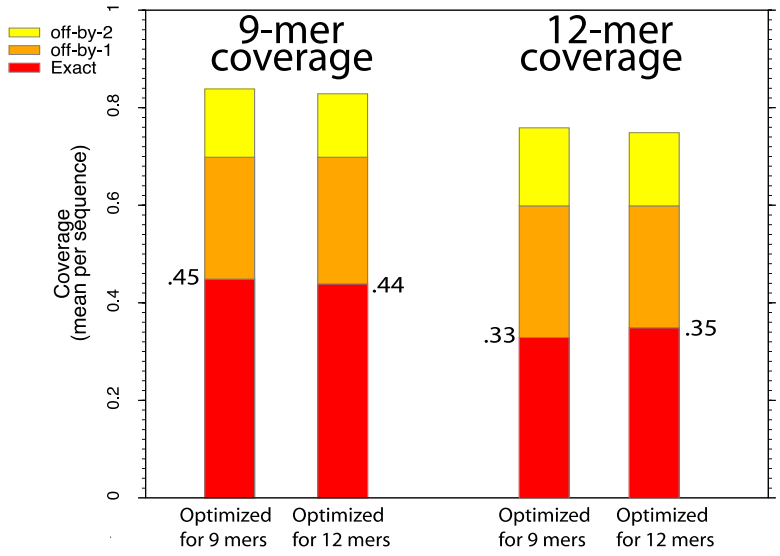

Figure 4-6. Comparison of the coverage of the 9-mers and 12-mers in the Los Alamos database using 9-mer and 12-mer optimized mosaics. The mosaic algorithm [17] requires the use of a string of specified length for optimization, and we typically use 9-mers, as that is the most common size of CD8+ T-cells epitopes, so 9 is a biologically motivated choice. However the optimal solution for 9-mers will be a near optimal solution for 8 to 12-mers [33]. Here we compare 9-mers and 12-mers in terms of the mosaic solution given the optimization criteria, versus the level of coverage obtained. CD4+ T cells tend to have longer epitopes, but 9–mer optimization provides excellent coverage of all epitope lengths.

4.4 GLA-SE adjuvant

The IDRI GLA-SE adjuvant is a glucopyranosyl lipid adjuvant-stable emulsion (synthetic lipid A derivative mixed with squalene oil). The Clinical studies section outlines previous experience with GLA-SE and other antigens (see Section 4.9). This adjuvant, however, has not yet been been administered to humans in combination with the EnvSeq-1 and the DNA Mosaic-Tre vaccines and therefore safety and tolerability of these products will be closely monitored.

4.5 Trial design rationale

An important goal for the vaccine field is to address the challenge of inducing broadly neutralizing antibodies against HIV-1. This study addresses this question by testing immunogens capable of recreating the natural evolution of Env that promote CD4 binding site specific antibody evolution. The first step in Part A of the study will be to compare the safety and immunogenicity of different doses of CH505TF gp120, including binding antibody responses and differential binding of antibodies to the wild type CH505 TF gp120 envelope compared to the IΔ371 mutant CH505 Env. These data will be used to determine the optimal dose of the protein to be used in the additive and sequential approach proposed in Part B. In the next step, we will build on the data from Part A to evaluate immune responses induced by the envelope variants which will be delivered either sequentially or with overlap (Table 3-1). This study aims to be the first of multiple iterative experimental trials to test the ability of these Envs to initiate bnAb lineages and to use the isolated B cells from the vaccinees to identify the lineages induced. Since it has been shown that DNA co-immunization with protein increased T-cell help and the

magnitude and quality of antibody responses, a trivalent mosaic DNA Env vaccine will be combined with the sequential immunization and the additive immunization schemes.

CH505TF gp120 protein will be available for clinical use approximately 6-7 months before the additional 3 proteins (CH505w53, w78, and w100 gp120s, representing variants from later timepoints in infection) are available. The IND will be established with the CH505TF gp120 protein information. Safety and immunogenicity data from after the third injection in Part A will be used to determine the protein doses to be used in Part B. Updated product documentation for the 3 later proteins and dose information for Part B will be submitted to the FDA prior to opening Part B for enrollment.

4.5.1 Dose (amount and number) and schedule

Part A, the dose-finding portion of the study, will involve 5 immunizations IM with the CH505TF gp120 at 0, 2, 4, 8, and 12 months. The doses of the protein will be 20 mcg (group 1), 100 mcg (group 2), and 400 mcg (group 3). GLA-SE will be admixed with all proteins and the total dose of GLA-SE will be 10 mcg at all timepoints. The total volume for protein plus adjuvant for injection will be 1 mL, mixed 1:1 by volume.

Part B will include an additive and a sequential approach to EnvSeq-1 protein administration, both with and without the DNA Mosaic-Tre *env* vaccine. The additive approach administers CH505TF, followed by CH505TF and w53 at month 2, CH505TF + w53 + w78 at month 4, CH505w53 + w78 + w100 at month 8, CH505w78 + w100 at month 12, and CH505w100 at month 16 as follows:

Month 0	**Month 2**	**Month 4**	**Month 8**	**Month 12**	**Month 16**
TF	TF	TF			
	w53	w53	w53		
		w78	w78	w78	
			w100	w100	w100

This additive immunization strategy mimics natural infection with proteins being administered in the order that they evolved naturally along with some overlap with preceding mutant Envs. This strategy also ensures that each protein is administered 3 times, which may be needed for optimal immune response to each immunogen to drive the desired B cell maturation.

The sequential approach will start with the CH505TF, then administer CH505w53 at month 2, CH505w78 at month 4, and CH505w100 at months 8, 12, and 16. The sequential approach also administers the Env mutants in the order that they evolved naturally, but does not include overlapping protein vaccinations. If the sequential approach is as effective as the additive approach, the simplified immunization schedule would be preferable. The immunization schedule is attempting to compress the process of recreating the evolution of antibody responses to the CD4 binding site in a much shorter timeframe than the 2 years it took for them to develop in natural infection. Data from NHP studies support the approach. This is the first clinical study evaluating this approach in humans. As mentioned above, the dose of the proteins will be determined by the safety and immunogenicity following the third immunization in Part A.

In Part B, the vaccines will be administered at months 0, 2, 4, 8, 12, and 16 and 10 mcg of GLA-SE adjuvant will be admixed with all proteins at all timepoints. In addition, some groups will also receive co-administration of 4 mg of DNA Mosaic-Tre *env* [17] to

compare the immunogenicity of the EnvSeq-1 vaccines with and without the DNA. The primary immunogenicity timepoints in Part B will occur after the fourth injection to permit sufficient time for the development of peak nAb titers and after the last vaccination to be able to evaluate whether the full regimens lead to the development of broadly neutralizing antibodies.

GLA-SE will be admixed with all proteins. The total dose of GLA-SE will be 10 mcg at all timepoints. The total volume for protein + adjuvant for injection is 1 mL, mixed 1:1 by volume.

If Part A indicates that the dose of each protein should be 20 mcg in Part B, the total dose of each protein in Groups 7 and 8 with the additive approach will also be 20 mcg. If the dose of each protein is determined to be 100 mcg or 400 mcg, the dose of each protein in Groups 7 and 8 will be 100 mcg to avoid going over 400 mcg of total protein at any timepoint.

All injections will be administered IM in the thigh. Part A injections will be administered as 1 mL of protein or placebo administered by needle and syringe. Part B injections will consist of 1 mL of protein or placebo administered by needle and syringe and 1 mL of DNA or placebo administered by needleless Biojector® into the same thigh. The rationale for injecting both products into the same limb is to ensure that both immunogens reach the same draining lymph nodes to optimize the amount of T-cell help available to promote B-cell differentiation and proliferation within the germinal center. Data from non-human primates (with different vaccine products) demonstrate that same limb co-immunization with DNA/gp120 protein/GLA-SE adjuvant vaccines resulted in enhanced humoral immune responses and polyfunctional cellular immune responses [28,34].

Adult immunizations are usually administered in 0.5 to 1 mL doses. There is, however, experience with the Hepatitis B vaccine Engerix-B, which has been safely given as a 2 mL injection in hemodialysis patients [29]. Similarly, multiple injections of licensed vaccines have been administered safely in the same limb, either the deltoid or the thigh [35,36]. As the 2 mL combined volume of the protein and DNA injections exceeds the typical volume administered in the deltoid, the larger vastus lateralis muscle will be used as the site of injection. To maintain comparability and blinding across groups, all groups will have all injections administered in the thigh.

4.5.2 Choice of control

Sodium chloride for injection USP, 0.9% will be used as placebo.

4.6 Plans for future product development and testing

This study will be the first trial of sequential Envs to attempt to initiate CD4-binding site neutralizing antibodies. The task is to begin the process of iterative human clinical trials to learn how to induce broadly neutralizing antibodies. Considerable data have been reported that bnAb precursors are rare and subdominant and, in some cases, are made so by tolerance controls of bnAb precursors in bone marrow [4,7,12]. Other types of bnAb such as membrane-proximal external region (MPER) antibodies (in addition to CD4 binding site antibodies) are also likely controlled in the periphery by peripheral immune tolerance control mechanisms because of bnAb acquisition of polyreactivity or autoreactivity [37]. Thus, this trial represents the first in a series of iterative phase 1

clinical studies to begin learning how to induce bnAbs in vaccinees. The goal of this trial is to be able to test immunogens capable of inducing HIV-1 bnAbs in humans.

4.7 Preclinical safety studies

Table 4-1 Summary of preclinical safety study

Study number	Product	Type of study	Animal	N	Dose groups	Route	Schedule
1726-031	CH505TF	Toxicity	New Zealand White Rabbits	10m, 10f/ group	Group 1: Saline Control Group 2: CH505TF +GLA-SE Group 3: DNA+CH505TF+GLA-SE Group 4: GLA-SE	IM	Day 1, 15, 29, 43, 57, 71, 85

4.7.1 Summary of preclinical safety studies related to the vaccines used in this study

Eighty New Zealand White Rabbits, 13-15 weeks in age, were randomly assigned to 1 of 4 groups (5/sex/group) as outlined in the table above. The test formulations were administered bi-weekly (ie, Days 1, 15, 29, 43, 57, 71, and 85) during the study via intramuscular injection in dose volumes of 1 mL of protein and 1 mL of DNA. Doses were withdrawn into the syringe in the animal room and administered via bolus intramuscular injection into the quadriceps or biceps femoris muscle groups of the hind legs, and were rotated between 4 injection sites. Animals were subjected to a full gross necropsy (5/group/sex) on SD 86. The remaining animals were necropsied on SD 113 following a 4-week no-treatment recovery period. Parameters evaluated during the study period included mortality, clinical and cage side observations, dermal grading observations, body weights, body temperatures, food consumption, ophthalmology, clinical pathology, immunology, immunogenicity, gross pathology, absolute and relative organ weights, and histopathology.

Eight animals died or were euthanized in extremis during the course of the study. None of these deaths were considered to be directly related to test article toxicity, as the distribution of morbidity was equal across all groups, including controls, and none of the findings associated with morbidity or mortality in these animals were seen in animals surviving to term.

Positive skin scores (very slight erythema and/or very slight edema) were observed following 6 out of 7 doses. The number of animals with positive dermal scores increased following the Day 57 dose administration, with a slight decrease following the Day 85 dose. Positive dermal scores were primarily noted in Group 3 animals with only a few animals in Groups 1 and 2 having positive scores which correlated with a higher incidence of injection site findings in Group 3 animals at terminal necropsy. The severity of the positive skin scores did not increase with additional doses. These observations may be observed with the administration of vaccines and were not considered adverse due to the magnitude, low severity of microscopic findings, and trend toward reversibility noted microscopically at the recovery necropsy.

Increased group mean body temperatures were observed across all groups (including controls) at 1 or more intervals, with a higher incidence observed in the vaccine-treated groups. In general, elevations (several outside of the protocol-specified normal range of

38.3º to 39.4ºC) were noted between 6 and 24 hours post dose. Body temperatures for most animals had returned to normal by 48 hours post dose. These elevations are a common finding following administration of a vaccine.

Test article-related macroscopic observations were seen in the terminal Group 3 males and females at injection sites 3 and 4 and consisted of swelling/thickening and red discoloration. These findings corresponded to microscopic findings of inflammation, edema, and/or fibrosis, or hemorrhage, for swelling/thickening and discoloration, respectively. There were no macroscopic observations in the recovery animals.

Test article-related microscopic findings were seen only at the injection sites for all terminal groups and all recovery groups and consisted of increased incidence and/or severity in all dosed groups compared to controls of a constellation of findings including: acute and chronic inflammation of the muscle; fibrosis; mixed cell infiltration/inflammation of the subcutaneous tissues; hemorrhage of the muscle and subcutaneous tissues; myofiber degeneration/necrosis; myofiber regeneration; and rare aggregates of vacuolated macrophages. While all groups had some degree of these findings, the DNA/CH505TF/GLA-SE-dosed group (Group 3), had the overall highest incidence and/or severity of hemorrhage, muscle inflammation (both chronic and acute), and myofiber degeneration/necrosis seen across terminal males and females at injection sites 1 and 2 combined. At injection sites 3 and 4 combined, Group 3 had the highest overall incidence and/or severity of the majority of the microscopic findings, including hemorrhage, edema, necrosis, vacuolated macrophages, muscle inflammation (both chronic and acute), subcutaneous infiltration/inflammation, and myofiber degeneration/necrosis and regeneration. Groups 2 and 4 were relatively similar to each other, with no clear trends in variation of incidence or severity of findings. At recovery, at all injection sites, there was a strong trend towards recovery in all groups, with generally reduced incidence and severity of most findings compared to terminal groups. Group 3 still had the highest overall incidence and/or severity of microscopic findings of vacuolated macrophages, chronic muscle inflammation, fibrosis, and subcutaneous infiltration/inflammation; with Group 2 and Group 4 being similar to each other and to Group 1 controls, albeit with slight increased incidence of vacuolated macrophages and, rarely, other findings consistent with resolving injection sites. The injection site findings were not considered to be adverse, given the generally low severity, lack of systemic findings, limited serum chemistry findings, and lack of associated clinical findings.

Mild, generally reversible and transient test article-related increases in monocytes, fibrinogen, globulin, and CRP and decreases in albumin and albumin/globulin ratio were observed after each dose administration in both sexes in all treatment groups. These changes were comparable in magnitude in all groups.

Potential test article-related statistically significant increases were seen in absolute and relative spleen weights of terminal Group 3 males and females. Notable absolute and relative spleen weight increases were also seen in the terminal Group 2 and 4 females. Spleen weight changes were similar between treatment groups and did not seem to vary to a notable degree between Groups 2, 3, and 4. However, these changes in spleen weight were not accompanied by any consistent corresponding macroscopic or microscopic observations and the toxicologic significance of this finding is unclear.

Seven biweekly intramuscular injections of 400 mcg CH505 TF gp120 with 20 mcg GLA-SE adjuvant with or without a 4 mg DNA booster injection or 20 mcg GLA-SE alone to New Zealand White rabbits for 13 weeks was well tolerated. Test article-related

changes in dermal scores, clinical pathology parameters, and microscopic injection site findings were resolved/partially resolved and/or trending toward recovery by the recovery necropsy, and none of the test article-related changes were deemed adverse.

4.8 Preclinical immunogenicity studies

Table 4-2 Summary of preclinical immunogenicity studies

Study number	Product	Animal	N	Dose groups	Route	Schedule	Assay
NHP 79	EnvSeq-1 gp120 Env with GLA-SE	RM	N=4 NHPs per group	• 100 μg CH505TF gp120 only • 100 μg CH505 sequential Envs • 100 μg CH505 additive Envs	IM	wk 0, 6, 12, 19, 24, 57	Neuts, binding, blocking
NHP 106	EnvSeq-1 gp120 Env with GLA-SE	RM	N=3 NHPs per group	• 5 μg CH505TF Env • 20 μg CH505TF Env • 100 μg CH505TF Env • 300 μg CH505TF Env • 600 μg CH505TF Env	IM	wk 0, 4, 12, 29, 37	Neuts, binding, blocking

4.8.1 Immunogenicity of the CH505 Env vaccines

NHP 79: Immunogenicity of the EnvSeq-1 gp120 Env proteins in rhesus macaques

The EnvSeq-1 immunogens have been administered to 3 groups of rhesus macaques (NHP 79) with the GLA-SE TLR4 agonist adjuvant from IDRI: the CH505TF Env gp120 alone, the Envs given in a sequential regimen (CH505TF, CH505w53, CH505w78, and CH505w100), and an additive-immunization regimen consisting of CH505TF gp120 in combination with the evolved Env variants (CH505TF, then CH505TF + CH505w53, then CH505TF + CH505w53 + CH505w78, then CH505TF + CH505w53 + CH505w78 + CH505w100).

From this study, the data indicate triggering of UCAs of lineages capable of binding HIV Envs with wild type sequences but not going into bnAb evolution is possible (Figure 4-7 and Figure 4-8). In this study of 12 monkeys, 11/12 monkeys had differential binding antibodies isolated and overall the Env differential binding antibodies (binds to wild type CH505 Env gp120 but not to CH505 with a deletion of isoleucine at position 371 indicating CD4 binding site antibodies and a trait of the CH103 bnAbs) were subdominant and were 15% of the total number of antibodies.

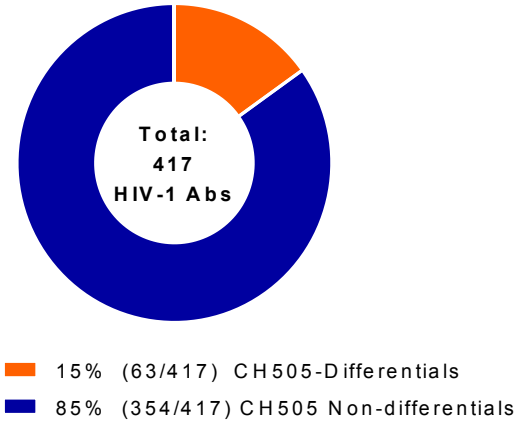

Figure 4-7. Binding specificities of 417 HIV-1 CH505 Env-induced antibodies isolated from rhesus macaques immunized with the CH505 4-valent sequential Envs (NHP79). Differential binders are antibodies that bind to the CH505 WT gp 120, but not the Δ371 gp120, which is indicative of putative CD4-binding site broadly neutralizing antibodies. Antibodies generated via small scale transient transfection were screened for binding in ELISA.

ID50 reciprocal dilutions	<20	20-100	101-1,000	1,001-10,000

Vaccine groups	Animal ID	Neutralization (ID50)							
		CH505 (Tier 2)	CH505.w4.3 (Tier 1b)	C.MW965 (Tier 1)	B.SF162 (Tier 1)	B.SS1196 (Tier 1b)	C.6644 (Tier 1b)	D.57128 (Tier 2)	MuLV
Group 1 – CH505 T/F Env alone	17	<20	359	958	20	<20	46	<20	<20
	18	<20	577	138	48	<20	<20	<20	<20
	19	<20	68	81	<20	<20	<20	<20	<20
	20	<20	1453	3470	302	49	67	*<20**	<20
Group 4 – CH505 Sequential Envs	21	<20	990	1775	49	24	37	*<20**	<20
	22	<20	539	335	<20	48	36	22	21
	23	<20	293	293	29	<20	<20	*<20**	<20
	24	<20	1908	1255	78	<20	34	22	<20
Group 5 - CH505 Additive Envs	25	<20	296	469	34	<20	<20	<20	<20
	26	<20	341	264	47	24	26	*<20**	<20
	27	<20	470	318	55	<20	25	<20	<20
	28	<20	130	329	<20	<20	<20	<20	<20

Figure 4-8. Plasma neutralization profile post sixth immunization in CH505-vaccinated macaques. Plasma was screened for neutralization of HIV-1 isolates via the TZM-bl assay. ID50 positivity cutoff was ≥20 or 3x> background neutralization of MuLV. *40-49% virus neutralization; less than 50% required to generate an ID50 value.

Using the following 4 criteria of the CH103 UCA characteristics, the Haynes team determined whether this 4-valent immunogen of CH505 Envs administered to rhesus macaques induced triggering of the UCA of a lineage with bnAb characteristics of: 1) no neutralization of the tier 2 CH505 TF virus; 2) neutralization of the tier 1B CH505 TF variant 4.3; 3) differentially binds to the CH505 TF gp120 but not to the mutated CH505 gp120 Env variants with a deletion of isoleucine at 371 (IΔ371); and 4) the lineage precursors are subdominant to other CH505 Env-binding lineages. Out of 412 Env-reactive antibodies isolated from rhesus macaques immunized with the CH505 4-valent sequential Envs, 60 (15%) antibodies fit this profile (Figure 4-7). Of the 60 CD4-binding site antibodies, the Haynes team has isolated a CH505 gp120/CH505 gp120IΔ371 differential-binding antibody, DH522, which not only neutralized the Tier 1b CH505 TF variant 4.3 but also neutralized other tier 1b viruses such as B.SS1196 C.6644, and AG.DJ263. Most importantly, this antibody is the first example of an antibody isolated in a vaccinated monkey that has tier 2 heterologous virus neutralization and neutralizes tier

2 HIV-1 strains; D.57128 and M.CON-S (Figure 4-9). Remarkably, the DH522 antibody VH in rhesus macaques is the VH4 gene that is most similar (92% identical) to the human VH4-59 that is used by CH103 CD4 binding site bnAb. This antibody was induced in the additive-immunization group in the non-human primate (NHP) study.

Additionally, further characterization of antibodies isolated from the additive immunization NHP group (macaque 5556) has led to the identification of 2 additional members of a DH522 clonal lineage (Figure 4-10). The DH522 antibody was found to block the binding of sCD4 as well as CH103 Abs to CH505 Env, and also exhibited differential binding to CH505 Env versus the Δ371/P363N mutant as well as YU2 wild-type versus YU2 D368R. DH522 also utilized the rhesus heavy chain most closely related to human VH4-59 (the VH used by CH103) and has a V_H mutation frequency of 3.4%. The frequency of the DH522 heterologous tier 2 neutralizing antibody lineage members is 2/412 (0.5%) of Env-specific antibodies.

IC50 (µg/mL)	0.01-1	1.0-10.0	10.0-50.0	>50

Virus Isolates		IC50 (µg/ml)					
ID	Tier	DH522 UCA	DH522IA1.2	DH522	DH564_ 565	CH103 UCA	CH103
CH505 T/F	2	>50	>50	>50	>50	>50	1.3
CH505.w4.3	1b	2.4	0.1	0.2	0.1	24.2	0.1
C.6644	1b	>50	2.0	4.3	9.5	>50	0.1
B.SS1196	1b	>50	14.6	22.9	19.4	>50	0.8
AG.DJ263	1b	>50	10.5	11.9	5.7	>50	0.9
D.57128	2	>50	14.0	15.6	13.8	>50	>50
M.CON-S	2	41	21.1	42.3	15.7	>50	3.0
MuLV	-	>50	>50	>50	>50	>50	>50

Figure 4-9. Neutralization profile of DH522 and other lineage members.

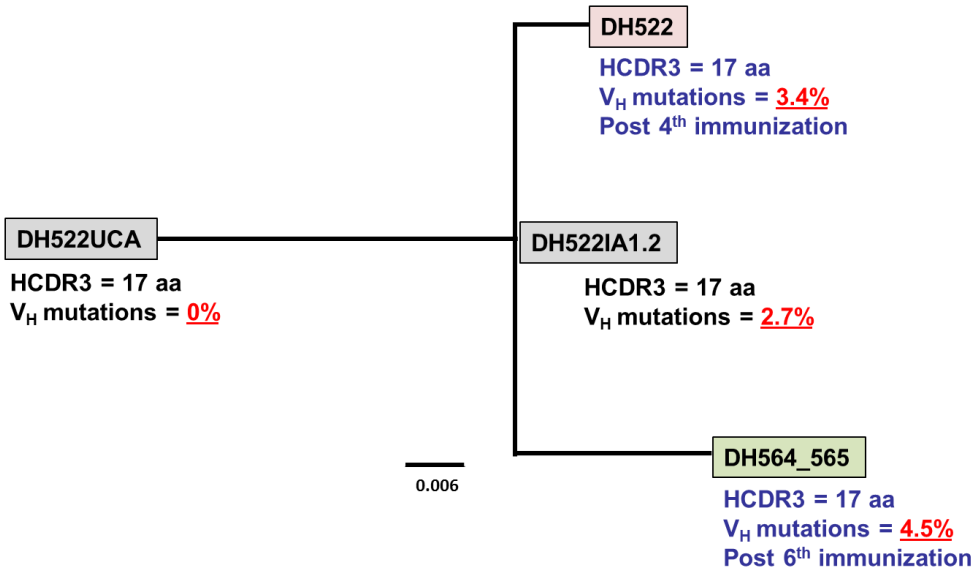

Figure 4-10. Three DH522 clonal lineage members from an NHP receiving the additive immunization regimen (NHP #79).

We determined the neutralization profile of DH522 and found that it neutralized 2 out of 31 primary isolates (6%), specifically D.57128 and M.CON-S (Figure 4-9).

We also performed an alignment of DH522 and CH103 and determined the light chain CH103 contacts that are shared with the vaccine-induced DH522 binding site antibody (Figure 4-11). We found that a shared mutation in contact region E50 in LCDR2 forms an H bond with N280 in loop D, and additionally shared contact K66 in light chain FR3 forms a salt bridge with D461 in V5 in CH103.

```
                 ***                          * ***             ****
CH103UCA_VL      SGDKLGDKYA C...WYQQKP GQSPVLVIYQ DSKRPSGIPE RFSGSNSGNT ATL
DH522UCA         T-TSSDIGGY NGVS----HS -TA-R-L--E V------VSD -----K---- -S-
DH522IA1.2       S-TSSDIGAY NGVS---HHS -TA-R-L--E V------VSD -----K---- -S-
DH522L           S-TSSDIGAY NGVS---HHS -TA-R-L--E V------VSD -----K---- -S-
DH564_565L       S-TTNDIGAY NGVS---HHS DTA-R-L--E VN-----VSD -----K---- -S-
CH103_VL         S-A...STNV -...---V-- ----EV--FE NY-------D -----K--S- ---
```

Figure 4-11. Alignment of DH522 and CH103: mutations to shared amino acids.

Thus, the CH103 human antibody and the DH522 rhesus antibody show convergent evolution with the same amino acids at gp120 contact sites. Finally, we characterized a fourth CH505 Env differential binder mAb (DH566) from post-fourth immunization sequential immunization in macaque 5553 (summarized in Table 4-3).

Table 4-3 Characteristics of CH505 Env differential binder DH566, post-fourth immunization (macaque 5553)

• VH3-30 (like HCDR3 binder CD4bs bnAb CH98)	• HCDR3 = 18
• Binds CH505 gp120, not CH505 Δ371; binds RSC3 but not RSC3Δ371, P363N gp120s	• Neutralization in TZM-bl: CH505TF = <20 CH505 4.3 = 0.2 mcg MW965 = 0. 2 mcg SF162 = 43 mcg/mL AG.DJ263 = 7.3 mcg/mL C.6644 = 3.0 mcg MuLV = <20
• V_H mutations = 8.5%, IgG1	

NHP 106 Study: Dose ranging study of EnvSeq-1 vaccine proteins administered with GLA-SE adjuvant in rhesus macaques

A dose ranging study was performed in rhesus macaques using CH505TF gp120 in 25 mcg GLA-SE with a dose of 5 to 600 mcg of Env with each dose administered 3 times. Env binding antibody (Figure 4-12) and CD4-binding site antibody (Figure 4-13) were determined and the CH505 TF gp120 was immunogenic after 2 immunizations. Moreover, it was noted that after 2 immunizations the 300 and 600 mcg doses of the protein were optimal with respect to inducing binding antibodies; after 3 immunizations the binding antibody responses were similar for all doses. With tier 1 neutralization, we found that while after 2 immunizations (Figure 4-14) there was a linear increase in neutralization titers with increasing Env dose, after 3 immunizations, there was no statistical difference between the tier 1 antibody neutralizing titers (Figure 4-15).

In the dose ranging portion of this clinical trial, (Part A) we will evaluate the binding Ab responses as the primary endpoint and quantify the frequency of the memory B cells that display differential binding to wild type CH505 TF gp120 envelope compared to mutant CH505 Env IΔ371 (Figure 4-16) induced after the third dose of CH505 TF gp120 vaccine to inform the protein dose for the second part of the study. The dose in Part A that will be

chosen for the dose in the arms of Part B will be determined by analysis of antibody binding data from a validated assay and will additionally be informed by the assay of memory B-cell receptor binding to CH505TF gp120 and differential binding to the CH505 IΔ371 gp120 Env. Preliminary data from the NHP 106 study included both plasma antibody and flow cytometric quantification of memory B cells that displayed differential binding; analysis of both measures indicated that 300 mgc/dose was optimal in NHP. For that reason, we expect that the validated assay primary outcome data and the secondary outcome data from flow cytometry will give a concordant answer.

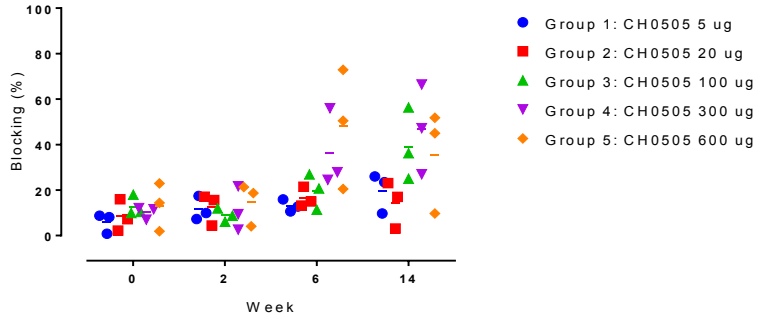

Figure 4-12. Inhibition of CH106 CD4 bs binding to B.63521 gp120 Env by NHP 106 plasma from immunization with 5 different doses of CH505TF gp120.

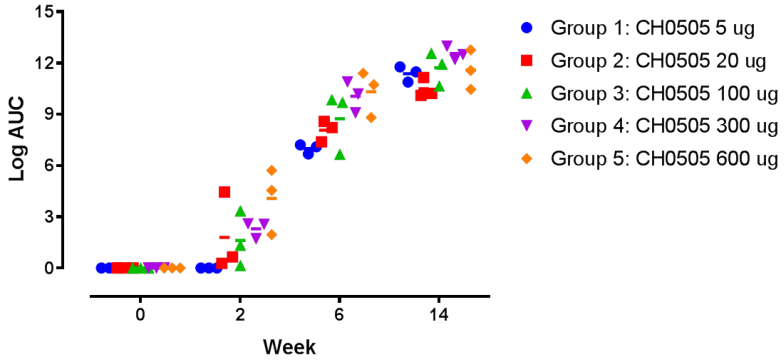

Figure 4-13. Binding of NHP 106 plasma to CHO-derived CH505TF gp120

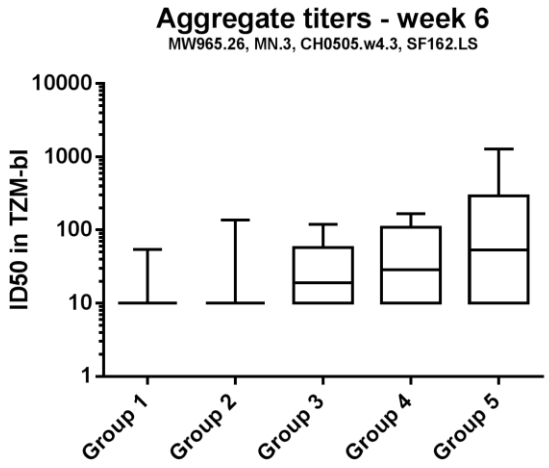

Figure 4-14. Tier 1 HIV neutralization in the TZM-Bl assay after 2 immunizations with CH505TF doses. Group 1, 5 mcg; Group 2, 20 mcg; Group 3, 100 mcg; Group 4; 300 mcg; Group 5, 600 mcg.

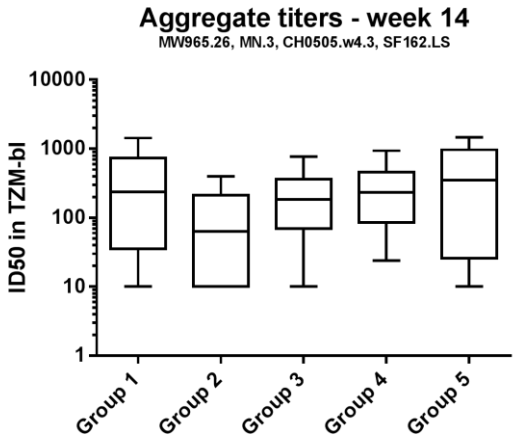

Figure 4-15. Tier 1 HIV neutralization in the TZM-Bl assay after 3 immunizations with CH505TF doses. Group 1, 5 mcg; Group 2, 20 mcg; Group 3, 100 mcg; Group 4; 300 mcg; Group 5, 600 mcg.

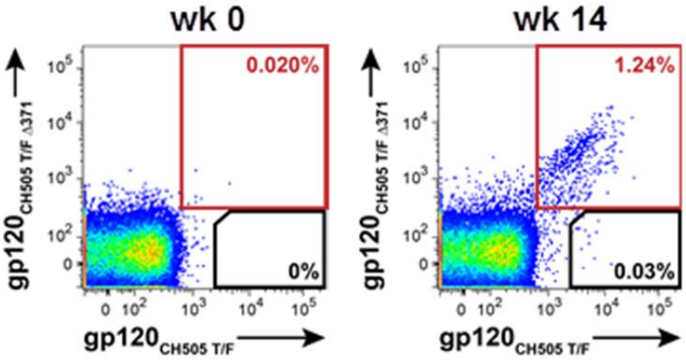

Figure 4-16 Gating strategy for B cell analysis. An example of NHP B cell data shown for preimmune and post third immunization samples. Few double positive (red gate gp120-reactive) or differential positive (black gate) cells are found in the preimmune sample; both double positive and differential B cells are found two weeks after the third immunization

4.9 Clinical studies

4.9.1 Clinical studies of gp120 Env protein vaccines

Although the EnvSeq-1 vaccines (CH505TF, CH505w53, CH505w78, and CH505w100 gp120 Env variants) have never been evaluated in humans before, there is extensive clinical trial experience with other gp120 Env proteins evaluated either alone or in combination with a prime or boost.

To date, there is considerable experience from phase 1 and phase 2 studies evaluating gp120/160 alone or in combination with pox vectors [38,39]. The AIDS Vaccine Evaluation Group conducted an analysis of safety data from over 15 clinical trials of gp120 and gp160 proteins administered to over 500 individuals demonstrating that overall, all HIV Env proteins at doses up to 640 mcg were well tolerated. Majority, if not all of the local and systemic reactions were deemed associated with adjuvant components of the vaccine rather than the Env proteins [40].

The monovalent subtype B and bivalent subtype B/E (CRF01_AE) recombinant glycoprotein 120 HIV-1 vaccine (AIDSVAX B/E) was evaluated in an efficacy study conducted among injection drug users in Thailand. A total of 600 mcg of AIDSVAX B/E was administered with alum as a stand-alone vaccination. The most commonly reported adverse event was tenderness at the injection site (71.0% vaccine recipients versus 65.7% placebo recipients), which did not increase with multiple injections. There were no differences between vaccine and placebo recipients with respect to the number of serious adverse events [41].

The RV144 Thai study evaluated the same gp120 vaccine in a heterologous prime-boost vaccination strategy consisting of a recombinant canarypox vector vaccine expressing *gag, pol,* and *env* (ALVAC-HIV) followed by the gp120 subunit boost. Over 8000 participants received multiple combined doses of 600 mcg of gp120 (300 mcg each of 2 different Clade B and E gp120s) in combination with ALVAC-HIV (vCP1521). This trial showed partial efficacy (31%) in protection from HIV-1 infection. Local and systemic reactogenicity noted after the gp120 protein (AIDSVAX B/E) was mostly mild and resulted in few serious adverse events [42].

Previous clinical experience comparing various doses of gp120 and gp160 proteins did not identify significant differences in safety profile when the protein dose was altered and adjuvant dose remained constant. For example, in a study evaluating AIDSVAX B/E gp120 at 100 mcg (n=31), 300 mcg (n=31) and 600 mcg (n=30) of protein in alum, majority (80%) of participants reported at least one reactogenicity event, most common one being injection site pain/tenderness, but all of the symptoms were mild to moderate and self-limited. Moreover, while there were fewer reactogenicty events at the lowest dose, the safety profile in the 300 mcg and 600 mcg groups was similar [43]. A study of recombinant gp160 VaxSyn formulated with an aluminium phosphate gel adjuvant compared two doses of 160 mcg (N=20) and 640 mcg (N=21) of the protein administered as 4 injections over 12 months. Both doses were generally well tolerated. Local reactogenicty (injection site erythema/induration) was frequent but self-limited, lasting less than 48 hours; and the frequency of local reactogenicity was similar between the two groups. There were no significant differences in severe local or systemic reactions between the dose groups. And importantly, there were no significant differences in laboratory abnormalities [hepatic, hematologic and renal function] between the different protein doses [44].

Taken together, the combined evidence from the large numbers of trial participants in these and other studies [45-49] demonstrates that gp120 recombinant protein vaccines administered in combined doses of up to 600 mcg were immunogenic and well tolerated. There was 1 case of anaphylaxis deemed related to the vaccine. There were no other unusual or serious vaccine-associated adverse events (SAE) reported. In Part A of the current study, the dose finding study for CH505TF gp120 will test doses ranging from 20, 100, to 400 mcg/dose with real-time safety evaluations. The protein dose for Part B of the study will be determined based on the safety and immunogenicity of the vaccine products in Part A of this trial.

4.9.2 Clinical studies of the GLA-SE adjuvant and protein combinations

Although the EnvSeq-1 vaccines in combination with GLA-SE has never been evaluated in humans, there is considerable clinical trial experience with the GLA-SE adjuvant in combination with other antigens. These data are summarized below and in Table 4-4 and Table 4-5.

Two previous studies evaluated the 10 mcg dose of the GLA-SE adjuvant proposed for this study. A phase 1 clinical trial was undertaken in Brazil with a *Schistosoma mansoni* antigen (Sm14; NCT01154049). Twenty healthy males received 3 doses of 50 mcg Sm14 + 10 mcg GLA-SE at 1-month intervals. There was no control group. The vaccine was safe and generally well tolerated with no SAEs or Grade 4 AEs (reported by the study PI). All AEs were rare with the exception of injection site pain (80%, 50%, and 41% after the first, second, and third dose, respectively). There were no abnormalities in physical exams, serum chemistries, and hematology values that were considered related to study vaccine.

The other trial is an ongoing phase 1, open-label evaluation of the safety, tolerability, and immunogenicity of the leishmaniasis vaccine (LEISH-F3) in combination with SLA-SE adjuvant compared to LEISH-F3 with GLA-SE in healthy adults (NCT02071758; Protocol IDRI-LVVPX-117). The SLA-SE adjuvant is the next generation TLR4 adjuvant formulation. Thirty-nine participants were randomized to 4 arms: high dose of antigen (20 mcg) and low dose of SLA-SE (5 mcg); high dose of antigen and high dose of GLA-SE adjuvant (10 mcg); low dose antigen (5 mcg) and high dose of GLA-SE adjuvant (10 mcg); and high dose of antigen and high dose of SLA-SE adjuvant (10 mcg). Participants received 3 injections at 1-month intervals. To date, all subjects have completed the treatment phase of the study and are in follow-up. There have been no Grade 3 or 4 AEs reported. Among the reported Grade 1 or 2 adverse events were:

- Local reactogenicity AEs: injection site tenderness (89%), pain (59%), induration (15%), erythema (15%), warmth (13%), pruritus (8%), and ecchymosis (5%)

- Systemic reactogenicity AEs: fatigue (41%), headache (26%), myalgia (21%), decreased appetite (18%), and chills (8%)

- Other AEs occurring in at least 2 subjects: hemoglobin decreased (15%), upper respiratory infection (13%), decreased potassium (5%), and emesis (5%)

GLA-SE at a 5 mcg dose has also been tested together with protein antigens in several other clinical trials which are included in Table 4-4 and Table 4-5.

Clinical experience with non-HIV protein antigens administered in combination with the GLA-SE adjuvant demonstrated that altering the dose of protein did not result in differences in the safety and tolerability of the vaccines [50,51] (Z Sagawa, personal communication). In a phase 1 study of a mycobacterium tuberculosis (Mtb) vaccine, sixty individuals received 3 doses of increasing dose of Mtb polyprotein from 2 mcg to 10 mcg in combination with GLA-SE from 2 mcg to 5 mcg [50]. Majority of adverse events were mild; most common were injection site pain, headache and fatigue. Importantly, there was no apparent increase in frequency or severity of adverse events with successive doses or higher doses of the antigen or GLA-SE.

Table 4-4. Completed clinical trials using IDRI GLA-SE adjuvant formulations in combination with other vaccines

Sponsor/Partner	Disease Area/Antigen	Adjuvant Formulation	Dose
Oswaldo Cruz Foundation **NCT01154049**	Schistosomiasis (Sm14)	GLA-SE	10 mcg
Rockefeller University/ IDRI **NCT01397604 and NCT01864876**	Adjuvant	GLA-SE, GLA-AF, SE	2 mcg 5 mcg
IDRI **NCT01484548**	Leishmaniasis (LEISH-F3)	GLA-SE	2 mcg 5 mcg
WRAIR **NCT01540474**	Malaria (CelTOS)	GLA-SE	2 mcg 5 mcg
Aeras / IDRI **NCT01599897**	TB (ID93)	GLA-SE	2 mcg 5 mcg
CONFIDENTIAL	Seasonal influenza	GLA-SE	0.5 mcg 1.0 mcg 2.5 mcg 5 mcg
Immune Design / Protein Sciences **NCT01147068**	Pandemic influenza (recombinant protein)	GLA-SE	1.0 mcg
Immune Design/Novavax **NCT01596725**	Pandemic influenza (H5-VLP)	GLA-SE	2.5 mcg
Immune Design / Medicago **NCT01991561**	Pandemic influenza (H5-VLP)	GLA-SE	5 mcg

Table 4-5. Ongoing clinical trials using IDRI GLA-SE adjuvant formulations in combination with other vaccines

European Vaccine Initiative (France) **NCT01949909**	Malaria (p27A)	GLA-SE	2.5 mcg 5 mcg
European Vaccine Initiative (France) **NCT02014727**	Malaria (AMA-1 DiCo)	GLA-SE	2.5 mcg
IDRI (US) / NIAID **NCT01751048**	Leishmaniasis (LEISH-F3)	GLA-SE MPL-SE	5 mcg
IDRI (US)	Leishmaniasis (LEISH-F3)	SLA-SE	10 mcg
Aeras / IDRI (South Africa) **NCT01927159**	TB (ID93)	GLA-SE	2 mcg 5 mcg
IDRI (South Africa) **NCT02465216**	TB (ID93)	GLA-SE	2 mcg 5 mcg

4.9.3 Clinical studies of the DNA Mosaic-Tre *env* vaccine

The DNA Mosaic-Tre *env* vaccine, administered via Biojector, is currently being tested in HVTN 106 to evaluate the breadth of induced CD4 and CD8 T-cell responses compared to group M consensus Env CON-S and a wild-type transmitted/founder Env B.1059 with the same DNA backbone as the DNA Mosaic-Tre *env*. HVTN 106 enrolled 105 participants randomized to receive 3 injections of DNA Mosaic-Tre *env* (previously known as DNA Mosaic *env*), DNA CON-S *env*, DNA WT *env*, or placebo, with 30 participants in each active group and 15 participants in the control group. Participants randomized to active groups also received 2 doses of an MVA HIV vaccine boost. The final product administration was in May 2016. As of June 2016, study follow-up is ongoing and the study remains blinded. The vaccines have been well tolerated with the most frequent reactogenicity symptoms being local pain and/or tenderness at the injection site, with the majority being mild (see Table 4-6). There have been 6 AEs deemed related to product, none of which necessitated discontinuation of vaccinations, and no serious adverse events attributed to the vaccines. All 6 AEs related to any of the 3 DNAs or placebo were mild and all have resolved. They included 1 lymphadenopathy, 3 alanine aminotransferase increases, 1 aspartate aminotransferase increase, and 1 localized cutaneous erythema. The SAEs or AEs that required expedited reporting to the trial sponsor that were not related to vaccine were appendicitis, severe head injury from a fall, alcohol withdrawal, acute systemic allergic reaction, demyelinating disorder, hepatic mesenchymal hamartoma, and uterine leiomyosarcoma.

Table 4-6. Summary of local site injection pain and/or tenderness reactogenicity after injections with DNA or placebo in HVTN 106

Deltoid pain and/or tenderness	Vac 1 N = 105 n (%)	Vac 2 N = 101 n (%)	Vac 3 N = 98 n (%)
None	13 (12%)	17 (17%)	27 (28%)
Mild	68 (65%)	73 (72%)	55 (56%)
Moderate	24 (23%)	11 (11%)	16 (16%)
Severe	0 (0%)	0 (0%)	0 (0%)
Potentially life-threatening	0 (0%)	0 (0%)	0 (0%)

In addition, DNA vaccines with the same plasmid backbones and similar HIV inserts have been administered to over 2100 human subjects. The results of human clinical trials with VRC DNA vaccines have been published for HIV DNA vaccines [plasmid DNA vaccines developed by VRC/NIAID/NIH] [52-58]. Table 4-7 summarizes previous human experience with the same DNA plasmid backbone as used for the proposed clinical trial.

Table 4-7. Clinical experience with same DNA plasmid backbone with different HIV gene inserts

Protocol Number	Clinical trials.gov NCT number	Number of subjects received DNA product
VRC 007	NCT00089531	15
VRC 008	NCT00109629	40
VRC 011	NCT00321061	60
VRC 101	NCT00270465	12
HVTN 204	NCT00125970	240
RV 172	NCT00123968	138
IAVI V001	NCT00124007	58
HVTN 077	NCT00801697	130
HVTN 096	NCT00179954	40
HVTN 092	NCT01783977	143
HVTN 505	NCT00865566	1252
Total number of participants who have received DNA HIV product		2128

Similar to the clinical study results with the exact DNA construct being used in this study, data from these DNA products that have the same plasmid backbone and related HIV inserts indicate that these products have been well tolerated and the most frequently reported local reactogenicity and AEs have been injection site pain and/or tenderness. The most frequently reported systemic symptoms were headache and malaise/fatigue. To date, there have been no serious adverse events (SAEs) attributable to these DNA vaccines.

4.10 Potential risks of study products and administration

Table 4-8. Summary of potential risks of study products and administration

Common	• Mild to moderate injection site pain, tenderness, 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
Less common	• Severe injection site pain or tenderness • Fever, chills, flu-like syndrome, arthralgia, rash, decreased appetite, 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 including decreases in hemoglobin, WBC, and neutrophils • Injection site hematoma, bruising/ecchymosis, laceration, other transient lesions, itching, 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 • Muscle damage at the injection site
Theoretical risks	• Autoimmune disease • 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 Part A

Primary objective 1:

To evaluate the safety and tolerability of different doses of CH505TF Env gp120 in HIV-uninfected healthy adults

Primary endpoint 1:

Local and systemic reactogenicity signs and symptoms, laboratory measures of safety, and AEs and SAEs

Primary objective 2:

To evaluate binding antibody responses elicited by different doses of the CH505TF gp120 vaccine

Primary endpoint 2:

HIV-specific binding Ab responses as assessed by binding Ab multiplex assay 2 weeks after the third vaccination with CH505TF gp120

5.2 Secondary objectives and endpoints Part A

Secondary objective 1:

To evaluate the ability of different doses of the vaccine regimen to elicit memory B cells that differentially bind CH505 wildtype gp120 TF Env vs the mutant CH505 Env IΔ371 gp120

Secondary endpoints 1:

Differential binding to CH505 gp120 compared to CH505 IΔ371 protein as assessed by flow cytometry analysis of the frequency of the differential binding memory B cells 2 weeks after the third vaccination with CH505TF gp120

Secondary objective 2:

To evaluate the ability of different doses of the vaccine regimen to elicit HIV-specific nAbs

Secondary endpoints 2:

- Magnitude and breadth of neutralizing antibody responses against autologous viral isolates as assessed by area under the magnitude-breadth curves 2 weeks after the third vaccination with CH505TF gp120 and at additional timepoints of interest following vaccination

- Magnitude and breadth of neutralizing antibody responses against a cross-clade panel of isolates induced 2 weeks after the third vaccination with CH505TF and at additional timepoints of interest following vaccination

Secondary objective 3:

To evaluate HIV-specific T-cell responses induced by different doses of the CH505TF vaccine

Secondary endpoint 3:

Response rate and magnitude of CD4+ T-cell responses as assessed by intracellular cytokine staining assays (ICS) 2 weeks after the third vaccination with CH505TF and at additional timepoints of interest following vaccination

Secondary objective 4:

To isolate single B cells with desired specificities and determine lineage characteristics

Secondary endpoint 4:

Monoclonal antibodies may be evaluated for binding and neutralization including tier 2 virus bnAb activity and CD4 binding site loop binding, neutralization of autologous TF or mutant CH505 viruses

5.3 Exploratory objectives Part A

Exploratory objective 1:

Based on the primary and secondary analyses, to further evaluate the immunogenicity of the different doses of CH505TF gp120 at additional timepoints of interest.

Exploratory objective 2:

To assess vaccine-induced follicular helper T cell (Tfh) responses

5.4 Primary objectives and endpoints Part B

Primary objective 1:

To evaluate the safety and tolerability of EnvSeq-1 vaccines administered with or without DNA Mosaic-Tre *env* in HIV-uninfected healthy adults

Primary endpoint 1:

Local and systemic reactogenicity signs and symptoms, laboratory measures of safety, and AEs and SAEs

Primary objective 2:

To evaluate and compare vaccine-induced humoral responses elicited by EnvSeq-1 vaccines administered in sequential and additive protein immunizations, with and without DNA

Primary endpoints 2:

- Magnitude and breadth of neutralizing antibody response against autologous viral isolates 2 weeks after the fourth and sixth (final) vaccinations with and without DNA

- Magnitude and breadth of neutralizing antibody responses against a panel of heterologous isolates induced 2 weeks after the fourth and sixth (final) vaccinations with and without DNA

5.5 Secondary objectives and endpoints Part B

Secondary objective 1:

To evaluate and compare HIV-1 specific binding Ab responses elicited by EnvSeq-1 vaccine administered in sequential and additive protein immunization, with and without DNA

Secondary endpoints 1:

- HIV-specific binding Ab responses as assessed by binding Ab multiplex assay 2 weeks after the fourth and sixth (final) vaccinations with and without DNA

- Differential binding to EnvSeq-1 gp120 compared to CH505 IΔ371 protein

Secondary objective 2:

To evaluate HIV-specific T-cell responses induced by different doses of the EnvSeq-1 vaccines

Secondary endpoint 2:

Response rate and magnitude of CD4+ T-cell responses as assessed by ICS assays 2 weeks after the fourth and sixth (final) vaccinations with EnvSeq-1 vaccines with and without DNA

Secondary objective 3:

To isolate single B cells with desired specificities and determine lineage characteristics

Secondary endpoint 3:

Monoclonal antibodies may be evaluated for binding and neutralization including tier 2 virus bnAb activity and CD4 binding site loop binding, neutralization of autologous TF or mutant CH505 viruses, and differential binding to CH505 gp120 compared to CH505 IΔ371 protein.

5.6 Exploratory objectives Part B

Exploratory objective 1:

To further evaluate the immunogenicity of the vaccine regimens at different timepoints

Exploratory objective 2:

To determine the B cell repertoire of HIV-specific B cells

Exploratory objective 3:

To assess vaccine-induced follicular helper T cell (Tfh) responses

Exploratory objective 4:

To correlate vaccine-induced B-cell and Tfh responses to EnvSeq-1 gp120s with responses to the gut microbiome using optionally-provided stool specimens

Exploratory objective 5:

To compare immune responses in groups 6 and 8 with DNA to groups 5 and 7 without DNA

Exploratory objective 6:

To further evaluate immunogenicity of each vaccine regimen, additional immunogenicity assays may be performed, including on samples from other timepoints, based on the HVTN Laboratory Assay Algorithm.

Exploratory objective 7:

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

6 Statistical considerations

6.1 Accrual and sample size calculations

Recruitment into Part A will target 42, and recruitment into Part B will target 90, healthy, HIV-uninfected adults aged 18 to 50 years old at low risk of HIV infection in regions where clade B is the predominant clade. Since enrollment is concurrent with receiving the first study vaccination, all participants will provide some safety data. However, for immunogenicity analyses, it is possible that data may be missing for various reasons, such as participants terminating from the study early, problems in shipping specimens, low cell viability of processed peripheral blood mononuclear cells (PBMCs) or high background. Immunogenicity data from 17 phase 1 and 2 phase 2a HVTN vaccine trials, which began enrolling after June 2005 (data as of September 2014), indicate that 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 ability of the study to identify SAEs can be expressed by the true event rate above which at least 1 event would likely be observed and the true event rate below which no events would likely be observed. Specifically, in each vaccine arm of Part A of the study (n = 12), there is a 90% chance of observing at least 1 event if the true rate of such an event is 17.5% or more; and there is a 90% chance of observing no events if the true rate is 0.8% or less. In all vaccine arms of the Part A of the study combined (n = 36), there is a 90% chance of observing at least 1 event if the true rate of such an event is 6.2% or more; and there is a 90% chance of observing no events if the true rate is 0.2% or less. In each vaccine arm of Part B of the study (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. In all vaccine arms of the Part B of the study combined (n = 80), there is a 90% chance of observing at least 1 event if the true rate of such an event is 2.9% or more; and there is a 90% chance of observing no events if the true rate is 0.1% or less.

Probabilities of observing 0, 1 or more, and 2 or more events among arms of size 12 and 20 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 12 and 20, for different true event rates

True event rate (%)	Pr(0/12)	Pr(1+/12)	Pr(2+/12)	Pr(0/20)	Pr(1+/20)	Pr(2+/20)
1	88.6	11.4	0.6	88.8	18.2	1.7
4	61.3	38.7	8.1	44.2	55.8	19.2
10	28.2	71.8	34.1	12.2	87.8	60.8
20	6.9	93.1	72.5	1.2	98.8	93.1
30	1.4	98.6	91.5	0.1	99.1	<0.1
40	0.2	99.8	98.0	<0.1	>99.9	<0.1

An alternative way of describing the statistical properties of the study design is in terms of the 95% confidence interval for the true rate of an adverse event based on the observed data. Table 6-2 shows the 2-sided 95% confidence intervals for the probability of an event based on a particular observed rate. Calculations are done using the score test method [59]. If none of the 36 participants receiving a vaccine regimen in Part A experience a safety event, the 95% 2-sided upper confidence bound for the true rate of such events in the total vaccinated population is 9.6%. For each individual Part A vaccine arm (n = 12), the 2-sided upper confidence bound for this rate is 24.2%. If none of the 80 participants receiving a vaccine regimen in Part B experience a safety event, the 95% 2-sided upper confidence bound for the true rate of such events in the total vaccinated population is 4.6%. For each individual Part B vaccine arm (n = 20), the 2-sided upper confidence bound for this rate is 16.1%.

Table 6-2 Two-sided 95% confidence intervals based on observing a particular rate of safety endpoints for arms of size 12 and 20

Observed event rate	95% Confidence interval (%)
0/12	[0, 24.2]
1/12	[1.5, 35.4]
2/12	[4.7, 44.8]
0/36	[0, 9.6]
1/36	[0.5, 14.2]
2/36	[1.5, 18.1]
0/20	[0, 16.1]
1/20	[0.9, 23.6]
2/20	[2.8, 30.1]
0/80	[0, 4.6]
1/80	[0.2, 6.7]
2/80	[0.7, 8.7]

6.1.2 Sample size calculations for immunogenicity

To address the humoral immunogenicity endpoints, the analysis will compare the area under the magnitude-breadth curve of the IgG Env binding Ab response, and will compare the frequency of memory B cells that bound to $gp120_{CH505\ TF}$ but not to $gp120_{CH505\ TF\ I\Delta371}$, 2 weeks after the third (Part A) or fourth and sixth (Part B) vaccinations with CH505TF gp120. To estimate power, binding magnitude-breadth data were simulated assuming that the proportion of non-zero responses was 0.94 in both comparison groups. This is based on pilot data for the IgG binding Ab response in HVTN 088. For these calculations, non-zero responses, modeled on the $\log_{10}$ scale, were drawn from a normal distribution. The mean non-zero response in group 1 was set to 4.06. This was the mean non-zero response in the HVTN 088 vaccine arm T2 (the treatment arm for vaccine naïve participants). The standard deviation (SD) of the non-zero response was set to 0.391 for both groups; this was the SD of the non-zero response in the HVTN 088 vaccine arm.

The differential binding rate is the fraction of class memory B cells that have the property of binding the two protein hooks in a differential manner (defined by flow gating). In the assay, two measurements are made simultaneously: binding to the transmitted founder CH505 virus, and binding to the IΔ371 variant. Each of these yields a measurement of the proportion of memory B cells that bind to a target virus. While the primary interest is in the differential binding to these two viruses, which is the difference of these proportions, we will also evaluate each measure separately to further describe and explore the relationship between these measures under the scenarios represented by the arms of this trial.

Power for this is high if we can pool the cells across participants, which would be possible under an assumption of a homogeneous rate of differential binders across participants within a treatment group. Our prior expectation is that this is at least approximately correct, though we will evaluate the homogeneity and if we find evidence for different rates across participants, we will revert to the more conservative approach of treating participant rates of differential binders as varying across participants around a common group mean. In case pooling is not justified, power is the same (on the per-SD scale) to that calculated for binding Abs, since preliminary data indicate that the log-transformed rates of differential binding are consistent with the normal distribution.

However, interpretation of these SDs differs between the differential binding rate power calculations and the power calculations for the primary antibody endpoints. In the case of binding Abs, the SDs are on the scale of log-transformed binding Ab response values (which are areas under the magnitude-breadth curve), so it is reasonable to describe these in terms of "fold changes". However for differential binding Abs, the values and SDs are fractions. So for instance, if the SD is .001 (equivalently, 0.1%) that means that there is 90% power to detect a 0.2% difference between the differential binding rates in the two arms. In the text below we provide example interpretations of the SDs for the context of the primary Ab binding endpoints only.

To address antibody endpoints in Part A, the analysis will descriptively summarize binding response positivity call rates, and test superiority of the frequency of differential memory B cell binding (to wild type vs mutant IΔ371 CH505) and of the magnitude and breadth of the IgG binding Ab response to a panel of gp120 proteins for each of 2 comparisons (Group 1 vs 2, and Group 2 vs 3), using a two- sided Wilcoxon rank sum test with 5%/2 type-I error rate per comparison. The sample size of 12 vaccinees per group will give 80% power to detect a true difference of 1.81 standard deviations (SDs) between the mean responses and 90% power to detect a true difference of 2.04 SDs. Using the mean and SD values from the HVTN 088 trial, the difference of 1.81 SDs translates to a difference of 0.708 units of $\log_{10}$ binding magnitude; if 1 of the comparison groups has the same mean as was seen among non-negative values among vaccine recipients in the HVTN 088 trial (4.06 units), then after incorporating the expected 6% zero-valued (negative responder) measures, this corresponds to observing an increase or decrease in the overall mean from 3.691 in group 1 to 4.336 in group 2. These calculations assume a 10% loss-to-follow-up rate and the (94%) response rate observed in the HVTN 088 vaccine recipients. The same approach will be used to test superiority of the magnitude of the IgG binding Ab response to each individual gp120 antigen in the panel.

To address antibody endpoints in Part B, the analysis will descriptively summarize binding and neutralization response positivity call rates and test superiority of the of the frequency of differential memory B cell binding and of magnitude and breadth of the IgG

binding Ab response to a panel of gp120 proteins and of the neutralization of tier 1 and tier 2 panels of HIV-1 viruses. Each analysis will be conducted separately for each of 2 pooled comparisons (additive vs sequential, pooling over groups with and without mosaic DNA: Groups 5 and 6 combined vs Groups 7 and 8 combined; effect of mosaic DNA, pooling over groups receiving additive and sequential proteins: Groups 5 and 7 combined vs Groups 6 and 8 combined), and for each of 6 pairwise comparisons among these groups using a two- sided Wilcoxon rank sum test with 5% type-I error rate per pooled comparison and a 5%/6 type-I error rate per pairwise comparison. The sample size of 20 vaccinees per group will give 80% power to detect a true difference of 1.63 standard deviations (SDs) between the mean non-zero responses and 90% power to detect a true difference of 1.81 SDs for the pairwise comparisons. Using the mean and SD values from the HVTN 088 trial, the difference of 1.63 SDs translates to a difference of 0.637 units of $\log_{10}$ binding magnitude; if 1 of the comparison groups has the same mean as was seen among non-negative values among vaccine recipients in the HVTN 088 trial (4.06 units), then after incorporating the expected 6% zero-valued (negative responder) measures, this corresponds to observing an increase or decrease in the overall mean from 3.609 in group 1 to 4.175 in group 2. The sample size of 40 vaccinees per pooled group will give 80% power to detect a true difference of 0.80 standard deviations (SDs) between the mean non-zero responses and 90% power to detect a true difference of 0.90 SDs (these 2 are each conducted at a 5% alpha level). Using the mean and SD values from the HVTN 088 trial, the difference of 0.80 SDs translates to a difference of 0.313 units of $\log_{10}$ binding magnitude; if 1 of the comparison groups has the same mean as was seen among non-negative values among vaccine recipients in the HVTN 088 trial (4.06 units), then after incorporating the expected 6% zero-valued (negative responder) measures, this corresponds to observing an increase or decrease in the overall mean from 3.722 in group 1 to 4.007 in group 2. These calculations assume a 10% loss-to-follow-up rate and the (94%) response rate observed in the HVTN 088 vaccine recipients. The same approach will be used to test superiority of the magnitude of the IgG binding Ab response to each individual gp120 antigen in the binding panel.

6.2 Randomization

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

6.3 Blinding

Participants and site staff (except for site pharmacists) will be blinded as to participant treatment arm/group assignments (eg, vaccine or control). 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 115 PSRT should be consulted before emergency unblinding occurs.

6.4 Statistical analyses

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

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

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

6.4.1 Analysis variables

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

6.4.2 Baseline comparability

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

6.4.3 Safety/tolerability analysis

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

6.4.3.1 Reactogenicity

The number and percentage of participants experiencing each type of reactogenicity sign or symptom will be tabulated by severity and treatment arm and the percentages displayed graphically by arm. For a given sign or symptom, each participant's reactogenicity will be counted once under the maximum severity for all injection visits. In addition, to the individual types of events, the maximum severity of local pain or

tenderness, induration or erythema, and of systemic symptoms will be calculated. Kruskal-Wallis tests will be used to test for differences in severity between arms.

6.4.3.2 AEs and SAEs

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

A listing of SAEs reported to the DAIDS Regulatory Support Center (RSC) Safety Office will provide details of the events including severity, relationship to study product, time between onset and last vaccination, and number of vaccinations received.

6.4.3.3 Local laboratory values

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

For each local laboratory measure, summary statistics will be presented by treatment arm and 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 Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (Section 9.10) 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 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

Discrete categorical assay endpoints (eg, response rates) will be analyzed by tabulating the frequency of positive response for each assay by antigen and treatment arm at each timepoint for which an assessment is performed. Crude response rates will be presented with their corresponding 95% confidence interval estimates calculated using the score test method [59]. Because of the small numbers of control participants, no adjustment will be made to the vaccine arm estimates for the false positive rates in the control arms. To address the aims of Part B, Barnard or Fisher's exact tests, as specified in the SAP, will be used to compare the response rates of any 2 vaccine arms and of pooled groups as described in Section 4.1.2, with a significant difference declared if the 2-sided p-value is

≤ 0.05. In general, Barnard's is preferred since under most circumstances it is more powerful than Fisher's [60].

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 confidence intervals, for each vaccine arm using maximum likelihood-based methods [61].

For quantitative assay data (eg, magnitudes and magnitude-breadth AUCs from the neutralizing antibody multiplex assay or percentage of positive cells from the ICS assay), graphical and tabular summaries of the distributions by antigen, treatment arm, and timepoint will be made. For all primary and secondary immunogenicity endpoints, box plots and plots of estimated reverse cumulative distribution curves will be used for graphical display of all of the study arms. Typically the results will be shown for each vaccine arm and for the set of control arms pooled into 1 group.

To test for differences among the vaccine arms in Part A and separately in Part B, first a Kruskal-Wallis rank test or an F-test (depending on the normality assumption) will be used to test for overall differences. Secondly, if the overall test is significant at the 2-sided 0.05 level, then individual tests comparing the 2 adjacent pairs of vaccine arms (Part A) and all 6 pairs of 4 vaccine arms (Part B) will be done unless pre-specified. If rank-based tests are used then the tests will be inverted to construct Hodges-Lehmann point estimates and 2-sided (1-0.05/k) × 100% CIs about the differences in location centers of the k = 2 (Part A) or k = 6 (Part B) pair-wise comparisons of vaccine arms. If actual-value tests are used then the Dunnett's procedure will be used to construct simultaneous confidence intervals about the pairs of mean differences for the many-to-one comparisons [62] when multiple vaccine arms are each compared with 1 common control arm. When all pair-wise comparisons between the multiple vaccine arms are of interest, the Tukey procedure [63] will be used. If only specific comparisons between pairs of the multiple vaccine arms are of interest, the Holm-Bonferroni procedure will be used. An appropriate data transformation (eg, $\log_{10}$ transformation) may be applied to better satisfy assumptions of symmetry and homoscedasticity (constant variance). Significance of the differences between pairs will be evaluated using 2 procedures, first based on whether the simultaneous 95% CIs exclude zero and secondly based on whether the nominal (unadjusted) 95% CIs exclude zero.

Some immunologic assays have underlying continuous or count-type readout that are dichotomized into responder/nonresponder categories (eg, nAb multiplex assay positivity). If treatment arm differences for these assays are best summarized by a mixture model, then Lachenbruch's test statistic [64] or an alternative two-part test [65] (as defined in the SAP) will be used to evaluate the composite null hypothesis of equal response rates in the 2 arms and equal response distributions. Lachenbruch's test statistic equals the square of a binomial Z-statistic for comparing the response rates plus the square of a Wilcoxon statistic for comparing the response distributions in the subgroup of responders. A permutation procedure is used to obtain a 2-sided p-value. For estimation, differences in response rates between arms will be estimated using the methods described above, and in the subgroup of positive responders, differences in location parameters between arms will be estimated using the methods described above.

Based upon previous 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 peripheral blood mononuclear cells (PBMCs). To achieve unbiased statistical estimation and inferences with standard methods applied in a complete-case manner (only including participants with observed data in the analysis), missing data need to be missing completely at random (MCAR). Following the most commonly used definition, MCAR assumes that the probability of an observation being missing does not depend on any participant characteristics (observed or unobserved). When missing data are minimal (specifically if no more than 20% of participants are missing any values), then standard complete-case methods will be used, because violations of the MCAR assumption will have little impact on the estimates and hypothesis tests.

If a substantial amount of immunogenicity data are missing for an endpoint (at least 1 value missing from more than 20% of participants), then using the methods that require the MCAR assumption may give misleading results. In this situation, analyses of the immunogenicity endpoints at a specific timepoint will be performed using parametric generalized linear models fit by maximum likelihood. These methods provide unbiased estimation and inferences under the parametric modeling assumptions and the assumption that the missing data are missing at random (MAR). MAR assumes that the probability of an observation being missing may depend upon the observed responses and upon observed covariates, but not upon any unobserved factors. Generalized linear models for response rates will use a binomial error distribution and for quantitative endpoints, a normal error distribution. For assessing repeated immunogenicity measurement, linear mixed effects models will be used. If the immunological outcomes are left- and/or right-censored, then the linear mixed effects models of Hughes [66] will be used, because they accommodate the censoring. In addition, secondary analyses of repeated immunogenicity measurements may be done using weighted GEE [67] methods, which are valid under MAR. All of the models described above in this paragraph will include as covariates all available baseline predictors of the missing outcomes.

Some "resource-intensive" immunogenicity endpoints are only measured in subset of participants, eg, immunogenicity endpoints based on mucosal samples. For such endpoints, exploratory analyses will be conducted to assess the correlation of participant characteristics measured in (nearly) all subjects with the resource-intensive endpoints. For example, if the same assay is performed on blood and mucosal samples, then a scatterplot and Spearman rank correlation coefficient (r) will be used to assess the correlation of responses. If at least moderate correlations exist (eg, $r \geq 0.3$), then the semiparametric efficient analysis method of Rotnitzky and Robins [68] will be used (described in Gilbert, Sato et al. for application to vaccine studies [69]) to estimate the mean of the resource-intensive endpoint for each group and to compare means between groups.

6.4.4.2 Primary analyses of neutralization magnitude-breadth curves

Tier 1 Screen of Vaccine Regimens versus Placebo

The area-under-the-magnitude-breadth curve (AUC-MB) to the tier 1 panel of 3 isolates will be computed for each participant with evaluable neutralization data, as described in [70]. Dunnett's procedure will be applied with 2-sided alpha = 0.05 to determine which of the k = 3 (Part A) or k = 4 (Part B) vaccine groups have a significantly higher mean AUC-MB than that of the pooled placebo groups, as described in [71] (see their formula (1.1)). This procedure will be applied to construct 95% CIs about the k 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.

6.4.4.3 Secondary analyses of neutralization magnitude-breadth curves

Superiority comparisons of vaccine regimens passing the tier 2 screen

For the set of vaccine regimens that passed the tier 2 screen, an F-test will be performed for whether any of the mean AUC-MBs differ. If this test is not significant (p-value > 0.05), then the conclusion will be that there were no significant differences in mean AUC-MBs among the advanced vaccine regimens. If the F-test is significant, then simultaneous 95% CIs about the mean-differences in AUC-MBs will be reported. These CIs are computed as the estimated mean-difference plus or minus $t_{N-m,025}$ multiplied by the square-root of S2 $(1/n_i + 1/n_j)$, where $t_{N-m,025}$ is the 97.5^{th} percentile of a t-distribution with N – m degrees of freedom, where N is the total number of vaccine recipients evaluated (summing over the advanced vaccine regimens) and m is the number of advanced vaccine regimens. In addition, S2 is an estimate of the common sample variance of the AUC-MB, whereas ni and nj are the sample sizes of evaluable participants for vaccine regimens i and j being compared. Following Fisher's least significant difference procedure, the pairs of vaccine regimens with this CI excluding zero are deemed to have a significant difference. Nominal (unadjusted) 95% CIs about pairs of vaccine arms will also be reported.

Omnibus comparison of magnitude-breadth distributions

The analyses of magnitude-breadth described above are based on the endpoint area-under-the-curve, which is interpreted as the average $\log_{10}$ IC50 to the set of isolates in the test panel. Use of this endpoint is maximally statistically powerful if 1 vaccine arm has greater magnitude and breadth than the comparator vaccine arm, but may miss an effect wherein 1 vaccine arm has greater magnitude and the comparator vaccine arm has greater breadth. Therefore, a secondary analysis may compare the distribution of magnitude-breadth curves among vaccine arms using the test statistic $\max|B_d^G|$ from Huang, et al [70] (see page 85), which is designed to detect general differences in magnitude-breadth curve distributions.

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

For each vaccine regimen that passes the tier 2 screen, the best vaccine regimen will be deemed as that with the greatest value of the $\max|B_d^G|$ test statistic comparing its distribution of magnitude-breadth curves versus the pooled placebo group.

Superiority comparisons of vaccine regimens passing the tier 2 screen

Similarly, the max$|B_d^G|$ test statistic will be used to compare the distribution of magnitude-breadth curves between each pair of advanced vaccine regimens. The Holm-Bonferroni procedure will be applied to determine the pairs of regimens with significant differences in distribution controlling the family-wise false positive error rate at no more than 0.05. Nominal (unadjusted) 95% CIs about pairs of vaccine arms will also be reported.

6.4.4.4 Analysis of CD4+ T-cell response as measured by the ICS assay

The analysis of CD4+ T-cell response rates as measured by the ICS assay will be evaluated and compared as described under the general approach. For each T-cell subset, the positivity call for each peptide pool will include a multiple comparison adjustment for the number of peptide pools used in the assay. In general, the Mixture Models for Single-cell Assays (MIMOSA) statistical framework [72] and/or the Fisher's exact test-based positivity criteria will be used. Details of the positivity criteria will be discussed in the SAP. The magnitude of marginal response will be analyzed as described for quantitative data in the general approach section. For each T-cell subset, graphs will be used to display the background-subtracted magnitudes for each participant by protein, treatment arm and timepoint. When 3 or more cytokines are being measured by the ICS assay, the polyfunctionality of ICS responses may also be analyzed as an exploratory endpoint. Besides descriptive plots of the magnitude of polyfunctional responses, the COMPASS (Combinatorial Polyfunctionality analysis of Antigen-Specific T-cell Subsets) statistical framework may also be used to perform joint modelling of multiple T-cell subsets of different cytokine combinations. For example, the functionality score (FS) and the polyfunctionality score (PFS) may be used to summarize the multi-parameter ICS responses.

6.4.4.5 Analysis of multiplexed immunoassay data

When a small panel of analytes (eg, ≤ 5) is being assessed in a multiplexed immunoassay, the analysis of response rates and response magnitudes will be evaluated and compared as described under the general approach. Details for calculating a positive response and response magnitude will be provided in the SAP. When a larger panel is being assessed, 2 approaches may be considered to evaluate the magnitude and breadth of these responses. First, Magnitude–Breadth (M-B) curves maybe employed to display individual- and group-level response breadth as a function of magnitude. Response breadth of neutralizing antibodies is defined as the average of the log10 NAb titer over the panel of isolates, where titers that are below the limit-of-detection are set to half of that limit. Response breadth of binding antibodies is similarly defined as the average of the response over the panel of antigens. Two choices are to compare the M-B curves among vaccine arms, as follows: a non-parametric Wilcoxon rank sum test on the subject-specific area-under-the M-B curve (AUC M-B) or a Kolmogorov-Smirnov type test on the 2 group-average M-B curves. Simulations can be used to obtain 2-sided p-values for the latter test. Second, a weighted-average score-like variable may be constructed to account for the correlations between analytes as an integrate magnitude of responses to multiple analytes. Similar group comparison methods described in the first approach may be adopted. Details of either approach will be described in the SAP.

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 115 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 1 of the primary immunogenicity endpoints of the same class (humoral, cell-mediated, innate or mucosal) or, if no primary endpoint of the same class, at least 1 of the primary immunogenicity endpoints reaches the aforementioned threshold. The Laboratory Program will review the analysis report prior to distribution to the protocol chairs, DAIDS, vaccine developer, and other key HVTN members and investigators. Distribution of reports will be limited to those with a need to know for the purpose of informing future trial-related decisions. The HVTN leadership must approve any other requests for HVTN immunogenicity analyses prior to the end of the scheduled follow-up visits.

7 Selection and withdrawal of participants

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

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

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

7.1 Inclusion criteria

General and Demographic Criteria

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

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

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

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

5. **Agrees not to enroll in another study** of an investigational research agent before the last required protocol clinic visit

6. **Willing to be contacted by phone**, text message, or e-mail 6 months after completion of the scheduled clinic visits

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

HIV-Related Criteria:

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

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

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

Laboratory Inclusion Values

Hemogram/CBC

11. **Hemoglobin** ≥ 11.5 g/dL for volunteers who were born female, ≥ 13.0 g/dL for volunteers who were born male

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

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

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

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

Chemistry

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

Virology

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

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

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

Urine

20. **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

21. **Volunteers who were born female**: negative serum or urine beta human chorionic gonadotropin (β-HCG) pregnancy test performed prior to vaccination on the day of initial vaccination. Persons who are NOT of reproductive potential due to having undergone

total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing.

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

    - Agree to consistently use effective contraception (see Appendix C) for sexual activity that could lead to pregnancy from at least 21 days prior to enrollment through the last required protocol clinic visit. Effective contraception is defined as using the following methods:
        - Condoms (male or female) with or without a spermicide,
        - Diaphragm or cervical cap with spermicide,
        - Intrauterine device (IUD),
        - Hormonal contraception, or
        - Any other contraceptive method approved by the HVTN 115 PSRT
        - Successful vasectomy in the male partner (considered successful if a volunteer reports that a male partner has [1] documentation of azoospermia by microscopy, or [2] a vasectomy more than 2 years ago with no resultant pregnancy despite sexual activity postvasectomy);
    - Or not be of reproductive potential, such as having reached menopause (no menses for 1 year) or having undergone hysterectomy, bilateral oophorectomy, or tubal ligation;
    - Or be sexually abstinent.

23. **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 or any other study that requires non-HVTN HIV antibody testing during the planned duration of the HVTN 115 study

5. **Pregnant or breastfeeding**

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

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 115 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 or by the national regulatory authority where the volunteer is enrolling. For volunteers who have received control/placebo in an experimental vaccine trial, the HVTN 115 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 115 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 exclusionary: [1] corticosteroid nasal spray; [2] inhaled corticosteroids; [3] topical corticosteroids for mild, uncomplicated dermatitis; or [4] a single course of oral/parenteral corticosteroids at doses < 2 mg/kg/day and length of therapy < 11 days with completion at least 30 days prior to enrollment.)

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

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

15. **Autoimmune disease**

16. **Immunodeficiency**

Clinically significant medical conditions

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

18. **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.

19. **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

20. **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.

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

22. **Asthma exclusion criteria:**

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

    Exclude a volunteer who:

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

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

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

25. **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.

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

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

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

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

30. 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 Delaying vaccinations for a participant

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

- Within 45 days prior to any study injection
  - Receipt of blood products or immunoglobulin

- Within 30 days prior to any study injection
  - Receipt of live attenuated vaccines other than influenza vaccine
  - Receipt of allergy treatment with antigen injections

- Within 14 days prior to any study injection
  - Receipt of influenza vaccine or any vaccines that are not live attenuated vaccines (eg, pneumococcal)

- Pre-vaccination abnormal vital signs or clinical symptoms that may mask assessment of vaccine reaction.

Vaccinations should not be administered outside the visit window period specified in the HVTN 115 Study Specific Procedures.

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

7.3.2 Participant departure from vaccination schedule

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

7.3.3 Discontinuing vaccination for a participant

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

- Co-enrollment in a study with an investigational research agent (rare exceptions allowing for the continuation of vaccinations may be granted with the unanimous consent of the HVTN 115 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 115 PSRT is required prior to subsequent vaccinations following any type 1 hypersensitivity reaction associated with study vaccination; or

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

- Participant misses more than 3 vaccinations(s) (see Section 7.3.2).

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

In addition, vaccinations will be stopped for participants diagnosed with HIV infection. HIV-infected participants will not continue in the trial (see Sections 7.3.4 and 9.7.1).

7.3.4 Participant termination from the study

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

- Participant refuses further participation,
- Participant relocates and remote follow-up or transfer to another HVTN CRS is not possible,
- HVTN CRS determines that the participant is lost to follow-up,
- Participant becomes HIV infected,
- Investigator decides, in consultation with Protocol Team leadership, to terminate participation (eg, if participant exhibits inappropriate behavior toward clinic staff), or
- 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. Study products are listed in Section 3, *Overview*, sub-section Study products and routes of administration. 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.

Part A

Group 1

Treatment 1 (T1): 20 mcg CH505TF gp120 admixed with 10 mcg GLA-SE, to be administered as a 1mL IM injection into either thigh at months 0, 2, 4, 8 and 12.

Group 2

Treatment 2 (T2): 100 mcg CH505TF gp120 admixed with 10 mcg GLA-SE, to be administered as a 1mL IM injection into either thigh at months 0, 2, 4, 8 and 12

Group 3

Treatment 3 (T3): 400 mcg CH505TF gp120 admixed with 10 mcg GLA-SE, to be administered as a 1mL IM injection into either thigh at months 0, 2, 4, 8 and 12.

Group 4

Control 4 (C4): Placebo for CH505TF/GLA-SE (labeled as Sodium Chloride for Injection, 0.9% USP) to be administered as a 1mL (IM) injection, into either thigh at months 0, 2, 4, 8 and 12.

Part B

Group 5

Treatment 5 (T5): TBD mcg CH505TF gp120 admixed with 10 mcg GLA-SE, to be administered as a 1mL IM injection, into either thigh at month 0.

AND

Placebo for DNA Mosaic-Tre *env* (labeled as Sodium Chloride for injection, 0.9% USP) 1mL IM by Biojector 2000® into the same thigh at month 0.

Then

TBD CH505w53 gp120 admixed with 10 mcg GLA-SE, to be administered as a 1mL IM injection, into either thigh at month 2.

AND

Placebo for DNA Mosaic-Tre *env* (labeled as Sodium Chloride for injection, 0.9% USP) 1mL IM by Biojector 2000® into the same thigh at month 2.

Then

TBD mcg CH505w78 gp120 admixed with 10 mcg GLA-SE, to be administered as a 1mL IM injection, into either thigh at month 4.

AND

Placebo for DNA Mosaic-Tre *env* (labeled as Sodium Chloride for injection, 0.9% USP) 1mL IM by Biojector 2000® into the same thigh at month 4.

Then

TBD mcg CH505w100 gp120 admixed with 10 mcg GLA-SE, to be administered as a 1mL IM injection, into either thigh at month 8.

AND

Placebo for DNA Mosaic-Tre *env* (labeled as Sodium Chloride for injection, 0.9% USP) 1mL IM by Biojector 2000® into the same thigh at month 8.

Then

TBD mcg CH505w100 gp120 admixed with 10 mcg GLA-SE, to be administered as a 1mL IM injection, into either thigh at month 12.

AND

Placebo for DNA Mosaic-Tre *env* (labeled as Sodium Chloride for injection, 0.9% USP) 1mL IM by Biojector 2000® into the same thigh at month 12.

Then

TBD CH505w100 gp120 admixed with 10 mcg GLA-SE, to be administered as a 1mL IM injection, into either thigh at month 16.

AND

Placebo for DNA Mosaic-Tre *env* (labeled as Sodium Chloride for injection, 0.9% USP) 1mL IM by Biojector 2000® into the same thigh at month 16.

Group 6

Treatment 6 (T6): TBD mcg CH505TF gp120 admixed with 10 mcg GLA-SE, to be administered as a 1mL IM injection, into either thigh at month 0.

AND

4 mg DNA Mosaic-Tre *env* (labeled as HV13284 + HV13285 + HV13286) 1mL IM by Biojector 2000® into the same thigh at month 0.

Then

TBD mcg CH505w53 gp120 admixed with 10 mcg GLA-SE, to be administered as a 1mL IM injection, into either thigh at month 2.

AND

4 mg DNA Mosaic-Tre *env* (labeled as HV13284 + HV13285 + HV13286) 1mL IM by Biojector 2000® into the same thigh at month 2.

Then

TBD mcg CH505w78 gp120 admixed with 10 mcg GLA-SE, to be administered as a 1mL IM injection, into either thigh at month 4.

AND

4 mg DNA Mosaic-Tre *env* (labeled as HV13284 + HV13285 +HV13286) 1mL IM by Biojector 2000® into the same thigh at month 4.

Then

TBD mcg CH505w100 gp120 admixed with 10 mcg GLA-SE, to be administered as a 1mL IM injection, into either thigh at month 8.

AND

4 mg DNA Mosaic-Tre *env* (labeled as HV13284 + HV13285 + HV13286) 1mL IM by Biojector 2000® into the same thigh at month 8.

Then

TBD mcg CH505w100 gp120 admixed with 10 mcg GLA-SE, to be administered as a 1mL IM injection, into either thigh at month 12.

AND

4 mg DNA Mosaic-Tre *env* (labeled as HV13284 + HV13285 + HV13286) 1mL IM by Biojector 2000® into the same thigh at month 12.

Then

TBD mcg CH505w100 gp120 admixed with 10 mcg GLA-SE, to be administered as a 1mL IM injection, into either thigh at month 16.

AND

4 mg DNA Mosaic-Tre *env* (labeled as HV13284 + HV13285 + HV13286) 1mL IM by Biojector 2000® into the same thigh at month 16.

Group 7

Treatment 7 (T7): TBD CH505TF gp120 admixed with 10 mcg GLA-SE, to be administered as a 1 mL IM injection into either thigh at month 0.

AND

Placebo for DNA Mosaic-Tre *env* (labeled as Sodium Chloride for injection, 0.9% USP) 1mL IM by Biojector 2000® into the same thigh at month 0.

Then

TBD mcg CH505TF gp120 + TBD mcg CH505w53 gp120 admixed with 10 mcg GLA-SE, to be administered as a 1 mL IM injection into either thigh at month 2.

AND

Placebo for DNA-Tre Mosaic *env* (labeled as Sodium Chloride for injection, 0.9% USP) 1mL IM by Biojector 2000® into the same thigh at month 2.

Then

TBD mcg CH505TF gp120 + TBD CH505w53 gp120 + TBD mcg CH505w78 gp120 admixed with 10 mcg GLA-SE, to be administered as a 1 mL IM injection into either thigh at month 4.

AND

Placebo for DNA Mosaic-Tre *env* (labeled as Sodium Chloride for injection, 0.9% USP) 1mL IM by Biojector 2000® into the same thigh at month 4.

Then

TBD mcg CH505w53 gp120 + TBD mcg CH505w78 + TBD mcg CH505w100 gp120 admixed with 10 mcg GLA-SE, to be administered as a 1 mL IM injection into either thigh at month 8.

AND

Placebo for DNA Mosaic-Tre *env* (labeled as Sodium Chloride for injection, 0.9% USP) 1mL IM by Biojector 2000® into the same thigh at month 8.

Then

TBD mcg CH505w78 gp120 + TBD mcg CH505w100 gp120 admixed with 10 mcg GLA-SE, to be administered as a 1 mL IM injection into either thigh at month 12.

AND

Placebo for DNA Mosaic-Tre *env* (labeled as Sodium Chloride for injection, 0.9% USP) 1mL IM by Biojector 2000® into the same thigh at month 12.

Then

TBD mcg CH505w100 gp120 admixed with 10 mcg GLA-SE, to be administered as a 1 mL IM injection into either thigh at month 16.

AND

Placebo for DNA Mosaic-Tre *env* (labeled as Sodium Chloride for injection, 0.9% USP) 1mL IM by Biojector 2000® into the same thigh at month 16.

Group 8

Treatment 8 (T8): TBD mcg CH505TF gp120 admixed with 10 mcg GLA-SE, to be administered as a 1 mL IM injection into either thigh at month 0.

AND

4 mg DNA Mosaic-Tre *env* (labeled as HV13284 + HV13285 + HV13286) 1mL IM by Biojector 2000® into the same thigh at month 0.

Then

TBD mcg CH505TF gp120 + TBD mcg CH505w53 gp120 admixed with 10 mcg GLA-SE, to be administered as a 1 mL IM injection into either thigh at month 2.

AND

4 mg DNA Mosaic-Tre *env* (labeled as HV13284 + HV13285 +HV13286) 1mL IM by Biojector 2000® into the same thigh at month 2.

Then

TBD mcg CH505TF gp120 + TBD mcg CH505w53 gp120 + TBD mcg CH505w78 gp120 admixed with 10 mcg GLA-SE, to be administered as a 1 mL IM injection into either thigh at month 4.

AND

4 mg DNA Mosaic-Tre *env* (labeled as HV13284 + HV13285 + HV13286) 1mL IM by Biojector 2000® into the same thigh at month 4.

Then

TBD mcg CH505w53 gp120 + TBD mcg CH505w78 gp120 + TBD mcg CH505w100 gp120 admixed with 10 mcg GLA-SE, to be administered as a 1 mL IM injection into either thigh at month 8.

AND

4 mg DNA Mosaic-Tre *env* (labeled as HV13284 + HV13285 + HV13286) 1mL IM by Biojector 2000® into the same thigh at month 8.

Then

TBD mcg CH505w78 gp120 + TBD mcg CH505w100 gp120 admixed with 10 mg GLA-SE, to be administered as a 1 mL IM injection into either thigh at month 12.

AND

4 mg DNA Mosaic-Tre *env* (labeled as HV13284 + HV13285 + HV13286) 1mL IM by Biojector 2000® into the same thigh at month 12.

Then

TBD mcg CH505w100 gp120 admixed with 10 mcg GLA-SE, to be administered as a 1 mL IM injection into either thigh at month 16.

AND

4 mg DNA Mosaic-Tre *env* (labeled as HV13284 + HV13285 + HV13286) 1mL IM by Biojector 2000® into the same thigh at month 16.

Group 9

Placebo for CH505TF gp120/GLA-SE (labeled as Sodium Chloride for injection, 0.9% USP) to be administered as a 1mL IM injection to either thigh at month 0.

AND

Placebo for DNA Mosaic-Tre *env* (labeled as Sodium Chloride for injection, 0.9% USP) 1mL by Biojector 2000® into the same thigh at month 0.

Then

Placebo for CH505TF or CH505w53 gp120/GLA-SE (labeled as Sodium Chloride for injection, 0.9% USP) to be administered as a 1mL IM injection to either thigh at month 2.

AND

Placebo for DNA Mosaic-Tre *env* (labeled as Sodium Chloride for injection, 0.9% USP) 1mL by Biojector 2000® into the same thigh at month 2.

Then

Sodium Chloride for injection, 0.9% USP for CH505TF or CH505w78 or CH505w100 or CH505w53, 78, or CH505w53,78,100 or CH505w78,100, to be administered as a 1mL IM injection to either thigh at months 4, 8 and 12.

AND

Placebo for DNA Mosaic-Tre *env* (labeled as Sodium Chloride for injection, 0.9% USP) 1mL by Biojector 2000® into the same thigh at months 4, 8 and 12.

Then

Placebo for CH505w100 gp120/GLA-SE (labeled as Sodium Chloride for injection, 0.9% USP) to be administered as a 1mL IM injection to either thigh at month 16.

AND

Placebo for DNA Mosaic-Tre *env* (labeled as Sodium Chloride for injection, 0.9% USP) 1mL by Biojector 2000® into the same thigh at month 16.

8.2 Study product formulation

8.2.1 CH505TF gp120

The CH505TF gp120 vaccine will be provided as a 0.8 mg/mL vial. Each sterile, single use vial contains 0.75 mL of product. Product should be stored at ≤ -65°C (+/-10°C). The study products are described in further detail within the Investigator's Brochure (IB).

8.2.2 CH505w53 gp120

More information will be provided prior to the implementation of Part B.

8.2.3 CH505w78 gp120

More information will be provided prior to the implementation of Part B.

8.2.4 CH505w100 gp120

More information will be provided prior to the implementation of Part B.

8.2.5 DNA Mosaic-Tre *env* (labeled as HV13284 + HV13285 + HV13286)

The DNA Mosaic-Tre *env* vaccine will be provided as a 4 mg/mL (3.9 mg/mL ± 0.2 mg/mL) vial. The product is a clear, colorless solution. Each sterile, single-use vial contains 1.33 mL of product. The product must be stored at -20°C +/- 5°C. The study products are described in further detail in the IB.

8.2.6 GLA-SE (glucopyranosyl lipid adjuvant-stable emulsion)

The GLA-SE adjuvant will be provided as vials containing 20 μg/mL GLA in a 4% oil-in-water emulsion. Each sterile, single use vial contains 0.6 mL of product. Product appears as a milky-white liquid. GLA-SE must be stored at 2-8°C and must not be frozen. The study product is described in further detail within the IB.

8.2.7 Placebo for DNA Mosaic-Tre *env*

Sodium Chloride for injection, 0.9% USP will be used as the placebo. It must be stored as recommended by the manufacturer.

8.2.8 Placebo for CH505TF, w53, w78, w100 gp120

Sodium Chloride for injection, 0.9% USP will be used as the placebo. It must be stored as recommended by the manufacturer.

8.3 Preparation of study products

Part A:

8.3.1 Group 1: 20 mcg CH505TF gp120 + 10 mcg GLA-SE

One vial of CH505TF gp120 0.8 mg/mL and one vial of GLA-SE (20 mcg/mL) will be needed to prepare the dose.

Prior to admixture, the pharmacist will remove a vial of CH505TF gp120 from the freezer and allow to thaw completely at room temperature. Once thawed completely, invert the vial 10 times to ensure a homogeneous product. Remove a vial of GLA-SE from the refrigerator and allow to equilibrate to room temperature.

Using aseptic technique, the pharmacist will add 0.6 mL of CH505TF gp120 and 1.8 mL of Sodium Chloride for injection, 0.9% USP to an empty vial. Mix the contents of this vial thoroughly using a vortexer machine at high speed for 3 seconds. The final concentration of CH505TF gp120 will be 200 mcg/mL.

Next, using aseptic technique, withdraw 0.5 mL from the diluted 200 mcg/mL vial of CH505TF gp120 and place into an empty vial. Add 2 mL of Sodium Chloride for injection, 0.9% USP to this vial for further dilution and mix the contents of this vial thoroughly using a vortexer machine at high speed for 3 seconds. The final concentration of CH505TF gp120 will be 40 mcg/mL.

Next, using aseptic technique, withdraw 0.6 mL of the diluted 40 mcg/mL CH505TF gp120 admixture and add to a vial of 20 mcg/mL GLA-SE and mix thoroughly using a vortexer machine at high speed for 3 seconds, yielding a concentration of 20 mcg/mL CH505TF gp120 and 10 mcg/mL GLA-SE.

Finally, using aseptic technique, withdraw 1 mL from the vial containing 20 mcg/mL CH505TF gp120 and 10 mcg/mL GLA-SE, using a 3 or 5 mL syringe. Remove the needle and cap syringe.

The final syringe for administration must be covered with an overlay and then labeled as "CH505TF/GLA-SE or placebo". The syringe must also be labeled for IM administration into thigh, with an expiration date and time of 8 hours following the last mixing procedure.

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

8.3.2 Group 2: 100 mcg CH505TF gp120 + 10 mcg GLA-SE

One vial of CH505TF gp120 0.8 mg/mL and one vial of GLA-SE (20 mcg/mL) will be needed to prepare the dose.

Prior to admixture, the pharmacist will remove a vial of CH505TF gp120 from the freezer and allow to thaw completely at room temperature. Once thawed completely, invert the vial 10 times to ensure a homogeneous product. Remove a vial of GLA-SE from the refrigerator and allow to equilibrate to room temperature.

Using aseptic technique, the pharmacist will add 0.6 mL of CH505TF gp120 and 1.8 mL of Sodium Chloride for injection, 0.9% USP to an empty vial. Mix the contents of this vial thoroughly using a vortexer machine at high speed for 3 seconds. The final concentration of CH505TF gp120 is 200 mcg/mL.

Next, using aseptic technique, withdraw 0.6 mL of the diluted 200 mg/mL CH505TF gp120 admixture and add to a vial of GLA-SE 20 mcg/mL and mix thoroughly using a vortexer machine at high speed for 3 seconds, yielding a concentration of 100 mcg/mL CH505TF gp120 and 10 mcg/mL GLA-SE.

Finally, using aseptic technique, withdraw 1 mL from the vial containing 100 mcg/mL CH505TF gp120 and 10 mcg/mL GLA-SE, using a 3 or 5 mL syringe. Remove the needle and cap syringe.

The final syringe for administration must be covered with an overlay and then labeled as "CH505TF/GLA-SE or placebo". The syringe must also be labeled for IM administration into thigh, with an expiration date and time of 8 hours following the last mixing procedure.

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

8.3.3 Group 3: 400 mcg CH505TF gp120 + 10 mcg GLA-SE

One vial of CH505TF gp120 0.8 mg/mL and one vial of GLA-SE (20 mcg/mL) will be needed to prepare the dose.

Prior to admixture, the pharmacist will remove a vial of CH505TF gp120 from the freezer and allow to thaw completely at room temperature. Once thawed completely, invert the vial 10 times to ensure a homogeneous product. Remove a vial of GLA-SE from the refrigerator and allow to equilibrate to room temperature.

Using aseptic technique, the pharmacist will add 0.6 mL of CH505TF gp120 to a vial of GLA-SE and mix thoroughly using a vortexer machine at high speed for 3 seconds. The final concentration of the admixture is 400 mcg/mL of CH505TF gp120 and 10 mcg/mL of GLA-SE.

Finally, using aseptic technique, withdraw 1 mL from the vial containing 400 mcg/mL CH505TF gp120 and 10 mcg/mL GLA-SE, using a 3 or 5 mL syringe. Remove the needle and cap syringe.

The final syringe for administration must be covered with an overlay and then labeled as "CH505TF/GLA-SE or placebo". The syringe must also be labeled for IM administration into thigh, with an expiration date and time of 8 hours following the last mixing procedure.

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

8.3.4 Group 4: Placebo for CH505TF gp120/GLA-SE

The pharmacist using aseptic technique will withdraw 1 mL of Sodium Chloride for Injection, 0.9% USP into a 3 or 5 mL syringe, remove the needle and cap the syringe.

The final syringe for administration must be covered with an overlay and then labeled as "CH505TF/GLA-SE or placebo". The syringe must also be labeled for IM administration into thigh, with an expiration date and time of 8 hours following the last mixing procedure.

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

Part B:

More information will be provided prior to the implementation of part B regarding CH505TF, w53, 78 and 100 gp120.

8.3.5 Placebo for CH505 Proteins- Groups 5-9

The pharmacist using aseptic technique will withdraw 1 mL of Sodium Chloride for Injection, 0.9% USP into a 3 or 5 mL syringe, remove the needle and cap the syringe.

The final syringe for administration must be covered with an overlay and then labeled as "CH505 Protein/GLA-SE or placebo". The syringe must also be labeled for IM administration into thigh, with an expiration date and time of 8 hours following the last mixing procedure.

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

8.3.6 Placebo for DNA Mosaic-Tre *env*

The pharmacist using aseptic technique will withdraw 1 mL of Sodium Chloride for Injection, 0.9% USP into the Biojector 2000® syringe and cap the syringe.

The syringe should be labeled as "DNA Mosaic env 4 mg or Placebo". The syringe must also be labeled for IM administration into thigh. The study product should be administered as soon as possible after preparation.

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

8.3.7 DNA Mosaic-Tre *env* (labeled as HV13284 + HV13285+ HV13286)

One vial of HV13284 + HV13285 + HV13286, 4 mg/mL will be needed to prepare the dose. Prior to dispensing, the pharmacist will remove the study product from the freezer and allow it to thaw at room temperature.

Once thawed, the pharmacist will gently swirl the contents of the vial and then, using aseptic technique, will withdraw 1 mL from the vial into the Biojector 2000® syringe and cap the syringe.

The syringe should be labeled as "DNA Mosaic env 4 mg or Placebo". The syringe must also be labeled for IM administration into thigh, with a preparation date and time. The study product should be administered as soon as possible after preparation.

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

8.4 Administration

All injections should be administered in the thigh of either leg.

At sites where registered pharmacists are legally authorized to administer drug, the HVTN CRS may choose to have the HVTN CRS pharmacist administer the injections.

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.

8.4.1 DNA Mosaic-Tre *env* vaccine or placebo

DNA vaccine or placebo will be administered IM as a 1 mL injection, using the Biojector 2000® at Months 0, 2, 4, 8, 12 and 16, to participants randomized into groups 5, 6, 7, 8 and 9.

The Biojector 2000® will be used as directed by Bioject Inc. Neither the material being injected nor injection site skin preparation require deviation from standard procedures. The injection site is disinfected and the area allowed to dry completely. The skin around the injection site is held firmly while the syringe is placed against the injection site at a 90° angle. The actuator is pressed and the material is released into the muscle and held firmly for 3 seconds. After the injection, the site is covered with a sterile covering and pressure applied with 3 fingers for 1 minute. Biojector 2000® utilizes sterile, single-use syringes for variable dose, up to 1mL, medication administration. The study product is delivered under pressure by a compressed CO2 gas cartridge that is stored inside the Biojector 2000®. When the Biojector 2000®'s actuator is depressed, CO2 is released, causing the plunger to push the study product out of the sterile syringe through the skin and into the underlying tissue. The study product is expelled through a micro-orifice at high velocity in a fraction of a second to pierce the skin. The CO2 does not come in contact with the injectate and the syringe design prevents any back splatter or contamination of the device by tissue from the subject.

8.4.2 CH505 gp120 proteins or placebo

Part A:

CH505TF gp120 protein admixed with GLA-SE adjuvant or placebo are to be given as a 1 mL IM injection, using a 3 mL or 5 mL syringe, at months 0, 2, 4, 8 and 12.

Part B:

CH505 gp120 proteins admixed with GLA-SE adjuvant or placebo are to be given as a 1 mL IM injection, using a 3 mL or 5 mL syringe, at months 0, 2, 4, 8, 12, and 16.

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

DNA and EnvSeq-1 vaccines will be provided by DAIDS. GLA-SE adjuvant will be provided by Infectious Disease Research Institute (IDRI). Placebo for DNA and Placebo for EnvSeq-1 proteins will not be provided through the protocol and must be obtained by the site.

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

8.6 Pharmacy records

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

8.7 Final disposition of study products

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

9 Clinical procedures

The schedule of clinical procedures for Part A is shown in Appendix K. The schedule of clinical procedures for Part B is shown in Appendix L.

9.1 Informed consent

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

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

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

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

9.1.1 Screening consent form

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

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

9.1.2 Protocol-specific consent forms

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

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

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

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

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

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

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

9.1.3 Assessment of Understanding

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

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

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

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

9.2 Pre-enrollment procedures for Parts A and B

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);
- HIV infection assessment including pretest counseling (see Section 9.7);
- Laboratory tests as defined in the inclusion and exclusion criteria, including:
  - Screening HIV test,
  - HBsAg,
  - Anti-HCV antibodies,
  - Syphilis test,
  - CBC with differential and platelets,
  - Chemistry panel (ALT, AST, alkaline phosphatase, and creatinine),
  - Urine dipstick (as described in Section 9.9), and
  - Urine or serum pregnancy test (participants who were born female)
- Administration of behavioral risk assessment questionnaire;
- Obtaining of volunteer demographics in compliance with the NIH Policy on Reporting Race and Ethnicity Data: Subjects in Clinical Research, Aug. 8, 2001 (available at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-01-053.html);

- Counseling on HIV testing and risk reduction, performed in compliance with the US Centers for Disease Control and Prevention (CDC)'s current guidelines or other local guidelines for HIV counseling, testing, and referral as described in Section 9.7; and

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

9.2.1 Use of screening results from another HVTN study

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

9.3 Enrollment and vaccination visits

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

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

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

- Assessment of baseline reactogenicity parameters;

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

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

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

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

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

Immediately following vaccination, the participant remains in the clinic for observation. An initial reactogenicity assessment is made at a target of 30 minutes after injection, with an acceptable range of 25-60 minutes. Before leaving the clinic, the participant is given the postvaccination memory tool and is instructed on how to complete it. The site will make arrangements to be in contact with the participant during the reactogenicity period (as described in Section 9.10).

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 9.7);
- Pregnancy prevention assessment (as described in Section 9.2 and 9.8); and
- Assessment of new or unresolved social impacts (site staff will ask participant about the status of any unresolved social impacts and if s/he has experienced any new social impacts as a result of the trial participation).

Additional procedures will be performed at scheduled visits as specified in Appendix K and Appendix L:

- 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;
- Confirm that participants received HIV test results from previous visit. If not, provide test results and post-test counseling as appropriate;
- Specimen collection (should be completed prior to vaccination); and
- **In Part B**, optional stool specimen collection (should be completed prior to vaccination) (see Appendix L). Collect dietary, antibiotic use, and gastrointestinal symptom information from participants providing stool specimen.

9.4 Follow-up visits

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

- Risk reduction counseling (as described in Section 9.7);
- Pregnancy prevention assessment (as described in Section 9.2 and 9.8); and
- Assessment of new or unresolved social impacts (site staff will ask participant about the status of any unresolved social impacts and if s/he has experienced any new social impacts as a result of the trial participation);

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

- Assessment of new or unresolved AEs/intercurrent illnesses.

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

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

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

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

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

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

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

- Specimen collection;

- **In Part B,** specimen collection includes optional stool sample (see Appendix L). Collect dietary, antibiotic use, and gastrointestinal symptom information from participants providing stool specimen.

- Clinical laboratory tests including:
  - CBC with differential,
  - Chemistry panel (see Section 9.2), and
  - Urine dipstick (urinalysis if appropriate; see Section 9.9); 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 Contact at 6 months after last scheduled visit

CRS staff will contact study participants at 6 months after the last scheduled visit to collect the information listed below. This contact may be accomplished by phone, text message, or email. If indicated, the participant may be asked to come in for a clinical assessment which may also include referrals for AESI assessment. AESIs are described further in Appendix M.

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

9.5.1 Interim contacts

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

9.6 Stool sample collection in Part B

Three stool samples will be collected from the study participants in Part B who agree to this procedure: 1 prior to enrollment (before the injection of the vaccine), 1 at the Month 8.5 timepoint, and 1 at the Month 16.5 timepoint. These samples will be collected using swabs, either via rectal swabs or by taking swabs from stool. If bulk stool is provided, a portion may be kept for further analysis.

9.7 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.

9.7.1 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 K and Appendix L. 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 (Appendix K and Appendix L). The Laboratory Program (or approved diagnostic laboratory) will follow the HVTN HIV testing algorithm (as described in the HVTN Laboratory Manual of Operations), which is able to distinguish vaccine-induced antibody responses from actual HIV infections.

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

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

9.7.2 VISP registry

Experimental HIV vaccines may induce antibody production to HIV antigens, producing reactive results on commercially available HIV test kits. This is called "vaccine-induced seropositivity" (VISP) (see Section 9.7.1). 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.

9.8 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.

9.9 Urinalysis

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

If the screening dipstick is transiently abnormal due to menses or infection, document this issue in the participant's source documentation. For infection, provide appropriate

treatment and/or referral. Following resolution, repeat the dipstick and, if within the eligibility limits specified in the protocol, the participant may be enrolled.

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

9.10 Assessments of reactogenicity

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

The reactogenicity assessment period is 7 full days following each vaccination per the assessment schedule shown in Table 9-1. Participants are instructed to record symptoms using a postvaccination memory tool. Contact between the participant and the site staff should take place at least once within 72 hours/3 days postvaccination. 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 lesions, and lymph nodes. Events not listed on a CRF, or with an onset after the reactogenicity assessment period (day of vaccination and 7 full days after), or those meeting SAE/adverse events requiring expedited reporting to DAIDS criteria, are recorded on an adverse experience log form.

Table 9-1 Schedule of reactogenicity assessments

Day	Time	Performed by
0[a]	Baseline: before vaccination	HVTN CRS staff
	Early: 25-60 minutes after vaccination	HVTN CRS staff
	Between early assessment and 11:59pm day 0	HVTN CRS staff or participant
1	Between 12:00am and 11:59pm day 1	HVTN CRS staff or participant
2	Between 12:00am and 11:59pm day 2	HVTN CRS staff or participant
3	Between 12:00am and 11:59pm day 3	HVTN CRS staff or participant
4	Between 12:00am and 11:59pm day 4	HVTN CRS staff or participant
5	Between 12:00am and 11:59pm day 5	HVTN CRS staff or participant
6	Between 12:00am and 11:59pm day 6	HVTN CRS staff or participant
7[b]	Between 12:00am and 11:59pm day 7	HVTN CRS staff or participant

[a] Day of vaccination

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

9.10.1 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.

9.10.2 Assessment of injection site

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

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

9.10.3 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, inguinal nodes on the same side of the body for injections given in the vastus lateralis). 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).

9.11 Visit windows and missed visits

Visit windows are defined in HVTN 115 Study Specific Procedures. For a visit not performed within the window period, a Missed Visit form is completed. If the missed visit is one that required safety assessments or local safety labs, HVTN CRS staff should attempt to bring the participant in for an interim visit as soon as possible.

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

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

9.12 Early termination visit

In the event of early participant termination, site staff should consider if the following assessments are appropriate: a final physical examination, clinical laboratory tests (including urine dipstick, CBC with differential, 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. Pregnancies and pregnancy outcomes will be reported.

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 I and Appendix J. 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 I and Appendix J. 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

Part A

The primary immunogenicity timepoint in Part A is at visit 8 (day 126) (ie, 2 weeks after the third vaccination visit). Endpoint assays for humoral and cellular responses are performed on participants at the primary immunogenicity timepoint 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 I.

Part B

The primary immunogenicity timepoints in in Part B are at visit 10 (day 238) (ie, 2 weeks after the fourth vaccination visit) and visit 15 (day 500) (2 weeks after the sixth vaccination visit). Endpoint assays for humoral and cellular responses are performed on participants at the primary immunogenicity timepoints and may be performed at baseline. Depending on the number of responders observed, assays for humoral and cellular responses may be performed on participants at other timepoints; the schedule is shown in Appendix J.

10.4 Endpoint assays: cellular

10.4.1 Flow cytometry

Flow cytometry will be used to examine vaccine-specific CD4+ and CD8+ T-cell responses following stimulation of PBMCs with synthetic HIV peptides that span the proteins encoded by the vaccine construct. ICS parameters 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. Additional cell surface markers, cytokines, or functional markers may also be analyzed.

Flow cytometry will also be used to phenotypically characterize peripheral follicular helper T cells (pTfh). Identification of pTfh will be based on expression of CXCR5 and PD-1 on CD4+ T cells, and may include additional markers.

10.5 Endpoint assays: humoral

10.5.1 Binding antibody assays

HIV-1 Env binding IgG and IgA antibodies to the vaccine immunogens (EnvSeq-1 gp120 TF, 53, 78, 100) will be assessed on plasma/serum samples from study participants taken at the primary immunogenicity timepoints and baseline. Conformational epitopes (ie CD4BS specificities using differential binding to EnvSeq-1 gp120 compared to CH505 IΔ371 protein and sCD4 blocking) will be determined. Specimens from other timepoints as well as other HIV antigens (ie other conformational epitopes and linear epitopes and profiling the antibody subclasses (IgG1, IgG2, IgG3, IgG4) 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. Assays will be performed with the 4 EnvSeq-1 vaccine isolates (CH0505.TF, CH0505.w53.e16, CH0505.w78.33, CH0505.w100.B6) and a tier 1 variant of the TF strain, CH0505.wk4.3. Additional assays will test neutralization of heterologous tier 1 and tier 2 isolates [73].

10.5.3 B-cell lineage and repertoire analysis

Single memory B cells or plasmablasts will be single cell sorted. For plasmablasts, VH and VL genes will be amplified and cloned into an IgG1 backbone for expression and determination of binding and neutralization. For memory B cells, Env reactive memory B cells will be sorted using CH505 Env hooks, and then single cell sorted into individual wells of 96 well plates. VH and VL genes will be amplified and cloned into an IgG1 backbone and tested for Env binding and HIV neutralization. In some cases, memory B cells will be cultured in limiting dilution cultures and screened for binding and neutralization before PCR and VH and VL rescue. Finally, next generation sequencing

for all VH and VL families will be performed on memory B cells or plasmablasts for VH and VL genes.

10.6 Lab assay algorithm

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

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 Fc-Mediated Antibody Effector Function

Additional assays to measure the induction of antibody Fc-mediated antibody functions (ie antibody dependent cellular cytotoxicity, phagocytosis, FcR binding, glycan modifications, recognition of virus particles and infected cells, etc.) may be performed on select samples and timepoints based on the primary analysis.

10.7.2 Microbiome analysis

Swabs of stool and/or bulk stool samples will be shipped to a central laboratory. Stool samples will be subjected to culture to obtain bacteria samples that are processed to yield a homogeneous stool flora lysate of each culture type. These lysates are then available to use to assess antibody binding by Western blot, ELISA, or other methods. In addition, stool samples will be subjected to chaotropic lysis and bead beating to liberate bacterial DNA, followed by DNA extraction. Purified DNA will be subjected to quality control qPCR assays to measure total bacterial load and assess for PCR inhibitors. Broad-range 16S rRNA PCR will be performed. Bacterial community composition will be assessed using taxonomic assignment of reads to a custom reference set of 16S rRNA gene sequences from the human gut. Some samples may be analyzed by shotgun metagenomics to create protein expression libraries to analyze the effect of stool composition on vaccine response. Finally, bulk stool samples will have antibodies extracted for analysis of the presence of mucosal antibodies elicited by the vaccine.

10.8 Other use of stored specimens

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

Other use of specimens is defined as studies not covered by the protocol or the informed consent form for the main study (Appendix A).

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

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

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

10.9 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 115 PSRT

The HVTN 115 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 115 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 115 PSRT meetings.

11.1.2 HVTN SMB

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

The SMB reviews safety data, unblinded as to treatment arm, approximately every 4 months. The reviews consist of evaluation of cumulative reactogenicity events, AE, laboratory safety data, and individual reports of adverse events requiring expedited reporting to DAIDS. To increase the sensitivity for detecting potential safety problems, the SMB will review safety data aggregated across multiple protocols that use the same or similar vaccine candidates. The SMB conducts additional special reviews at the request of the HVTN 115 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 115 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 115 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 115 PSRT.

11.2 Safety reporting

11.2.1 Submission of safety forms to SDMC

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

11.2.2 AE reporting

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

- Weight loss is required to be reported as an AE only if it is considered to be deleterious to the participant's health (see *HVTN 115 Study Specific Procedures*);
- Injection Site Erythema or Redness and Injection Site Induration or Swelling will not consider interference with usual social and functional activities such that:
  - Grade 1 is: 2.5 to < 5 cm in diameter OR 6.25 to < 25 cm^2 surface area;
  - Grade 2 is: ≥ 5 to < 10 cm in diameter OR ≥ 25 to < 100 cm^2 surface area;
  - Grade 3 is: ≥ 10 cm in diameter OR ≥ 100 cm^2 surface area OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage;
  - Grade 4 is: Potentially life-threatening consequences (eg, abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue);

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

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

All AEs are reported to the SDMC on the appropriate CRF. Clinic staff should evaluate every AE to determine if (1) the AE meets the requirements for expedited reporting to DAIDS (Section 11.2.3), (2) if the AE meets the criteria for a safety pause/prompt AE review (Section 11.4) and (3) if the AE is a potential immune-mediated disease that may be listed as an AE of special interest (AESI). A sample list of AESI is provided in Appendix M.

Sites are expected to notify the CSS of any serious safety concern requiring their attention (see Table 11-1). Telephone numbers and email addresses are listed in the Key Resource Guide of the HVTN 115 Study Specific Procedures. Telephone numbers and email addresses are found on the protocol home page on the HVTN Members' site (https://members.hvtn.org/protocols/hvtn115). 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 Experience Reporting System (DAERS) must be used for expedited AE reporting to DAIDS. In the event of system outages or technical difficulties, expedited AE reports may be submitted via the DAIDS EAE Form. For

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

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

- CH505TF HIV gp120 with GLA-SE or placebo
- CH505w53 HIV gp120 with GLA-SE or placebo
- CH505w78 HIV gp120 with GLA-SE or placebo
- CH505w100 HIV gp120 with GLA-SE or placebo
- DNA Mosaic-Tre *env* or placebo

While the participant is in the main 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 routinely be unblinded to study treatment assignment when there is an assessment of relatedness of the SAE with the study product(s); and the safety report will be sent to the FDA based on the blinded attribution assessment.

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

11.3 Safety reviews

11.3.1 Initial safety evaluation

Enrollment across all participating HVTN CRSs will be restricted to a maximum of 1 participant per day and restricted to US sites until 10 participants have been enrolled. All

groups in Part A will enroll simultaneously. The HVTN 115 PSRT will review the cumulative safety data including at minimum local and systemic reactogenicity data reported for the first 72 hours postvaccination on each of these 10 participants, and will determine whether it is safe to proceed with full enrollment in Part A.

11.3.2 Safety evaluation for moving from Part A to Part B

In addition to monitoring participant safety throughout the study period, the HVTN 115 PSRT will review all cumulative safety data available from groups 1 through 4 up to and including the 2-week visit after the third vaccination. Based on the assessment of this safety data, the HVTN 115 PSRT will make a decision regarding the appropriateness of moving to Part B from a safety perspective. The HVTN SMB may perform an additional unblinded review of this safety data to make the final determination based on safety for proceeding to part B.

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 115 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 115 PSRT, participant safety may be threatened. Criteria for an individual participant's departure from the schedule of vaccinations are listed in Section 7.3.

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

Event and relationship to study products	Severity	HVTN CRS action[a]	HVTN Core action
SAE, related	Grade 5 or Grade 4	Phone immediately, email and submit forms immediately	Immediate pause
SAE, not related	Grade 5	Phone immediately, email and submit forms immediately	Immediate HVTN 115 PSRT notification
SAE, related	Grade 3	Email and submit forms immediately	Prompt HVTN 115 PSRT AE review to consider pause
AE[b], related	Grade 4 or 3	Email and submit forms immediately	Prompt HVTN 115 PSRT AE review to consider pause

[a] Phone numbers and email addresses are found on the Protocol home page on the HVTN Members' site (https://members.hvtn.org/protocols/hvtn115).

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

For all safety pauses, HVTN Core notifies the HVTN 115 PSRT, HVTN Regulatory Affairs, DAIDS Pharmaceutical Affairs Branch (PAB), DAIDS Regulatory Affairs Branch (RAB), DAIDS Safety and Pharmacovigilance Team (SPT), and participating HVTN CRSs. When an immediate safety pause is triggered, HVTN Core notifies the SMB.

Once a trial is paused, the HVTN 115 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 115 PSRT assessment, DAIDS RAB notifies the FDA as needed.

If an immediate HVTN 115 PSRT notification or prompt HVTN 115 PSRT AE review is triggered, HVTN Core notifies the HVTN 115 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 115 PSRT AE review cannot be completed within 72 hours of notification (excluding weekends and US federal holidays), an automatic safety pause occurs.

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

In addition, all other AEs are reviewed routinely by the HVTN 115 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 115 PSRT AE review criteria.

11.5.2 Weekly review

During the injection phase of the trial, the HVTN 115 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 115 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 115 PSRT, a pertinent national regulatory authority, NIH, FDA, Office for Human Research

Protections (OHRP), or vaccine developer(s). In addition, the conduct of this study at an individual HVTN CRS may be terminated by the determination of the IRB/EC and any applicable RE.

12 Protocol conduct

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

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

Any policies or procedures that vary from DAIDS and HVTN standards or require additional instructions (eg, instructions for randomization specific to this study) will be described in the HVTN 115 *Study Specific Procedures.*

12.1 Social impacts

Participants in this study risk experiencing discrimination or other personal problems, resulting from the study participation itself or from the development of VISP. The HVTN

CRS is obliged to provide advocacy for and assistance to participants regarding these negative social impacts associated with the vaccine trial. If HVTN CRS staff have questions regarding ways to assist a participant dealing with a social impact, a designated NIAID or HVTN Core representative can be contacted.

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

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

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

Because this study is evaluating products containing recombinant or synthetic DNA, it must comply with regulations set forth in the NIH's *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (April 2016).* Information about the study must be submitted to site Institutional Biosafety Committees (IBCs). Investigators at each site are responsible for obtaining IBC approval per NIH guideline *section IV-B7-a-(1).* IBC review and approval must be documented by the investigator and submitted as part of DAIDS initial protocol registration for this trial before participants are enrolled at the site. If this protocol is amended, investigators should follow the requirements of their IBC.

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 115 are described below.

Protocol history and modifications

Date: May 2, 2017

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 https://www.niaid.nih.gov/research/daids-clinical-research-policies-standard-procedures
- Division of AIDS Protocol Registration Manual. Available at https://www.niaid.nih.gov/sites/default/files/documents/prmanual.pdf
- Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events. Version 2.0, November 2014. Available at http://rsc.tech-res.com/docs/default-source/safety/daids_ae_grading_table_v2_nov2014.pdf?sfvrsn=8
- The Manual for Expedited Reporting of Adverse Events to DAIDS. Version 2.0, January 2010. Available at http://rsc.tech-res.com/clinical-research-sites/safety-reporting/manual
- HVTN Certificate of Confidentiality. Accessible through the HVTN website.
- HVTN 115 Special Instructions. Accessible through the HVTN protocol-specific website.
- HVTN 115 Study Specific Procedures. Accessible through the HVTN protocol-specific website.
- HVTN Laboratory Manual of Operations. Accessible through the HVTN website.
- HVTN Manual of Operations. Accessible through the HVTN website.
- Dangerous Goods Regulations (updated annually), International Air Transport Association. Available for purchase at http://www.iata.org/publications/dgr/Pagesindex.aspx
- Lab assay algorithm
- HVTN algorithm for diagnosis of HIV infections. Part of the HVTN Laboratory Manual of Operations (see above).

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

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

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

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

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

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

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

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

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

15 Acronyms and abbreviations

Ab	antibody
Ad	adenovirus
AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC-MB	area-under-the-magnitude-breadth curve
β-HCG	beta human chorionic gonadotropin
BMI	body mass index
bnAb	broadly neutralizing antibody
bs	binding site
CAB	Community Advisory Board
CBC	complete blood count
CDC	US Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CHAVI-ID	Scripps Center for HIV/AIDS Vaccine Immunology & Immunogen Discovery
CIOMS	Council for International Organizations of Medical Sciences
CI	confidence intervals
CRF	case report form
CRPMC	NIAID Clinical Research Products Management Center
CRS*	clinical research site
DAERS	DAIDS Adverse Experience Reporting System
DAIDS	Division of AIDS (US NIH)
DHHS	US Department of Health and Human Services
EAE	adverse events requiring expedited reporting to DAIDS
EC	Ethics Committee
EIA	enzyme immunoassay
ELISA	enzyme-linked immunosorbent assay
FDA	US Food and Drug Administration
FHCRC	Fred Hutchinson Cancer Research Center
GCP	Good Clinical Practice
GEE	generalized estimating equation
HCV	hepatitis C virus
HVTN	HIV Vaccine Trials Network
IB	Investigator's Brochure
IBC	Institutional Biosafety Committee
ICH	International Conference on Harmonisation
ICS	intracellular cytokine staining
IFN-γ	interferon gamma
IND	Investigational New Drug
IRB	Institutional Review Board

IUD	intrauterine device
MAR	missing at random
MCAR	missing completely at random
MMR	measles, mumps, and rubella
nAb	neutralizing antibody
NHP	nonhuman primate
NIAID	National Institute of Allergy and Infectious Diseases (US NIH)
NICD	National Institute for Communicable Diseases (Johannesburg, South Africa)
NIH	US National Institutes of Health
OHRP	US Office for Human Research Protections
OPV	oral polio vaccine
PAB	DAIDS Pharmaceutical Affairs Branch
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PI	Principal Investigator
PSRT	Protocol Safety Review Team
RAB	DAIDS Regulatory Affairs Branch
RE	regulatory entity
RSC	DAIDS Regulatory Support Center
SAE	serious adverse event
SCHARP	Statistical Center for HIV/AIDS Research and Prevention
SDMC	statistical and data management center
SIV	simian immunodeficiency virus
SMB	Safety Monitoring Board
SPT	DAIDS Safety and Pharmacovigilance Team
TB	tuberculosis
TF	transmitter/founder
Tfh	T follicular helper T cells
UCA	unmutated common ancestor
UW-VSL	University of Washington Virology Specialty Laboratory
VISP	Vaccine induced seropositivity
VRC	Vaccine Research Center (NIAID)

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

16 Literature cited

1. UNAIDS. Ethical considerations in biomedical HIV prevention trials. **2007**.

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Appendix A Sample informed consent form for Part A

Title: A phase 1 clinical trial to evaluate the safety and immunogenicity of EnvSeq-1 Envs adjuvanted with GLA-SE, administered alone or with DNA Mosaic-Tre env, in healthy, HIV-uninfected adult participants

HVTN protocol number: HVTN 115

Site: [Insert site name]

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

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

About the study

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

Up to 132 people will take part in this study at multiple sites in the U.S. 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 divided into 2 parts, part A and part B. Forty-two (42) people will take part in part A of this study. After we see the results from part A, we will decide what amount of vaccine will be used for part B of the study. If we decide to do part B, 90 more people will join. Part B will look at different combinations of vaccines.

You are being invited to join part A of the study.

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

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

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

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

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

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

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

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

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

4. These study vaccines are experimental.

The study vaccine in part A is called CH505TF gp120. The study vaccines in part B are CH505TF gp120, CH505w53 gp120, CH505w78 gp120, CH505w100 gp120, and DNA Mosaic-Tre *env*. From here on, we will call them the study vaccines. They are experimental HIV vaccines. That means we do not know whether the vaccines will be safe to use in people, or whether they will work to prevent HIV infection. These vaccines are used only in research studies.

The vaccines were developed by the Division of AIDS (DAIDS) at the National Institutes of Health (NIH). The EnvSeq-1 vaccines have man-made pieces of protein that look like part of the protein found in HIV. Your body's immune system might learn to recognize these proteins and prepare itself to fight HIV. This is called an immune response.

The CH505 vaccines are mixed with an adjuvant. An adjuvant is a substance that should help the immune system respond better. The adjuvant in this study is called GLA-SE. GLA-SE was made by the Infectious Disease Research Institute (IDRI).

In part A of the study, we are looking to see what amount of vaccine will create the best immune response.

The study vaccine in part A has not been given to people before. The vaccine has been tested in animals and appears safe. Even if something looks like it is safe or works in animals, it may not be true for people.

General risks of vaccines:

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

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

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

Risks of the study vaccines:

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

The EnvSeq-1 vaccine has not been given to people before, but similar vaccines have been used in other studies. The most common complaints were pain or tenderness at the injection site.

The adjuvant, GLA-SE, has been tested in humans with vaccines for other diseases. The most common complaints were pain and tenderness at the injection site and feeling tired.

Joining the study

5. 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. You cannot be in this study while you are in another study where you get a study product. Also during the study, you should not donate blood or tissue.

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

Site: Remove item 6 if you use a separate screening consent that covers these procedures.

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

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

- Checking your weight, temperature, and blood pressure
- Looking in your mouth and throat
- Listening to your heart and lungs
- Feeling your abdomen (stomach and liver)

We will also do blood and urine tests. These tests tell us about some aspects of your health, such as how healthy your kidneys, liver, and immune system are. We will also test you for Hepatitis 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. The screening results may show you are not eligible to join the study, even if you want to.

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

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

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

8. **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 to join this study.**

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

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

Being in the study

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

9. **You will come to the clinic for scheduled visits about [#] times over 18 months.**

   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. We may need to ask you for your Social Security number for tax reasons.

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

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

Not everyone in this study will get the study vaccines. 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 vaccines. In this study, the placebo is sterile salt water.

You have a 1-in-7 chance of getting the placebo, which means you have a 6-in-7 chance of receiving the study vaccine.

Site: Modify the randomization metaphor in the next sentence as appropriate to your local culture. Whether you get the study vaccines or the placebo is completely random, like flipping a coin.

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

You will have to wait until everyone completes their final study visits to find out whether you got the study vaccines 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 1 of 4 groups. You will not know which group you will be assigned to. You will get 1 injection with needle and syringe into the upper thigh at each of 5 separate visits.

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

			Injection Schedule				
	Group	Vaccine dose	First injection	2 months later	4 months later	8 months later	12 months later
Part A	1	Low	EnvSeq-1 TF	EnvSeq-1 TF	EnvSeq-1 TF	EnvSeq-1 TF	EnvSeq-1 TF
	2	Medium	EnvSeq-1 TF	EnvSeq-1 TF	EnvSeq-1 TF	EnvSeq-1 TF	EnvSeq-1 TF
	3	High	EnvSeq-1 TF	EnvSeq-1 TF	EnvSeq-1 TF	EnvSeq-1 TF	EnvSeq-1 TF
	4	None	Placebo	Placebo	Placebo	Placebo	Placebo

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 7 more days, you will need to keep track of how you are feeling and if you have any symptoms. To help you do this, we will give you tools and show you how to use them. Contact the clinic staff if you have any issues or concerns after getting 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;
- Do physical exams;
- Do pregnancy tests if you were born female;
- Ask questions about your health, including medications you may be taking;
- Ask questions about any personal problems or benefits you may have from being in the study; and
- Take urine and blood samples.

When we take blood, the amount will depend on the lab tests we need to do. It will be some amount between 10 mL and 210 mL (2 teaspoons to about 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: Insert Appendix I, Table of procedures (for informed consent form) in this section or distribute it as a separate sheet if it is helpful to your study participants. You are not required to do either.

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

14. We will counsel you on avoiding HIV infection.

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

15. We will test your samples for this study

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

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

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

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

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

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

16. After your clinic visits end, we will contact you about 6 months later.

We will contact you by phone, email, or text message *[Site: Modify mode of contact as appropriate; consult IRB/EC if necessary]* 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 (about 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 contact information changes, if you are moving away, or if you do not want us to contact you anymore.

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

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

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

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

Where are the samples stored? Extra samples are stored in a secure central place called a repository. *[Site: insert specific information if your regulatory authority requires it.]* Your samples will be stored in the HVTN repository in the United States.

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

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

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

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

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

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

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

Researchers may also do genetic testing on your samples.

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

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

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

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

People who may see your information are:

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

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

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

Sites: Check HIPAA authorization for conflicts with this section.

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

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

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

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

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

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

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

All reviewers will take steps to keep your records private.

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

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

- [Item 1]
- [Item 2]

- [Item 3]

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

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

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

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

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

This may happen if:

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

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

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

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

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

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

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

Other Risks

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

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

Risks of routine medical procedures:

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

Personal problems/discrimination/testing HIV antibody positive:

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

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

If you have a positive test result caused by the study vaccines at any time, we can arrange free HIV testing for as long as you need it. If this happens, we do not know how long you will test positive due to the study vaccines. If you receive a positive HIV test result and we determine it is because you have HIV, we will refer you for follow-up care.

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

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

If you become pregnant during or after the study and have VISP, we don't know if the antibodies could be passed to your baby. We know that this happens with other vaccines, like tetanus vaccine. These antibodies from the mother are not a danger to the baby, and they go away over time. For most babies antibodies from the mother last for about 6 months.

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

Embarrassment/anxiety:

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

Risks of disclosure of your personal information:

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

Risks of genetic testing:

The genetic testing could show you may be at risk for certain diseases. If others found out, it could lead to discrimination or other problems. However, it is almost impossible for you or others to know your test results from the genetic testing. The results are not part of your study records and are not given to you.

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

Unknown risks:

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

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

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

Benefits

23. The study may not benefit you.

We do not know whether getting the study vaccines 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 vaccines later become approved and sold, there are no plans to share any money with you.

Your rights and responsibilities

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

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

Leaving the study

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

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

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

Injuries

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

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

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

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

The HVTN has limited funds to pay medical costs that it determines are reasonable. *(Sites: insert locale- appropriate medical insurance language in the following sentence)* If the injury is not study related, then you and your health insurance will be responsible for treatment costs.

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

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

Questions

27. 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

28. In Section 17 of this form, we told you about possible other uses of your extra samples and limited information, outside this study. Please choose only one of the options below and write your initials or make your mark in the box next to it. Whatever you choose, the HVTN keeps track of your decision about how your samples and information can be used.

☐ I allow my extra samples combined with limited information to be used for other studies related to HIV, vaccines, the immune system, and other diseases. This may include genetic testing and keeping my cells growing over time.

OR

☐ I agree to the option above *and* also to allow my extra samples combined with limited information to be used in genome wide studies.

OR

☐ I do not allow my extra samples to be used in any other studies. This includes not allowing genetic testing, growing more of my cells, or genome wide studies.

29. If you agree to join this study, you will need to sign or make your mark below. Before you sign or make your mark on this consent form, make sure of the following:

- You have read this consent form, or someone has read it to you.
- You feel that you understand what the study is about and what will happen to you if you join. You understand what the possible risks and benefits are.
- You have had your questions answered and know that you can ask more.
- You agree to join this study.

You will not be giving up any of your rights by signing this consent form.

Participant's name (print)	Participant's signature or mark	Date	Time

Clinic staff conducting consent discussion (print)	Clinic staff signature	Date	Time

For participants who are unable to read or write, a witness should complete the signature block below:

Witness's name (print)	Witness's signature	Date	Time

*Witness is impartial and was present for the consent process.

Appendix B Sample informed consent form for Part B

Title: A phase 1 clinical trial to evaluate the safety and immunogenicity of EnvSeq-1 Envs adjuvanted with GLA-SE, administered alone or with DNA Mosaic-Tre env, in healthy, HIV-uninfected adult participants

HVTN protocol number: HVTN 115

Site: [Insert site name]

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

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

About the study

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

Up to 132 people will take part in this study at multiple sites in the U.S. 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 divided into 2 parts, part A and part B. Forty-two (42) people joined part A of this study. You are being invited to join part B of the study. Up to 90 people will join part B.

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

   - Are the study vaccines safe to give to people?
   - Are people able to take the study vaccines without becoming too uncomfortable?
   - How do people's immune systems respond to the different combinations of study vaccines? (Your immune system protects you from disease.)

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

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

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

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

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

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

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

4. These study vaccines are experimental.

The study vaccines are called CH505TF gp120, CH505w53 gp120, CH505w78 gp120, CH505w100 gp120, and DNA Mosaic-Tre *env*. From here on, we will call them the study vaccines. They are experimental HIV vaccines. That means we do not know whether the vaccines will be safe to use in people, or whether they will work to prevent HIV infection. These vaccines are used only in research studies.

The vaccines were developed by the Division of AIDS (DAIDS) at the National Institutes of Health (NIH). The CH505 vaccines have man-made pieces of protein that look like part of the protein found in HIV. Your body's immune system might learn to recognize these proteins and prepare itself to fight HIV. This is called an immune response. By giving many injections of the CH505 vaccines, we hope the immune system develops antibody responses that can fight against many strains of HIV. The CH505 vaccines are mixed with an adjuvant. An adjuvant is a substance that should help the immune system respond better. The adjuvant in this study is called GLA-SE. GLA-SE was made by the Infectious Disease Research Institute (IDRI).

The DNA in the study vaccine will tell the body to make small amounts of proteins that look like the ones found in HIV.

The CH505TF gp120 vaccine was given to people in part A and was found to be safe. The other CH505 gp120 vaccines are very similar to the CH505TF gp120 vaccine, but have not been given to people. The DNA vaccine has been given to people in another study. The CH505TF gp120 and DNA vaccines have been tested in animals and appear safe. Even if something looks like it is safe or works in animals, it may not be true for people.

General risks of vaccines:

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

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

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

Risks of the study vaccines:

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

The CH505TF gp120 vaccine has been given to people before. The most common complaints were pain or tenderness where people were injected.

The adjuvant, GLA-SE, has been tested in humans with vaccines for other diseases. The most common complaints were pain and tenderness and feeling tired.

The DNA vaccine has been tested in 30 people. The most common complaints were pain and tenderness where people were injected. Similar DNA vaccines have been given to more than 1,000 people in other studies. In studies with similar DNA vaccines, the most common complaints were pain or itching at the injection site, headache, and feeling tired.

Joining the study

5. 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. You cannot be in this study while you are in another study where you get a study product. Also during the study, you should not donate blood or tissue.

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

Site: Remove item 6 if you use a separate screening consent that covers these procedures.

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

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

- Checking your weight, temperature and blood pressure
- Looking in your mouth and throat

- Listening to your heart and lungs
- Feeling your abdomen (stomach and liver)

We will also do blood and urine tests. These tests tell us about some aspects of your health, such as how healthy your kidneys, liver, and immune system are. We will also test you for: Hepatitis 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. The screening results may show you are not eligible to join the study, even if you want to.

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

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

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

8. **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 to join this study.**

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

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

Being in the study

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

9. **You will come to the clinic for scheduled visits about [#] times over 22 months.**

   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. We may need to ask you for your Social Security number for tax reasons.

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

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

Not everyone in this study will get the study vaccines. 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 vaccines. In this study, the placebo is sterile salt water.

You have a 1-in-9 chance of getting the placebo, which means you have an 8-in-9 chance of receiving the study vaccines.

Site: Modify the randomization metaphor in the next sentence as appropriate to your local culture. Whether you get the study vaccines or the placebo is completely random, like flipping a coin.

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

You will have to wait until everyone completes their final study visits to find out whether you got the study vaccines 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 1 of 5 groups. You will not know which group you will be assigned to. You will get 2 injections at each of 6 separate visits. The EnvSeq-1 injections will be given with needle and syringe into the muscle of your upper thigh. The DNA injections will be given by a device called a Biojector 2000 into the muscle of your upper thigh. The Biojector 2000 delivers the vaccine or placebo through the skin without using a needle. The FDA has approved it for delivering injections into muscles. The placebo will be given either with a needle and syringe or Biojector 2000.

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

		Injection Schedule					
	Group	First injection	2 months later	4 months later	8 months later	12 months later	16 months later
Part B	5	EnvSeq-1 TF + placebo	EnvSeq-1 53 + placebo	EnvSeq-1 78 + placebo	EnvSeq-1 100 + placebo	EnvSeq-1 100 + placebo	EnvSeq-1 100 + placebo
	6	EnvSeq-1 TF + mosaic DNA	EnvSeq-1 53 + mosaic DNA	EnvSeq-1 78 + mosaic DNA	EnvSeq-1 100 + mosaic DNA	EnvSeq-1 100 + mosaic DNA	EnvSeq-1 100 + mosaic DNA
	7	EnvSeq-1 TF + placebo	EnvSeq-1 TF, 53 + placebo	EnvSeq-1 TF, 53, 78 + placebo	EnvSeq-1 53, 78, 100 + placebo	EnvSeq-1 78, 100 + placebo	EnvSeq-1 100 + placebo
	8	EnvSeq-1 TF + mosaic DNA	EnvSeq-1 TF, 53 + mosaic DNA	EnvSeq-1 TF, 53. 78 + mosaic DNA	EnvSeq-1 53, 78, 100 + mosaic DNA	EnvSeq-1 78, 100 + mosaic DNA	EnvSeq-1 100 + mosaic DNA
	9	Placebo + placebo	Placebo + placebo	Placebo + placebo	Placebo + placebo	Placebo + placebo	Placebo + placebo

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 7 more days, you will need to keep track of how you are feeling and if you have any symptoms. To help you do this, we will give you tools and show you how to use them. Contact the clinic staff if you have any issues or concerns after getting 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;
- Do physical exams;
- Do pregnancy tests if you were born female;
- Ask questions about your health, including medications you may be taking;
- Ask questions about any personal problems or benefits you may have from being in the study; and
- Take urine and blood samples.

When we take blood, the amount will depend on the lab tests we need to do. It will be some amount between 10 mL and 210 mL (2 teaspoons to about 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: Insert Appendix J, Table of procedures (for informed consent form) in this section or distribute it as a separate sheet if it is helpful to your study participants. You are not required to do either.

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

14. If you agree, we will also collect stool samples.

At the end of this form we will ask if you allow us to collect stool samples. You can decide not to give these samples and still be in the study.

We would like to collect a small sample of your stool to look at the bacteria living in your stomach. We want to learn if your immune response to the study vaccines is influenced by these bacteria. We will ask you to do this 3 times during this study. You may provide a stool sample at home or at the clinic by pooping into a cup we will give you. The clinic must receive the stool sample within 4 hours after it is collected. Another option is that we can collect a stool sample by doing rectal swabs. If that is what you choose, we will briefly insert a sterile swab into your rectum. We will do this up to 4 times.

15. We will counsel you on avoiding HIV infection.

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

16. We will test your samples for this study

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

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

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

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

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

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

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

We will contact you by phone, email, or text message *[Site: Modify mode of contact as appropriate; consult IRB/EC if necessary]* 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 (about 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 contact information changes, if you are moving away, or if you do not want us to contact you anymore.

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

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

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

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

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

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

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

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

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

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

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

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

Researchers may also do genetic testing on your samples.

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

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

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

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

People who may see your information are:

- Researchers who use your stored samples and limited information for other research

- Government agencies that fund or monitor the research using your samples or information

- The researcher's Institutional Review Board or Ethics Committee

- The people who work with the researcher

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

19. We will do our best to protect your private information.

Sites: Check HIPAA authorization for conflicts with this section.

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

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

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

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

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

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

- The US National Institutes of Health and its study monitors,
- The US Food and Drug Administration,
- [Insert name of local IBC],
- [Insert name of local IRB/EC] ,
- [Insert name of local and/or national regulatory authority as appropriate],

- IDRI and people who work for them,
- The HVTN and people who work for them,
- The HVTN Safety Monitoring Board, and
- The US Office for Human Research Protections.

All reviewers will take steps to keep your records private.

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

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

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

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

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

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

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

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 more injections.

This may happen if:

- you do not follow instructions,
- we think that staying in the study might harm you,
- you get HIV,

- you enroll in a different research study where you get another study product, or
- the study is stopped for any reason.

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

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

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

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

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 if there are other studies you may want to join. *Site: Modify the following sentence as appropriate.* We will not provide or pay for any of your HIV care directly.

Other Risks

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

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

Risks of routine medical procedures:

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

Risks of Biojector:

Injection of the DNA vaccine with the Biojector 2000® device may cause a small red bump and then a scab on the thigh. This usually goes away within a few days. Tell the study nurse or doctor if you have a bump or scab.

Risks of rectal swab:

If you choose to provide a stool sample by rectal swabbing, you may feel pressure as the swab is inserted into your rectum, but it is usually not painful. Some people might have a little bit of bleeding.

Personal problems/discrimination/testing HIV antibody positive:

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

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

If you have a positive test result caused by the study vaccines at any time, we can arrange free HIV testing for as long as you need it. If this happens, we do not know how long you will test positive due to the study vaccines. If you receive a positive HIV test result and we determine it is because you have HIV, we will refer you for follow-up care.

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

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

If you become pregnant during or after the study and have VISP, we don't know if the antibodies could be passed to your baby. We know that this happens with other vaccines, like tetanus vaccine. These antibodies from the mother are not a danger to the baby, and they go away over time. For most babies antibodies from the mother last for about 6 months.

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

Embarrassment/anxiety:

You may feel embarrassed when we ask about your HIV risks, such as having sex and using drugs. Also, waiting for your HIV test results or other health test results could

make you feel anxious. You could feel worried if your test results show that you are infected with HIV. If you feel embarrassed or anxious, please tell us and we will try to help you.

Risks of disclosure of your personal information:

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

Risks of genetic testing:

The genetic testing could show you may be at risk for certain diseases. If others found out, it could lead to discrimination or other problems. However, it is almost impossible for you or others to know your test results from the genetic testing. The results are not part of your study records and are not given to you.

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

Unknown risks:

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

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

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

Benefits

24. The study may not benefit you.

We do not know whether getting the study vaccines 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 vaccines 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 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. We will also ask about any personal problems or benefits you have experienced from being in the study. We believe these steps are important to protecting your health, but it is up to you whether to complete them.

Injuries

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

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

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

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

The HVTN has limited funds to pay medical costs that it determines are reasonable. *(Sites: insert locale- appropriate medical insurance language in the following sentence)* If the injury is not study related, then you and your health insurance will be responsible for treatment costs.

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

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

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 14 of this form, we told you about collecting stool samples. Please write your initials or make your mark in the box next to the options you choose.

☐ I agree to provide stool samples.

☐ I do not agree to provide stool samples.

30. In Section 18 of this form, we told you about possible other uses of your extra samples and limited information, outside this study. Please choose only one of the options below and write your initials or make your mark in the box next to it. Whatever you choose, the HVTN keeps track of your decision about how your samples and information can be used.

☐ I allow my extra samples combined with limited information to be used for other studies related to HIV, vaccines, the immune system, and other diseases. This may include genetic testing and keeping my cells growing over time.

OR

☐ I agree to the option above *and* also to allow my extra samples combined with limited information to be used in genome wide studies.

OR

☐ I do not allow my extra samples to be used in any other studies. This includes not allowing genetic testing, growing more of my cells, or genome wide studies.

31. If you agree to join this study, you will need to sign or make your mark below. Before you sign or make your mark on this consent form, make sure of the following:

- You have read this consent form, or someone has read it to you.
- You feel that you understand what the study is about and what will happen to you if you join. You understand what the possible risks and benefits are.
- You have had your questions answered and know that you can ask more.
- You agree to join this study.

You will not be giving up any of your rights by signing this consent form.

Participant's name (print)	Participant's signature or mark	Date	Time

Clinic staff conducting consent discussion (print)	Clinic staff signature	Date	Time

For participants who are unable to read or write, a witness should complete the signature block below:

Witness's name (print)	Witness's signature	Date	Time

*Witness is impartial and was present for the consent process.

Appendix C 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 vaccines could affect the developing baby.

You must agree to use effective birth control from 21 days before your first injection until 6 months after your last study injection.

Effective birth control means using any of the following methods every time you have sex:

- Birth control drugs that prevent pregnancy—given by pills, shots, patches, vaginal rings, or inserts under the skin;
- Male or female condoms, with or without a cream or gel that kills sperm;
- Diaphragm or cervical cap with a cream or gel that kills sperm;
- Intrauterine device (IUD); or
- Any other contraceptive method approved by the researchers.

You do not have to use birth control if:

- You are only having sex with a partner or partners who have had a vasectomy. (We will ask you some questions to confirm that the vasectomy was successful.);
- You have reached menopause, with no menstrual periods for 1 year;
- You have had a hysterectomy (your uterus removed);
- You have had your ovaries removed;
- You have a tubal ligation (your “tubes tied”) or confirmed successful placement of a product that blocks the fallopian tubes;
- You are having sex only with a female partner or partners;
- You only have oral sex; or,
- You are sexually abstinent (no sex at all).

Remember: If you are having sex, you need to use male or female condoms to protect yourself from HIV infection.

If you join the study, we will test you for pregnancy at some visits, including before each study injection

Appendix D 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 EnvSeq-1 Envs adjuvanted with GLA-SE, administered alone or with DNA Mosaic-Tre env, in healthy, HIV-uninfected adult participants

HVTN protocol number: HVTN 115

Site: [Insert site name]

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

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

1. Do I have to agree?

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

2. Where are the samples stored?

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

3. How long will the samples be stored?

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

4. Will I be paid for the use of my samples?

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

5. Will I benefit from allowing my samples to be used in other studies?

Probably not. Results from these other studies are not given to you, this clinic, or your doctor. They are not part of your medical record. The studies are only being done for research purposes.

6. Will the HVTN sell my samples and information?

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

7. How do other researchers get my samples and information?

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

8. What information is shared with other researchers?

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

9. What kind of studies might be done with my extra samples and information?

- The studies will be related to HIV, vaccines, the immune system and other diseases.

Researchers may also do genetic testing on your samples.

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

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

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

10. What are the risks of genetic testing?

The genetic testing could show you may be at risk for certain diseases. If others found out, it could lead to discrimination or other problems. However, it is almost impossible for you or others to know your test results from the genetic testing. The results are not part of your study records and are not given to you.

In the very unlikely event that your genetic information becomes linked to your name, a federal law called the Genetic Information Nondiscrimination Act (GINA) helps protect you. GINA keeps health insurance companies and employers from seeing results of genetic testing when deciding about giving you health insurance or offering you work.

GINA does not help or protect you against discrimination by companies that sell life, disability or long-term care insurance.

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

People who may see your information are:

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

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

Questions

12. If you have questions or problems about allowing your samples and information to be used in other studies, use the following important contacts.

If you have questions about the use of your samples or information or if you want to change your mind about their use, contact
[name and telephone number of the investigator or other study staff].

If you think you may have been harmed because of studies using your samples or information, contact [name and telephone number of the investigator or other study staff].

If you have questions about your rights as a research participant, contact
[name/title/phone of person on IRB or other appropriate organization].

13. Please choose only one of the options below and write your initials or make your mark in the box next to it. Whatever you choose, the HVTN keeps track of your choice about how your samples and information can be used.

☐ I allow my extra samples combined with limited information to be used for other studies related to HIV, vaccines, the immune system, and other diseases. This may include genetic testing and keeping my cells growing over time.

OR

☐ I agree to the option above *and* also to allow my extra samples combined with limited information to be used in genome wide studies.

OR

☐ I do not allow my extra samples to be used in any other studies. This includes not allowing genetic testing, growing more of my cells, or genome wide studies.

Participant's name (print)	Participant's signature or mark	Date	Time

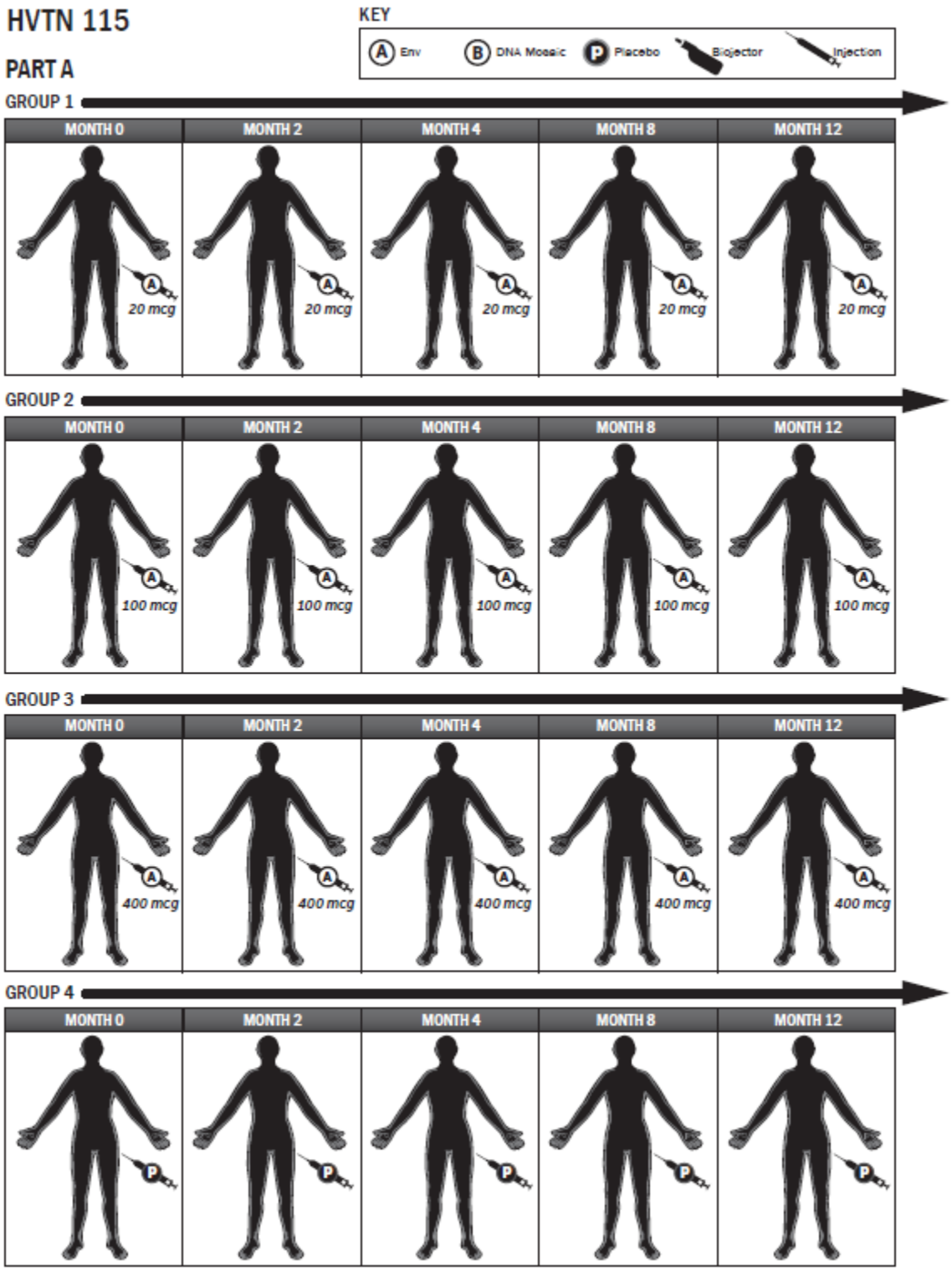
Clinic staff conducting consent discussion (print)	Clinic staff signature	Date	Time

For participants who are unable to read or write, a witness should complete the signature block below:

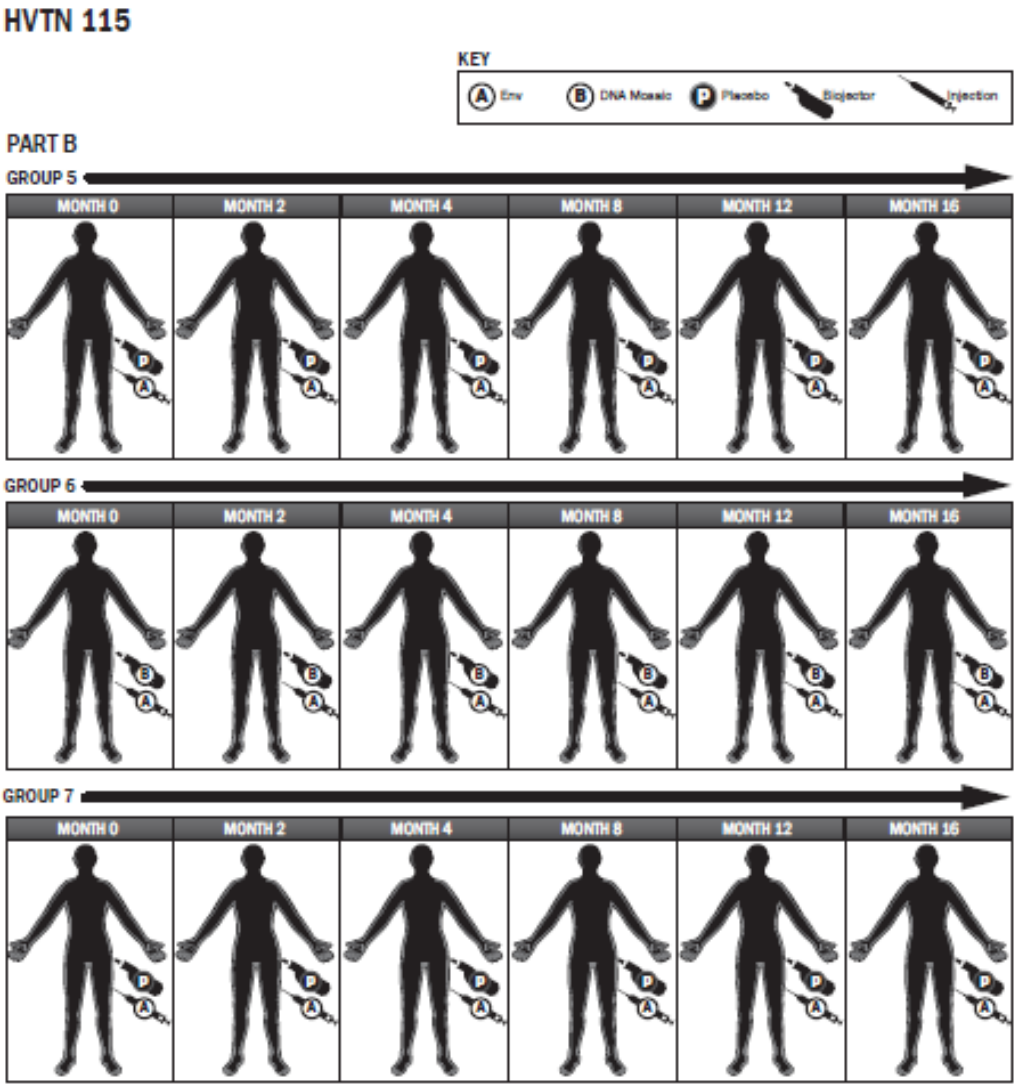
Witness's name (print)	Witness's signature	Date	Time

*Witness is impartial and was present for the consent process

Appendix E Injection schedule for part A sample informed consent form



Appendix F Injection schedule for part B sample informed consent form



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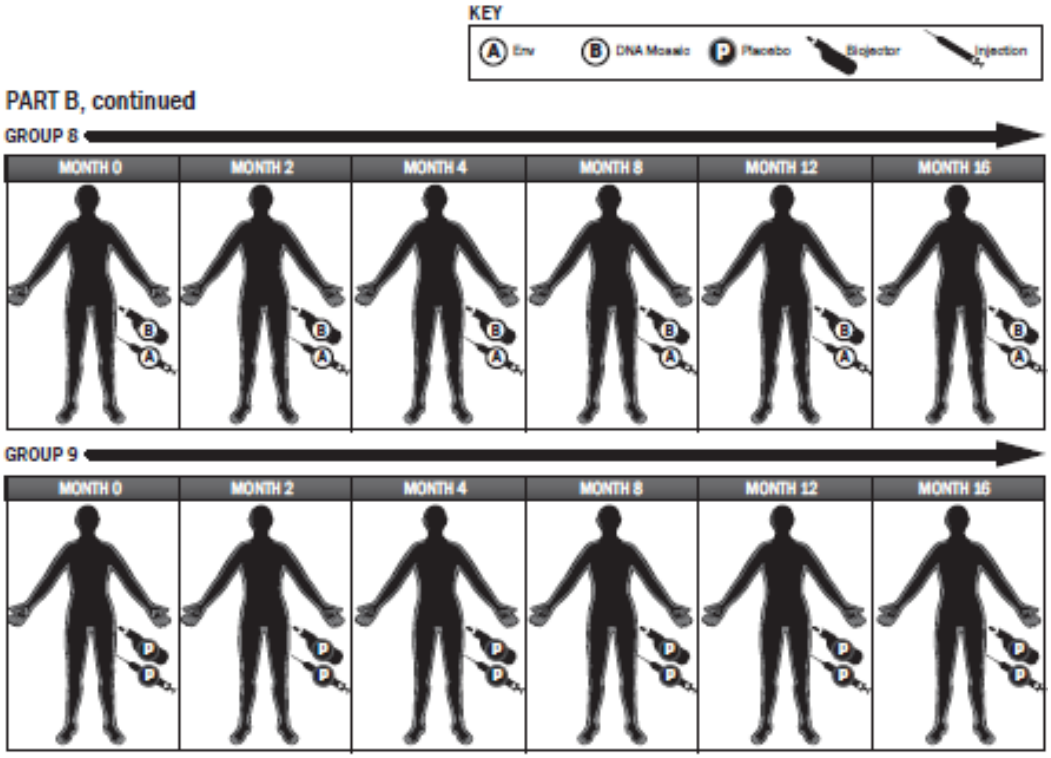
KEY
A Env
B DNA Mosaic
P Placebo
Biojector
Injection
PART B, continued
GROUP 8
MONTH 0
MONTH 2
MONTH 4
MONTH 8
MONTH 12
MONTH 16
GROUP 9
MONTH 0
MONTH 2
MONTH 4
MONTH 8
MONTH 12
MONTH 16

Appendix G Table of procedures for Part A (for sample informed consent form)

			Time after first injection visit (in months)													
Procedure	Screening visit(s)	First injection visit	0.25	0.5	2	2.5	4	4.5	8	8.5	12	12.25	12.5	15	18	24
Injection		√			√		√		√		√					
Medical history	√															
Complete physical	√														√	
Brief physical		√	√	√	√	√	√	√	√	√	√	√	√	√		
Urine test	√			√									√			
Blood drawn	√	√	√	√		√		√		√	√	√	√	√	√	
Pregnancy test (participants born female)*	√	√			√		√		√		√			√		
HIV testing & pretest counseling	√					√		√		√	√			√	√	
Risk reduction counseling	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	
Interview/questionnaire	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	
Health contact																√

Not shown in this table is a time after all study participants have completed their last scheduled visit when you can find out what products you received.
* Persons who had a complete hysterectomy (removal of the uterus and ovaries, verified by medical records), are not required to have a pregnancy test.

Appendix H Table of procedures for Part B (for sample informed consent form)

			Time after first injection visit (in months)															
Procedure	Part B Eligibility visit(s)	First injection visit	0.25	0.5	2	2.5	4	4.5	8	8.5	12	12.5	16	16.25	16.5	19	22	28
Injection		√			√		√		√		√		√					
Medical history	√																	
Complete physical	√																√	
Brief physical		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√		
Urine test	√			√											√			
Blood drawn	√	√	√	√		√		√		√	√	√	√	√	√	√	√	
Pregnancy test (participants born female)*	√	√			√		√		√		√		√			√		
HIV testing & pretest counseling	√					√		√		√	√		√			√	√	
Risk reduction counseling	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	
Interview/questionnaire	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	
Stool sample (optional)		√								√					√			
Health contact																		√

Not shown in this table is a time after all study participants have completed their last scheduled visit when you can find out what products you received.

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

Appendix I Laboratory procedures for Part A

					Tube volume (mL)															
				Visit:	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**[9]	
				Day:	Screening visit[3]	D0	D7	D14	D56	D70	D112	D126	D224	D238	D364	D371	D378	D455	D546	D728	
				Week:		W0	W1	W2	W8	W10	W16	W18	W32	W34	W52	W53	W54	W65	W78	W104	
				Month:		M0	M0.25	M0.5	M2	M2.5	M4	M4.5	M8	M8.5	M12	M12.25	M12.5	M15	M18	M24	
						VAC1			**VAC2**		**VAC3**		**VAC4**		**VAC5**						
Procedure	**Ship to**[1, 2]	**Assay Location**[2]	**Tube**[4]	**Tube size (vol. capacity)**[4]																	**Total**
BLOOD COLLECTION																					
Screening or diagnostic assays																					
Screening HIV test	Local Lab	Local Lab	SST	5mL	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5
HBsAg/anti-HCV	Local Lab	Local Lab	SST	5mL	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5
Syphilis	Local Lab	Local Lab	SST	5mL	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5
HIV diagnostics[8]	UW-VSL	UW-VSL	EDTA	10mL	—	—	—	—	—	10	—	10	—	10	10	—	—	10	20	—	70
Safety labs																					
CBC/ Diff/ platelets	Local lab	Local lab	EDTA	5mL	5	—	—	5	—	5	—	5	—	5	—	—	5	5	—	—	35
Chemistry panel[5]	Local lab	Local lab	SST	5mL	5	—	—	5	—	5	—	5	—	5	—	—	5	5	—	—	35
Immunogenicity assays[6]																					
Cellular assays																					
ICS	CSR	FHCRC	ACD	8.5mL	—	42.5	—	42.5	—	42.5	—	*42.5*	—	42.5	—	—	42.5	—	42.5	—	297.5
Phenotyping (pTfh)	CSR	FHCRC	ACD	8.5mL	—	—	17	—	—	—	—	—	—	—	—	17	—	—	—	—	34
Humoral assays																					
Binding Ab	CSR	Duke	SST	8.5mL	—	8.5	—	8.5	—	8.5	—	*8.5*	—	8.5	—	—	8.5	—	8.5	—	59.5
Neutralizing Ab	CSR	Duke	SST	8.5mL	—	8.5	—	8.5	—	8.5	—	*8.5*	—	8.5	—	—	8.5	—	8.5	—	59.5
B-cell lineage	CSR	Duke	ACD	8.5mL	—	34	34	34	—	34	—	*34*	—	34	—	34	68	—	34	—	340
Specimen storage																					
PBMC	CSR		ACD	8.5mL	—	42.5	—	42.5	—	42.5	—	42.5	—	42.5	—	—	42.5	—	68	—	323
Plasma	CSR		ACD	8.5mL	—	z	z	z	—	z	—	z	—	z	—	z	z	—	z	—	0
Serum	CSR		SST	8.5mL	—	25.5	—	17	—	17	—	17	—	17	—	—	17	—	17	—	127.5
Visit total					**25**	**161.5**	**51**	**163**	**0**	**173**	**0**	**173**	**0**	**173**	**10**	**51**	**197**	**20**	**198.5**	**0**	**1396**
56-Day total					**25**	**186.5**	**237.5**	**400.5**	**400.5**	**336**	**173**	**346**	**0**	**173**	**10**	**61**	**258**	**20**	**198.5**	**0**	
URINE COLLECTION																					
Urinalysis	Local lab	Local lab			X	—	—	X	—	—	—	—	—	—	—	—	X	—	—	—	
Pregnancy test[7]	Local lab	Local lab			X	X	—	—	X	—	X	—	X	—	X	—	—	X	—	—	

[1]CSR = central specimen repository

[2]HVTN Laboratory Program includes laboratories at UW-VSL, FHCRC, and Duke. UW-VSL = University of Washington Virology Specialty Laboratory (Seattle, Washington, USA); FHCRC = Fred Hutchinson Cancer Research Center (Seattle, Washington, USA); Duke = Duke University Medical Center (Durham, North Carolina, USA).

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

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

[5]Chemistry panels are defined in Section 9.2 (pre-enrollment) and Section 9.4 (follow-up visits).

[6]Immunogenicity assays will be performed at M0 (for binding Ab assay) and M4.5. 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

[7]For a participant who was born female (ie, assigned female sex at birth), pregnancy test must be performed on urine or blood specimens on the day of vaccination with negative results received prior to vaccination. Persons who have undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing.

[8]At an early termination visit for a withdrawn or terminated participant (Section 9.12), blood should be drawn for HIV diagnostic testing, as shown for visit 15 above.

[9]Visit 16 is a health contact visit. Clinic visits are not required unless a participant indicates symptoms that require further assessment. See Section 9.5.

z = 5 x 1mL aliquots of ACD plasma will be harvested for storage during PBMC processing; no separate blood draw is needed.

Appendix J Laboratory procedures for Part B

					Tube volume (mL)																	
				Visit:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18[10]	
				Day:	Screening visit[3]	D0	D7	D14	D56	D70	D112	D126	D224	D238	D364	D378	D485	D493	D500	D576	D667	D849	
				Week:		W0	W1	W2	W8	W10	W16	W18	W32	W34	W52	W54	W69	W70	W71	W82	W95	W121	
				Month:		M0	M0.25	M0.5	M2	M2.5	M4	M4.5	M8	M8.5	M12	M12.5	M16	M16.25	M16.5	M19	M22	M28	
						VAC1			**VAC2**		**VAC3**		**VAC4**		**VAC5**		**VAC6**						
Procedure	**Ship to[1, 2]**	**Assay Location[2]**	**Tube[4]**	**Tube size (vol. capacity)[4]**																			**Total**
BLOOD COLLECTION																							
Screening or diagnostic assays																							
Screening HIV test	Local Lab	Local Lab	SST	5mL	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5
HBsAg/anti-HCV	Local Lab	Local Lab	SST	5mL	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5
Syphilis	Local Lab	Local Lab	SST	5mL	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5
HIV diagnostics[8]	UW-VSL	UW-VSL	EDTA	10mL	—	—	—	—	—	10	—	10	—	10	10	—	10	—	—	10	20	—	80
Safety labs																		—	—				
CBC/ Diff/ platelets	Local lab	Local lab	EDTA	5mL	5	—	—	5	—	5	—	5	—	5	—	5	—	—	5	5	—	—	40
Chemistry panel[5]	Local lab	Local lab	SST	5mL	5	—	—	5	—	5	—	5	—	5	—	5	—	—	5	5	—	—	40
Immunogenicity assays[6]																		—	—				
Cellular assays																		—	—				
ICS	CSR	FHCRC	ACD	8.5mL	—	42.5	—	42.5	—	42.5	—	42.5	—	*42.5*	—	42.5	—	—	*42.5*	—	42.5	—	340
Phenotyping (pTfh)	CSR	FHCRC	ACD	8.5mL	—	—	17	—	—	—	—	—	—	—	—	—	—	17	—	—	—	—	34
Humoral assays																		—	—				
Binding Ab	CSR	Duke	SST	8.5mL	—	8.5	—	8.5	—	8.5	—	8.5	—	*8.5*	—	8.5	—	—	*8.5*	—	8.5	—	68
Neutralizing Ab	CSR	Duke	SST	8.5mL	—	8.5	—	8.5	—	8.5	—	8.5	—	*8.5*	—	8.5	—	—	*8.5*	—	8.5	—	68
B-cell repertoire analysis	CSR	Duke	ACD	8.5mL	—	34	34	34	—	34	—	34	—	*34*	—	34	—	34	*68*	—	34	—	374
Specimen storage																		—	—				
PBMC	CSR		ACD	8.5mL	—	42.5	—	42.5	—	42.5	—	42.5	—	42.5	—	42.5	—	—	42.5	—	68	—	365.5
Plasma	CSR		ACD	8.5mL	—	z	z	z	—	z	—	z	—	z	—	z	—	z	z	—	z	—	0
Serum	CSR		SST	8.5mL	—	25.5	—	17	—	17	—	17	—	17	—	17	—	—	17	—	17	—	144.5
Visit total					**25**	**161.5**	**51**	**163**	**0**	**173**	**0**	**173**	**0**	**173**	**10**	**163**	**10**	**51**	**197**	**20**	**198.5**	**0**	**1569**
56-Day total					**25**	**186.5**	**237.5**	**400.5**	**400.5**	**336**	**173**	**346**	**0**	**173**	**10**	**173**	**10**	**61**	**258**	**20**	**198.5**	**0**	
URINE COLLECTION																							
Urinalysis	Local lab	Local lab			X	—	—	X	—	—	—	—	—	—	—	—	—	—	X	—	—	—	
Pregnancy test[7]	Local lab	Local lab			X	X	—	—	X	—	X	—	X	—	X	—	X	—	—	X	—	—	
STOOL COLLECTION (OPTIONAL)[9]																							
Stool	CSR	Duke			—	X	—	—	—	—	—	—	—	X	—	—	—	—	X	—	—	—	

[1]CSR = central specimen repository

[2]HVTN Laboratory Program includes laboratories at UW-VSL, FHCRC, and Duke. UW-VSL = University of Washington Virology Specialty Laboratory (Seattle, Washington, USA); FHCRC = Fred Hutchinson Cancer Research Center (Seattle, Washington, USA); Duke = Duke University Medical Center (Durham, North Carolina, USA).

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

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

[5]Chemistry panels are defined in Section 9.2 (pre-enrollment) and Section 9.4 (follow-up visits).

[6]Immunogenicity assays will be performed at M0 (for binding Ab assay), M8.5 and M 16.5. 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

[7]For a participant who was born female (ie, assigned female sex at birth), pregnancy test must be performed on urine or blood specimens on the day of vaccination with negative results received prior to vaccination. Persons who have undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing.

[8]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 17 above.

[9]Optional stool specimens may be collected at or before enrollment and at visits 10 and 15 (2 weeks after the fourth and sixth vaccinations, respectively).

[10]Visit 18 is a health contact visit. Clinic visits are not required unless a participant indicates symptoms that require further assessment. See Section 9.5.

z = 5 x 1mL aliquots of ACD plasma will be harvested for storage during PBMC processing; no separate blood draw is needed.

Appendix K Procedures at HVTN CRS for Part A

Visit:	01[a]	02[h]	03	04	05	06	07	08	09	10	11	12	13	14	15	16[b]	Post
Day:		D0	D7	D14	D58	D70	D112	D126	D224	D238	D364	D371	D378	D455	D546	D728	
Month:		M0	M0.25	M0.5	M2	M2.5	M4	M4.5	M8	M8.5	M12	M12.25	M12.5	M15	M18	M24	
Procedure	Scr.	VAC1			VAC2		VAC3		VAC4		VAC5					AESI[c]	
Study procedures[d]																	
Signed screening consent (if used)	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Assessment of understanding	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Signed protocol consent	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Medical history	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Complete physical exam	X	—	—	—	—	—	—	—	—	—	—	—	—	—	X	—	—
Abbreviated physical exam	—	X	X	X	X	X	X	X	X	X	X	X	X	X	—	—	—
Risk reduction counseling	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	—
Pregnancy prevention assessment[e]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	—	—
Behavioral risk assessment	X	—	—	—	—	X	—	—	—	X	—	—	—	X	—	—	—
Confirm eligibility, obtain demographics, randomize	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Social impact assessment	—	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	—
Social impact assessment questionnaire	—	—	—	—	—	X	—	—	—	X	—	—	—	—	X	—	—
Outside testing and belief questionnaire	—	—	—	—	—	—	—	—	—	X	—	—	—	—	X	—	—
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	—	—
Intercurrent illness/adverse experience	—	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	—
HIV infection assessment[f]	X	—	—	—	—	X	—	X	—	X	X	—	—	X	X	—	—
Confirm HIV test results provided to participant	—	X	—	—	—	—	X	—	X	—	X	X	—	—	X	X	X
Local lab assessment																	
Urine dipstick	X	—	—	X	—	—	—	—	—	—	—	—	X	—	—	—	—
Pregnancy (urine or serum HCG)[g]	X	X	—	—	X	—	X	—	X	—	X	—	—	X	—	---	—
CBC, differential	X	—	—	X	—	X	—	X	—	X	—	—	X	X	—	---	—
Chemistry panel (see Section 9.2 and 9.3)	X	—	—	X	—	X	—	X	—	X	—	—	X	X	—	---	—

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

[b] Clinic visits are not required unless a participant indicates symptoms that require further assessment. See Section 9.5.

[c] See Section 9.5.

[d] For specimen collection requirements, see Appendix I.

[e] Pregnancy prevention assessment is required only for participants who were born female and are capable of becoming pregnant.

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

[g] For a participant who was born female (ie, assigned female sex at birth), pregnancy test must be performed on urine or blood specimens on the day of vaccination with negative results received prior to vaccination. Pregnancy test to determine eligibility may be performed at screening, but must also be done on Day 0 prior to vaccination. Persons who have undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing.

Appendix K, continued

Visit:	01[a]	02[d]	03	04	05	06	07	08	09	10	11	12	13	14	15	16	Post
Day:		D0	D7	D14	D58	D70	D112	D126	D224	D238	D364	D371	D378	D455	D546	D728	
Month:		M0	M0.25	M0.5	M2	M2.5	M4	M4.5	M8	M8.5	M12	M12.25	M12.5	M15	M18	M24	
Procedure	Scr.	VAC1			VAC2		VAC3		VAC4		VAC5					AESI	
Syphilis, Hepatitis B, Hepatitis C	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Vaccination procedures																	
Vaccination[b]	—	X	—	—	X	—	X	—	X	—	X	—	—	—	—	—	—
Reactogenicity assessments[c]	—	X	—	—	X	—	X	—	X	—	X	—	—	—	—	—	—
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] Blood draws required at vaccination visits must be performed prior to vaccination; however, it is not necessary to have results prior to vaccination, except for results of a urine or serum pregnancy test, if indicated.

[c] Reactogenicity assessments performed daily for at least 7 days postvaccination (see Section 9.10).

Appendix L Procedures at HVTN CRS for Part B

Visit:	01[a]	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18[b]	Post
Day:		D0	D7	D14	D58	D70	D112	D126	D224	D238	D364	D378	D485	D493	D500	D576	D667	D849	
Month:		M0	M0.25	M0.5	M2	M2.5	M4	M4.5	M8	M8.5	M12	M12.5	M16	M16.25	M16.5	M19	M22	M28	
Procedure	Scr.	VAC1			VAC2		VAC3		VAC4		VAC5		VAC6					AESI[c]	
Study procedures[d]																			
Signed screening consent (if used)	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Assessment of understanding	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Signed protocol consent	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Medical history	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Complete physical exam	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	X	—	—
Abbreviated physical exam	—	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	—	X	—
Stool collection (optional)[e]	—	X	—	—	—	—	—	—	—	X	—	—	—	—	X	—	—	—	—
Risk reduction counseling	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	—	—
Pregnancy prevention assessment[f]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	—	—
Behavioral risk assessment	X	—	—	—	—	X	—	—	—	X	—	—	—	X	—	—	—	—	—
Confirm eligibility, obtain demographics, randomize	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Social impact assessment	—	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	—
Social impact assessment questionnaire	—	—	—	—	—	X	—	—	—	X	—	—	—	—	—	—	X	—	—
Outside testing and belief questionnaire	—	—	—	—	—	—	—	—	—	X	—	—	—	—	X	—	—	—	—
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	—	—
Intercurrent illness/adverse experience	—	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	—
HIV infection assessment[g]	X	—	—	—	—	X	—	X	—	X	X	—	X	—	—	X	X	—	—
Confirm HIV test results provided to participant	—	X	—	—	—	—	X	—	X	—	X	X	—	X	—	—	X	X	X
Local lab assessment																			
Urine dipstick	X	—	—	X	—	—	—	—	—	—	—	—	—	—	X	—	—	—	—

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

[b] Clinic visits are not required unless a participant indicates symptoms that require further assessment. See Section 9.5.

[c] See Section 9.5.

[d] For specimen collection requirements, see Appendix J.

[e] Optional stool specimens must be collected prior to first vaccination.

[f] Pregnancy prevention assessment is required only for participants who were born female and are capable of becoming pregnant.

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

Appendix L, continued

Visit:	01[a]	02[f]	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	Post
Day:		D0	D7	D14	D58	D70	D112	D126	D224	D238	D364	D378	D485	D493	D500	D576	D667	D849	
Month:		M0	M0.25	M0.5	M2	M2.5	M4	M4.5	M8	M8.5	M12	M12.5	M16	M16.25	M16.5	M19	M22	M28	
Procedure	Scr.	VAC1			VAC2		VAC3		VAC4		VAC5		VAC6					AESI	
Study procedures[b]																			
Pregnancy (urine or serum HCG)[c]	X	X	—	—	X	—	X	—	X	—	X	—	X	—	—	X	—	—	—
CBC, differential	X	—	—	X	—	X	—	X	—	X	—	X	—	—	X	X		—	—
Chemistry panel (see Section 9.2 and 9.3)	X	—	—	X	—	X	—	X	—	X	—	X	—	—	X	X	—	—	—
Syphilis, Hepatitis B, Hepatitis C	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Vaccination procedures																			
Vaccination[d]	—	X	—	—	X	—	X	—	X	—	X	—	X	—	—	—	—	—	—
Reactogenicity assessments[e]	—	X	—	—	X	—	X	—	X	—	X	—	X	—	—	—	—	—	—
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 specimen collection requirements, see Appendix J.

[c] For a participant who was born female (ie, assigned female sex at birth), pregnancy test must be performed on urine or blood specimens on the day of vaccination with negative results received prior to vaccination. Pregnancy test to determine eligibility may be performed at screening, but must also be done on Day 0 prior to vaccination. Persons who have undergone total hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing.

[d] Blood draws required at vaccination visits must be performed prior to vaccination; however, it is not necessary to have results prior to vaccination, except for results of a urine or serum pregnancy test, if indicated.

[e] Reactogenicity assessments performed daily for at least 7 days postvaccination (see Section 9.10).[f]

Appendix M Adverse events of special interest

AEs of special interest (AESI) for this protocol include but are not limited to potential immune-mediated diseases; representative examples of AESI are listed below. Updates to AESI will be provided as an appendix to the *HVTN 115 Study Specific Procedures.*

Gastrointestinal disorders	Liver disorders	Metabolic diseases
• Celiac disease • Crohn's disease • Ulcerative colitis • Ulcerative proctitis	• Autoimmune cholangitis • Autoimmune hepatitis • Primary biliary cirrhosis • Primary sclerosing cholangitis	• Addison's disease • Autoimmune thyroiditis (including Hashimoto thyroiditis) • Diabetes mellitus type I • Grave's or Basedow's disease
Neuroinflammatory disorders	**Musculoskeletal disorders**	**Skin disorders**
• Acute disseminated encephalomyelitis, including site specific variants (eg, non-infectious encephalitis, encephalomyelitis, myelitis, myeloradiculomyelitis) • Cranial nerve disorders, included paralyses/paresis (eg, Bell's palsy) • Guillain-Barré syndrome, including Miller Fisher syndrome and other variants • Immune-mediated peripheral neuropathies and plexopathies, including chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy and polyneuropathies associated with monoclonal gammopathy • Multiple sclerosis • Narcolepsy • Optic neuritis • Transverse Myelitis	• Antisynthetase syndrome • Dermatomyositis • Juvenile chronic arthritis (including Still's disease) • Mixed connective tissue disorder • Polymyalgia rheumatic • Polymyositis • Psoriatic arthropathy • Relapsing polychondritis • Rheumatoid arthritis • Scleroderma, including diffuse systemic form and CREST syndrome • Spondyloarthritis, including ankylosing spondylitis, reactive arthritis (Reiter's Syndrome) and undifferentiated spondyloarthritis • Systemic lupus erythematosus • Systemic sclerosis	• Alopecia areata • Autoimmune bullous skin diseases, including pemphigus, pemphigoid and dermatitis herpetiformis • Cutaneous lupus erythematosus • Erythema nodosum • Morphoea • Lichen planus • Psoriasis • Sweet's syndrome • Vitiligo
Vasculitides	**Others**	
• Large vessels vasculitis including: giant cell arteritis such as Takayasu's arteritis and temporal arteritis • Medium sized and/or small vessels vasculitis including: polyarteritis nodosa, Kawasaki's disease, microscopic polyangiitis, Wegener's granulomatosis, Churg-Strauss syndrome (allergic granulomatous angiitis), Buerger's disease thromboangiitis obliterans, necrotizing vasculitis and anti-neutrophil cytoplasmic antibody (ANCA) positive vasculitis (type unspecified), Henoch-Schonlein purpura, Behcet's syndrome, leukocytoclastic vasculitis	• Antiphospholipid syndrome • Autoimmune hemolytic anemia • Autoimmune glomerulonephritis (including IgA nephropathy, glomerulonephritis rapidly progressive, membranous glomerulonephritis, membranoproliferative glomerulonephritis, and mesangioproliferative glomerulonephritis) • Autoimmune myocarditis/cardiomyopathy • Autoimmune thrombocytopenia • Goodpasture syndrome • Idiopathic pulmonary fibrosis • Pernicious anemia • Raynaud's phenomenon • Sarcoidosis • Sjögren's syndrome • Stevens-Johnson syndrome • Uveitis	