HIV VACCINE
TRIALS NETWORK

PROTOCOL

HVTN 139

A phase 1 clinical trial to evaluate the safety and immunogenicity of HIV-1 vaccines based on chimpanzee serotypes of adenovirus expressing clade C gp140 and a CH505TF gp120 protein boost in healthy, HIV-uninfected adult participants

DAIDS DOCUMENT ID 12052

A non-IND study

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

STUDY PRODUCTS PROVIDED BY

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

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Contents

1 Overview 5
1.1 Protocol Team 9
2 Ethical considerations 10
3 IRB/EC review considerations 12
3.1 Minimized risks to participants 12
3.2 Reasonable risk/benefit balance 12
3.3 Equitable participant selection 13
3.4 Appropriate informed consent 13
3.5 Adequate safety monitoring 13
3.6 Protect privacy/confidentiality 14
4 Background 15
4.1 Rationale for trial concept 15
4.2 AdC6-HIVgp140 17
4.3 AdC7-HIVgp140 18
4.4 CH505TF gp120 19
4.5 GLA-SE adjuvant 19
4.6 Trial design rationale 20
4.7 Plans for future product development and testing 21
4.8 Preclinical safety studies 22
4.9 Preclinical immunogenicity studies 30
4.10 Clinical studies 41
4.11 Potential risks of study products and administration 49
5 Objectives and endpoints 50
5.1 Primary objectives and endpoints 50
5.2 Secondary objectives and endpoints 50
5.3 Exploratory objectives 52
6 Statistical considerations 53
6.1 Accrual and sample size calculations 53
6.2 Randomization 55
6.3 Blinding 55
6.4 Statistical analyses 56
7 Selection and withdrawal of participants 62
7.1 Inclusion criteria 62
7.2 Exclusion criteria 65
7.3 Participant departure from vaccination schedule or withdrawal 69
8 Study product preparation and administration 72
8.1 Vaccine regimen 72
8.2 Study product formulation 73
8.3 Preparation of study products 74
8.4 Administration 77
8.5 Acquisition of study products 78
8.6 Pharmacy records 79

8.7 Final disposition of study products 79
9 Clinical procedures 80
9.1 Informed consent 80
9.2 Pre-enrollment procedures 82
9.3 Enrollment and vaccination visits 84
9.4 Follow-up visits 85
9.5 Health Contacts 86
9.6 HIV counseling and testing 88
9.7 Contraception status 89
9.8 Urine testing 90
9.9 Assessments of reactogenicity 90
9.10 Visit windows and missed visits 92
9.11 Early termination visit 92
9.12 Pregnancy 92
9.13 HIV infection during the study 93
10 Laboratory 94
10.1 HVTN CRS laboratory procedures 94
10.2 Total blood volume 94
10.3 Primary immunogenicity timepoint 94
10.4 Endpoint assays: cellular 95
10.5 Endpoint assays: humoral 95
10.6 HVTN Lab Center assay portfolio 97
10.7 Exploratory studies 97
10.8 Specimen storage and other use of specimens 97
10.9 Biohazard containment 98
11 Safety monitoring and safety review 99
11.1 Safety monitoring and oversight 99
11.2 Safety reporting 100
11.3 Safety reviews 103
11.4 Safety pause and prompt PSRT AE review 104
11.5 Review of cumulative safety data 105
11.6 Study termination 106
12 Protocol conduct 108
12.1 Social impacts 109
12.2 Compliance with NIH guidelines for research involving products containing recombinant or synthetic nucleic acid molecules 109
12.3 Emergency communication with study participants 109
13 Version history 111
14 Document references (other than literature citations) 112
15 Acronyms and abbreviations 115
16 Literature cited 118
Appendix A Sample informed consent form for Part A 124
Appendix B Sample informed consent form for Part B 144

Appendix C Approved birth control methods for persons assigned female sex at birth (for sample informed consent form) 165
Appendix D Sample consent form for use of samples and information in other studies 166
Appendix E Table of procedures for Part A (for sample informed consent form) 171
Appendix F Table of procedures for Part B (for sample informed consent form) 172
Appendix G Laboratory procedures Part A 173
Appendix H Laboratory procedures Part B 175
Appendix I Procedures at HVTN CRS for Part A 177
Appendix J Procedures at HVTN CRS for Part B 179
Appendix K HVTN 139 Visit Windows for Part A 181
Appendix L HVTN 139 Visit Windows for Part B 182
Appendix M HVTN low risk guidelines for Southern Africa 183
Appendix N Adverse events of special interest 185
Appendix O Protocol Signature Page 186

1 Overview

Title

A phase 1 clinical trial to evaluate the safety and immunogenicity of HIV-1 vaccines based on chimpanzee serotypes of adenovirus expressing clade C gp140 and a CH505TF gp120 protein boost in healthy, HIV- uninfected adult participants

Primary objective

To evaluate the safety and tolerability of AdC6-HIVgp140 and AdC7-HIVgp140 at doses of 1 x 10^{10} virus particles (vp) and 5 x 10^{10} vp, alone and in combination with CH505TF gp120 adjuvanted with GLA-SE in HIV- uninfected adults.

Study products and routes of administration

- **AdC6-HIVgp140**: a chimpanzee-derived replication-defective adenovirus (Ad) vector expressing codon optimized gp140 of clade C isolate Du422 administered at a dose of 1 x 10^{10} vp or 5 x 10^{10} vp. The vaccine dose will be divided equally into two separate 1mL intramuscular (IM) injections, administered into the deltoid of the non-dominant arm unless medically contraindicated.

- **AdC7-HIVgp140**: a chimpanzee-derived replication-defective Ad vector expressing codon optimized gp140 of clade C isolate Du172 administered at a dose of 1 x 10^{10} vp or 5 x 10^{10} vp. The vaccine dose will be divided equally into two separate 1mL IM injections administered into the deltoid of the non-dominant arm unless medically contraindicated.

- **CH505TF gp120**: CH505 transmitted/founder gp120 mixed with GLA-SE [the immunological adjuvant Glucopyranosyl Lipid A (GLA) in an oil-in-water stable emulsion (SE)], administered at a dose of 400 mcg with 10 mcg GLA-SE as a 1 mL injection in the thigh unless medically contraindicated.

- Placebo control for AdC6 -HIVgp140 and AdC7-HIVgp140 vaccine: Sodium Chloride for Injection, 0.9% , administered as two separate 1 mL IM injections into the deltoid of the non-dominant arm unless medically contraindicated.

- Placebo control for CH505TF gp120: Sodium Chloride for Injection, 0.9% administered as a 1 mL IM injection in the thigh unless medically contraindicated.

Table 1-1 Schema

Study arm	N	AdC dose (vp)	M0	M3	M6
Part A: Low Dose					
Group 1	5	1x 10^{10}	AdC6-HIVgp140	-	-
Group 2	5	1x 10^{10}	AdC7-HIVgp140	-	-
Group 3	2	0	Placebo	-	-
Part B: High Dose					
Group 4	10	5 x 10^{10}	AdC6-HIVgp140	AdC7-HIVgp140	400 mcg CH505TF gp120/GLA-SE
Group 5	10	5 x 10^{10}	AdC7-HIVgp140	AdC6-HIVgp140	400 mcg CH505TF gp120/GLA-SE
Group 6	2	0	Placebo	Placebo	Placebo
Total	34 (30 vaccinees / 4 placebos)				

Notes:
Study is blinded as to Group assignment (Part assignment is not blinded). Enrollment will be stepwise starting with Part A. To ensure the safety of participants, a series of pre-planned enrollment pauses will occur.

Safety Review #1: Low-dose Initial Vaccination. Enrollment for Part A will be restricted to a maximum of 1 participant per day across all participating HVTN Clinical Research Sites (CRSs) until a total of 5 participants have been enrolled. The HVTN Protocol Safety Review Team (PSRT) will review available safety and reactogenicity data reported for each of these 5 participants, up to and including the data reported for the first 72 hours postvaccination for the 5th enrolled participant, and determine whether it is safe to proceed with full enrollment in Part A.

Safety Review #2: Low-dose (Part A) Safe-to-Proceed. The HVTN PSRT will review cumulative safety data, including at a minimum the 2-weeks following the vaccination visit, available on all 12 participants in Part A to determine whether dose-escalation may occur from Part A to Part B.

Safety Review #3: Full Enrollment at Targeted Dose. The HVTN PSRT will also review cumulative safety data available on the first 12 participants enrolled in Part B, including the 2-week post Month 0 vaccination visit, to determine whether it is safe to proceed with full enrollment.

Participants

34 healthy, HIV– uninfected volunteers aged 18 through 50 years, inclusive; 30 vaccinees, 4 placebo recipients

Design

Multicenter, randomized, controlled, double-blind trial

Duration per participant

Part A participants: 6 months of scheduled clinic visits (main study) followed by AESI (Adverse Events of Special Interest) health contacts at month 12, and then annual health contacts at month 24 and 36.

Part B participants: 12 months of scheduled clinic visits (main study) followed by an AESI health contact at month 18, and then annual health contacts at month 24 and 36.

Estimated total study duration

48 months, includes enrollment, planned safety holds, AESI and annual health contacts.

Clinical trial sponsor

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

Study product providers

- AdC6-HIVgp140: provided by DAIDS, NIAID, NIH, DHHS (Bethesda, Maryland, USA)
- AdC7-HIVgp140: provided by DAIDS, NIAID, NIH, DHHS (Bethesda, Maryland, USA)
- CH505TF gp120: DAIDS, NIAID, NIH, DHHS (Bethesda, Maryland, USA)
- GLA-SE adjuvant: DAIDS, NIAID, NIH, DHHS (Bethesda, Maryland, USA)

HVTN Leadership Operations Center (LOC) operations

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

HVTN Statistical and Data Management Center (SDMC)

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

HVTN Laboratory Center (LC)

HIV diagnostic laboratories

HIV Sero-Molecular Laboratory-National Institute for Communicable Diseases (HSML-NICD) (Johannesburg, South Africa)

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

Endpoint assay laboratories

- Cape Town HVTN Immunology Laboratory (CHIL, Cape Town, Africa);
- South African Immunology Laboratory-National Institute for Communicable Diseases (SAIL-NICD, Johannesburg, South Africa)

- Duke University Medical Center (Durham, North Carolina, USA)
- Fred Hutch/University of Washington (Seattle, Washington, USA)
- The Ertl Lab at the Wistar Institute Vaccine and Immunotherapy Center (Philadelphia, Pennsylvania, USA)

Study sites

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

Safety monitoring

HVTN 139 PSRT; HVTN Safety Monitoring Board (SMB)

1.1 Protocol Team

Protocol leadership

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Project coordinator	Haven Wilvich HVTN LOC, Fred Hutch

2 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 International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) and/or other 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, in accordance with Good Participatory Practices (GPP) and all local and national guidelines.

- 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. If a program for antiretroviral therapy (ART) provision is not available at a site and ART is needed, a privately established fund will be used to pay for access to treatment to the fullest extent possible.

- 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.

3 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.

This trial is being conducted in countries outside of the United States, with funding from the US NIH among others. Due to this, the trial is subject to both US and local regulations and guidelines on the protection of human research subjects and ethical research conduct. Where there is a conflict in regulations or guidelines, the HVTN strives towards maximum protection of human research participants.

In compliance with international and local (as appropriate) ICH and/or other GCP guidelines, each research location has a locally-based Principal Investigator (PI) who is qualified to conduct (and supervise the conduct of) the research. The investigators take responsibility for the conduct of the study and the control of the study products, including obtaining all appropriate regulatory and ethical reviews of the research.

3.1 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 persons assigned female at birth); and (f) providing safety monitoring.

3.2 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.

3.3 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.

3.4 Appropriate informed consent

45 CFR 46.111 (a) 4 and 5 and 21 CFR 56.111 (a) 4 and 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). As part of entering the HVTN, each site is provided training in informed consent by the HVTN and is required to have an SOP on the informed consent process. The HVTN requires a signed consent document for documentation, in addition to chart notes or a consent checklist.

3.5 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 LOC and routinely by the HVTN 139 Protocol Safety

Review Team (PSRT). In addition, the HVTN Safety Monitoring Board (SMB) periodically reviews study data.

3.6 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 they/their 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 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. In some cases, a comparable confidentiality agreement process may be acceptable. 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.

4 Background

4.1 Rationale for trial concept

Adenovirus ('Ad' for short) vectors have been tested extensively, are well tolerated at immunogenic doses, induce very potent cellular responses, and are able to elicit potent and sustained humoral responses to a wide variety of transgene inserts. Nevertheless, pre-existing neutralizing antibodies (nAbs) to human serotypes of Ad vectors can impair immune responses to the transgene product to which a protective response is sought. This protocol will study replication-deficient Ad vectors derived from simian serotypes (AdC vectors), as a strategy of circumventing any pre-existing nAbs. Derived from Ad viruses isolated from a chimpanzee and known as AdC6 and AdC7 vectors (1), these products provide a novel approach to overcoming reduced vaccine efficacy and immunogenicity due to pre-existing nAbs. This trial is designed to test the safety and tolerability of two of these vectors, while also assessing immunogenicity using a relevant HIV-1 insert, gp140 of subtype C. The specific overall goal of this proposed trial is therefore to test two Ad vectors derived from chimpanzee serotypes 6 (AdC6) and 7 (AdC7) for safety and immunogenicity for the first time in humans.

Adenoviral vectors have been used extensively as a vaccine platform for HIV-1. Human Ad serotype 5 (AdHu5) was tested as a single vaccine modality given repeatedly in the STEP/Phambili trials and combined with a DNA vaccine prime in HVTN505; both trials were designed to test for vaccine efficacy. The former vaccine was designed to induce T-cell responses to HIV-1, the latter to induce T- and B-cell responses. Neither trial achieved protection against HIV-1 acquisition or lowering of peak or setpoint viral loads in vaccinated individuals with breakthrough infection. In the STEP trial, uncircumcised male individuals with pre-existing AdHu5-specific nAbs showed a significant increase in HIV-1 acquisition rates (2).

Any theoretical risk of HIV acquisition related to the adenoviral vector (as observed in the STEP/Phambili trials) is mitigated by several important factors in the current protocol: 1.) The AdC6 and AdC7 vectors in this trial are biologically different from AdHu5 and derived from chimpanzees; 2.) .) this trial will enroll individuals at low risk for HIV infection; and 3.) the majority of humans lack nAbs to AdC6 and AdC7 viruses.

As indicated above, one of the critical problems of using human adenoviral vectors as a vaccine platform is pre-existing humoral immunity. Extensive studies have been done with human sera to assess for the presence and titers of nAbs to AdC6 and AdC7 in comparison to human serotypes Ad vectors, eg, serotypes 5 (AdHu5), and 26 (AdHu26) (3-5). These data show that nAbs to AdC viruses are rare in humans and people who carry such Abs generally have low titers. In contrast, nAbs to AdHu5 or AdHu26 are common in people living in developing

countries, and nAb titers to either of these viruses exceed those to the AdC viruses (3-5). T cells, as well as binding non-neutralizing Abs, cross-react between different Ad vectors of human or simian origin (6) as they are phylogenetically closely related (7), but neither affects vaccine immunogenicity (8-11). As we have shown (1), rhesus macaques (RMs) with pre-existing immunity to heterologous Ad viruses can readily be protected against chronic SIV infection by AdC vector immunization (see Section 4.9.1.7). AdC6 and AdC7 have not yet undergone clinical testing. As such they represent a novel vaccine platform. The combination of two distinct serotypes allows for fully circumventing any pre-existing nAbs including those elicited upon priming.

The rationale for this protocol is also informed by the goal to combine the two serologically distinct AdC vectors in a heterologous prime-boost regimen. Such combinations are expected to outperform homologous prime-boost regimens in which nAbs elicited against the vector after priming may impair immune responses to a subsequent boost. Studies in nonhuman primates showed that priming with AdC7 followed by a boost with AdC6 resulted in slightly better protection against SIVmac challenge (12) than the reverse sequence of AdC6 prime followed by an AdC7 boost.

It is unknown if the human immune system will mirror these results. In this phase 1 trial, human volunteers will therefore be primed with AdC6-HIVgp140 followed by a boost with AdC7-HIVgp140. The reverse sequence will also be tested (AdC7-HIVgp140 followed by a boost with AdC6-HIVgp140).

Another mechanism for circumventing pre-existing immunity to the vector is to boost with a heterologous immunogen, such as a recombinant protein. Studies in mice showed that a recombinant protein boost given to AdC7-gp140-immunized animals induces higher antibody responses than a prime with protein followed by an AdC7gp140 boost (13). We have additional evidence that the inclusion of a protein boost after sequential AdC priming (similar to the proposed regimen) leads to antibodies directed to the V1V2 region that were identified as a correlate of immunity in RV144 (14, 15). To maximize the potential immunogenicity of the regimen, we have included an additional boost with the GLA-SE adjuvanted CH505TF gp120 protein.

AdC6 and AdC7 belong to the subfamily E of Ad, such as Ad of human serotype 4 (which has been used as a vaccine platform in human trials (16, 17)). AdC vectors readily induce innate immune responses (18, 19) and thereby do not require addition of adjuvant.

We developed recombinant AdC6 and AdC7 vectors expressing Env of HIV-1 clade C. Du422 and Du172 were chosen as inserts because they are early infection clade C African isolates. The protein used for boosting is derived from yet another clade C isolate. We intentionally are using heterologous clade C Env immunogens to focus the immune response onto epitopes that are conserved between different HIV-1 clade C isolates.

HVTN 139 is a protocol designed to test whether a series of novel replication incompetent Chimpanzee adenoviruses could be advanced as a vaccine platform in the context of HIV. Pre-existing immunity to adenoviral vectors is associated with diminished immune responses and therefore the protocol is designed to test two chimpanzee adenoviruses (AdC6 and AdC7) given as a heterologous sequence. Evaluating the immune response to AdC6 and AdC7 vectors expressing clade C derived HIVgp140 Env followed by administration of CH505TF gp120 Env protein is directly relevant to the South African population where the major circulating clade is subtype C. A vaccine based upon a Chimpanzee adenoviral vector from a different family (Chimpanzee Adenovirus 25) has recently had success within the context of COVID-19, as the ChadOx1 vaccine was recently licensed in the UK for the prevention of SARS-CoV-2 infection.

4.2 AdC6-HIVgp140

The E1- and E3-deleted viral molecular clone of AdC6 was constructed by assembling gene fragments generated by restriction enzyme digest or PCR from the purified DNA of the AdC6 virus (ATCC-VR-592) into the pNEB193 vector using conventional cloning techniques (see Table 4-1, (20). AdC6-HIVgp140 is an E1- and partially E3-deleted AdC6 vector expressing gp140 of subtype C Du422. The insert Du422 (GenBank number: AAL05330.1) was chosen because subtype C is the dominant strain that circulates in Africa and the virus represents an early isolate of subtype C viruses. The transgene was cloned into pShuttle, which contains the cytomegalovirus promoter and other regulatory elements and from there into the parent AdC6 molecular clones using routine cloning procedures (see Figure 4-1).

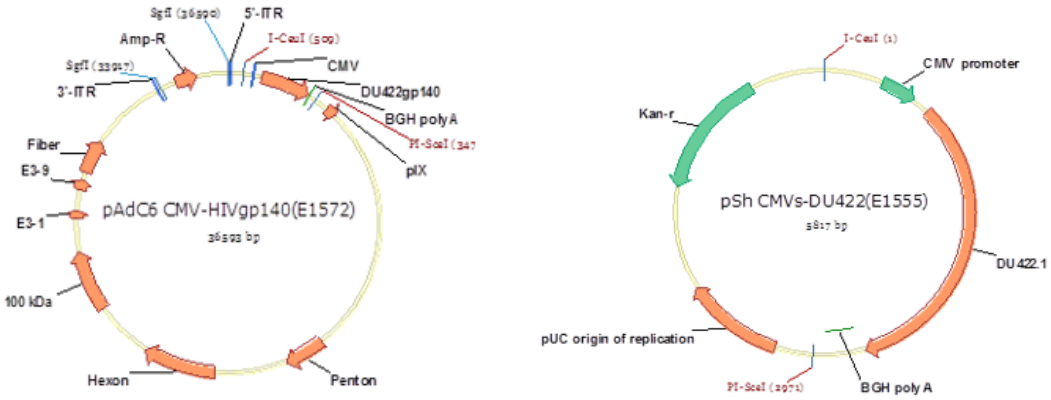

Figure 4-1 Maps of AdC6 recombinant virus (left) and AdC6-gp140Du422 pShuttle vector (right)

Table 4-1 Nucleotide Numbers of the Deleted Portion of E1 and E3 Domain in the AdC6 Vector

Vector Name	Nucleotide Numbers of E1 Deleted Region*	Nucleotide Numbers of E3 Deleted Region*

AdC6-HIVgp140	455 – 3022 bp	27835 – 31052 bp

Note: * Nucleotide numbers are based on GenBank accession numbers that are AY530877 for AdC6 (wildtype) virus

The DNA plasmids encoding the viral vectors were purified by 3 rounds of phenol/chloroform extraction prior to transferring to SAFC Pharma Carlsbad (Carlsbad, California) for manufacturing the vaccines. The vaccine is vialed in 2.5% Glycerol / 25 mM NaCl / 20 mM TRIS, pH 8.0. The vaccine was filled by IDT Biologika (Rockville, MD).

4.3 AdC7-HIVgp140

AdC7-HIVgp140 is an E1- and partially E3-deleted AdC7 vector expressing gp140 of subtype C Du172. The parent virus was obtained from ATCC (AdC7 – ATCC-VR-593). To create an E1- and E3-deleted viral molecular clone of AdC7 and then a recombinant virus expressing gp140, a similar cloning strategy as described above for AdC6 was adopted. (see Table 4-2 and Figure 4-2).

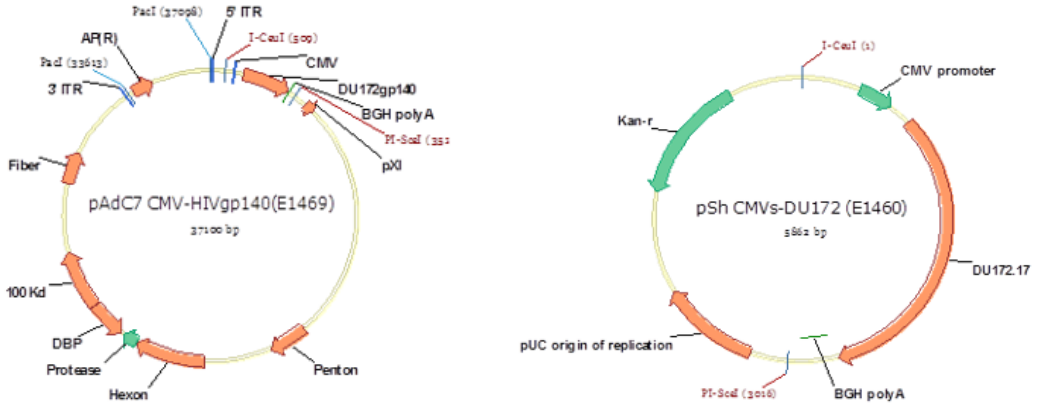

Figure 4-2 Maps of AdC7 recombinant virus (left) and AdC7-gp140Du422 pShuttle vector (right)

Table 4-2 Nucleotide Numbers of the Deleted Portion of E1 and E3 Domain in the rAdC Vectors

Vector Name	Nucleotide Numbers of E1 Deleted Region*	Nucleotide Numbers of E3 Deleted Region*
AdC7-HIVgp140	455 – 3028 bp	27775 – 31298 bp

Note: * Nucleotide numbers are based on GenBank accession number AY530878 for AdC7 (wildtype) virus

Manufacturing of the recombinant vector (AdC7-HIVgp140) was performed at SAFC Pharma Carlsbad (Carlsbad, California). The vaccine is vialed in 2.5% Glycerol / 25 mM NaCl / 20 mM TRIS, pH 8.0. The vaccine was filled by IDT Biologika (Rockville, MD).

Both serotypes can be grown in cells transformed with E1 region of AdHu5 (such as HEK 293 cells). Differences in the E1 flanking region between E1 of AdC viruses and E1 of AdHu5 prevents outgrowth of replication competent Ad, which can be a problem upon growth of AdHu5 vectors on HEK 293 cells.

Vectors were tested by western blotting of transduced cells and shown to express high levels of gp140. Genetic stability testing showed that the genomes of both AdC6 vectors expressing Du422 and AdC7 vectors expressing Du172 were stable after 12 sequential passages.

4.4 CH505TF gp120

CH505TF gp120 protein is the transmitted/founder (TF) gp120 Env isolated from the HIV-infected African individual CH505 by Bart Haynes's group (21). CH505TF is the first immunogen of the CH505 sequential envelope vaccine (EnvSeq-1) made up of 4 sequential Env gp120 components: the transmitted/founder (TF) CH505 Env (CH505TF gp120), the week 53 CH505 Env (CH505w53 gp120), the week 78 CH505 Env (CH505w78 gp120), and the week 100 gp120 CH505 Env (CH505w100 gp120).

CH505TF gp120 adjuvanted with GLA-SE is currently being evaluated in HVTN 115 and HVTN 123 (see Section 4.10.2).

4.5 GLA-SE adjuvant

GLA-SE is a synthetic TLR4 agonist formulated in a stable nano-emulsion of squalene oil and promotes a robust Th1-type immune response to vaccine antigens (22, 23). GLA-SE has been tested in over 2700 individuals in phase 1 and 2 clinical trials with a variety of antigens, including CH505TF gp120 in Part A of HVTN 115 and HVTN 123 (see Section 4.10.2). No significant safety concerns were reported. Previous clinical trial experience with GLA-SE and other antigens is described in Section 4.10.4.

The GLA-SE adjuvant has been used extensively in human phase 1 trials for influenza and leishmaniasis vaccines (24, 25). 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).

CH505TF gp120 combined with 10 mcg GLA-SE adjuvant has been administered to 36 participants in HVTN 115 Part A and 30 participants in HVTN 123. Based on the interim safety data (see Section 4.10.2) and immunogenicity data from 2 weeks after the 3rd immunization in HVTN 115 Part A, the dose for the protein vaccines in Part B will be 400 mcg. HVTN 115 Part A reached full enrollment with 42 participants in May 2018. As of January 27, 2020, vaccinations are complete and the study remains blinded.

4.6 Trial design rationale

With evidence that limitations in efficacy of human-derived Ad vector-based vaccines may be related to pre-existing nAbs to human Ad serotypes, this trial is designed to test safety and tolerability of two distinct replication-defective Ad vector vaccines derived from chimpanzee Ads. The trial is designed with a one-dose vaccine regimen given into two intramuscular sites that provides for safety assessments prior to proceeding with second priming and a protein boost. Specifically, the two low dose groups (1 x 10^{10} vp) in Part A will receive a single dose of vaccine. As this is the first time AdC will be tested in humans, there are two planned enrollment pauses to ensure that it is safe to proceed to a higher dose (see Table 1-1 Schema). There is an additional planned enrollment pause with interim safety review prior to full enrollment in Part B (high dose).

Group 4 and Group 5 will be given one dose of 5 x 10^{10} vp AdC6-HIVgp140 or AdC7-HIVgp140, respectively, and will be distributed equally between two injection sites in the same arm. Three months later Group 4 will be injected with one dose of 5 x 10^{10} vp of AdC7-HIVgp140 given to two sites in the same arm, and Group 5 will be injected with one dose of 5 x 10^{10} vp of AdC6-HIVgp140 given to two sites in the same arm. Groups 4 and 5 will then receive (CH505TF gp120/GLA-SE) 6 months after the initial AdC6-HIVgp140 or AdC7-gp140 injection. Injections of CH505 TF gp120/GLA-SE will be in the thigh..

4.6.1 Dose (amount and number)

Part A

Group 1: Dose is 1 x 10^{10} vp of AdC6-HIVgp140

Group 2: Dose is 1 x 10^{10} vp of AdC7-HIVgp140

Group 3: Placebo control

While the AdC6-HIVgp140 and AdC7-HIVgp140 have not been tested in humans, chimpanzee adenoviral vectors have been used as a vaccine platform in a variety of clinical trials (see Table 4-7). The dose of viral particles selected as the initial dose in Part A are as low as any dose selected for other chimpanzee adenoviral vectors.

Part B

Group 4: Dose is 5 x 10^{10} vp of AdC6-HIVgp140 followed by 5 x 10^{10} vp of AdC7-HIVgp140 (month 3) and 400 mcg CH505TF with 10 mcg GLA-SE (month 6)

Group 5: Dose is 5 x 10^{10} vp of AdC7-HIVgp140 followed by 5 x 10^{10} vp of AdC6-HIVgp140 (month 3) and 400 mcg CH505TF with 10 mcg GLA-SE (month 6)

Group 6: Placebo control

The target dose selected for immunogenicity analysis is in keeping with targeted doses of other chimpanzee adenoviral vectors (Table 4-7). The dose mirrors preclinical immunogenicity studies performed in non-human primates (see Section 4.9.1 below).

4.6.2 Schedule

The primary objective of this study is safety. Part A begins with a low dose of both products and includes two planned safety reviews prior to dose escalation. These reviews include an enrollment limit of 1 participant per day for the first 5 participants receiving either AdC6-HIVgp140 or AdC7-HIVgp140 because the products are very similar structurally and manufactured via nearly identical processes (please see the Investigator's Brochure (IB) for more information); a second review occurs after all 12 participants have been enrolled in Part A. An additional safety review will occur after 12 participants have received the higher dose of AdC vaccines in Part B.

The schedule in Part B is designed to also test the immunogenicity of a heterologous prime with different adenoviral vectors followed by a protein boost. It has been designed to mimic preclinical studies that demonstrate that a late vaccination with recombinant protein leads to increased antibody titers (see Section 4.9.2 below).

4.6.3 Prime-boost regimen

Participants in Part B will receive a prime boost vaccine regimen consisting of two Ad vector primes followed by a protein boost with CH505TF.

4.6.4 Choice of control

Control for AdC6-HIVgp140 or AdC7-HIVgp140, Sodium Chloride for Injection, 0.9%

Control for CH505TF gp120/GLA-SE adjuvanted protein: Sodium Chloride for Injection, 0.9%

4.7 Plans for future product development and testing

Our long-term goal is to combine Env-expressing AdC vectors with AdC vectors expressing Gag for induction of cellular and humoral immune responses. Furthermore, to enhance immune responses we will combine the AdC7gag and AdC6gag vector mixtures in a prime-boost regimen. If the current study reveals promising immune responses, boosting with alternate recombinant HIV proteins would be natural follow-on studies. For example, we conducted studies with a trimeric Env protein (with the US Military HIV Research Program) used for priming or boosting of AdC vector-induced immune responses and our data showed that such regimens induce neutralizing antibodies in mice, especially if

the protein is used for boosting (13). Therefore, our long-term goal is to combine AdC vectors expressing Gag and Env of HIV-1 which are followed by protein vaccines.

4.8 Preclinical safety studies

Table 4-3 Summary of preclinical safety studies

Study number	Product	Type of study	Animal	N	Dose groups	Route	Schedule
S14487	AdC6-HIVgp140 or AdC7-HIVgp140	Toxicity and Biodistribution	New Zealand White Rabbits	5M, 5F/ group	Group 1: formulation buffer Group 2: AdC6-HIVgp140 (1 x 10^{11} vp) Group 3: AdC7-HIVgp140 (1 x 10^{11} vp)	IM	Day 1:one dose split equally
1726-031	CH505TF gp120/ GLA-SE	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

4.8.1 Toxicity and biodistribution study of AdC6-HIVgp140 and AdC7-HIVgp140 in rabbits

Toxicity studies in rabbits were conducted by Sinclair Research SRC Study No. S14487. The study contained 3 cohorts to assess early toxicity, toxicity after recovery, and extended biodistribution. Each cohort had three groups of 5 male and 5 female animals each, which received either placebo (formulation buffer), 1 x 10^{11} vp of AdC6-HIVgp140 or 1 x 10^{11} vp of AdC7-HIVgp140 (see Table 4-3 and Table 4-4).

Table 4-4 Experimental study design, toxicity and biodistribution study in rabbits

Group	Dose Route	Treatment Dose Level (vp)[A]	Main Cohort[B]	Recovery Cohort[C]	Extended Biodistribution Cohort[D]
1	IM	Vehicle Control	5M + 5F	5M + 5F	5M + 5F
2	IM	1 x 10^{11} AdC6-HIVgp140	5M + 5F	5M + 5F	5M + 5F
3	IM	1 x 10^{11} AdC7-HIVgp140	5M + 5F	5M + 5F	5M + 5F

A All animals were IM-administered 0.84 mL (two sites of ~ 0.42 mL) of their designated treatment. (Study Day (SD) 1). The total administered dose was equally administered between two separate injection locations in the same hind limb (left) spaced ~2.5 cm apart.

B Main cohort euthanized four days after immunization (SD5).

C Recovery cohort euthanized 28 days after immunization (SD29).

D Extended biodistribution cohort 112 days after immunization (SD113).

The majority of finding during routine follow up were minor and indicative of a mild inflammatory reaction to the vaccines. There were no significant pathological findings in tissues or tissue sections in the main or recovery groups. Two animals died post-dosing due to respiratory distress following phlebotomy. Clinical observations in all animals were superficial and unrelated to test article. Draize scores were no higher than 2 for any animals except 4 control animals on SD3 through SD7. Slight to moderate decreases in food consumption were observed on SD2 and SD3 in both treated groups, returning to normal by SD4 and remaining normal thereafter. Some animals had random episodes of low food consumption and were supplemented as necessary. These findings are not unusual in the species. There was no impact on body weights or gains or losses, with no significant differences between the control and treated animals. There were no ocular abnormalities observed. At 3 h and one day post-dosing, treated animals had increased temperatures relative to the control group, returning to normal thereafter.

Several hematology parameters were changed in association with treatment including total white cells, neutrophils, lymphocytes, monocytes, eosinophils, and basophils, attributed to receipt of a vaccine and considered clinically insignificant. Changes in coagulation factors of prothrombin time (PT) and activated partial thromboplastin time (APTT) were mild and insignificant, while changes in fibrinogen were considered due to an inflammatory reaction of vaccination and considered clinically insignificant. Serum chemistry changes included albumin to globulin ratio (A/G) and globulin, which are typical following vaccination. Changes in creatinine kinase (CK) were considered to be due to intramuscular injections, were mild, and considered to be clinically insignificant. No differences were observed in urinalysis. Immunogenicity was shown by ELISA at SD29 (Figure 4-3), demonstrating that all treated animals responded positively to gp140, the transgene, post-vaccination, showing they were immunized with vaccine. Control animals did not have increased values at post-dosing timepoints (data not shown). These results verify dosing.

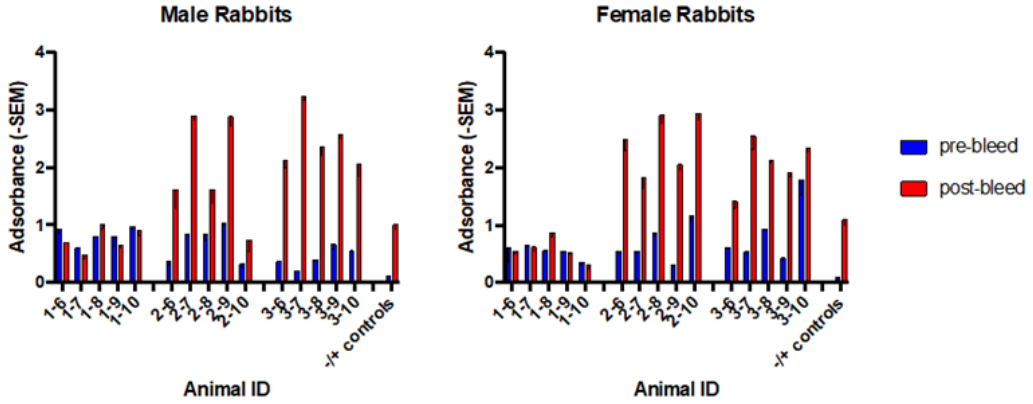

Figure 4-3 Anti-Ad antibodies in rabbits after single AdC6-HIVgp140 or AdC7-HIVgp140 vectors. Antibody titer in rabbit serum measured by ELISA (1:100 serum dilution) in rabbits immunized with the Chimp Ad vectors. Data obtained with pre-immunization sera are shown in blue, data obtained with sera collected 28 days after immunization are shown in red. In all animals of groups 2 and 3 differences between pre- and post-immunization sera are statistically significant by multiple type 1 error corrected student t-test.

Gross findings were not considered to be related to vaccination. Organ weights in group 2 females in the main cohort included adrenal glands that were statistically significantly decreased (28%) compared to group 1 females, as were adrenal to body (22%) and adrenal to brain (28%) weight ratios. These differences were not observed in the recovery cohort. However, in the recovery cohort, thymus gland weights were slightly increased compared to controls in group 2 females, as were thymus to body and thymus to brain weight ratios. These were not associated with microscopic lesions. The only relevant histopathological finding in the main or recovery cohorts were injection site local inflammation seen in the treated animals, as would be expected from vaccination and were considered normal reactions to injections.

Biodistribution analyses were performed on the following tissues on SD5: lungs, liver, spleen, kidneys, bone marrow from the sternum, heart, testes, ovaries, injection site muscle, inguinal lymph nodes, brain (cerebellum), and blood. Positive tissues were tested from cohorts at SD29 and SD113. Copy numbers are presented per mg of tissue. Results showed that some vector DNA or RNA derived from transcription of the vector could be detected at some sites on SD5, SD29, and SD113 (4, 28, and 112 days after vector injection, respectively) (Figure 4-4). However, the number of animals with positive tissues and the copy numbers were decreasing throughout the study. Specifically, on SD5 low levels of DNA were detected in spleens of group 2 (10/10 animals, group mean 82.57 copies) and group 3 (10/10 animals, group mean 332 copies), testis of group 3 (1M [male]/5 animals, copy number of 756.12), ovaries of group 3 (1F [female]/5 animals, copy number of 228.74), injection site of group 2 (3F [female], group mean of 97.08 copies) and group 3 (9/10 animals, group mean of 2001.83), lung of group 3 (1F with 21.72 copies), liver of group 3 (7/10 animals, group mean 33.25 copies), and bone marrow of group 3 (2M). By SD29, vector DNA was detected in spleen of 10/10 animals of group 2 (group mean of 38.64) and 1M and

1F animal each of group 3 (copy numbers of 25.63 and 22.17, respectively, resulting in a group mean less than the LOD of the assay), as well as at the injection site in group 2 in 1F animal (5.51 copies) and in inguinal lymph nodes in group 2 in 1F animal (7.00 copies). By SD113, DNA was cleared from most animals with residual levels present as follows: spleen of 2F of group 2 (group mean below the limit of detection of the assay) and 8/10 animals of group 3 (group mean of 34.45 copies), liver of 8/10 animals of group 3 (group mean of 40.07 copies), and injection site of 2/10 animals of group 3 (group mean below assay LOD).

On SD5, low levels of RNA were detected as follows: liver of group 2 (5/10 animals, group mean of 18.85 copies) and group 3 (8/10 animals, group mean of 53.77 copies), spleen of group 2 (10/10 animals, group mean of 260.40) and group 3 (5/10 animals, group mean of 11.49), testes of group 2 (3/5 M, group mean of 10.50 copies), ovaries of group 2 (1/5 F with copy number of 29.45), injection site of group 2 (9/10 animals, group mean of 5942.27 copies) and group 3 (6/10 animals, group mean of 601.81). By SD29 RNA was detected in liver of 1M animal of group 2 (77.43 copies) and 1M of group 3 (72.67 copies), in spleens of 8/10 animals of group 2 (group mean of 75.04) and 5/10 animals of group 3 (group mean of 43.82), at the injection site of 1F animal of group 2 (copy number of 14.09), in inguinal lymph nodes of 4/10 animals of group 2 (group mean of 157.65) and 1M animal of group 3 (47.65 copies). By SD113 RNA was detected in spleen of 3/10 animals of group 2 (group mean of 24.26), inguinal lymph node of 1M of group 2 (9.23 copies), and liver of 1M of group 3 (12.26 copies).

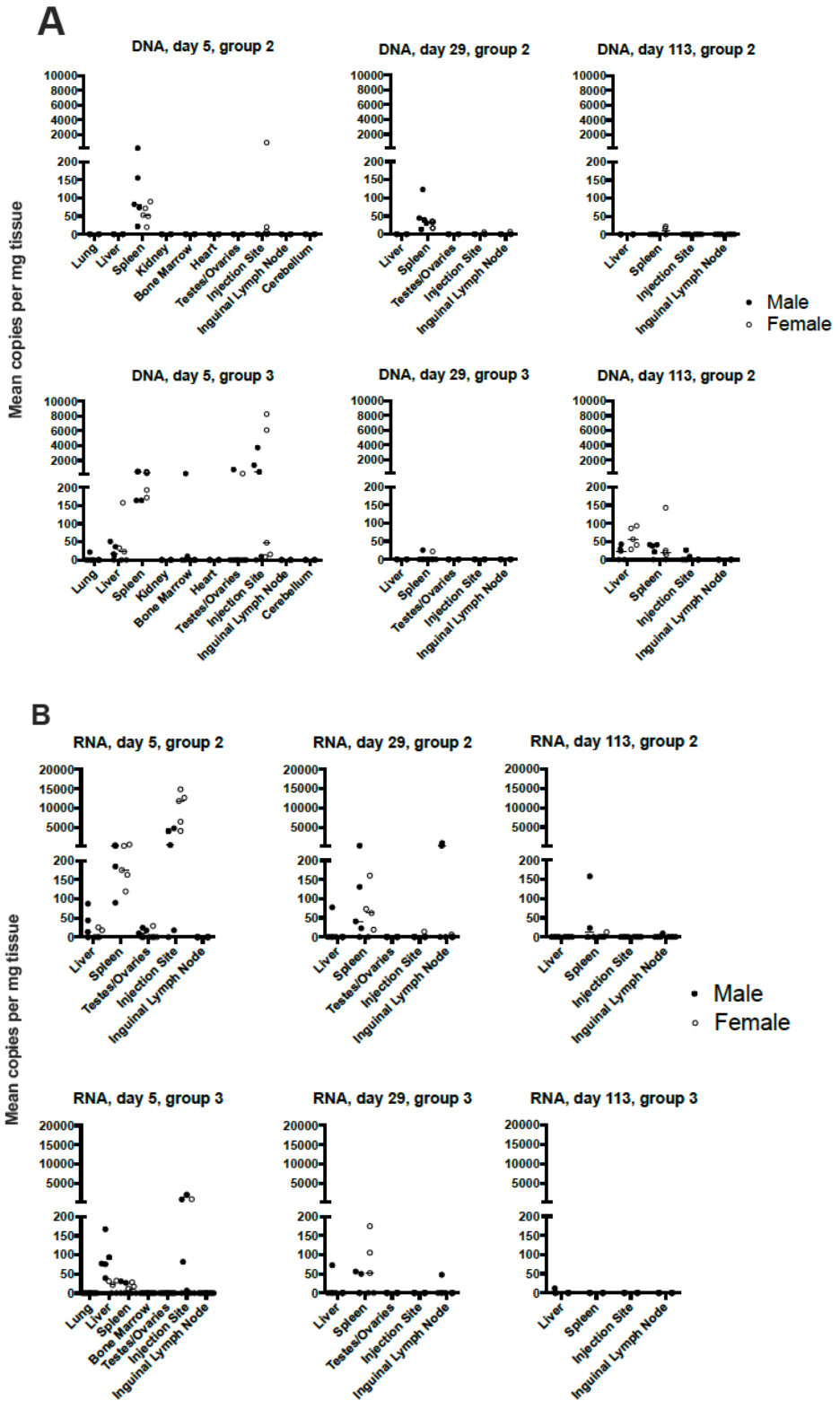

Figure 4-4 Vaccine-derived DNA (A) or RNA (B) tissue distribution. Mean copy number per mg of tissue shown for individual male (solid circles) or female (open circles) rabbits on days 5, 29 or 113 after vaccination with 10^{11} vp of AdC6-HIVgp140 (group 2) or AdC7-HIVgp140 (group 3) vectors.

4.8.2 Summary of a preclinical toxicity study of CH505TF gp120

Eighty New Zealand White Rabbits, 13-15 weeks in age, were randomly assigned to 1 of 4 groups (5/sex/group) as outlined in Table 4-3. Group 3 included a DNA vaccine proposed for use in another clinical trial. 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, with rotation 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 gp120/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 CH505TF gp120 with 20 mcg GLA-SE adjuvant with or without a 4 mg DNA 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.

4.9 Preclinical immunogenicity studies

Table 4-5 Summary of preclinical immunogenicity studies

Study number	Product	Animal	N	Dose groups	Route	Schedule	Assay
Study M1 (13)	gp145 protein + AdC6-HIVgp140 + AdC7-HIVgp140	mice	10M, 10F /group	Group 1: gp145 protein + 10^{10} vp AdC7-HIVgp140 Group 2: gp145 protein + 10^{9} vp AdC7-HIVgp140 Group 3: 10^{10} vp AdC7-HIVgp140 + gp145 protein Group 4: 10^{9} vp AdC7-HIVgp140 + gp145 protein Group 5: 10^{10} vp AdC7-HIVgp140 + 10^{10} vp AdC6 Group 6: 10^{9} vp AdC7-HIVgp140 + 10^{9} vp AdC6	IM	Group 1 and 2: 0, 4, 8, 12 weeks Group 3 and 4: 0, 8, 12, 18 weeks Group 5 and 6: 0 and 8 weeks	Gp140-, V2-specific Abs
Study M2	AdC6-HIVgp140 + AdC7-HIVgp140 + gp140 protein	mice	10M, 10F /group	Group 1: 10^{10} vp AdC7-HIVgp140 + 10^{10} vp AdC6-HIVgp140 + gp140 protein	IM	0, 8, 16 weeks	Gp140-, V1V2-specific Abs
NHP #1 (26)	AdC7gag37 + AdC6gag37 + AdHu5gag37	RM	4/group 2/control	Group 1: 10^{12} vp AdC7gag37 + 10^{12} vp AdC6gag37 + 10^{12} vp AdHu5gag37 Group 2: 10^{12} vp AdHu5gag37 + 10^{12} vp AdC6gag37 + 10^{12} vp AdC7gag37 Control: rabies virus glycoprotein	IM	0, 12, 24, 36 weeks	Gag-specific Abs
NHP #2 (8)	AdC6-HIVgag + AdC7-HIVgag AdHu5-HIVgag	RM	6/group 2/control	Group 1, 3: AdHu5-HIVgag pre-exposed Group 2, 4: AdHu5-HIVgag non pre-exposed Regimen 1: 10^{11} vp AdC6-HIVgag + 10^{11} vp AdC7-HIVgag + 10^{11} vp AdC6-HIVgag + 10^{11} vp AdC7-HIVgag (groups 1 and 2) Regimen 2: 10^{11} vp AdHu5-HIVgag + 10^{11} vp AdHu5-HIVgag Control: AdHu5rab.gp	IM	Regimen 1: 0, 32, 58 weeks Regimen 2: 0, 12 weeks	T-cell response Gag specific Ab

Study number	Product	Animal	N	Dose groups	Route	Schedule	Assay
NHP #3 (27)	AdC6-SIVgag + AdC7-SIVgag + MVASIVgagtat	RM	8/group; 8/control	10^{11}vp AdC6-SIVgag + 10^{11}vp AdC7-SIVgag + MVASIVgagtat Control: AdHu5rab.gp (2), no vector (6)	IM	0, 32, 58 weeks	Gag-specific T cells
PSIV #1 (12)	AdC6-SIVgag/tat + AdC7-SIVgag/tat	RM	10/group; 10/control	Group 1: AdC6-SIVgag/tat+AdC7-SIVgag/tat Group 2: AdC7-SIVgag/tat+AdC6-SIVgag/tat Group 3: control	IM	Ad vector: 0, 24, SIVmac239: 42 weeks, 1 dose/2wks x 15	T-cell response Viral loads
PSIV #2 (1)	Vectors: AdC6, AdC7 AdH5, AdHu26 Inserts: SIV Gag and gp140	RM	12/group	All groups: AdHu control pre-exposure Group 1: AdC7-gag/gp140 + AdC6-gag/gp140 Group 2: AdHu26-gag/gp140 + AdHu5-gag/gp140 Group 3: control	IM	Ad vector:0, 4, 28, SIVmac239: 52 weeks, 1 dose/week x 10	T-cell response Viral loads

F: female; M: male; IM: intramuscular; RM: rhesus macaques.

4.9.1 Immunogenicity and SIV protection studies of AdC6 and AdC7 vectors expressing HIV-1 or SIV proteins

We conducted a number of immunogenicity studies in mice and nonhuman primates. Antibody responses were tested using prime boost regimens.

4.9.1.1 Study M1: Immunogenicity of AdC6-HIVgp140 and AdC7-HIVgp140 vectors with and without boosting with gp145 protein in mice

This study has been published (13). Results show that priming with AdC7-HIVgp140 induced HIV-1 Env-specific antibody responses, which further increased after a subsequent boost with AdC6-HIVgp140 or a partially trimeric clade C protein. Multiple booster immunizations with the alum-adjuvanted protein did not enhance antibody titers beyond those reached after the 1st protein boost. The Ab response was primarily composed of IgG2a and IgG2b isotypes, indicative of responses driven by type 1 T helper (Th1) cells.

To assess Ab function, we performed TZM-bl neutralization assays against three tier 1 pseudoviruses (PVs), MW965 (subtype C), GS015 (subtype C), and TH023 (CRF01_AE), and one tier 2 PV, TZBD 9/11 (subtype C). A PV containing the Env from murine leukemia virus (MuLV) was tested as a non-specific control. Sera from mice that had been boosted neutralized tier 1 but not tier 2 viruses. There was a trend towards higher titers of nAbs in mice primed with AdC7-HIVgp140 and boosted with protein compared to mice that were sequentially immunized with the two AdC vectors.

The results from the RV144 Thai trial showed that Abs targeting the V2 region of gp120 correlated with decreased risk of HIV-1 acquisition. Anti-V2 Abs were detected in all vaccine groups.

Conclusions: Priming with AdC followed by a protein boost or sequential immunizations with two AdC vectors induced HIV-1 Env-specific binding Abs including those to the V2 region as well as Abs that cross-neutralized tier 1 HIV-1 from different subtypes.

4.9.1.2 Study M2: Immunogenicity of AdC6-HIVgp140 and AdC7-HIVgp140 vectors with and without gp140 protein boost in mice

In an unpublished study in mice (Study M2) we tested if a prime-boost regimen with AdC vector and protein induced antibodies to V1V2. We primed mice with 10^{10} vp of AdC7-HIVgp140, boosted them 8 weeks later with the same dose of AdC6-HIVgp140 and then at week 16 of the study, boosted them with an alum-adjuvanted baculovirus-derived gp140 protein of Du172. Mice were tested for antibodies to gp140 as above and for antibodies to the V1V2 loop on gp70-V1V2, a structurally confined V1V2 of a consensus clade C sequence on a scaffold protein composed of murine leukemia virus (MLV) glycoprotein which was also used to assess antibody responses in the RV144 trial. As shown in Figure 4-5 A, mice developed robust responses to gp140 after the boost with AdC6-HIVgp140, which only slightly increased after the protein boost. The protein boost achieved a marked increase in antibodies to the V1V2 sequence (Figure 4-5 B. Most importantly, although all regimens induced long-lasting responses to gp140 (> 1 year, Figure 4-5 C) responses to the V1V2 loop only remained most significant in groups that have been primed with the AdC7-HIVgp140 vector, boosted with the AdC6-HIVgp140 vector, and then received a final boost with the gp140 protein (Figure 4-5 D).

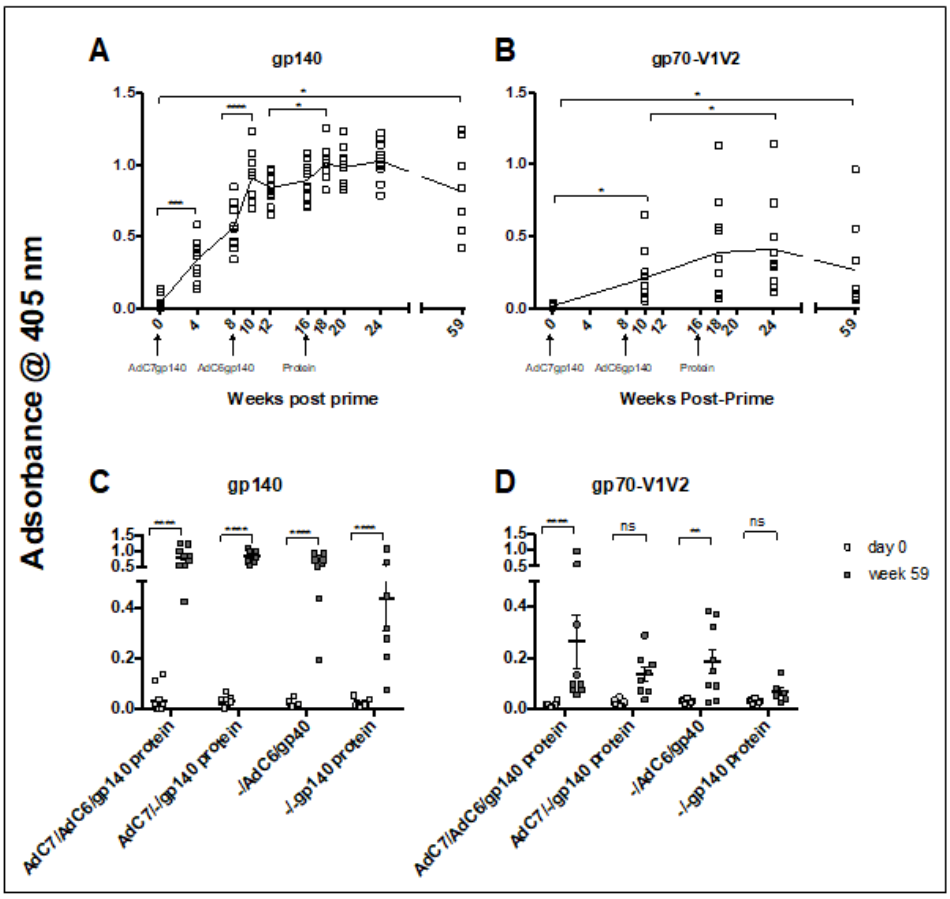

Figure 4-5 Induction of antibodies to V1V2. Mice were primed with 10^{10} vp of AdC7-HIVgp140. Eight weeks later they were boosted once with 10^{10} vp of AdC6-HIVgp140 and then after an additional 8 weeks they were boosted with a clade C gp140 protein. Animals were bled before and after vaccination and sera were tested by ELISA for antibodies to gp140 clade C (A, C) or gp70-V1V2 (B, D). The graphs show adsorbance at a 1:100 serum dilution. (A, B) show responses over time, (C, D) compare responses at baseline and at week 59 after priming. Significant differences were calculated by 2-way Anova (A, B) or multiple t-tests (C, D). Stars above line indicate degree of significance (*) – p >0.05, (**) p > 0.01, (***) p >0.001, (****) p > 0.0001.

In summary, these data support the notion that induction of HIV-1 envelope-specific antibodies that are high in magnitude, sustained over time, recognize crucial residues such as the V1V2 loop, and achieve cross-specific neutralization can best be achieved by multiple sequential immunizations.

We conducted several studies in rhesus macaques (RM) to assess the efficacy of AdC vector immunization in inducing immune responses and providing protection against SIVmac challenges.

4.9.1.3 NHP #1: Immunogenicity of AdC7gag37 and AdC6gag37 vectors in NHPs

In the NHP #1 study (26) two groups of 4 NHPs each were immunized with 10^{12} vp of AdC7gag37 (a truncated form of gag), followed 8 months later by an injection of 10^{12} vp of AdC6gag37, and after an additional 4 month by a final boost with 10^{12} vp of AdHu5gag37. The 2nd group of 4 NHPs received the same

vectors but in the reverse order. Control animals were immunized with vectors expressing the rabies virus glycoprotein. Results showed that gag-specific antibody responses markedly increased after booster immunizations, demonstrating that AdC can serve as a vaccine platform capable of inducing robust humoral responses (26).

4.9.1.4 NHP #2: Immunogenicity of AdC6-HIVgag and AdC7-HIVgag vectors in NHPs

In a second NHP study (NHP#2, (8)) we compared two vaccine regimens based on sequential use of AdHu5 vectors or two different chimpanzee-derived Ad vectors (AdC6 and AdC7) expressing HIV-1 gag in rhesus macaques that were AdHu5 seropositive or seronegative at the onset of vaccination. Our results show that heterologous booster immunizations with the chimpanzee-derived Ad vectors induced higher T- and B-cell responses than did repeated immunizations with the AdHu5 vector, especially in AdHu5-preexposed macaques (8).

4.9.1.5 NHP #3: Immunogenicity of AdC6-SIVgag and AdC6-SIVgag vectors in NHPs

In a third NHP study (NHP #3, (27)) rhesus macaques were vaccinated intramuscularly with AdC6 and then boosted intramuscularly with AdC7 both expressing Gag of SIVmac239. Animals were subsequently boosted intramuscularly with a modified vaccinia Ankara (MVA) virus expressing Gag and Tat of the homologous SIV before mucosal challenge with a high dose of SIVmac239 given rectally. Animals developed robust T-cell responses after the boosts and the breadth of the response increased.

4.9.1.6 PSIVP #1: Preclinical SIV protection study using AdC6-SIV gag/tat and AdC7-SIV gag/tat vectors

In the SIV protection study (12) we utilized two regimens of prime-boost immunizations with AdC6 and AdC7 expressing SIV Gag/Tat fusion protein to test their immunogenicity and ability to protect rhesus macaques (RMs) from a repeated low-dose SIVmac239 challenge. No significant protection from SIV transmission was observed in either AdC7/6- or AdC7/6-vaccinated RMs.

4.9.1.7 PSIVP #2: Preclinical SIV protection study using AdC6 and AdC7 vectors expressing Gag and gp160 of SIVmac239

We tested two vaccine regimens based on prime-boosting with AdC7 and AdC6, or two distinct human serotype Ad vectors, AdHu5 and AdHu26, expressing Gag and gp160 of SIVmac239 in a nonhuman primate model (1).

Role of pre-existing adenoviral immunity: To assess effects of pre-existing Abs to AdHu vectors, we first injected 36 Indian-origin RMs with AdHu vectors expressing an unrelated transgene. RMs of group 1, the AdC group, were vaccinated 4 weeks after the last AdHu exposure with 5×10^{10} virus particles (vp) of AdC7 expressing Gag (AdC7-gag) mixed with 5×10^{10} vp of AdC7 expressing

gp160 (AdC7-gp160). RMs of group 2, the AdHu group, were vaccinated with the same doses of AdHu26 vectors expressing the same inserts. Six months later RMs of the AdC7 and AdHu26 groups were boosted with AdC6 and AdHu5 vectors, respectively, expressing the same inserts and used at the same doses as the priming vectors. The control group was not vaccinated.

Antibody-dependent cell-mediated cytotoxicity responses are improved in absence of pre-existing immunity: Env-specific Ab responses were low post-priming, increased post-boost, and contracted by the time of challenges. Sera from MamuA*01/B17^{+} vaccinated RMs and from 6 control RMs were tested for antibody-dependent cell-mediated cytotoxicity (ADCC). In the AdC group most RMs scored positive while only 2/7 AdHu -vaccinated RMs showed low activity (<10% killing). Data for the AdC but not the AdHu groups were significantly different from those of the control group ($p = 0.019$ by 2-way Anova).

Cellular Responses: Both vaccine regimens induced Gag-specific CD4+ and CD8+T-cell responses. Frequencies of the two CD4+T-cell subsets were comparable at all timepoints.

Overall the NHP studies show that the AdC vectors are immunogenic and induce transgene product-specific T- and B-cell responses even in the presence of nAbs to human serotype Ad vectors.

4.9.2 Immunogenicity of the CH505 EnvSeq-1 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 unmutated common ancestors (UCAs) of lineages capable of binding HIV Envs with wild type sequences but not going into bnAb evolution is possible (Figure 4-6 and Figure 4-7). In this study of 12 monkeys, 11/12 monkeys had differential binding antibodies isolated and overall the Env differential binding antibodies (bind 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.

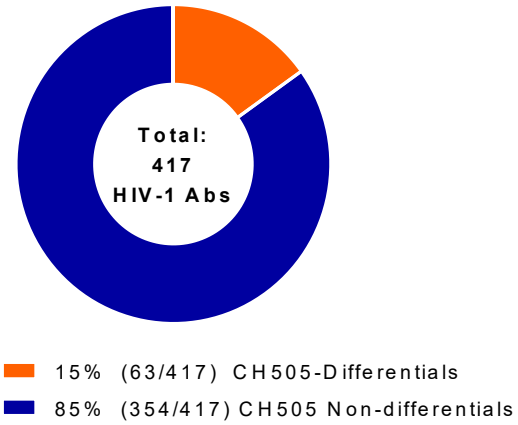

Figure 4-6 Binding specificities of 417 HIV-1 CH505 Env-induced antibodies isolated from rhesus macaques immunized with the CH505 4-valent sequential Envs (NHP79). Differential binders are antibodies that bind to the CH505 wild type gp120, but not the ΔI371 gp120, which is indicative of putative CD4-binding 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 4-7 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 1A 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 (ΔI371); 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 4-6). Of the 60 CD4-binding site antibodies, the Haynes team has isolated a CH505 gp120/CH505 gp120ΔI371 differential-binding antibody, DH522, which not only neutralized the tier 1A CH505 TF variant 4.3 but also neutralized 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 4-8). 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 4-9). The DH522 antibody was found to block the binding of soluble CD4 as well as CH103 Abs to CH505 Env, and also exhibited differential binding to CH505 Env versus the ΔI371/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 4-8 Neutralization profile of DH522 and other lineage members.

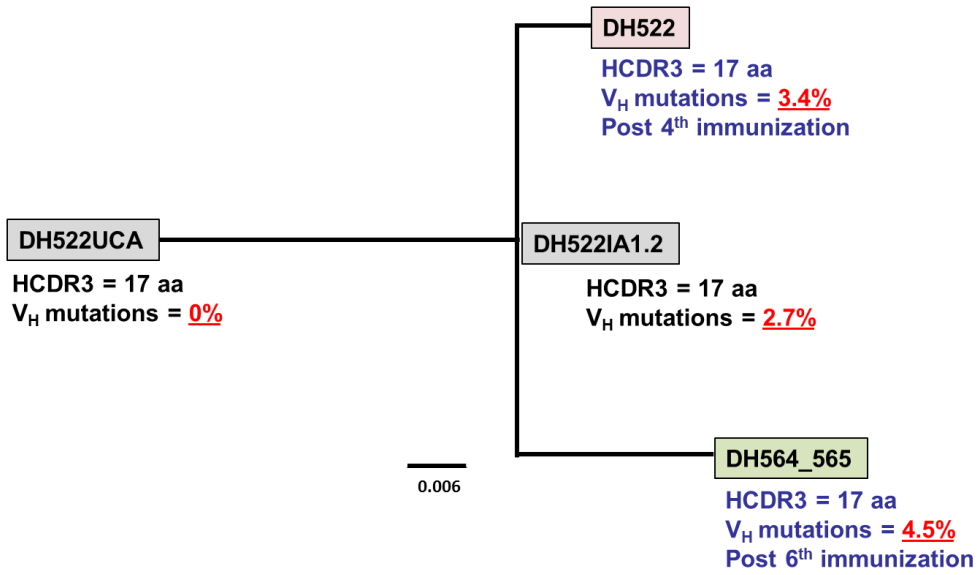

Figure 4-9 Three DH522 clonal lineage members from an NHP receiving the additive immunization regimen (NHP #79).

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 4-8).

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 4-10). 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 4-10 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 4-6).

Table 4-6 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 ΔI371; binds RSC3 but not RSC3ΔI371, 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	

Note: HCDR3: long heavy chain third complementarity determining regions

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 gp120 Env with each dose administered 3 times. Env binding antibody (Figure 4-11) and CD4-binding site antibody (Figure 4-12) were determined and the CH505TF 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 4-13) there was a linear increase in neutralization titers with increasing gp120 dose, after 3 immunizations, there was no statistical difference between the tier 1 antibody neutralizing titers (Figure 4-14).

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 mcg/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.

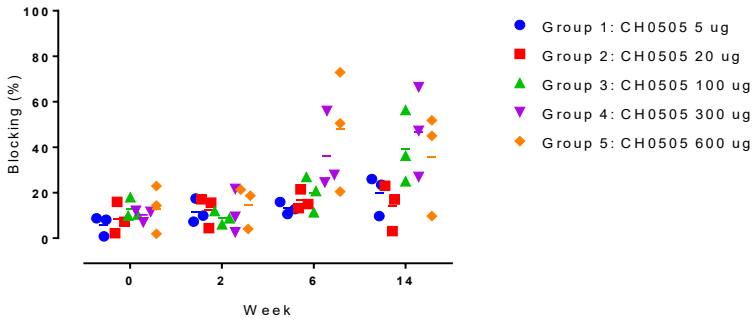

Figure 4-11 Inhibition of CH106 CD4bs binding to B.63521 gp120 Env by NHP 106 plasma from immunization with 5 different doses of CH505TF gp120.

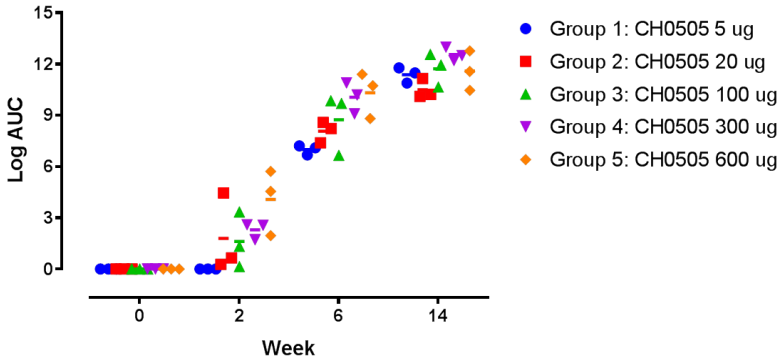

Figure 4-12. Binding of NHP 106 plasma to CHO-derived CH505TF gp120.

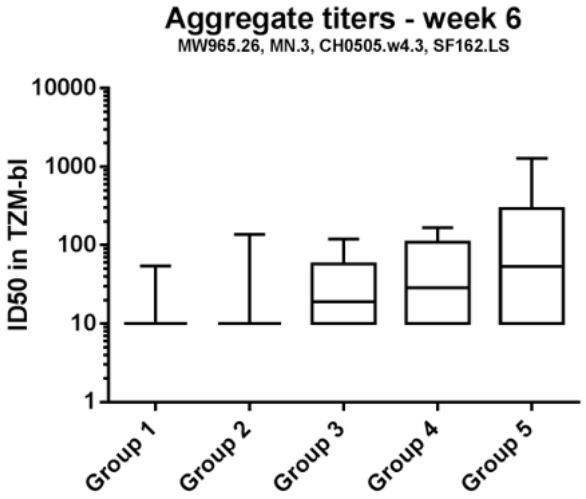

Figure 4-13 Tier 1 HIV neutralization in the TZM-Bl assay after 2 immunizations with CH505TF gp120 doses. Group 1, 5 mcg; Group 2, 20 mcg; Group 3, 100 mcg; Group 4; 300 mcg; Group 5, 600 mcg.

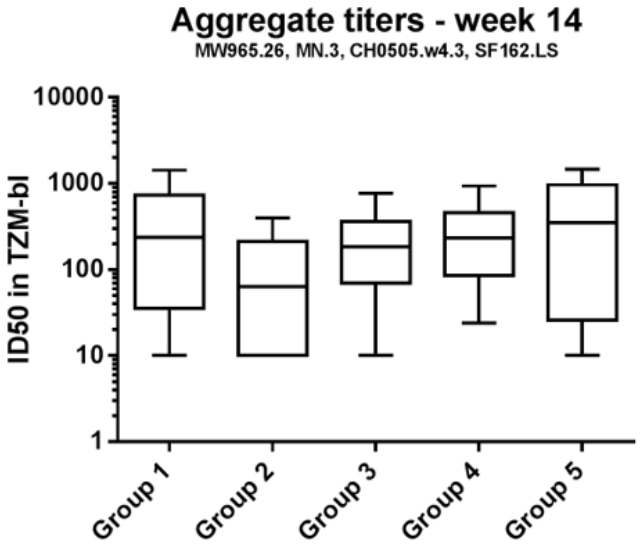

Figure 4-14 Tier 1 HIV neutralization in the TZM-BI assay after 3 immunizations with CH505TF gp120 doses. Group 1, 5 mcg; Group 2, 20 mcg; Group 3, 100 mcg; Group 4; 300 mcg; Group 5, 600 mcg.

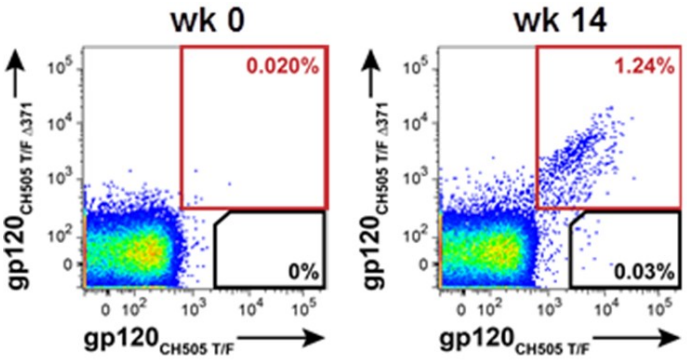

Figure 4-15 Gating strategy for B cell analysis. An example of NHP B-cell data shown for pre-immune and post third immunization samples. Few double positive (red gate gp120-reactive) or differential positive (black gate) cells are found in the pre-immune sample; both double positive and differential B cells are found two weeks after the third immunization.

4.10 Clinical studies

4.10.1 Clinical studies of Chimpanzee Ad vectors targeting diverse pathogens

The AdC6-HIVgp140 and AdC7-HIVgp140 vectors have not yet been tested in humans.

Chimpanzee Ad vectors of different serotypes (AdC63, AdC3) with inserts targeting other pathogens have been tested in human adults administered intramuscularly (see Table 4-7). None of the trials reported serious adverse events, even in trials with vectors used at higher doses than those planned in this trial.

Table 4-7 Summary of clinical trials that used chimpanzee Ad vectors of different serotypes

Vaccine	Doses (vp)	Number of subjects	Disease	Ref.
AdC63	5×10^9	8	Malaria	(28)
	5×10^{10}	8		
AdC3	2.5×10^{10}	5	Hepatitis C	(29)
	7.5×10^{10}	5		
AdC3	2.5×10^{10}	9	Hepatitis C	(30)
AdC3	1×10^{10}	10	Ebola	(31)
	2.5×10^{10}	35		
	5×10^{10}	20		
	1×10^{11}	11		
AdC63	1×10^{10}	4	Malaria	(32)
	5×10^{10}	8		
	2×10^{11}	10		
AdC3	2×10^{10}	10	Ebola	(33)
	2×10^{11}	10		
AdC63	5×10^{10}+Matrix-M 50 mcg	10	Malaria	(34)
	5×10^{10}+Matrix-M 50 mcg	10		
AdC63	5×10^9	3	Malaria	(35)

4.10.2 Clinical studies of CH505TF gp120 /GLA-SE vaccine

4.10.2.1 HVTN 115, Part A

The CH505TF gp120/GLA-SE vaccine has been administered at different doses in the HVTN 115 Part A trial. This was a dose escalation study, evaluating CH505TF gp120 /GLA-SE at protein doses of 20, 100, and 400 mcg adjuvanted with 10 mcg of GLA-SE adjuvant, respectively.

Table 4-8 Schema of HVTN 115, Part A

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)					

Based on the interim safety data and immunogenicity data from 2 weeks after the 3rd immunization in Part A, the dose for the protein vaccines in Part B will be 400 mcg, the same as in this study. HVTN 115 Part A reached full enrollment with 42 participants in May 2018. As of January 27, 2020, vaccinations are complete and the study remains blinded. Most participants reported no or mild local signs and symptoms after vaccine administrations. There have been 2 cases of severe (grade 3) erythema and induration, which resolved within days, and one case of severe (grade 3) pain. One of those participants experienced moderate injection site cellulitis, which resolved with oral antibiotics. Most participants reported no or mild systemic reactogenicity signs or symptoms after vaccinations. Two cases of severe (grade 3) headache were reported during the reactogenicity period. Beyond the reactogenicity period, there have been no severe adverse events related to study product.

4.10.2.2 HVTN 123

The HVTN 123 study is a double-blind, randomized clinical trial comparing the safety, tolerability and immunogenicity of CH505TF gp120 produced from stably transfected cells to CH505TF gp120 produced from transiently transfected cells. In this study CH505TF gp120 is used at 100 mcg dose in combination with 10 mcg GLA-SE.

HVTN 123 reached full enrollment with 30 participants in January 2020. As of 4 March 2020, vaccinations are ongoing, all 30 participants have received their 1st vaccination, 22 participants have received their 2nd vaccination and 13 participants have received their 3rd vaccination. The study remains blinded. Most participants experienced none or mild reactogenicity after vaccine administrations. There was no severe or potentially life-threatening reactogenicity. As of March 4, 2020, 17 mild and moderate adverse events have occurred in 10 of 30 participants. Of those, 1 moderate adverse event was designated as related to study product. This participant had grade 2 axillary lymphadenopathy that resolved by day 4 after vaccination.

Table 4-9 Schema of HVTN 123

Study arm	n	Month 0	Month 2	Month 6
Group 1	15	100 mcg CH505TF gp120 **Stable**	100 mcg CH505TF gp120 **Stable**	100 mcg CH505TF gp120 **Stable**
Group 2	15	100 mcg CH505TF gp120 **Transient**	100 mcg CH505TF gp120 **Transient**	100 mcg CH505TF gp120 **Transient**
Total	30			

4.10.3 Clinical studies of gp120 Env protein vaccines

Although CH505TF gp120 has already been evaluated in humans without identification of safety issues, there is additional 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 (36, 37). 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. The majority, if not all of the local and systemic reactions were deemed associated with adjuvant components of the vaccine rather than the Env proteins (38).

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 (39).

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 8,000 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 (40).

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, the most common being injection site pain/tenderness, but all of the symptoms were mild to moderate and self-limited. Moreover, while there were fewer reactogenicity events at the lowest dose, the safety profile in the 300 mcg and 600 mcg groups was similar (41). 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 reactogenicity (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 (42).

Taken together, the combined evidence from the large numbers of trial participants in these and other studies (43-47) 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.

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

GLA-SE is a synthetic TLR4 agonist formulated in a stable nano-emulsion of squalene oil and promotes a strong Th1-type immune response to vaccine antigens (22, 23). CH505TF gp120 has been tested in combination with 10 mcg GLA-SE in part A of HVTN 115, HVTN 123 and HVTN124 (see Section 4.10.2). There is considerable clinical trial experience with the GLA-SE adjuvant in combination with other antigens. These data are summarized below and in Table 4-10 and Table 4-11.

Over 2700 individuals have received at least one dose of the GLA-SE adjuvant at doses ranging from 0.5 to 20 mcg with no significant safety concerns identified to date. Please see the Investigator's Brochure (IB) for additional details of the clinical trials performed with GLA-SE.

In addition to HVTN 115, HVTN 123, and HVTN 124 five other studies have included the 10 mcg dose of the GLA-SE adjuvant, the same dose as proposed for this study. The first was an open-label phase 1 clinical trial conducted in Brazil with a *Schistosoma mansoni* antigen (Sm14) (48). Twenty healthy males received 3 IM doses of 50 mcg Sm14 + 10 mcg GLA-SE at one-month intervals. The vaccine was safe and generally well tolerated with no serious adverse events (SAEs) or Grade 4 adverse events (AEs) (48). Injection site pain was commonly reported (80%, 50%, and 41% after the first, second, and third dose, respectively), but generally mild and self-limited. There were no abnormalities in physical exams, serum chemistries, and hematology values related to study product. Humoral and CD4+ T-cell responses to Sm14 were reported (48).

The second clinical trial using the 10 mcg dose of GLA-SE was a phase 1, open-label evaluation of the safety, open-label evaluation of the safety, tolerability, and immunogenicity of a Leishmania vaccine (LEISH-F3) in combination with SLA-SE (second generation glucopyranosyl lipid A in stable oil-in-water emulsion) adjuvant compared to LEISH-F3 with GLA-SE in healthy adults (NCT02071758; Protocol IDRI-LVVPX-117). The SLA-SE adjuvant is a next generation TLR4 adjuvant formulation. Thirty-nine participants were randomized to 4 arms: high dose LEISH-F3 (20 mcg) and low dose SLA-SE (5 mcg); high dose LEISH-F3

and high dose GLA-SE adjuvant (10 mcg); low dose LEISH-F3 (5 mcg) and high dose GLA-SE adjuvant (10 mcg); and high dose LEISH-F3 and high dose SLA-SE adjuvant (10 mcg). Participants received 3 injections at one-month intervals. The LEISH-F3 + GLA-SE and LEISH-F3 + SLA-SE vaccines were safe and well tolerated in adult subjects. No deaths, no dose-limiting toxicities (DLTs), no Grade 3 or 4 AEs, no SAEs, and no AE of special interest (AESIs) occurred during the study. All study injection reactions were Grade 1 or Grade 2. The most frequently reported reactions were injection site tenderness/pain and fatigue.

Three other clinical trials are evaluating 10 mcg or higher doses of GLA-SE in oncology. Two ongoing trials are investigating GLA-SE alone as an immunotherapy and one trial tested GLA-SE in combination with a prostate cancer antigen. No data are currently available from these trials.

Clinical experience with GLA-SE as an adjuvant for other vaccines at doses ranging from 1 mcg to 5 mcg suggests that it is safe and well-tolerated. Treanor et al. (24) tested an avian influenza hemagglutinin (H5) subunit vaccine at a range of doses with and without a fixed dose of GLA-SE (1 mcg) and reported mild to moderate injection site pain and/or tenderness in 50–70% of H5 + GLA-SE recipients, with myalgias and headaches reported by a minority (25-27%) of vaccinees. No other safety findings were noted, and the GLA-SE adjuvant substantially increased the immunogenicity of the vaccine (24).

A recent phase 1 clinical trial of a respiratory syncytial virus (RSV) vaccine in older adults (≥ 60 years of age) found that a combination of the RSV fusion (F) protein at three different doses with 2.5 mcg of GLA-SE was safe, well-tolerated, and immunogenic. Compared with the unadjuvanted vaccine, GLA-SE increased local reactogenicity, with 40-65% of subjects reporting mild-to-moderate, self-limited injection site pain and/or tenderness (49). No other safety concerns were reported. Immune responses were F protein dose-dependent, and the adjuvant enhanced both humoral and cellular immune responses (49). A follow-up study using a higher dose of F protein and three doses of GLA-SE has been completed (NCT02289820). The vaccine was safe, tolerable, and immunogenic and the data supported the selection of 120 mcg F protein /5 mcg of GLA-SE for further evaluation (50).

GLA-SE was also found to be safe and well-tolerated at doses of 2 mcg and 5 mcg in a phase 1 trial of LEISH-F3 given three times at one-month intervals (25). Injection site pain and/or tenderness was quite common (90-100%) with fatigue noted by 40-60% of vaccinees receiving the adjuvanted product versus 33% of subjects who received unadjuvanted vaccine (25). The unadjuvanted LEISH-F3 was essentially non-immunogenic while antibody and cytokine responses were noted in vaccinees in both dose groups of GLA-SE (25).

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

Table 4-10 Completed clinical trials using IDRI GLA-SE adjuvant formulations in combination with other vaccines (as of February 14, 2020)

Sponsor/Partner	Disease Area/Antigen	GLA-SE Dose	# Receiving GLA-SE	# Total in Study
Oswaldo Cruz Foundation / IDRI NCT01154049	Schistosomiasis (Sm14)	10 mcg	20	20
Oswaldo Cruz Foundation / IDRI NCT03041766	Schistosomiasis (Sm14)	5 mcg	30	30
Oswaldo Cruz Foundation / IDRI NCT03799510	Schistosomiasis (Sm14)	2.5 mcg	64	95
IDRI / Rockefeller University NCT01397604 and NCT01864876	Adjuvant only	2 mcg 5 mcg	10 7	49
IDRI NCT01484548	Leishmaniasis (LEISH-F3)	2 mcg 5 mcg	12 12	36
NIAID / IDRI NCT01751048	Leishmaniasis (LEISH-F3)	5 mcg	16	48
IDRI NCT02071758	Leishmaniasis (LEISH-F3)	10 mcg	18	39
IDRI NCT03302897	Leprosy (LEP-F1)	5 mcg	24	24
WRAIR / IDRI NCT01540474	Malaria (CelTOS)	2 mcg 5 mcg	10 20	30
European Vaccine Initiative / IDRI NCT01949909	Malaria (p27A)	2.5 mcg 5 mcg	24 8	56
European Vaccine Initiative / IDRI NCT02014727	Malaria (AMA-1 DiCo)	2.5 mcg	33	66
University Hospital Tuebingen / IDRI NCT02647489	Malaria (PAMVAC)	5 mcg	21	63
Institut National de la Santé et de la Recherche Médicale / IDRI NCT02658253	Malaria (PRIMVAC)	2.5 mcg	29	68
IDRI / Aeras NCT01599897	TB (ID93)	2 mcg 5 mcg	24 24	60
IDRI / Aeras NCT01927159	TB (ID93)	2 mcg 5 mcg	39 15	66
IDRI NCT02465216	TB (ID93)	2 mcg 5 mcg	20 28	60
NIH/NIAID/DMID / IDRI NCT02508376	TB (ID93)	5 mcg	20	70
CONFIDENTIAL	Seasonal influenza	0.5 mcg 1 mcg 2.5 mcg 5 mcg	6 12 36 4	96
Protein Sciences / Immune Design NCT01147068	Pandemic influenza (recombinant protein)	1 mcg	220	392
Novavax / Immune Design NCT01596725	Pandemic influenza (H5-VLP)	2.5 mcg	169	333

Sponsor/Partner	Disease Area/Antigen	GLA-SE Dose	# Receiving GLA-SE	# Total in Study
Medicago / Immune Design NCT01991561	Pandemic influenza (H5-VLP)	5 mcg	130	390
Immune Design NCT02015416	Cancer (NY-ESO-1)	2 mcg 5 mcg 10 mcg	3 3 6	12
Immune Design NCT02035657	Merkel cell carcinoma	5 mcg	9	9
Immune Design NCT02387125	Cancer (CMB305, G305)	Not Available		
Immune Design NCT02609984	Cancer (CMB305)	Not Available		
Immune Design NCT02501473	Non-Hodgkins lymphoma	5 mcg 10 mcg 20 mcg	Unknown	Unknown
Immune Design NCT02180698	Sarcoma	5 mcg 10 mcg 20 mcg	4 4 4	12
Immune Design NCT02320305	Melanoma (MART1)	Not Available		
Medimmune / Immune Design NCT02115815	Respiratory syncytial virus (sF)	2.5 mcg	60	144
Medimmune / Immune Design NCT02289820	Respiratory syncytial virus (sF)	1 mcg 2.5 mcg 5 mcg	39 99 79	261
Medimmune / Immune Design NCT02508194	Respiratory syncytial virus (sF)	5 mcg	946	1894

Table 4-11 Ongoing clinical trials using IDRI GLA-SE adjuvant formulations in combination with other vaccines (as of February 14, 2020)

Sponsor/Partner	Disease Area/Antigen	GLA-SE Dose	# Receiving GLA-SE	# Total in Study
IDRI NCT03722472	TB (ID93)	5 mcg	48	48
Quratis / IDRI NCT03806686	TB (ID93)	5 mcg	96	144
Quratis / IDRI NCT03806699	TB (ID93)	5 mcg	24	36
Immune Design NCT03450122	Sarcoma (CMB305)	Not Available		
Immune Design NCT02406781	Sarcoma (G100)	Not Available		
HVTN 115 / IDRI NCT03220724	HIV Vaccine (CH505TF gp120)	10 mcg	96	107
HVTN 123 / IDRI NCT03856996	HIV Vaccine (CH505TF gp120)	10 mcg	30	30
HVTN 124 / IDRI NCT03409276	HIV Vaccine (PDPHV)	10 mcg	52	60

4.11 Potential risks of study products and administration

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

Common	• Mild to moderate injection site pain, tenderness, 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, nausea, or dizziness in the first few days following injection • Vasovagal reaction/lightheadedness/dizziness related to the injection procedure • Transient changes in clinical laboratory values • Injection site hematoma, bruising/ecchymosis, 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

Primary objective 1:

To evaluate the safety and tolerability of AdC6-HIVgp140 and AdC7-HIVgp140 at doses of 1 x 10^{10} virus particles (vp) and 5 x 10^{10} vp, alone and in combination with CH505TF gp120 adjuvanted with GLA-SE in HIV- uninfected adults.

Primary endpoint 1:

Local and systemic reactogenicity signs and symptoms for a minimum of seven days following receipt of any study product. All adverse events for thirty days after any receipt of study vaccination. All serious adverse events (SAEs), medically attended adverse events (MAAEs), and adverse events of special interest (AESIs) will be collected for twelve months following any receipt of study product.

5.2 Secondary objectives and endpoints

Secondary objective 1:

To evaluate and compare immune responses elicited by single administration of AdC6-HIVgp140 or AdC7-HIVgp140 at two different dose levels

Secondary endpoints 1:

- Response rate, magnitude, and breadth of HIV-specific serum IgG binding antibodies assessed 4 weeks after a single vaccination (Part A) or the first vaccination (Part B)

- Response rate, magnitude, and polyfunctionality of HIV-specific CD4+ and CD8+ T-cell responses assessed 4 weeks after a single vaccination (Part A) or the first vaccination (Part B)

- Response rate, magnitude, and breadth of serum neutralizing antibodies against tier 1 and if applicable, other heterologous tier 2 HIV-1 isolates assessed 4 weeks after a single vaccination (Part A) or the first vaccination (Part B)

Secondary objective 2:

To evaluate and compare immune responses elicited by sequential administration of AdC7-HIVgp140 followed by AdC6-HIVgp140 and AdC7-HIVgp140 followed by a dose of AdC6-HIVgp140 at 5 x 10^{10} vp

Secondary endpoints 2:

- Response rate, magnitude, and breadth of HIV-specific serum IgG binding antibodies assessed 4 weeks after the second vaccination (Part B)

- Response rate, magnitude, and polyfunctionality of HIV-specific CD4+ and CD8+ T-cell responses assessed 4 weeks after the second vaccination (Part B)

- Response rate, magnitude, and breadth of serum neutralizing antibodies against tier 1 and if applicable, other heterologous tier 2 HIV-1 isolates assessed 4 weeks after the second vaccination (Part B)

Secondary objective 3:

To evaluate and compare immune responses elicited by heterologous AdC7-HIVgp140 followed by AdC6-HIVgp140 or AdC7-HIVgp140 followed by AdC6-HIVgp140 prime followed with CH505TF gp120 / GLA-SE boost

Secondary endpoints 3:

- Response rate, magnitude, and breadth of HIV-specific serum IgG binding antibodies assessed 2 weeks after the third vaccination (Part B)

- Response rate, magnitude, and polyfunctionality of HIV-specific CD4+ and CD8+ T-cell responses assessed 2 weeks after the third vaccination (Part B)

- Response rate, magnitude, and breadth of serum neutralizing antibodies against tier 1 and if applicable, other heterologous tier 2 HIV-1 isolates assessed 2 weeks after the third vaccination (Part B)

Secondary objective 4:

To evaluate vector-specific antibodies both before and after vaccination.

Secondary endpoint 4:

Magnitude of serum antibody neutralization of AdC6 and AdC7 vectors as assessed by adenovirus neutralization assay at baseline (Parts A and B), 4 weeks after the first vaccination (Parts A and B), and 4 weeks after the second vaccination (Part B)

5.3 Exploratory objectives

Exploratory objective 1:

To characterize antibody avidity and/or Fc-mediated antibody functions (infected cells antibody binding assay [ICABA], antibody-dependent cellular cytotoxicity [ADCC], antibody dependent cellular phagocytosis [ADCP], antibody dependent neutrophil phagocytosis [ADNP], and FcR-binding)

Exploratory objective 2:

To assess the impacts of vaccination on B-cell subset distribution in blood

Exploratory objective 3:

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

Exploratory objective 4:

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 Center assay portfolio

6 Statistical considerations

6.1 Accrual and sample size calculations

Recruitment will target enrolling 34 healthy, HIV-uninfected adult participants aged 18 through 50 years. Part A and B are single- and multiple-administration groups, respectively. Enrollment to the groups in the two parts will have a series of pre-planned enrollment pauses as indicated in Section 1.

To ensure balance of both sexes assigned at birth, the trial will enroll at least approximately 40% of each sex assigned at birth across all treatment groups. The study team will work closely with the sites to monitor enrollment progress to ensure appropriate balance.

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 assay 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% enrolled participants having missing data for the primary immunogenicity endpoint.

6.1.1 Sample size calculations for safety

The goal of the safety evaluation for this study is to identify safety concerns associated with product administration. The ability of the study to detect serious adverse events (SAEs) (see Section 10, Table 6-1) can be expressed by the true event rate above which at least 1 SAE would likely be observed and the true event rate below which no events would likely be observed. Specifically, for each vaccine arm of the study in Part A (n =5), there is at least an 83% chance of observing at least 1 event if the true rate of such an event is 30% or more; and there is at least a 95% chance of observing no events if the true rate is 1% or less. As a reference, in HVTN vaccine trials from April 2008 through March 2018, about 1.6% of participants who received placebos experienced an SAE.

Binomial probabilities of observing 0, 1 or more, and 2 or more events among arms of size 5 and 10 are presented in Table 6-1 for a range of possible true adverse event rates. Ten participants does not account for historical potential dropout because every enrolled subject provides some safety data. These calculations provide a more complete picture of the sensitivity of this study design to identify potential safety problems with the vaccine.

Table 6-1 Probability of observing 0 events, 1 or more events, and 2 or more events, among arms of size 5 or 10, for different true event rates

True event rate (%)	Pr(0/5)	Pr(1+/5)	Pr(2+/5)	Pr(0/10)	Pr(1+/10)	Pr(2+/10)
1	0.95	0.05	<0.01	0.90	0.10	<0.01
3.5	0.84	0.16	0.01	0.70	0.30	0.05
5	0.77	0.23	0.02	0.60	0.40	0.09
10	0.59	0.41	0.08	0.35	0.65	0.26
20	0.33	0.67	0.26	0.11	0.89	0.62
30	0.17	0.83	0.47	0.03	0.97	0.85
40	0.08	0.92	0.66	0.01	0.99	0.95

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 (51, 52). If none of the 10 participants receiving a vaccine regimen experience a safety event, the 95% 2-sided upper confidence bound for the true rate of such events in the total vaccinated population is 27.8%.

Table 6-2 Two-sided 95% confidence intervals for the probability of observing a safety event based on observing a particular rate of safety endpoints for arms of sizes 5 and 10

Observed event rate	95% Confidence interval (%)
0/5	0.0 – 43.4
1/5	1.0 – 62.4
2/5	11.8 – 76.9
0/10	0.0 – 27.8
1/10	0.5 – 40.4
2/10	5.7 – 51.0

6.1.2 Sample size calculations for immunogenicity

The main goals of this trial regarding immunogenicity outcomes involve a preliminary estimation of response rates based on data from the BAMA assays among vaccinees. No adjustment for multiple comparisons will be made for the use of multiple assays. The precision with which the true response rate can be estimated from the observed data depends on the true underlying response rate and the sample size. Two-sided 95% confidence intervals for the response rate based on observing a particular rate of responses in the vaccinees is shown in Table 6-3. Calculations are done using the score test method (51). The n = 9 assumes 10% missing immunogenicity data.

Table 6-3 Two-sided 95% confidence intervals for the true response rate based on observing a particular rate of responses in the vaccinees (n = 9, assuming 10% missing data)

No. of responses	Observed response rate (%)	95% Confidence interval
5	56	[26.7, 81.1]
6	67	[35.4, 87.9]
7	78	[45.3, 93.7]
8	89	[56.5, 99.4]
9	100	[70.1, 100.0]

6.2 Randomization

A participant's randomization assignment will be computer generated and provided to the HVTN CRS pharmacist through a Web-based randomization system. 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 and other staff designated for pharmacy quality control) will be blinded as to participant treatment group assignments (eg, vaccine or control) but not to study part. Safety assessments will be made in a blinded fashion until the planned unblinding occurs (see Appendix E, Appendix F, Appendix I, and Appendix J).

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 excepted for designated staff used solely for the purposes of quality control. 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.

In some cases, the CRS, PSRT, or study sponsor may believe unblinding of the site PI and participant would be appropriate to facilitate the clinical management of an AE or SAE. The HVTN Unblinding MOP specifies procedures for emergency unblinding, and for early unblinding for medical reasons.

6.4 Statistical analyses

This section describes the final study analyses, unblinded as to treatment arm assignment. All data from enrolled participants will be analyzed according to the initial randomization assignment regardless of how many vaccinations they received. In the rare instance that a participant receives the wrong treatment at a specific vaccination time, the Statistical Analysis Plan (SAP) will address how to analyze the participant's safety and immunogenicity 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 post-enrollment values. In addition, the number (percentage) of participants with local laboratory values recorded as meeting Grade 1 AE criteria or above as specified in the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (see Section 9.9) will be tabulated by treatment arm for each postvaccination timepoint. Reportable clinical laboratory abnormalities without an associated clinical diagnosis will also be included in the tabulation of AEs described above.

6.4.3.4 Reasons for vaccination discontinuation and early study termination

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

6.4.4 Immunogenicity analysis

6.4.4.1 General approach

For the statistical analysis of immunogenicity endpoints, data from enrolled participants will be used according to the initial randomization assignment regardless of how many injections they received. Additional analyses may be

performed, limited to participants who received all scheduled injections per protocol. Assay results that are unreliable are excluded; results from specimens collected outside of the visit window, or from HIV-infected participants post-infection, may be excluded. Since the exact date of HIV infection is unknown, any assay data from blood draws 4 weeks prior to an infected participant's last seronegative sample and thereafter may be excluded. If an HIV-infected participant does not have a seronegative sample post-enrollment, then all data from that participant may be excluded from the analysis.

Discrete categorical assay endpoints (eg, response rates) will be analyzed by tabulating the frequency of positive response for each assay by antigen and treatment arm at each timepoint for which an assessment is performed. Crude response rates and difference between treatment arms will be presented with their corresponding 95% confidence interval estimates calculated using the score test method (51).

For quantitative assay data, 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 study arms. Typically, the results will be shown for each vaccine arm and for the set of control arms pooled into one group.

Mean or median (if normality assumption severely violated) assay readouts will be compared between arms and bootstrap confidence intervals will be estimated.

More sophisticated analyses employing repeated measures methodology (for example, linear mixed models or marginal mean models fit by generalized estimating equations) may be utilized to incorporate immune responses over several timepoints and to test for differences over time. However, inference from such analyses would be limited by the small sample size of this study. All statistical tests will be 2-sided and will be considered statistically significant if $p \leq 0.05$.

6.4.4.2 Multivariate display of immunogenicity endpoints

Data visualization techniques may be used to explore the relationship among immunogenicity readouts. The set of readouts may be based on one of the primary endpoints (eg, ICS), on the set of primary endpoints, or on immunogenicity endpoints that also include secondary or exploratory endpoints. To understand the relationship between pairs of readouts, scatter plots may be used when the number of readouts is small or for a larger number of readouts, a heatmap showing the degree of correlation between any two pairs. Principal component analysis (PCA) and associated 'biplots' of the scores and loadings are particularly useful to understand associations between readouts, especially when readouts are correlated (53). PCA is a method to reduce the dimensionality of the number of readouts to a smaller set of values (principal components) that are normalized linear combinations of the readouts in such a way that the first principal component

accounts for the most variability in the data and subsequent components, while maximizing variability, are uncorrelated with each other. A 'biplot' displays the first and second principal component scores and principal component loadings. The x-axis is the value from the first principal component and the y-axis is the second principal component, where each axis label includes the percentage of variation in the total set of readouts captured by the principal component. The top axis is the first principal component loadings and the right axis is the second principal component loadings. An arrow is drawn for each immunogenicity readout (eg, Env-specific CD4+ T-cell polyfunctionality score, Env-specific CD8+ T-cell total magnitude) from the origin to the point defined by its first two principal component loadings. The length of the arrow represents the amount of total variation of the set of readouts captured by the given readout. The direction of an arrow conveys the extent to which the variation of a readout is in the direction of the first or second principal component. The angle between two arrows conveys information about the correlation of the two readouts, with a zero-degree angle denoting perfect correlation and a 90-degree angle denoting no correlation. Each arrow on the biplot is labeled by the immunogenicity readout it represents. A biplot is annotated with key meta-information such as the treatment arm (most common application) or a demographic category. Depending on the application, K-means clustering and hierarchical clustering may also be applied for multivariate graphical display of immunogenicity readouts.

6.4.4.3 Primary analyses of neutralization magnitude-breadth curves

The area-under-the-magnitude-breadth curve (AUC-MB) to a global panel of viral isolates (54) will be computed for each participant with evaluable neutralization data, as described in (55). AUC-MB to a vaccine-matched panel may also be computed when the panel consists of at least 3 isolates. Tier 1A viral isolates will not be included in AUC-MB analyses. Dunnett's procedure will be applied with 2-sided alpha = 0.05 to determine which of the [n] vaccine groups have a significantly higher mean AUC-MB than that of the pooled placebo groups, as described in (52) (see their formula (1.1)). This procedure will be applied to construct 95% CIs about the [*n*] differences in mean AUC-MB for each vaccine regimen versus the pooled placebo groups (vaccine – placebo), which simultaneously have at least 95% coverage probability.

6.4.4.4 Analysis of CD4+ and CD8+ T-cell responses as measured by the ICS assay

The analysis of CD4+ and CD8+ T-cell response rates as measured by the ICS assay will be evaluated and compared as described under the general approach. For each T-cell subset, the positivity call for each peptide pool will include a multiple comparison adjustment for the number of peptide pools used in the assay. In general, the Mixture Models for Single-cell Assays (MIMOSA) statistical framework (56) 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 (57) 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, two 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 is defined as the number of antigens, peptides, and/or isolates with associated magnitudes above the assay-specific positivity threshold. 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 AUC-MB 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 and data sharing 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 in accordance with Sections 6.4.5.1. Interim blinded safety and immunogenicity data should not be shared outside of the SMB, HVTN 139 PSRT, the protocol team leadership, the HVTN Executive Management Team, the study product developer, and the study sponsor and/or its designee(s) for their regulatory reporting unless approved by the protocol leadership and the HVTN leadership.

6.4.5.1 Safety analyses

During the course of the trial, unblinded analyses of safety data will be prepared approximately every 4 months during the main study, as defined in Section 1, for

review by the SMB. Ad hoc safety reports may also be prepared for SMB review at the request of the HVTN 139 PSRT. Refer to the process described in the HVTN Unblinding MOP for any requests for unblinded safety data prior to the end of the scheduled follow-up visits.

6.4.5.2 Immunogenicity analyses

An unblinded statistical analysis by treatment assignment of a primary immunogenicity endpoint may be performed when all participants have completed the corresponding primary immunogenicity visit and data are available for analysis from at least 80% of these participants. Similarly, an unblinded statistical analysis by treatment assignment of a secondary or exploratory immunogenicity endpoint may be performed when all participants have completed the corresponding immunogenicity visit and data are available for analysis from at least 80% of these participants. However, such analyses for a secondary or exploratory immunogenicity endpoint will only take place after at least one of the primary immunogenicity endpoints reaches the aforementioned threshold. The HVTN Laboratory Center will review the analysis report prior to distribution to the protocol chairs, DAIDS, study product developer, and other key HVTN members and investigators. Reports for distribution or presentation should use PubIDs and not PTIDs for individual responses. 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 information available at the time of enrollment, including results of screening 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 through 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 and that in a previous trial with an adenovirus type 5 (Ad5) vector there was an association of increased HIV acquisition with receipt of that study product; completes a questionnaire prior to first vaccination with verbal demonstration of understanding of all questionnaire items answered incorrectly.

5. **Willing to be contacted annually** after completion of scheduled clinic visits for a total of 3 years following initial study injection.

6. **Agrees not to enroll in another study** of an investigational research agent until the last scheduled clinic visit.

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 as "low risk" for HIV acquisition per low risk guidelines (see Appendix M), agrees to discuss HIV infection risks, agrees to risk reduction counseling, and agrees to avoid behavior associated with high risk of HIV exposure through the final study visit. Low risk may include persons stably taking PrEP as prescribed for 6 months or longer.

Laboratory Inclusion Values

Hemogram/Complete blood count (CBC)

11. **Hemoglobin**

    ≥ 11.0 g/dL for volunteers who were assigned female sex at birth

    ≥ 13.0 g/dL for volunteers who were assigned male sex at birth and transgender male who have been on hormone therapy for more than 6 consecutive months

    ≥ 12.0 g/dL for transgender female who have been on hormone therapy for more than 6 consecutive months

For transgender participants who have been on hormone therapy for less than 6 consecutive months, determine hemoglobin eligibility based on the sex assigned at birth

12. **White blood cell count** = 2,500 to 12,000 cells/mm^3 with normal differential, or differential approved by Investigator of Record (IoR) or designee as not clinically significant

13. **Total lymphocyte count** ≥ 650 cells/mm^3 with normal differential, or differential approved by Investigator of Record (IoR) or designee as not clinically significant

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

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

Chemistry

16. **Alanine aminotransferase (ALT)** < 1.25 times the institutional upper limit of normal;

17. **Creatinine** <1.1 times the institutional upper limit of normal

Virology

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

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

20. **Negative anti-Hepatitis C virus antibodies (anti-HCV)**, or negative HCV nucleic acid test if the anti-HCV is positive

Urine

21. **Normal urine**:

    - Negative or trace urine protein, and
    - Negative, trace, or 1+ blood/hemoglobin on urine dipstick. If 1+ hemoglobin is present on dipstick, a microscopic urinalysis with red blood cells levels within institutional normal range is required.

Reproductive Status

22. **Volunteers who were assigned female sex at birth**: negative serum or urine beta human chorionic gonadotropin (β-HCG) pregnancy test at screening (ie, prior to randomization) and prior to study product administration on the day of study product administration. Persons who are NOT of reproductive potential due to having undergone hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing.

23. **Reproductive status**: A volunteer who was assigned female sex at birth:

    Must agree to use effective contraception for sexual activity that could lead to pregnancy from at least 21 days prior to enrollment until two months following final product administration.

    - Condoms (male or female) with or without a spermicide,
    - Diaphragm or cervical cap with spermicide,
    - Intrauterine device (IUD),
    - Hormonal contraception,
    - Tubal ligation, or
    - Any other contraceptive method approved by the HVTN 139 PSRT

- Successful vasectomy in any partner assigned male sex at birth (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, or bilateral oophorectomy;

- Or be sexually abstinent.

24. **Volunteers who were assigned female sex at birth must also agree not to seek pregnancy through alternative methods**, such as artificial insemination or in vitro fertilization until two months after the last product administration

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 tobacco 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 139 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 139 PSRT will determine eligibility on a case-by-case basis.

8. **Previous receipt of monoclonal antibodies (mAbs)**, whether licensed or investigational. Exceptions may be made by the HVTN 139 PSRT on a case-by-case basis.

9. **Non-HIV experimental vaccine(s) received within the last 1 year** in a prior vaccine trial. Exceptions may be made by the HVTN 139 PSRT for vaccines that have subsequently undergone licensure by the FDA. For volunteers who have received control/placebo in an experimental vaccine trial, the HVTN 139 PSRT will determine eligibility on a case-by-case basis. For volunteers who have received an experimental vaccine(s) greater than 1 years ago, eligibility for enrollment will be determined by the HVTN 139 PSRT on a case-by-case basis.

10. **Live attenuated vaccines** received within 30 days before first vaccination or scheduled within 28 days after injection (eg, measles, mumps, and rubella [MMR]; oral polio vaccine [OPV]; varicella; yellow fever; live attenuated influenza vaccine).

11. **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).

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

Immune System

13. **Immunosuppressive medications** received within 168 days before first vaccination (Not exclusionary: [1] corticosteroid nasal spray; [2] inhaled corticosteroids; [3] topical corticosteroids for mild, uncomplicated dermatologic condition; or [4] a single course of oral/parenteral prednisone or equivalent at doses $\leq$ 60 mg/day and length of therapy < 11 days with completion at least 30 days prior to enrollment).

14. **Serious adverse reactions to vaccines or to vaccine components such as** glycerol, sodium chloride, tris (hydroxymethyl)aminomethane 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).

15. **Immunoglobulin** received within 90 days before first vaccination (for mAb see criterion 8 above).

16. **Autoimmune disease, current or history.** (Not exclusionary: well-controlled psoriasis that does not require systemic therapy)

17. **AESIs**

    Volunteers who currently have, or have a history of, any condition that could be considered an AESI for the products administered in this protocol (representative examples are listed in Appendix N). The exception to Appendix N is well-controlled psoriasis, which is not exclusionary (as above)

18. **Immunodeficiency.**

Clinically significant medical conditions

19. **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.

20. **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.

21. **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.

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

23. **Asthma exclusion criteria:**

    Asthma is excluded if the participant has ANY of the following:

    - Required either oral or parenteral corticosteroids for an exacerbation two or more times within the past year; OR
    - Needed emergency care, urgent care, hospitalization, or intubation for asthma within the past year; OR
    - Uses a short-acting rescue inhaler more than 2 days/week; OR
    - Uses medium-to-high dose inhaled corticosteroids (greater than 250 mcg fluticasone or therapeutic equivalent) or more than one medication for

maintenance therapy daily. For example, potential participants taking long acting bronchodilator/inhaled corticosteroid combinations for daily maintenance are excluded. [Note: Maintenance monotherapy with cromolyn, leukotriene receptor antagonist, or theophylline is not exclusionary.]; OR

- Meets any other asthma-related criteria that, in the judgement of the investigator, could lead to interference with study participation.

24. **Diabetes mellitus** type 1 or type 2. (Not exclusionary: type 2 cases controlled with diet alone or a history of isolated gestational diabetes)

25. **Thyroidectomy, or thyroid disease** (Not exclusionary: well-controlled non-autoimmune thyroid disease as defined in HVTN 139 *Study Specific Procedures* (SSP))

26. **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 in this protocol 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.

27. **Bleeding disorder** (eg, factor deficiency, coagulopathy, or platelet disorder requiring special precautions)

28. **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).

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

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

31. History of **angioedema**, or **anaphylaxis**. (Not exclusionary: angioedema or anaphylaxis with known trigger and no episodes within five years.)

32. History of **generalized urticaria** within past five years.

7.3 Participant departure from vaccination schedule or withdrawal

This section concerns an individual participant's departure from the study product administration schedule. Pause rules for the trial 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
  - Receipt of allergy treatment with antigen injections
- Within 14 days prior to any study injection
  - Receipt of any vaccines that are not live attenuated vaccines (eg, pneumococcal)
- Prevaccination abnormal vital signs or clinical symptoms that may mask assessment of vaccine reaction

Vaccinations should not be administered outside the visit window HVTN 139.

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 products 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 139 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)
  - HIV infection
  - 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 that is subsequently considered to be related to vaccination
  - Other grade 3 clinical AE that is subsequently considered to be related to vaccination with the exception of fever and subjective local and systemic symptoms. For grade 3 injection site erythema and/or induration, upon review, the PSRT may allow continuation of vaccination
  - SAE that is subsequently considered to be related to vaccination
  - Clinically significant type 1 hypersensitivity reaction associated with study vaccination. Consultation with the HVTN 139 PSRT is required prior to subsequent vaccinations following any type 1 hypersensitivity reaction associated with study vaccination
- Investigator determination in consultation with Protocol Team leadership (eg, for repeated nonadherence to study staff instructions)

Participants discontinuing study product for reasons other than HIV infection 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 (see HVTN 139 SSP).

Participants diagnosed with HIV infection during the study should be encouraged to participate in follow-up visits as indicated in Section 9.13.

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 declines 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, or
- Investigator decides, in consultation with Protocol Team leadership, to terminate participation (eg, if participant exhibits inappropriate behavior toward clinic staff).
- Any condition where termination from the study is required by applicable regulations.

8 Study product preparation and administration

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

8.1 Vaccine regimen

The schedule of vaccination is shown in Section 1 and additional information is given below.

Part A: Low Dose Arm

Treatment 1 (T1): 1 x 10^{10} vp AdC6-HIVgp140 to be administered as two separate 1 mL IM injections into the deltoid of the non-dominant arm unless medically contraindicated at month 0.

Treatment 2 (T2): 1 x 10^{10} vp AdC7-HIVgp140 to be administered as two separate 1 mL IM injections into the deltoid of the non-dominant arm unless medically contraindicated at month 0.

Control 3 (C3): Placebo for AdC6-HIVgp140 or AdC7-HIVgp140 (labeled as Sodium Chloride for Injection, 0.9%) to be administered as two separate 1 mL IM injections into the deltoid of the non-dominant arm unless medically contraindicated at month 0.

Part B: High Dose Arm

Treatment 4 (T4): 5 x 10^{10} vp AdC6-HIVgp140 to be administered as two separate 1 mL IM injections into the deltoid of the non-dominant arm unless medically contraindicated at month 0.

Then

5 x 10^{10} vp AdC7-HIVgp140 to be administered as two separate 1 mL IM injections into the deltoid of the non-dominant arm unless medically contraindicated at month 3.

Then

400 mcg CH505TF gp120 admixed with 10 mcg GLA-SE administered as a 1mL IM injection into either thigh unless medically contraindicated at month 6.

Treatment 5 (T5): 5 x 10^{10} vp AdC7-HIVgp140 to be administered as two separate 1 mL IM injections into the deltoid of the non-dominant arm unless medically contraindicated at month 0.

Then

5 x 10^{10} vp AdC6-HIVgp140 to be administered as two separate 1 mL IM injections into the deltoid of the non-dominant arm unless medically contraindicated at month 3.

Then

400 mcg CH505TF gp120 admixed with 10 mcg GLA-SE administered as a 1mL IM injection into either thigh unless medically contraindicated at month 6.

Control 6 (C6): Placebo for AdC6-HIVgp140and AdC7-HIVgp140 (labeled as Sodium Chloride for Injection, 0.9%) to be administered as two separate 1 mL IM injections into the deltoid of the non-dominant arm unless medically contraindicated at month 0 and 3. Placebo for CH505TF/GLA-SE (labeled as Sodium Chloride for Injection, 0.9%) to be administered as 1 mL IM injection into either thigh at month 6.

8.2 Study product formulation

8.2.1 AdC6-HIVgp140 and AdC7-HIVgp140

Both products are diluted in 2.5% Glycerol/25 mM NaCl/20 mM TRIS, pH 8.0 formulation buffer to a concentration of 1.2 x 10^{11} vp/mL and filled at 0.3 mL for AdC6-HIVgp140 and 0.6 mL for AdC7-HIVgp140 into a 2 mL Type 1 glass vial and stoppered with a gray chlorobutyl rubber stopper. Both products are clear to slightly cloudy liquid, essentially free of visible particles.

Both AdC6-HIVgp140 and AdC7-HIVgp140 should be stored at ≤ -65°C prior to use/preparation.

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

8.2.2 CH505TF gp120

CH505TF gp120 is formulated in 20 mM sodium phosphate, 150 mM NaCl, 0.02% polysorbate 80 (PS80), pH 6.5, supplied as a frozen liquid in 2 mL glass vials. Each 2 mL vial contains 0.75 mL of formulated gp120 at a concentration of 0.8 mg/mL and is stored at ≤ -65°C.

8.2.3 GLA-SE (glucopyranosyl lipid A – stable emulsion; [labeled as AP 10-201])

The GLA-SE adjuvant will be provided as vials containing 20 mcg/mL GLA in a 4% oil-in-water emulsion. Each sterile, single use vial contains 0.4 mL of product.

Product appears as a milky-white liquid. GLA-SE must be stored at 2° to 8°C and must not be frozen. The study product is described in further detail within the IB.

8.2.4 Placebo will be Sodium Chloride for Injection, 0.9%, will be used as the placebo. It must be stored as recommended by the manufacturer.

8.3 Preparation of study products

Aseptic technique should be used for the preparation of all study products described in this section.

8.3.1 AdC6-HIVgp140

One vial of AdC6-HIVgp140 and Sodium Chloride for Injection, 0.9% , will be needed to prepare the low dose of 1 x 10^{10} vp for Part A.

Two vials of AdC6-HIVgp140 and Sodium Chloride for Injection, 0.9% , will be needed to prepare the high dose of 5 x 10^{10} vp for Part B.

Prior to admixture, the pharmacist will remove the appropriate number of vials of AdC6-HIVgp140 from the freezer and allow to thaw completely at room temperature. Once thawed, mix using a vortexer machine at high speed for one second.

Low Dose:

1. Withdraw 0.25 mL of AdC6-HIVgp140 from 1 vial, and add to an empty sterile vial.

2. Add 5.75 ml of sodium chloride for injection to the vial containing 0.25 mL of AdC6-HIVgp140.

3. Vortex the preparation at high speed for one second.

High Dose:

1. Withdraw 0.5 mL of AdC6-HIVgp140 from the two vials and add to an empty sterile vial.

2. Add 1.9 ml of sodium chloride for injection to the vial containing 0.5 mL of AdC6-HIVgp140.

3. Vortex the preparation at high speed for one second.

Finally, using two 3 or 5 mL syringes, withdraw 1 mL of the diluted vaccine into each syringe, remove the needle and cap the syringe.

The final syringe(s) for administration must be covered with an overlay and labeled as:

"AdC6-HIVgp140 or placebo syringe 1 of 2 and AdC6-HIVgp140 or placebo syringe 2 of 2". The syringe must also be labeled for IM administration into deltoid, with an expiration date and time of 2 hours following thawing.

The preparation must be stored at 2° to 8°C in the refrigerator immediately after labeling each syringe, until the time of administration.

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

8.3.2 AdC7-HIVgp140

One vial of AdC7-HIVgp140 and Sodium Chloride for Injection, 0.9% will be needed to prepare the dose.

Prior to admixture, the pharmacist will remove one vial of AdC7-HIVgp140 from the freezer and allow to thaw completely at room temperature. Once thawed, mix using a vortexer machine at high speed for one second.

Low Dose:

1. Withdraw 0.25 ml of AdC7-HIVgp140 from 1 vial,. and add to an empty sterile vial.

2. Add 5.75 ml of sodium chloride for injection to the vial containing 0.25 mL of AdC7-HIVgp140.

3. Vortex the preparation at high speed for one second.

High Dose:

1. Withdraw 0.5 mL of AdC7-HIVgp140 from 1 vial and add to an empty sterile vial.

2. Add 1.9 ml of sodium chloride for injection to the vial containing 0.5 mL of AdC7-HIVgp140.

3. Vortex the preparation at high speed for one second.

Finally, using two 3 or 5 mL syringes, withdraw 1 mL of the diluted vaccine into each syringe, remove the needle and cap the syringe**.**

The final syringe(s) for administration must be covered with an overlay and labeled as:

"AdC7-HIVgp140 or placebo syringe 1 of 2 and AdC7-HIVgp140 or placebo syringe 2 of 2". The syringe must also be labeled for IM administration into deltoid, with an expiration date and time of 2 hours following thawing.

The preparation must be stored at 2° to 8°C in the refrigerator immediately after labeling each syringe, until the time of administration.

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

8.3.3 CH505TF gp120 + GLA-SE

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

Prior to admixture, the pharmacist will remove the CH505TF gp120 vial 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 two vials of GLA-SE from the refrigerator and allow to equilibrate to room temperature.

1. Add 0.2 ml of GLA-SE to another vial of GLA-SE. This vial now contains 0.6 mL of GLA-SE.

2. Add 0.6 mL of CH505TF gp120 to the vial containing 0.6 mL 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.

3. 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 either 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 Placebo for AdC6-HIVgp140 and AdC7-HIVgp140

Withdraw 1 mL of Sodium Chloride for Injection, 0.9% into two separate 3 or 5 mL syringes, remove the needle and cap each syringe.

The final syringes for administration must be covered with an overlay and labeled as:

"AdC6-HIVgp140/placebo or AdC7-HIVgp140/placebo" (depending on treatment allocation) syringe 1 of 2 and syringe 2 of 2. The syringe must also be labeled for IM administration into deltoid, with an expiration date and time of 2 hours.

The preparation must be stored at 2°C to 8°C in the refrigerator immediately after labeling each syringe, until the time of administration.

8.3.5 Placebo for CH505TF/GLA-SE

Withdraw 1 mL of Sodium Chloride for Injection, 0.9% 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 either thigh, with an expiration date and time of 8 hours following the last mixing procedure.

8.4 Administration

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.

Any administrator of study product will be blinded to the individual participant's treatment assignment.

8.4.1 **AdC6-HIVgp140:** will be administered at a dose of 1 x 10^{10} vp in Part A and a dose of 5 x 10^{10} vp in Part B of the study. The vaccine dose will be divided equally into two separate 1 mL intramuscular (IM) injections, administered into the deltoid of the non-dominant arm unless medically contraindicated.

8.4.2 **AdC7-HIVgp140:** will be administered at a dose of 1 x 10^{10} vp in Part A and a dose of 5 x 10^{10} vp in Part B of the study. The vaccine dose will be divided equally into two separate 1 mL IM injections administered into the deltoid of the non-dominant arm unless medically contraindicated.

8.4.3 **CH505TF gp120:** will be administered at a dose of 400 mcg protein with 10 mcg GLA-SE as a 1mL intramuscular (IM) injection, into either thigh unless medically contraindicated.

8.4.4 **Placebo control for AdC6-HIVgp140 or AdC7-HIVgp140 vaccine:** Sodium Chloride for Injection, 0.9%, administered as two separate 1 mL IM injections into the deltoid of the non-dominant arm unless medically contraindicated.

8.4.5 **Placebo control for CH505TF gp120:** Sodium Chloride for Injection, 0.9%, administered as a 1 mL IM injection into either thigh unless medically contraindicated.

8.5 Acquisition of study products

AdC6-HIVgp140 was manufactured by IDT Biologika (Rockville, Maryland, USA) and will be provided by DAIDS, NIAID, NIH, DHHS (Bethesda, Maryland, USA).

AdC7-HIVgp140 was manufactured by IDT Biologika (Rockville, Maryland, USA) and will be provided by DAIDS, NIAID, NIH, DHHS (Bethesda, Maryland, USA).

CH505TF gp120 was manufactured by Berkshire Sterile Manufacturing (Lee, Massachusetts, USA) and will be provided by DAIDS, NIAID, NIH, DHHS (Bethesda, Maryland, USA).

GLA-SE adjuvant: was manufactured by Infectious Disease Research Institute (IDRI) (Seattle, Washington, USA) and will be provided by DAIDS, NIAID, NIH, DHHS (Bethesda, Maryland, USA).

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 outlined 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

For US clinical research sites, all unused study products must be returned to the CRPMC after the study is completed or terminated unless otherwise instructed by the study sponsor. For non-US clinical research sites, all unused study products must be destroyed after the study is completed or terminated unless otherwise instructed by the study sponsor. The procedures are included in the Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks.

9 Clinical procedures

The schedule of clinical procedures is shown in Appendix I and Appendix J.

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, as well as per the CRS's SOP on the informed consent process.

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 as directed by the IRB/EC.

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 entity/body that has the power to regulate which includes authorities that review submitted clinical data and those that conduct inspections. These are sometimes referred to as competent authorities. These are entities/bodies whose approval/authorization/acknowledgment of a clinical trial is required for conducting a clinical trial. Any organization whose approval is required prior to a CRS's participation in DAIDS funded and/or Sponsored Clinical Trial. Includes but not limited to approvals from state/national health systems and administrative bodies, drug agencies etc. (DAIDS adopted from ICH E6(R2)". 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. Sample protocol-specific consent forms for the main study of Part A and Part B are located in Appendix A and Appendix B, respectively. 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 and Appendix B. 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 ICH E6(R2), Good Clinical Practice: Consolidated Guidance 4.8.

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

The sample informed consent form(s) include instructions for developing specific content.

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 and that in a previous trial with an adenovirus type 5 (Ad5) vector there was a statistically significant increase of HIV acquisition with receipt of that study product. The participant must complete the Assessment of Understanding before enrollment. Staff may provide assistance in reading and understanding the questions and responses, if necessary. Participants must verbalize understanding of all questions answered incorrectly. This process and the participant's understanding of the key concepts should be recorded in source documentation at the site.

IRB/EC and any applicable RE may require that a participant has signed either a screening or protocol-specific consent document prior to administering the Assessment of Understanding. The consent process (including the use of the Assessment of Understanding) should be explained thoroughly to the IRB/EC and any applicable RE, whose recommendations should be followed.

9.2 Pre-enrollment procedures

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

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 (see Appendix M).
- 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

- Laboratory tests, including:
  - Screening HIV test
  - Complete blood count (CBC) with differential and platelets
  - Limited chemistry panel (ALT and creatinine)
  - Hepatitis B surface antigen (HBsAg)
  - Anti-Hepatitis C (HCV) antibodies
  - Urine or serum pregnancy test
- Administration of behavioral risk assessment questionnaire
- Obtaining of volunteer demographics in compliance with the NIH Policy and Guidelines on the Inclusion of Women and Minorities as Subjects in Clinical Research, November 28, 2017 Amendment (available at https://grants.nih.gov/grants/guide/notice-files/NOT-OD-18-014.html)
- Counseling on HIV testing and risk reduction, performed in compliance with the US Centers for Disease Control and Prevention (CDC)'s current guidelines or other local guidelines for HIV counseling, testing, and referral as described in Section 9.6
- Discussion with participants regarding need to refrain from blood or tissue donation while being enrolled in this trial due to potential for inadvertent unblinding from HIV-antibody testing.
- Discussion of reproductive status and contraception. A pregnant or breastfeeding person may not be enrolled in this trial. Specific criteria and assessment of contraception and reproductive status are described in study inclusion criteria. Discussion of contraception includes advising a participant who was assigned female sex at birth 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

Once a volunteer has consented to trial participation and is found to meet all eligibility criteria (see Sections 7.1 and 7.2), the HVTN CRS requests the randomization assignment via a Web-based randomization system. Enrollment is simultaneous with first vaccination. In general, the time interval between randomization and enrollment should not exceed 4 working days. However, circumstances may require a participant's enrollment visit to be changed. This may exceed the 4-day randomization time limit. Clinically important lab results will be reviewed with the participant at any visit, as needed.

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
- Urine or serum pregnancy test (for participants who were assigned female sex at birth). Persons who are NOT of reproductive potential due to having undergone hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing. For pregnant participants, see Section 9.12

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

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 a minimum of 60 minutes for observation and initial reactogenicity assessment. Before leaving the clinic, the participant is given the Participant Diary and is instructed on how to complete it. The site will make arrangements to be in contact with the participant during the reactogenicity period (as described in Section 9.9).

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

- HIV risk reduction counseling (as described in Section 9.6)

- Contraception status assessment (as described in Section 9.2 and 9.7). During follow-up in persons who are confirmed pregnant, contraception status assessment is not required

- 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 I and Appendix J:

- 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)

9.4 Follow-up visits

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

- HIV risk reduction counseling (as described in Section 9.6);

- 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 I and Appendix J:

- Contraception status assessment (as described in Section 9.2 and 9.7). During follow-up in persons who are confirmed pregnant, contraception status assessment is not required

- 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 pretest counseling and HIV testing. 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
- Clinically important lab results will be reviewed with the participant, as needed
- Abbreviated physical examination including weight, vital signs, and a symptom-directed evaluation by history and/or appropriate physical exam based on participant self-reported symptoms or complaints
- Complete physical examination, including weight, vital signs, and clinical assessments of head, ears, eyes, nose, and throat; neck; lymph nodes; heart; chest; abdomen; extremities; neurological function; and skin
- Specimen collection
- Clinical laboratory tests including:
    - CBC with differential
    - Chemistry panel (see Section 9.2)
    - Urine dipstick (urinalysis if appropriate; see Section 9.4)
- Urine or serum pregnancy test (for participants who were assigned female sex at birth). Persons who are NOT of reproductive potential due to having undergone hysterectomy or bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing. During follow-up in persons who are confirmed pregnant, pregnancy testing is not required, unless clinically indicated.

9.5 Health Contacts

9.5.1 AESI Contact

CRS staff will contact study participants 12 months after the final study product administration to collect the information listed below. Clinic visits will only be required if HIV confirmatory testing is necessary (see Section 9.6.1); however, a clinic visit may be arranged for other reasons (eg, AESI assessment and referral).

Confirmation of vital status:

If deceased, attempt to learn cause and date of death.

If participant is alive, record the following events:

- SAEs
- AEs of special interest (AESI, see Section 11.2.2). A sample list of AESI is provided in Appendix N
- New diagnosis of HIV infection
- Pregnancies and outcomes, including congenital anomalies/birth defects
- Medically attended adverse events (MAAE), defined as any adverse events leading to an unscheduled visit to a healthcare professional (see HVTN 139 SSP)

All such events will be recorded.

9.5.2 Annual Health Contacts

In addition to the AESI contact, participants will be contacted annually at 2 years and 3 years (month 24 and month 36) following initial study injection (see and Appendix I and Appendix J). At these contacts, CRS staff will collect the information listed below. Clinic visits will only be required if HIV confirmatory testing is necessary (see Section 9.6.1); however, a clinic visit may be arranged for other reasons.

- Confirmation of vital status. If deceased, attempt to learn cause and date of death
- If participant is alive, record the participant's responses to questions regarding any occurrence of the following events since the last HVTN study contact:
  - 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
  - New diagnosis of HIV infection
  - 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.

9.5.3 Interim contacts

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

9.6 HIV counseling and testing

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

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

Study staff will take particular care to inform study participants of the likelihood of routine HIV testing being offered or performed outside the study CRS at emergency rooms, clinics, and medical offices. Such testing has become more likely due to the CDC's revised guidelines for HIV counseling and testing, as well as policy changes in many countries to make HIV testing more frequent and routine. CRS staff should inform participants of their right to opt out of HIV testing outside the study site. CRS staff should inform study participants if local and/or state policies and regulations permit medical providers to perform HIV testing without first informing patients. If this is the case, then CRS staff should advise study participants that they may decline testing preemptively. CRS staff should also inform participants if positive results must be reported to local public health authorities. CRS staff should also inform participants of the need to maintain study blinding by getting HIV testing only at the study CRS. CRS staff should provide participants with CRS contact information and should encourage participants to ask medical providers to contact the CRS. The CRS can verify that the participant is in an HIV vaccine clinical trial and should only be tested at the study CRS.

Potential participants identified as being HIV-infected during screening are not enrolled. Potential and enrolled participants identified as being HIV-infected will be referred for medical treatment, counseling, and management of the HIV infection. Participants who are found to be HIV-infected after enrollment will not receive any additional study product but will continue to be followed in the study for safety assessments. These individuals may also be referred to appropriate ongoing clinical trials or observational studies.

9.6.1 Distinguishing intercurrent HIV infection from vaccine-induced positive serology

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

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

- HIV testing will be performed at multiple timepoints throughout the study (see Appendix G, Appendix H, Appendix I, and Appendix J). The Laboratory Program (or approved diagnostic laboratory) will follow the HVTN HIV testing algorithm (see HVTN 139 SSP), which is able to distinguish vaccine-induced antibody responses from actual HIV infections.

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

9.6.2 VISP registry

Experimental HIV vaccines may induce antibody production to HIV antigens, producing reactive results on commercially available HIV test kits. This is called "vaccine-induced seropositivity" (VISP) (see Section 9.6.1). In order to provide 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.

9.7 Contraception status

Contraception status is assessed and documented as shown in Appendix I and Appendix J for a participant who was assigned female sex at birth 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

assigned female sex at birth and is sexually active in a way that could cause that participant to become pregnant should be reminded at scheduled clinic visits of the importance of using contraception and should be referred to specific counseling, information, and advice as needed. (Specific contraception requirements are listed in Section 7.1.) This reminder should be documented in the participant's study record.

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

9.8 Urine testing

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 non-urinary bleeding (eg, menstruation) or infection, document this issue in the participant's source documentation. For infection, provide appropriate treatment and/or referral and document this in the participant's chart. Following resolution, repeat the dipstick and, if within the eligibility limits specified in the protocol, the participant may be enrolled.

Follow-up visit dipstick testing should be deferred if a participant is experiencing non-urinary bleeding (eg, menstruation), but should be performed as soon as possible. If a follow-up visit dipstick is abnormal due to a participant's non-urinary bleeding (eg, menstruation), document in the comment section of the case report form (CRF) and repeat the dipstick once the participant is no longer experiencing non-urinary bleeding. A micro-urinalysis is not required. If a follow-up visit dipstick or micro-urinalysis is abnormal due to infection, provide appropriate treatment and/or referral and document this in the participant's source documentation. See the Urine Testing MOP for further details.

9.9 Assessments of reactogenicity

For all participants, baseline assessments are performed before and reactogenicity assessments are performed after each vaccination. All reactogenicity symptoms are followed until resolution and graded according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1, July 2017, 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 Participant Diary. Contacts between the participant and the site staff should take place at least once between 1-3 days postvaccination (for Part A

participants, please refer to HVTN 139 SSP). In general, a participant who self-reports any postvaccination reaction greater than mild is seen by a clinician within 48 hours after onset, unless the reaction is improving and/or has completely resolved. Clinic staff will follow new or unresolved reactogenicity symptoms present at day 7 to resolution.

Reactogenicity events are reported using CRFs that correspond to the time of assessment in Table 9-1. Reactogenicity assessments include assessments of systemic and local symptoms, and vaccine-related lesions. 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 according 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 clinician
	Early: At least 60 minutes after vaccination	HVTN CRS clinician
	Between early assessment and 11:59pm day 0	HVTN CRS clinician or participant
1-7[b]	Between 12:00am and 11:59pm on the respective day	HVTN CRS clinician or participant

[a] Day of vaccination

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

9.9.1 Assessment of systemic and local symptoms

Systemic symptoms include increased body temperature, malaise and/or fatigue, myalgia, headache, chills, arthralgia, and nausea. Local symptoms include pain and/or tenderness at 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. All temperatures must be measured by non-axillary thermometry. This includes temperatures taken in the clinic, as well as temperatures taken by participants during the reactogenicity period.

Temperature is reported in degrees Celsius. A measurement is taken once daily during the assessment period and should be repeated if participant is feeling feverish.

9.9.2 Assessment of injection site

Typical injection site reactions are erythema/redness and induration/swelling. The maximum diameter for all injection site reactions is recorded.

All injection site reactions are monitored until resolution. Reactions with diameters ≥5 cm are followed daily; otherwise, the frequency of follow-up is based on clinician judgment. See HVTN 139 SSP for detail.

9.10 Visit windows and missed visits

Visit windows are included in Appendix K (Part A) and Appendix L (Part B). The procedures for documenting missed visits and out of window visits are described in HVTN 139 SSP. If the missed visit is one that required safety assessments or local safety labs, HVTN CRS staff should attempt to bring the participant in for an interim visit as soon as possible.

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

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

9.11 Early termination visit

In the event of early participant termination, site staff should consider if the following assessments are appropriate: a final physical examination, clinical laboratory tests (including urine dipstick, CBC with differential, and chemistry panel), pregnancy testing (note: for persons who are confirmed pregnant, pregnancy testing is not required, unless clinically indicated), social impact assessment, and HIV test. For participants who have a confirmed diagnosis of HIV infection, see Section 9.13.

9.12 Pregnancy

If a participant becomes pregnant during the course of the study, no more injections of study product will be given, but remaining visits and study procedures should be completed unless medically contraindicated or applicable regulations require termination from the study. During follow-up in persons who are confirmed pregnant, pregnancy testing is not required, unless clinically indicated. 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 as described in the HVTN 139 SSP section on Pregnancy Management and Reporting. If the participant is no longer pregnant, refer to Section 7.3.3.

9.13 HIV infection during the study

If a participant becomes HIV-infected during the course of the study, no additional study product will be administered. Participants will be encouraged to continue scheduled study visits for 12-weeks following their last study product administration. Follow-up duration for participants diagnosed with HIV infection may be adjusted in consultation with the CRS investigator and the HVTN 139 PSRT (eg, to avoid interference with participant initiation of HIV treatment). At post-infection follow-up visits, only specimens required for protocol-specified safety laboratory tests, urinalysis, and pregnancy tests will be collected (note: for persons who are confirmed pregnant, pregnancy testing is not required, unless clinically indicated); in addition, some clinic procedures may be modified or discontinued (see Appendix I and Appendix J).

10 Laboratory

10.1 HVTN CRS laboratory procedures

The HVTN 139 Site Processing Lab Instructions and SSP provide further guidelines for operational issues concerning the clinical and processing laboratories. These documents include 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 G and Appendix H. 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.

Of note, all assays described below are performed as research assays to evaluate the ability of the vaccine to induce immune responses, and are not approved for use in medical care. Results from these assays are not made available to participants or medical professionals to guide treatment decisions

10.2 Total blood volume

Required blood volumes per visit are shown in Appendix G and Appendix H. 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 timepoint

The primary immunogenicity timepoints for Part A occur at 1 month post-vaccination; and for Part B at 1 month post first vaccination, 1 month post-second vaccination, and 2 weeks after the protein boost. Endpoint assays may be performed on specimens collected from participants at the primary immunogenicity timepoint and samples collected at baseline and other timepoints.

10.4 Endpoint assays: cellular

10.4.1 Flow cytometry

Intracellular cytokine staining (ICS) 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 vaccines, or with peptides that encompass HIV sequence diversity (such as PTE peptides). Data will be reported as percentages of CD4+ or CD8+ T cells responding to a specific peptide pool. Additional cell surface markers, cytokines, or functional markers may also be analyzed.

10.4.2 Antigen-specific B cell and plasmablast phenotyping

HIV-1 antigen-specific memory B cells and plasmablasts induced by vaccination will be identified and characterized using fluorescently-labeled recombinant proteins in combination with a flow cytometry phenotyping panel. In particular, HIV Env-reactive B cells and plasmablasts will be enumerated and may be further characterized for expression of memory, activation, inhibitory or other markers of interest.

10.5 Endpoint assays: humoral

10.5.1 Binding antibody multiplex assay (BAMA)

HIV-1–specific total IgG binding antibodies to vaccine-matched antigens as well as non-matched antigens will be assessed on serum samples from study participants. In addition, HIV-1–specific IgA and IgG subclass (IgG1, IgG2, IgG3, and IgG4) binding antibodies may also be assessed.

10.5.2 Antibody avidity

Antibody avidity may be measured using BAMA with the addition of a dissociation step to calculate the antibody avidity index (BAMA-AI). Biolayer Interferometry (BLI) and/or Surface Plasmon Resonance (SPR) technologies may also be used to define antibody avidity.

10.5.3 HIV-1- specific neutralizing antibody assay (NAb)

HIV-1–specific nAb assays will be performed on serum samples from study participants. The TZM-bl assay will test neutralization of the vaccine strain(s) Du422, Du172, CH505TF and a single highly neutralization-sensitive tier 1 virus as a positive control (eg, MW965.25). The global panel and/or clade-specific panels may be used to assess tier 2 neutralization (54, 58).

10.5.4 Antibody-dependent cellular cytotoxicity assay (ADCC)

ADCC activity may be assessed using serum samples from study participants using one or both of the assays listed here. For the Granzyme B flow-based cytotoxicity assay, participant sera are incubated with effector cells sourced from human PBMCs and gp120-coated CEM.NKR.$_{CCR5}$ target cells. ADCC is quantified as net percent granzyme B activity which is the percent of target cells positive for GranToxiLux (GTL), an indicator of granzyme B uptake, minus the percent of target cells positive for GTL when incubated with effector cells but without sera. For the Luciferase-based cytotoxicity assay, participant sera are incubated with infectious molecular clone (IMC)-infected CEM.NKR.$_{CCR5}$ cells and percent killing is measured through the use of Viviren luminescence.

10.5.5 Antibody-dependent cellular phagocytosis (ADCP)

To assess the ability of vaccine-elicited antibodies to engage cellular FcR for potential antiviral function, ADCP may be measured using serum samples from study participants taken at baseline and at a primary immunogenicity timepoint. ADCP is measured by assessing the ability of vaccine elicited antibodies to mediate monocyte phagocytosis of HIV-1 antigen coated fluorescent beads by flow cytometry (59, 60). An array of antigens or viruses in addition to specimens from other timepoints may also be analyzed at the discretion of the HVTN Laboratory Center, which may be contingent on the results of the primary immunogenicity timepoint.

10.5.6 Antibody-dependent neutrophil phagocytosis (ADNP)

To assess the ability of vaccine-elicited antibodies to engage cellular FcR for potential antiviral function, ADNP may be measured using serum samples from study participants at a primary immunogenicity timepoint and compared to placebo controls. ADNP is measured by assessing the ability of vaccine elicited antibodies to mediate neutrophil cell line phagocytosis of HIV-1 antigen coated fluorescent beads by flow cytometry (61). An array of antigens or viruses in addition to specimens from other timepoints may also be analyzed at the discretion of the HVTN Laboratory Center, which may be contingent on the results of the primary immunogenicity timepoint.

10.5.7 Fc receptor binding

The ability of vaccine elicited antibodies to bind to cellular Fc receptors enables characterization of the antibody Fc profile that results in antiviral function (ie, includes subclass and glycans). HIV-specific antibody binding to FcγR proteins will be assessed by the FcR BAMA. The FCR BAMA is a modification of the binding assay where fluorescently labeled FcR proteins are utilized as the detection reagent for serum antibodies bound to HIV proteins on microspheres. The readout may include the magnitude and/or avidity for an array of Fc receptors involved in mediating antiviral activity.

10.5.8 Infected cells antibody binding assay (ICABA)

The capacity of vaccine elicited antibodies to recognize epitopes exposed on the surface of infected cells may be assessed using serum samples from study participants. This assay measures the capacity of vaccine elicited antibodies to recognize HIV envelope on the surface of infected cells (ie, the first step in mediating antibody Fc effector function) with a readout by flow cytometry.

10.5.9 Antivector antibody studies (AdC6- and AdC7-specific neutralizing antibodies)

Adenovirus neutralization assays will be performed on serum samples from study participants. These assays will be conducted by incubating a dose of diluted GFP-expressing AdC6 or AdC7 vector with diluted sera. The vector-serum mixture will be mixed with HEK 293 cells and transferred into culture wells to incubate; the cells will be screened visually for green fluorescence by microscopy. The titer of neutralizing antibodies to AdC6 and AdC7 will be determined as the reciprocal serum dilution that causes an ~50% reduction of fluorescence in comparison to fluorescence of the control wells infected with vector only.

10.6 HVTN Lab Center assay portfolio

Additional assays may be performed per the HVTN Laboratory Center assay portfolio, which includes immune assessments such as those for cellular, humoral, and innate immune responses and host genetics. The assay portfolio will be updated periodically to include new assays and adjust qualification levels of existing 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.

10.8 Specimen storage and other use of 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 (see Appendix A and Appendix B).

This research may relate to HIV, vaccines, the immune system, and other diseases. This could include 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 research on specimens ("other use") will occur only after review and approval by the HVTN, the IRB/EC of the researcher requesting the specimens, and the IRBs/ECs/REs of the CRSs if required.

As part of consenting for the study, participants document their initial decision to allow or not allow their specimens to be used in other research, and they may change their decision at any time. The participant's initial decision about other use of their specimens, and any later change to that decision, is recorded by their CRS in a Web-based tool that documents their current decisions for other use of their specimens. The HVTN will only allow other research to be done on specimens from participants who allow such use.

CRSs must notify HVTN Regulatory Affairs if institutional or local governmental requirements pose a conflict with or impose restrictions on specimen storage or 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 139 PSRT

The HVTN 139 PSRT is composed of the following members:

- DAIDS medical officer representative
- Protocol chair / medical monitor
- Protocol cochair
- Protocol Team leader
- Regional Medical Liaison
- Clinical safety specialist

The clinician members of HVTN 139 PSRT are responsible for decisions related to participant safety.

The Protocol Team clinic coordinator, clinical data manager, vaccine developer representative, clinical trial manager, and others may also be included in HVTN 139 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 during the main study (as defined in Section 1); for safety reviews during the annual health contacts period, see Section 11.5.3. The reviews consist of evaluation of cumulative reactogenicity events, AE, laboratory safety data, and individual reports of adverse events requiring expedited reporting to DAIDS. The SMB conducts additional special reviews at the request of the HVTN 139 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 139 PSRT (see Section 11.1.1) and HVTN SMB (see Section 11.1.2)

11.1.4 HVTN LOC roles and responsibilities in safety monitoring

- Daily monitoring of clinical data for events that meet the safety pause and HVTN 139 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
- Providing support to the HVTN 139 PSRT

11.2 Safety reporting

11.2.1 Submission of safety forms to SDMC

Site staff must submit all safety forms (eg, reactogenicity, adverse experience, urinalysis, local lab results, and concomitant medications) within 3 business days of the site being notified, or sooner per Table 11-1. 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. For the case of a longer site holiday closure, site staff must submit the data by the end of the 5th day (local time) after receiving the information even if this day is a holiday.

For example: If the site becomes aware of an AE on Thursday (Day 0), the site must submit the data by the end of the next business day, on Friday. If there is a longer site holiday closure, then this AE must be reported no later than the end of the fifth day, Monday (Day 4). If Monday is a holiday as well, all safety forms still need to be submitted by the end of Monday (Day 4).

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 (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1, July 2017, available on the RSC website at https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables, except:

- Unintentional Weight Loss is required to be reported as an AE only if it is considered to be potentially deleterious to the participant's health (see HVTN 139 Study Specific Procedures);
- Injection Site Erythema or Redness and Injection Site Induration or Swelling will not consider surface area and interference with usual social and functional activities such that:
  - Grade 1 is: 2.5 to < 5 cm in diameter;
  - Grade 2 is: ≥ 5 to < 10 cm in diameter;
  - Grade 3 is: ≥ 10 cm in diameter 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);
- Creatinine is required to be reported as an AE only if it is gradable per the increase from local lab ULN parameter. Do not grade elevated creatinine based on the change from the baseline parameter;
- Creatinine clearance or estimated glomerular filtration rate (eGFR) is required to be reported based only on the reported value or if dialysis is needed. Do not grade Creatinine clearance or eGFR based on the change from the baseline parameter.

Unsolicited AEs will be collected over a period of 30 days after each vaccination visit. All collected 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 (see Section 11.2.3), (2) if the AE meets the criteria for a safety pause/prompt AE review (see 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 N.

Certain AEs will be collected and reported throughout the study until 12 months following final study product administration:

- SAEs/EAEs,
- AESIs,

- Medically Attended Adverse Events (MAAEs)
- New chronic conditions requiring medical intervention for ≥ 30 days,
- Newly diagnosed or treated STIs,
- AEs leading to early participant withdrawal or early discontinuation of study product(s) administration.

Report the subset of AEs bulleted in Section 9.5.1 until the AESI health contact is complete and the subset of AEs bulleted in Section 9.5.2 until the last annual health contact is complete.

Sites are expected to notify HVTN clinical safety staff of any serious safety concern requiring their attention (Table 11-1). Telephone numbers and email addresses are found on the protocol home page on the HVTN Members' site (https://members.hvtn.org/protocols/hvtn139). Concerns requiring immediate attention should be communicated by calling the clinical safety phone.

In the case of email notification, clinical safety staff will reply within one business day. Serious events that meet pause rule criteria will be addressed immediately (as outlined in Table 11-1). If email service is not available, the CRS should notify clinical safety staff of the event by telephone, and 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 https://rsc.niaid.nih.gov/clinical-research-sites/manual-expedited-reporting-adverse-events-daids.

The internet-based DAIDS Adverse Experience Reporting System (DAERS) must be used for expedited AE (EAE) reporting to DAIDS. In the event of system outages or technical difficulties, expedited AE reports may be submitted via the DAIDS EAE Form. This form is available on the DAIDS RSC website at https://rsc.niaid.nih.gov/clinical-research-sites/paper-eae-reporting.

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

For questions about EAE reporting, please contact the DAIDS RSC Safety Office at (DAIDSRSCSafetyOffice@tech-res.com).

The study products for which expedited reporting are required are:

- AdC6-HIVgp140
- AdC7-HIVgp140
- CH505TF gp120
- GLA-SE adjuvant

From enrollment until 12 months following final study product administration, the SAE Reporting Category will be used.

From 12 months following final study product administration until completion of the month 36 annual health contact, the SUSAR Reporting Category will be used.

After the participant has completed the month 36 annual health contact and is off study, sites must report SUSARs if the study site staff becomes aware of the events on a passive basis (eg, from publicly available information).

The NIAID/DAIDS will report all unexpected SAEs related to the study products observed in this clinical trial to the South African Health Products Regulatory Authority (SAHPRA). However, because safety is a primary study endpoint, the study 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 SAHPRA based on the blinded attribution assessment.

In some cases, the PSRT or CRS may believe unblinding of the site PI or their designee and participant would be appropriate to facilitate the clinical management of an AE or SAE. The HVTN MOP specifies procedures for emergency unblinding, and for early unblinding for medical reasons.

11.3 Safety reviews

11.3.1 Safety review # 1: Low-dose Initial Vaccination

Enrollment across all participating HVTN CRSs will be restricted to a maximum of 1 participant per day and restricted until 5 participants have been enrolled in Part A (Groups 1-3). Enrollment will then pause. The HVTN 139 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 5 participants, and will determine whether it is safe to proceed with full enrollment in Part A.

11.3.2 Safety review # 2: Low-dose (Part A) Safe-to-Proceed

In addition to monitoring participant safety throughout the study period, the HVTN 139 PSRT will review cumulative safety data available on all 12 Part A participants up to and including the 2-week visit after the final participant receives a vaccination to determine whether dose escalation and enrollment in Part B may occur. The HVTN 139 PSRT may consult with the HVTN SMB on an ad hoc basis for these evaluations.

11.3.3 Safety review # 3: Full Enrollment at Targeted Dose

Enrollment will start in Part B for Groups 4 -6 and pause after the first 12 participants have enrolled. In addition to monitoring participant safety throughout the study period, the HVTN 139 PSRT will review all cumulative safety data available up to and including the 2-week visit after the vaccination in the first 12 participants enrolled in Part B. Based on the assessment of this safety data, the HVTN 139 PSRT will determine whether it is safe to proceed with full enrollment in Part B. The HVTN SMB may perform an additional unblinded review of this safety data to make the final determination based on safety for proceeding with full enrollment in Part B.

11.4 Safety pause and prompt PSRT AE review

When a trial is placed on an unplanned safety pause, all enrollment and vaccinations 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 139 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 139 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 LOC 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 PSRT notification
SAE, related	Grade 3, 2, or 1	Email and submit forms immediately	Immediate PSRT notification and prompt PSRT AE review to consider pause
AE[b], related	Grade 4 or 3	Email and submit forms immediately	Immediate PSRT notification and prompt 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/hvtn139).

[b] Does not include the following Grade 3 subjective reactogenicity symptoms: injection site pain, tenderness, fatigue/malaise, myalgia, arthralgia, chills, headache, nausea (unless IV rehydration required).

For all safety pauses, HVTN LOC notifies the HVTN 139 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 LOC notifies the SMB.

Once a trial is paused, the HVTN 139 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 LOC 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 139 PSRT assessment, DAIDS RAB notifies the SAHPRA as needed.

If an immediate HVTN 139 PSRT notification or prompt HVTN 139 PSRT AE review is triggered, HVTN LOC notifies the HVTN 139 PSRT as soon as possible during working hours (local time)—or, if the information was received during off hours, by the morning of the next workday. If a prompt HVTN 139 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, unanticipated problems involving risks to participants or others, 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 139 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 LOC for events requiring expedited reporting to DAIDS, and events that meet safety pause criteria or prompt HVTN 139 PSRT AE review criteria.

11.5.2 Weekly review

During the injection phase of the trial, the HVTN 139 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 139 PSRT. HVTN LOC 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.

11.5.3 Annual Health Contacts quarterly review

After the main study period, a monitoring team reviews safety reports quarterly during the annual health contacts period. This monitoring team comprises a DAIDS Medical Officer, LOC medical monitor, and an HVTN clinical safety staff member.

11.6 Study termination

NIAID reserves the right to terminate or curtail a clinical study for any reason, including but not limited to the following (reference: https://grants.nih.gov/grants/guide/rfa-files/RFA-AI-12-012.html):

- risk to subject safety
- the scientific question is no longer relevant or the objectives will not be met (ie slow accrual)
- failure to comply with GCP, US Federal regulations, or Terms and Conditions of Award
- occurrence of unforeseen drug safety issues or data from preclinical studies indicate a presence of unanticipated toxicity
- risks that cannot be adequately quantified
- ethical concerns raised by the local community or local medical care/health care authorities
- failure to remedy deficiencies identified through site monitoring
- substandard data
- reaching a major study endpoint substantially before schedule with persuasive statistical significance

This study may also be terminated early by the determination of the HVTN 139 PSRT, a pertinent national regulatory authority, SAHPRA, NIH, Office for Human Research Protections (OHRP), or study product developers. 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 (ICH E6 (R2)), 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;
- Exploratory and ancillary studies and sub-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 139 SSP.

12.1 Social impacts

Participants in this study risk experiencing discrimination or other personal problems that may result from study participation itself or from the development of VISP: these are known collectively as negative social impacts. 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 negative social impact, a designated NIAID or HVTN Core representative can be contacted.

Participants may also experience positive social impacts as a benefit of study participation. When asked, participants say that being in a study made them feel good about helping others, increased their knowledge about HIV, and improved their self-esteem.

Negative social impacts are tabulated by the SDMC and are subjected to descriptive analysis. The goals are to reduce the incidence of negative social impacts and enhance the ability of study staff to mitigate them when possible. Reports of positive social impacts may assist sites in providing participants with a favorable experience.

Summary tables of all negative 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 nucleic acid molecules, it must comply with regulations set forth in the NIH's *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (April 2019). Information about the study must be submitted to the Institutional Biosafety Committee (IBC) for each CRS. Investigators at each CRS 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's initial protocol registration for this trial before participants are enrolled at the CRS. If this protocol is amended, investigators should follow the requirements of their respective 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 can contact the participant without IRB/EC approval if such communication is necessary to avoid imminent harm to the study participant. The CRS must 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 139 are described below.

Protocol history and modifications

Date: February 23, 2021

Protocol version: Version 1.0
Protocol modification:

Original protocol

14 Document references (other than literature citations)

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

- Assessment of Understanding. Accessible through the HVTN protocol-specific website.
- Current CDC Guidelines:
  - Revised Recommendations for HIV Testing of Adults, Adolescents, and Pregnant Women in Health-Care Settings. Available at http://www.cdc.gov/mmwr/PDF/rr/rr5514.pdf
  - Revised Guidelines for HIV Counseling, Testing, and Referral. Available at http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5019a1.htm
- 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/prmanual.pdf
- Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events. Corrected Version 2.1, July 2017. Available at https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables
- The Manual for Expedited Reporting of Adverse Events to DAIDS. Version 2.0, January 2010. Available at https://rsc.niaid.nih.gov/clinical-research-sites/manual-expedited-reporting-adverse-events-daids
- HVTN Certificate of Confidentiality. Accessible through the HVTN website.
- HVTN 139 Special Instructions. Accessible through the HVTN protocol-specific website.
- HVTN 139 Study Specific Procedures. Accessible through the HVTN protocol-specific website.
- HVTN 139 Site Processing Laboratory Instructions. Accessible through the HVTN protocol-specific website.
- HVTN Manual of Operations. Accessible through the HVTN website.

- Dangerous Goods Regulations (updated annually), International Air Transport Association. Available for purchase at https://www.iata.org/publications/dgr/Pages/index.aspx

- HVTN Lab Center assay portfolio (available upon request).

- International Council on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) E6(R2), 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/office-biotechnology-activities/biosafety/nih-guidelines

- NIH Policy and Guidelines on the Inclusion of Women and Minorities as Subjects in Clinical Research, November 28, 2017 Amendment (available at https://grants.nih.gov/grants/guide/notice-files/NOT-OD-18-014.html)

- Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks, March 2016.

- Requirements for Source Documentation in DAIDS Funded and/or Sponsored Clinical Trials. Available at https://www.niaid.nih.gov/sites/default/files/daids-sourcedocpolicy.pdf

- 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 (2018 requirements). Current requirements available at https://www.hhs.gov/ohrp/regulations-and-policy/regulations/45-cfr-46/index.html

- Guidelines for Phase I clinical trials, The Association of the British Pharmaceutical Industry. Available at https://www.abpi.org.uk/publications/guidelines-for-phase-i-clinical-trials-2018-edition/

- The Protection of Personal Information Act (POPIA, South Africa, 2013). Available at https://www.gov.za/sites/default/files/gcis_document/201409/3706726-11act4of2013protectionofpersonalinforcorrect.pdf

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

15 Acronyms and abbreviations

A/G	albumin/globulin (ratio)
Ab	antibody
Ad	adenovirus
AdC6	Ad vectors derived from chimpanzee serotypes 6
AdC7	Ad vectors derived from chimpanzee serotypes 7
ADCC	antibody-dependent cellular cytotoxicity
ADCP	antibody-dependent cellular phagocytosis
ADNP	antibody-dependent neutrophil phagocytosis
AE	adverse event
AESI	adverse events of special interest
ALT	alanine aminotransferase
ANOVA	analysis of variance
ART	antiretroviral therapy
AUC-MB	area-under-the-magnitude-breadth
AVEG	AIDS Vaccine Evaluation Group
β-HCG	beta human chorionic gonadotropin
BMI	body mass index
CAB	Community Advisory Board
CBC	complete blood count
CDC	US Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CI	confidence intervals
CIOMS	Council for International Organizations of Medical Sciences
COMPASS	Combinatorial Polyfunctionality analysis of Antigen-Specific T-cell Subsets
CRF	case report form
CRPMC	NIAID Clinical Research Products Management Center
CRS	clinical research site
CTL	cytotoxic T lymphocyte
DAERS	DAIDS Adverse Experience Reporting System
DAIDS	Division of AIDS (US NIH)
DHHS	US Department of Health and Human Services
DSMB	NIAID Data and Safety Monitoring Board
EAE	adverse events requiring expedited reporting to DAIDS
EC	Ethics Committee
eGFR	estimated glomerular filtration rate
EIA	enzyme immunoassay
ELISA	enzyme-linked immunosorbent assay

Fc	fragment crystallizable
FcR	Fc receptor
FDA	US Food and Drug Administration
FPR	false positive rate
Fred Hutch	Fred Hutchinson Cancer Research Center
GLA	glucopyranosyl lipid A
GCP	Good Clinical Practice
GPP	Good Participatory Practices
HBsAg	hepatitis B surface antigen
HCDR3	heavy chain complementarity-determining region 3
HCV	hepatitis C virus
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HVTN	HIV Vaccine Trials Network
IB	Investigator's Brochure
IBC	Institutional Biosafety Committee
ICH	International Council on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICABA	infected cells antibody binding assay
ICS	intracellular cytokine staining
IDRI	Infectious Disease Research Institute
IgG	immunoglobulin subtype G
IM	intramuscular (injection)
IND	Investigational New Drug
IRB	Institutional Review Board
IUD	intrauterine device
LOC	HVTN Leadership Operations Center
LOD	limit of detection
mAb	monoclonal antibody
MAR	missing at random
MCAR	missing completely at random
MIMOSA	Mixture Models for Single-cell Assays
MMR	measles, mumps, and rubella
nAb	neutralizing antibody
NHP	nonhuman primate
NIAID	National Institute of Allergy and Infectious Diseases (US NIH)
NIH	US National Institutes of Health
OBA	NIH Office of Biotechnology Activities

OHRP	US Office for Human Research Protections
OPV	oral polio vaccine
PAB	DAIDS Pharmaceutical Affairs Branch
PBMC	peripheral blood mononuclear cell
PBS	phosphate-buffered saline
PCA	principal component analysis
PCR	polymerase chain reaction
PFS	polyfunctionality score
PI	Principal Investigator
PrEP	pre-exposure prophylaxis
PSRT	Protocol Safety Review Team
PTE	potential T-cell epitope
PV	pseudovirus
RAB	DAIDS Regulatory Affairs Branch
RAC	NIH Recombinant DNA Advisory Committee
RE	regulatory entity
RM	Rhesus Macaque
RSC	DAIDS Regulatory Support Center
SAE	serious adverse event
SAHPRA	South African Health Products Regulatory Authority
SAP	Statistical Analysis Plan
SCHARP	Statistical Center for HIV/AIDS Research and Prevention
SD	Study Day
SDMC	statistical and data management center
SIV	simian immunodeficiency virus
SMB	Safety Monitoring Board
SPT	DAIDS Safety and Pharmacovigilance Team
SSP	Study Specific Procedures
SUSAR	Suspected Unexpected Serious Adverse Reaction
TB	tuberculosis
UCA	unmutated common ancestor
UW-VSL	University of Washington Virology Specialty Laboratory
VISP	Vaccine induced seropositivity
VLP	virus-like particle
VP	virus particle
VRC	Vaccine Research Center (NIAID)

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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 HIV-1 vaccines based on chimpanzee serotypes of adenovirus expressing clade C gp140 and a CH505TF gp120 protein boost in healthy, HIV- uninfected adult participants

HVTN protocol number: HVTN 139

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.

Key information

- Being in this research study is voluntary. It is your choice.
- You are being asked to take part in this study because you are 18-50 years old, HIV negative and healthy.
- The purpose of this study is to see if the study vaccines are safe, if people are able to take them without becoming too uncomfortable, and to see how a person's immune system responds to the study vaccines.
- Your participation in this study will include about 6 months of clinic visits. After that, there will be annual health contacts by phone or message at one year, two years, and three years after you got the study injection.
- Procedures will include blood draws and two injections of a study vaccine or placebo given at one study visit.
- There are risks from participating.
    - We will tell you more information about risks later in this consent form.
    - This is the first study to give these study vaccines to people. Because these study vaccines have not been given to people yet, we do not know what all of the risks may be.
    - We do not expect the study vaccines to benefit you in any way.

About the study

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

About 34 people will take part in this study at multiple sites. The researcher in charge of this study at this clinic is [Insert name of site PI]. The US National Institutes of Health (NIH) is paying for the study.

This study is divided into two parts, Part A and Part B. About 12 people will take part in Part A of this study. After we see the results from Part A, we will decide whether or not to do Part B of the study. If we decide to do Part B, 22 more people will join.

You are being invited to join Part A of the study, which will only test 2 of the 3 study products.

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 getting HIV if you are exposed to the virus.**

Site: Any change to the following boxed text requires approval from HVTN Regulatory Affairs at vtn.core.reg@hvtn.org. You can remove the box around the text.

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.

The studies that found a higher risk of HIV infection were testing a vaccine made from a weakened common cold virus called adenovirus type 5 (Ad5).

In one study, the group of men at higher risk had some things in common. In addition to getting the vaccine, they either:

- had antibodies to Ad5 (your body makes antibodies to fight infection),
- or they were uncircumcised (still had the foreskin on their penises),
- or both.

Men who were circumcised and did not have these antibodies did not have higher risk when they got the vaccine. In the group with higher risk after vaccination, the risk seemed to lessen after about a year and a half.

Only a few women in that study got HIV, so we can't tell if the vaccine affected their risk.

In another study, men who got the vaccine had higher risk whether or not they were circumcised or had antibodies to Ad5. Many women got HIV during the study but we can't tell if the vaccine affected their risk.

This study differs from the studies in which people who got the vaccine had a higher or lower risk of getting HIV. We 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 in Part A are called AdC6-HIVgp140 and AdC7-HIVgp140. From here on, we will call them AdC6 and AdC7 vaccines or the study vaccines. The study vaccines have not been given to people before. They are experimental HIV vaccines. That means we do not know if the study vaccines will be safe to use in people, or if they will work to prevent HIV infection. These vaccines are used only in research studies.

The study 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.

The vaccines were developed by the Division of AIDS (DAIDS) at the US National Institutes of Health (NIH).

The AdC6 and AdC7 study vaccines are "adenoviral vector" vaccines. They are made from two kinds of adenovirus. Adenoviruses cause colds, coughs, and

diarrhea. The adenoviruses used in these study vaccines are from chimpanzees so they should not cause infections in people. The adenoviruses in the vaccines have been changed so that they will not spread in your body or to people around you. You cannot become infected with HIV or AIDS from the study vaccines.

The AdC6 and AdC7 study vaccines have been made in a lab to tell your body to make a small amount of some proteins that are found in HIV. Your body's immune system might recognize these proteins and prepare itself to fight HIV by making antibodies and T cells. Antibodies and T cells are parts of an immune response. Immune responses are part of the body's natural defense against disease.

Some people will get a placebo, a substance that does not contain vaccine. In this study, the placebo is sterile salt water.

In Part A of the study, we are looking to see if the AdC6 or AdC7 study vaccines are safe when given in a small amount. We will also measure your body's immune responses.

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 have 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 trouble 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. Anyone can get an autoimmune disease. It is possible but unlikely that a vaccine can lead to an autoimmune disease or make one worse.

Risks of the AdC6 and AdC7 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.

These AdC6 and AdC7 study vaccines have not been given to people before. Studies in animals showed that these vaccines are safe. Even if something looks like it is safe or works in animals, it may not be true for people. Other chimpanzee adenovirus vaccines have been used in other research studies for other diseases

and did not cause serious health problems, even when given at higher doses than will be used in this study.

Some people who get a study product may have changes in their blood or urine that can only be seen in laboratory tests. These changes usually go away on their own in a few days but sometimes require more testing.

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. Being in more than one study may not be safe. We check to make sure that you are not in more than one study by taking your **fingerprint** on an electronic system. This information is only accessed by a few members of the study team using a secure password.

   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 ask you about medications you are taking, including HIV pre-exposure prophylaxis (PrEP). We will ask you about behaviors that might put you at risk

for getting HIV. If you were assigned female sex at birth, 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.

Site: 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 assigned female sex at birth and could become pregnant, you must agree to use 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 2 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 6 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 will contact you after the main study ends to check your health, and 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).

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

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

Not everyone in this study will get a study vaccine. Some people will get a placebo. We will compare the results from people who got the placebo with results from people who got the study vaccines.

You have about an 83% chance of getting one of the study vaccines. *Site: Modify the randomization metaphor in the next sentence as appropriate to your local culture.* Whether you get a study vaccine or the placebo is completely random, like flipping a coin.

We have no say in whether you get a study vaccine 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 **in Part A** completes their final main 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 one of 3 groups. You will get 2 injections into the same upper arm at one clinic visit.

Part A Group	N	Dose	Enrollment Visit
1	5	Low dose	AdC6
2	5	Low dose	AdC7
3	2	None	Placebo

You will have to wait in the clinic for at least an hour after the injections 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. *Site: Customize the next sentence based on how you collect reactogenicity information.* You will bring this information back to the clinic at your next visit. Within 3 days of the injection, we will also need to be in contact with you to ask how you are doing.

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 assigned female sex at birth
- 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
- 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 190 mL (about 1 tablespoon to a little less than 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 E, 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 about protecting yourself from HIV.

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. The HVTN will test your samples to see how your immune system responds to the study products.

We will send your samples (without your name) to labs approved by the HVTN for this study, which are located in South Africa and in the United States. 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. The genetic testing will only involve some of your genes, not all of your genes (your genome). The researchers will study only the genes related to the immune system and HIV and those that affect how people get HIV.

If you get HIV, 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 products.

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.

These tests done on your samples are for research purposes, not to check your health. The labs will not give the results to you or this clinic because their tests are not approved for use in making health care decisions. These labs are only approved to do research tests.

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

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

16. When samples are no longer needed for this study, the HVTN wants to use them in other studies and share them with other researchers.

The HVTN calls these samples "extra samples". The HVTN will only allow your extra samples to be used in other studies if you agree to this. You will mark your decision at the end of this 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 South Africa.

How long will the samples be stored? There is no limit on how long your extra samples will be stored. *[Site: Revise the previous sentence to 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 HVTN or other researchers. Once we share your samples and information, we may 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 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 HVTN or other researchers? The samples and information will be labeled with a code number. The key to the code will stay at this clinic. It will not be shared with anyone who does not need to know your name. 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.

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

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, but your name and other personal information will not be included. 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. There may be other unknown risks.

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

People who may see your information are:

- Researchers who use your extra samples and information for other research
- Government agencies that fund or monitor the research using your extra samples and information
- The researcher's Institutional Review Board or Ethics Committee
- Any regulatory agency that reviews clinical trials
- 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 and information may be published. No publication will use your name or identify you personally.

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

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.

Site: Any change to the following boxed text requires approval from HVTN Regulatory Affairs. You can remove the box around the text.

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,
- Any regulatory agency that reviews clinical trials,
- [Insert name of local IRB/EC] ,
- [Insert name of local and/or national regulatory authority as appropriate],
- Wistar Institute and people who work for them,
- Duke University and people who work for them,
- IDRI and people who work for them,
- The Division of AIDS and people who work for them,
- The HVTN and people who work for them,
- The 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. If you are found to have a medical condition that we are required to report by law, then some of your information may be shared. 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.) If your site does not have public health or legal reporting requirements, you may delete the last sentence in the paragraph above, along with the bullets below.

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

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.

18. If you become pregnant or get HIV during the study, we will discuss your options for participation with you.

We will encourage you to stay in the study if you choose. This means that we may still take blood and urine samples and ask you to come to scheduled clinic visits. We will also call you once a year for 3 years after your injection to check on your safety.

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

If you get HIV, we will help you get care and support. We will counsel you about having HIV and about telling your partner(s). *Site: Modify the following sentence as appropriate.* We will not provide or pay for any of your HIV care directly.

19. We may take you out of the study at any time.

We may take you out of the study if:

- you do not follow instructions,
- we think that staying in the study might harm you,or
- the study is stopped for any reason.

Other Risks

20. 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:

Most people who join HVTN studies report no problems or discrimination as a result of their participation in an HIV vaccine study. Most problems that were reported were with personal relationships. These problems can include family or friends worrying, getting upset or angry, or assuming that you have HIV or are at high risk and treating you unfairly as a result. Most problems that were reported by participants had minimal impact on their lives. Rarely, a person has lost a job because the study took too much time away from work, or because their employer thought they had HIV.

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

If you have a positive test result caused by a study vaccine 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 a study vaccine. If you receive a positive HIV test result and we determine it is because you have HIV, we will refer you for follow-up care.

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

Site: Modify the following paragraph if applicable. If someone believes you have HIV even if you do 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. We may also show you a video to help you think about these VISP issues.

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

You should always tell the delivery staff if you have VISP. However, you may still be tested for HIV using the antibody test when you deliver your baby. If your test is positive and the delivery staff believes you have HIV, 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 have 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:

It is unlikely, but the genetic tests done on your samples 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 getting HIV if exposed. If you get 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 the study vaccines will affect how you respond to any future approved HIV vaccine. 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

21. The study may not benefit you.

We do not expect the study vaccines to 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.

When asked, most study participants say that participating in a study made them feel good about helping others, increased their knowledge about HIV, and improved their self-esteem.

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

22. 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 Bill of Rights and Responsibilities (BRR) for HIV Research. We will give you a copy of it.

Leaving the study

23. 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.

Previously collected information about you will remain in the study records and will be included in the analysis of results. Your information can not be removed from the study records.

We may 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 may 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: Approval from HVTN Regulatory Affairs (at vtn.core.reg@hvtn.org) is needed for any change (other than those that the instructions specifically request or those previously approved by HVTN Regulatory Affairs) to the boxed text. You can remove the box around the text.

24. **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, the HVTN has 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.

In this study, our clinic has insurance to cover your medical treatment in the case of a study-related injury. We will follow the **Association of the British Pharmaceutical Industry guidelines** for payment of study-related injury. We can give you a copy of these guidelines. In rare cases, the insurance funds may not be enough.

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/or 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.

Annual health contacts

25. After your clinic visits end, we will contact you once a year until 3 years after your injection to check your health

We will contact you once a year to ask questions about your health.

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 have HIV, we will ask you to come back to the clinic for another HIV test. We will draw about 15 mL (1 tablespoon) of blood. We may ask you to come back more than once for this testing.

Because we will want to contact you once a year, please tell us if your contact information changes, if you are moving away, or if you do not want us to contact you anymore.

You can tell us at any time that you don't want any more annual health contacts. If you do so, you will not lose any benefits or rights you would normally have.

Questions

26. 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 or title 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 or title and telephone number of the investigator or other study staff].

This study has been reviewed and approved by a committee called the
[name of local IRB/EC]. 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 or title and telephone number of person on IRB/EC] , at the committee.

The study has been structured in accordance with the Declaration of Helsinki (last updated October 2013) which deals with the recommendations guiding doctors in biomedical research involving human participants, the Ethics in Health Research: Principles, Structures and Processes Second Edition 2015, and Guidelines for Good Practice in the Conduct of Clinical Trials in Human Participants in South Africa. We can provide you with copies of these guidelines if you wish to review them. In addition, the recent Protection of Personal Information Act (POPIA) ensures that all South African institutions conducts themselves in a responsible manner when collecting, processing, storing and sharing another entity's personal information by holding them accountable should they abuse or compromise your personal information in any way.

If you want to leave this study, contact
[name or title and telephone number of the investigator or other study staff].

You can reach a study staff member 24 hours a day at [telephone number].

If you have questions about this trial you should first discuss them with your doctor or the ethics committee (contact details as provided on this form). After you have consulted your doctor or the ethics committee and if they have not provided you with answers to your satisfaction, you should write to the South African Healthcare Products Regulatory Authority (SAHPRA) at:

The Chief Executive Officer
Dr Boitumelo Semete-Makokotlela
South African Health Products Regulatory Authority Private Bag X828
PRETORIA
0001
Tel: (012) 501 0410/13
e-mail: Boitumelo.Semete@sahpra.org.za

Your permissions and signature

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

27. In Section 16 of this form, we told you about possible other uses of your extra samples and 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. You can change your mind after signing this form.

☐ I allow my extra samples and 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 and 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.

28. 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 entire discussion of this consent form.

Appendix B Sample informed consent form for Part B

Title: A phase 1 clinical trial to evaluate the safety and immunogenicity of HIV-1 vaccines based on chimpanzee serotypes of adenovirus expressing clade C gp140 and a CH505TF gp120 protein boost in healthy, HIV- uninfected adult participants

HVTN protocol number: HVTN 139

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.

Key information

Being in this research study is voluntary. It is your choice.

- You are being asked to take part in this study because you are 18-50 years old, HIV negative and healthy.
- The purpose of this study is to see if the study products are safe, if people are able to take them without becoming too uncomfortable, and to see how a person's immune system responds to the study products.
- Two of the study vaccines were tested in Part A at a lower dose and were found to be safe to continue testing in this part of the study.
- Your participation in this study will include about 12 months of clinic visits. After that, there will be health contacts by phone or message at 18 months, 2 years, and 3 years after your first study injection.
- Procedures will include blood draws and injections of the study products or a placebo at three study visits.
- There are risks from participating.
  - We will tell you more information about risks later in this consent form.
  - Part A of this study was the first time two of the study vaccines have been given to people. Because these vaccines have not been given to very many people yet, we do not know what all of the risks may be.
  - We do not expect the study vaccines to benefit you in any way.

About the study

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

About 34 people will take part in this study at multiple sites. The researcher in charge of this study at this clinic is [Insert name of site PI]. The US National Institutes of Health (NIH) is paying for the study.

This study is divided into two parts, Part A and Part B. About 12 people took part in Part A of this study. The safety data from participants in Part A was reviewed, and we believe it is safe to continue with Part B. In Part B, 22 more people will join.

You are being invited to join Part B of the study.

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

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

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

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

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

Site: Any change to the following boxed text requires approval from HVTN Regulatory Affairs at vtn.core.reg@hvtn.org. You can remove the box around the text.

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.

The studies that found a higher risk of HIV infection were testing a vaccine made from a weakened common cold virus called adenovirus type 5 (Ad5).

In one study, the group of men at higher risk had some things in common. In addition to getting the vaccine, they either:

- had antibodies to Ad5 (your body makes antibodies to fight infection),
- or they were uncircumcised (still had the foreskin on their penises),
- or both.

Men who were circumcised and did not have these antibodies did not have higher risk when they got the vaccine. In the group with higher risk after vaccination, the risk seemed to lessen after about a year and a half.

Only a few women in that study got HIV, so we can't tell if the vaccine affected their risk.

In another study, men who got the vaccine had higher risk whether or not they were circumcised or had antibodies to Ad5. Many women got HIV during the study but we can't tell if the vaccine affected their risk.

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

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

4. These study products are experimental.

The study products in Part B are called AdC6-HIVgp140, AdC7-HIVgp140, and CH505TF gp120. From here on, we will call them AdC6, AdC7, CH505TF or the study vaccines. The AdC6 and AdC7 study vaccines have not been given to people before Part A of this study. They are experimental HIV vaccines. That means we do not know if the study vaccines will be safe to use in people, or if 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 AdC6 and AdC7 study 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.

The AdC6 and AdC7 study vaccines are "adenoviral vector" vaccines. They are made from two kinds of adenovirus. Adenoviruses cause colds, coughs, and diarrhea. The adenoviruses used in these study vaccines are from chimpanzees so they should not cause infections in people. The adenoviruses in the vaccines have

been changed so that they will not spread in your body or to people around you. You cannot become infected with HIV or AIDS from the study vaccines.

The AdC6 and AdC7 study vaccines have been made in a lab to tell your body to make a small amount of some proteins that are found in HIV. Your body's immune system might recognize these proteins and prepare itself to fight HIV by making antibodies and T cells. Antibodies and T cells are parts of an immune response. Immune responses are part of the body's natural defense against disease.

The CH505TF study vaccine has man-made pieces of protein that look like part of a protein found in HIV. Your body's immune system might learn to recognize this protein and prepare itself to fight HIV. This is called an immune response.

The CH505TF study vaccine is 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 CH505TF study vaccine and GLA-SE study adjuvant have been given to 72 people in two other ongoing HVTN studies. So far they have not made people too uncomfortable or caused serious health problems.

Some people will get a placebo, a substance that does not contain vaccine. In this study, the placebo is sterile salt water.

In part A of the study, we looked to see if the AdC6 and AdC7 study vaccines were safe when given once at a low dose, and then we measured people's immune responses. There were about 12 people in Part A and the results showed that it is safe to move ahead with Part B of the study.

In part B of the study, we are looking to see if the AdC6 and AdC7 study vaccines are safe when given at a higher dose, in combination, and when followed by the CH505TF study vaccine. We will measure your body's immune responses. By giving the combination of the 3 study vaccines, we hope the immune system develops responses that can better teach the body to fight against HIV.

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 have 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 trouble 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. Anyone can get an autoimmune disease. It is possible but unlikely that a vaccine can lead to an autoimmune disease or make one worse.

Risks of the AdC6 and AdC7 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.

Studies in animals showed that these vaccines are safe. Even if something looks like it is safe or works in animals, it may not be true for people. Other chimpanzee adenovirus vaccines have been used in other research studies for other diseases and did not cause serious health problems, even when given at higher doses than will be used in this study.

Risks of the CH505TF study vaccine:

In one study, the CH505TF study vaccine and GLA-SE adjuvant were given to 12 people at the same dose that will be given to people in this study. Thirty other people in that study got different doses of the CH505TF study vaccine and GLA-SE adjuvant or placebo. That study is still ongoing and we don't know who got the vaccine and adjuvant, or who got the placebo. The most common complaints were pain or tenderness where people were injected. One person had a skin infection where they got the injection, which went away within 5 days after taking medication (antibiotics). It did not affect that person's daily routine. Two people had bad headaches that went away in a few days. In a second study, 30 participants received CH505TF at a lower dose than we are testing in this study. That study is also ongoing so we do not know the results, but we do know that as of March 4, 2020, there have been no serious health problems reported.

The study adjuvant, GLA-SE, has also been tested in people with vaccines for other diseases. The most common complaints were pain and tenderness at the place where you got the injection, and feeling tired.

Some people who get a study product may have changes in their blood or urine that can only be seen in laboratory tests. These changes usually go away on their own in a few days but sometimes require more testing.

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. Being in more than one study may not be safe. We check to make sure that you are not in more than one study by taking your **fingerprint** on an electronic system. This information is only accessed by a few members of the study team using a secure password.

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 ask you about medications you are taking, including HIV pre-exposure prophylaxis (PrEP). We will ask you about behaviors that might put you at risk for getting HIV. If you were assigned female sex at birth, 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.

Site: 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 assigned female sex at birth and could become pregnant, you must agree to use 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 products could affect the developing baby. You must agree to use effective birth control from 21 days before your first injection until 2 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 12 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 will contact you after the main study ends to check your health, and 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).

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

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

Not everyone in this study will get the study products. Some people will get a placebo. We will compare the results from people who got the placebo with results from people who got the study products.

You have about a 90% chance of getting the study products. *Site: Modify the randomization metaphor in the next sentence as appropriate to your local culture.* Whether you get the study products or the placebo is completely random, like flipping a coin.

We have no say in whether you get the study products or the placebo. We will not know which 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 in Part B completes their final main study visits to find out whether you got the study products or the placebo. This could be several years. But, if you have a serious medical problem and need to know what you got before the end of the study, we can tell you.

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

You will be in one of 3 groups. At the first two injection visits, you will get 2 injections into the same upper arm. At the third injection visit, you will get 1 injection into the upper thigh.

Part B Group	N	Dose	Enrollment visit	3 months	6 months
4	10	Higher dose	AdC6	AdC7	CH505TF + GLA-SE
5	10	Higher dose	AdC7	AdC6	CH505TF+ GLA-SE
6	2	None	Placebo	Placebo	Placebo

You will have to wait in the clinic for at least an 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. *Site: Customize the next sentence based on how you collect reactogenicity information.* You will bring this information back to the clinic at your next visit. Within 3 days of each injection, we will also need to be in contact with you to ask how you are doing. 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 assigned female sex at birth
- 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

- 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 190 mL (1 tablespoon to a little less than 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 F, 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 about protecting yourself from HIV.

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. The HVTN will test your samples to see how your immune system responds to the study products.

We will send your samples (without your name) to labs approved by the HVTN for this study, which are located in South Africa and the United States. 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. The genetic testing will only involve some of your genes, not all of your genes (your genome). The researchers will study only the genes related to the immune system and HIV and those that affect how people get HIV.

If you get HIV, 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 products.

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.

These tests done on your samples are for research purposes, not to check your health. The labs will not give the results to you or this clinic because their tests are not approved for use in making health care decisions. These labs are only approved to do research tests.

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

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

16. When samples are no longer needed for this study, the HVTN wants to use them in other studies and share them with other researchers.

The HVTN calls these samples "extra samples". The HVTN will only allow your extra samples to be used in other studies if you agree to this. You will mark your decision at the end of this 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 South Africa.

How long will the samples be stored? There is no limit on how long your extra samples will be stored. *[Site: Revise the previous sentence to 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 HVTN or other researchers. Once we share your samples and information, we may 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 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 HVTN or other researchers? The samples and information will be labeled with a code number. The key to the code will stay at this clinic. It will not be shared with anyone who does not need to know your name. 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.

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

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, but your name and other personal information will not be included. 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. There may be other unknown risks.

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

People who may see your information are:

- Researchers who use your extra samples and information for other research
- Government agencies that fund or monitor the research using your extra samples and information
- The researcher's Institutional Review Board or Ethics Committee
- Any regulatory agency that reviews clinical trials

- 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 and information may be published. No publication will use your name or identify you personally.

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

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.

Site: Any change to the following boxed text requires approval from HVTN Regulatory Affairs. You can remove the box around the text.

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,
- Any regulatory agency that reviews clinical trials,
- [Insert name of local IRB/EC] ,
- [Insert name of local and/or national regulatory authority as appropriate],
- Wistar Institute and people who work for them,
- Duke University and people who work for them,

- IDRI and people who work for them,
- The Division of AIDS and people who work for them,
- The HVTN and people who work for them,
- The 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. If you are found to have a medical condition that we are required to report by law, then some of your information may be shared. 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.) If your site does not have public health or legal reporting requirements, you may delete the last sentence in the paragraph above, along with the bullets below.

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

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.

18. There are several reasons why we may stop your injections. We may stop them even if you want to stay in the study and even if you were scheduled for more injections.

We will stop your injections if you become pregnant. 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.

We will stop your injections if you get HIV. We will also take fewer samples, and we will help you get care and support. We will encourage you to stay in the study, including annual contacts to check your health for 3 years after your first injection if you choose. We will discuss your study options with you. We will counsel you about having HIV and about telling your partner(s). *Site: Modify the following sentence as appropriate.* We will not provide or pay for any of your HIV care directly.

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

19. We may take you out of the study at any time.

We may take you out of the study if:

- you do not follow instructions,
- we think that staying in the study might harm you, or
- the study is stopped for any reason.

Other Risks

20. 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:

Most people who join HVTN studies report no problems or discrimination as a result of their participation in an HIV vaccine study. Most problems that were reported were with personal relationships. These problems can include family or friends worrying, getting upset or angry, or assuming that you have HIV or are at high risk and treating you unfairly as a result. Most problems that were reported by participants had minimal impact on their lives. Rarely, a person has lost a job because the study took too much time away from work, or because their employer thought they had HIV.

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

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

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

Site: Modify the following paragraph if applicable. If someone believes you have HIV even if you do 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. We may also show you a video to help you think about these VISP issues.

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

You should always tell the delivery staff if you have VISP. However, you may still be tested for HIV using the antibody test when you deliver your baby. If your test is positive and the delivery staff believes you have HIV, 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 have 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:

It is unlikely, but the genetic tests done on your samples 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 products will increase, decrease, or not change your risk of getting HIV if exposed. If you get 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 the study products will affect how you respond to any future approved HIV vaccine. Currently, no HIV vaccine has been approved for use.

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

Benefits

21. The study may not benefit you.

We do not expect the study products to 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.

When asked, most study participants say that participating in a study made them feel good about helping others, increased their knowledge about HIV, and improved their self-esteem.

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

Your rights and responsibilities

22. 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 Bill of Rights and Responsibilities (BRR) for HIV Research. We will give you a copy of it.

Leaving the study

23. 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.

Previously collected information about you will remain in the study records and will be included in the analysis of results. Your information can not be removed from the study records.

We may 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 may 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: Approval from HVTN Regulatory Affairs (at vtn.core.reg@hvtn.org) is needed for any change (other than those that the instructions specifically request or those previously approved by HVTN Regulatory Affairs) to the boxed text. You can remove the box around the text.

24. **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, the HVTN has 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.

In this study, our clinic has insurance to cover your medical treatment in the case of a study-related injury. We will follow the **Association of the British Pharmaceutical Industry guidelines** for payment of study-related injury. We can give you a copy of these guidelines. In rare cases, the insurance funds may not be enough.

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/or 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.

Annual health contacts

25. **After your clinic visits end, we will contact you at 18 months, two years, and 3 years after your first injection to check your health.**

We will contact you once a year or to ask questions about your health

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 have HIV, we will ask you to come back to the clinic for another HIV test. We will draw about 15 mL (1 tablespoon) of blood. We may ask you to come back more than once for this testing.

Because we will want to contact you once a year, please tell us if your contact information changes, if you are moving away, or if you do not want us to contact you anymore.

You can tell us at any time that you don't want any more annual health contacts. If you do so, you will not lose any benefits or rights you would normally have.

Questions

26. 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 or title 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 or title and telephone number of the investigator or other study staff].

This study has been reviewed and approved by a committee called the
[name of local IRB/EC]. 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 or title and telephone number of person on IRB/EC] , at the committee.

The study has been structured in accordance with the Declaration of Helsinki (last updated October 2013) which deals with the recommendations guiding doctors in biomedical research involving human participants, the Ethics in Health Research: Principles, Structures and Processes Second Edition 2015, and Guidelines for Good Practice in the Conduct of Clinical Trials in Human Participants in South Africa. We can provide you with copies of these guidelines if you wish to review them. In addition, the recent Protection of Personal Information Act (POPIA) ensures that all South African institutions conducts themselves in a responsible manner when collecting, processing, storing and sharing another entity's personal information by holding them accountable should they abuse or compromise your personal information in any way.

If you want to leave this study, contact
[name or title and telephone number of the investigator or other study staff].

You can reach a study staff member 24 hours a day at [telephone number].

If you have questions about this trial you should first discuss them with your doctor or the ethics committee (contact details as provided on this form). After you have consulted your doctor or the ethics committee and if they have not provided you with answers to your satisfaction, you should write to the South African Healthcare Products Regulatory Authority (SAHPRA) at:

The Chief Executive Officer
Dr Boitumelo Semete-Makokotlela
South African Health Products Regulatory Authority Private Bag X828
PRETORIA
0001
Tel: (012) 501 0410/13
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Your permissions and signature

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

27. In Section 16 of this form, we told you about possible other uses of your extra samples and 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. You can change your mind after signing this form.

☐	I allow my extra samples and 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 and 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.

28. 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 entire discussion of this consent form.

Appendix C Approved birth control methods for persons assigned female sex at birth (for sample informed consent form)

Site: Any change to the following boxed text requires approval from HVTN Regulatory Affairs at vtn.core.reg@hvtn.org. You can remove the box around the text.

You should not become pregnant during the study because we do not know how the study products could affect the developing baby.

You must agree to use effective birth control from 21 days before your first injection until 2 months after your last study injection. This includes not seeking pregnancy through alternative methods such as artificial insemination or in vitro fertilization.

Effective birth control means using any of the following methods every time you have sex in a way that could lead to pregnancy:

- Birth control drugs that prevent pregnancy—given by pills, shots, patches, vaginal rings, or inserts under the skin;
- Internal or external condoms, with or without a cream or gel that kills sperm;
- Diaphragm or cervical cap with a cream or gel that kills sperm;
- Intrauterine device (IUD); or
- Any other contraceptive method approved by the researchers.

You do not have to use birth control if:

- You are only having sex with a partner or partners who have had a vasectomy. (We will ask you some questions to confirm that the vasectomy was successful.);
- You have reached menopause, with no menstrual periods for one year;
- You have had a hysterectomy (your uterus removed);
- You have had your ovaries removed;
- You have a tubal ligation (your “tubes tied”) or confirmed successful placement of a product that blocks the fallopian tubes;
- You are sexually abstinent (no sex at all).

Remember: If you are having sex, male and female condoms are the only birth control methods that also provide protection against HIV and other sexually transmitted infections.

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 HIV-1 vaccines based on chimpanzee serotypes of adenovirus expressing clade C gp140 and a CH505TF gp120 protein boost in healthy, HIV- uninfected adult participants

HVTN protocol number: HVTN 139

Site: [Insert site name]

Key Information

These are some of the things you should know about the use of your samples and information for other studies:

- The extra samples will be labeled with a code number. They will not be labeled with your name. The extra samples are stored in a secure place. At your request, the HVTN will destroy all your extra samples. You can still join the main study even if you do not agree to use of your extra samples in other studies.

- Researchers may do genetic testing on your samples, which could include genome wide studies. It is unlikely, but these tests could show you may be at risk for certain diseases. In the very unlikely event that others found out, this could lead to discrimination or other problems.

- You will not be paid or otherwise benefit from allowing your extra samples to be used in other studies.

The rest of this form gives more information about use of your extra samples for other studies. Please read it carefully.

When samples are no longer needed for this study, the HVTN wants to use them in other studies and share them with other researchers. The HVTN calls these samples “extra samples.” The HVTN will only allow your extra samples to be used in other studies if you agree to this. You will mark your decision at the end of this 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 South Africa.

3. How long will the samples be stored?

There is no limit on how long your extra samples will be stored. *[Site: Revise the previous sentence to 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 HVTN or other researchers. Once we share your samples and information, we may 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 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 HVTN or other researchers?

The samples and information will be labeled with a code number. The key to the code will stay at this clinic. It will not be shared with anyone who does not need to know your name. 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.

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

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, but your name and other personal information will not be included. 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. There may be other unknown risks.

10. What are the risks of genetic testing?

It is unlikely, but the genetic tests done on your samples 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.

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 extra samples and information for other research
- Government agencies that fund or monitor the research using your extra samples and information
- The researcher’s Institutional Review Board or Ethics Committee
- Any regulatory agency that reviews clinical trials
- 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 and 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 or title 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 or title and telephone number of the investigator or other study staff].

If you have questions about your rights as a research participant, contact
[name or title and telephone number of person on IRB/EC .

The study has been structured in accordance with the Declaration of Helsinki (last updated October 2013) which deals with the recommendations guiding doctors in biomedical research involving human participants, the Ethics in Health Research: Principles, Structures and Processes Second Edition 2015, and Guidelines for Good Practice in the Conduct of Clinical Trials in Human Participants in South Africa. We can provide you with copies of these guidelines if you wish to review them. In addition, the recent Protection of Personal Information Act (POPIA) ensures that all South African institutions conducts themselves in a responsible manner when collecting, processing, storing and sharing another entity's personal information by holding them accountable should they abuse or compromise your personal information in any way.

You can reach a study staff member 24 hours a day at [telephone number].

If you have questions about this trial you should first discuss them with your doctor or the ethics committee (contact details as provided on this form). After you have consulted your doctor or the ethics committee and if they have not provided you with answers to your satisfaction, you should write to the South African Healthcare Products Regulatory Authority (SAHPRA) at:

The Chief Executive Officer
Dr Boitumelo Semete-Makokotlela
South African Health Products Regulatory Authority Private Bag X828
PRETORIA
0001
Tel: (012) 501 0410/13
e-mail: Boitumelo.Semete@sahpra.org.za

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. You can change your mind after signing this form.

☐ I allow my extra samples and 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 and 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 entire discussion of this consent form.

Appendix E Table of procedures for Part A (for sample informed consent form)

Procedure	Screening visit	Enrollment visit	2 weeks	1 month	6 months	1 year	2 years	3 years
Injection		√						
Medical history	√							
Complete physical	√				√			
Brief physical		√	√	√				
Urine test	√		√					
Blood drawn	√	√	√	√	√			
Pregnancy test (participants assigned female sex at birth)*	√	√		√				
HIV testing and pretest counseling	√				√			
Risk reduction counseling	√	√	√	√	√			
Interview/questionnaire	√	√	√	√	√			
Health contact**						√	√	√
Unblinding***					√			

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

** Phone contact about one, two, and three years after injection to check on certain aspects of participant health.

*** Participants find out if they got the vaccines or placebo as soon as the data are available after the 6 month visit.

Appendix F Table of procedures for Part B (for sample informed consent form)

Procedure	Screening visit	Enrollment visit	2 weeks	1 month	3 months	3½ months	4 months	6 months	6½ months	1 year	1½ years	2 years	3 years
Injection		√			√			√					
Medical history	√												
Complete physical	√									√			
Brief physical		√	√	√	√	√	√	√	√				
Urine test	√		√			√			√				
Blood drawn	√	√	√	√	√	√	√	√	√	√			
Pregnancy test (participants assigned female sex at birth)*	√	√			√			√					
HIV testing and pretest counseling	√				√			√		√			
Risk reduction counseling	√	√	√	√	√	√	√	√	√	√			
Interview/questionnaire	√	√	√	√	√	√	√	√	√	√			
Health contact**											√	√	√
Unblinding***										√			

* Persons who had a hysterectomy (removal of the uterus) or removal of both ovaries (verified by medical records) are not required to have a pregnancy test.
** Phone contact about one and a half, two, and three years after the first injection to check on certain aspects of participant health.
*** Participants find out if they got the vaccines or placebo as soon as the data are available after the 1 year visit.

Appendix G Laboratory procedures Part A

Procedure	Send to[1,2]	Assay location[2]	Tube Type[4]	Tube size (vol. capacity)[4]	Visit	1	2[10]	3	4	5	AESI[11]	Total
					Day	Screening visit[3]	D0	D14	D28	D168	D364	
					Week		W0	W2	W4	W24	W52	
					Month		M0	M0.5	M1	M6	M12	
							AdC6HIVgp140 OR AdC7HIVgp140 OR Placebo					
BLOOD COLLECTION												
Screening/Diagnostic												
HIV Screening test	Local lab	Local lab	SST	5mL		5	—	—	—	—	—	**5**
HBsAg/anti-HCV	Local lab	Local lab	SST	5mL		5	—	—	—	—	—	**5**
HIV diagnostics[7]	HSML-NICD / UW-VSL	HSML-NICD / UW-VSL	EDTA	10mL		—	—	—	—	20	—	**20**
Safety labs[8]												
CBC/ Diff/ platelets	Local lab	Local lab	EDTA	5mL		5	—	5	—	—	—	**10**
Chemistry panel[5]	Local lab	Local lab	SST	5mL		5	—	5	—	—	—	**10**
Immunogenicity & Virologic assays												
Cellular assays												
ICS	CSR	HVTN Labs	ACD	8.5mL		—	42.5	—	***42.5***	42.5	—	**128**
Humoral assays												
Binding Ab Assay	CSR	HVTN Labs	SST	8.5mL		—	***8.5***	—	***8.5***	8.5	—	**26**
Neutralizing Ab Assay	CSR	HVTN Labs	SST	8.5mL		—	8.5	—	***8.5***	8.5	—	**26**
AdC6 and AdC7 Titer												
AdC6 and AdC7 Neutralizing Ab Assay	CSR	Non-HVTN Labs	SST	8.5mL		—	***8.5***	—	***8.5***	—	—	**17**
Storage												
PBMC	CSR		ACD	8.5mL		—	59.5	—	42.5	68	—	**170**
Serum	CSR		SST	8.5mL		—	17	—	17	17	—	**51**
Visit total						**20**	**145**	**10**	**128**	**165**	**0**	**467**
56-Day total						**20**	**165**	**175**	**302**	**165**	**0**	
URINE COLLECTION[8]												
Urine dipstick[9]	Local lab	Local lab				X	—	X	—	—	—	—
Pregnancy Test[6]	Local lab	Local lab				X	X	—	X	—	—	—

[1] CSR = Central Specimen Repository; HSML-NICD = HIV Sero-Molecular Laboratory-National Institute for Communicable Diseases (Johannesburg, South Africa); UW-VSL = University of Washington Virology Specialty Laboratory (Seattle, Washington, USA)

[2] HVTN Laboratories include: Cape Town HVTN Immunology Laboratory (CHIL, Cape Town, Africa); South African Immunology Laboratory-National Institute for Communicable Diseases (SAIL-NICD, Johannesburg, South Africa); Fred Hutchinson Cancer Research Center (Seattle, Washington, USA); Duke University Medical Center (Durham, North Carolina, USA). Non-HVTN Laboratories include: Wistar Institute's Vaccine and Immunotherapy Center (Philadelphia, PA).
[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 (post-enrollment).
[6] For a participant who was assigned female sex at birth, pregnancy test must be performed on urine or blood specimens within 24 hours 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.
[7] At an early termination visit for a withdrawn or terminated participant who is not HIV-infected (see Section 9.11), blood should be drawn for HIV diagnostic testing, as shown for visit 5 above. If a participant has a confirmed diagnosis of HIV infection, do not collect blood for HIV diagnostic testing (see Section 9.13).
[8] For participants with confirmed diagnosis of HIV infection, only specimens required for protocol-specified safety laboratory tests, urinalysis and pregnancy tests will be collected.
[9] And microscopy if needed.
[10] Specimens indicated for Day 0 (except the pregnancy test) may be obtained within the 14 days prior to vaccination.
[11] Clinic visit is not required for AESI Health Contact except for conditions specified in Protocol Section 9.5.1.

Appendix H Laboratory procedures Part B

				Visit	**1**	**2**[11]	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**AESI**[10]	
				Day	Screening visit[3]	D0	D14	D28	D84	D98	D112	D168	D182	D364	D546	
				Week		W0	W2	W4	W12	W14	W16	W24	W26	W52	W78	
				Month		M0	M0.5	M1	M3	M3.5	M4	M6	M6.5	M12	M18	
						AdC6HIVgp140 OR AdC7HIVgp140 OR Placebo			**AdC7HIVgp140 OR AdC6HIVgp140 OR Placebo**			**CH505TF gp120/ GLA-SE OR Placebo**				
Procedure	**Send to**[1,2]	**Assay location**[2]	**Tube Type**[4]	**Tube size (vol. capacity)**[4]												**Total**
BLOOD COLLECTION																
Screening/Diagnostic																
HIV Screening test	Local lab	Local lab	SST	5mL	5	—	—	—	—	—	—	—	—	—	—	**5**
HBsAg/anti-HCV	Local lab	Local lab	SST	5mL	5	—	—	—	—	—	—	—	—	—	—	**5**
HIV diagnostics[7]	HSML-NICD / UW-VSL	HSML-NICD / UW-VSL	EDTA	10mL	—	—	—	—	10	—	—	10	—	20	—	**40**
Safety labs[8]																
CBC/ Diff/ platelets	Local lab	Local lab	EDTA	5mL	5	—	5	—	5	5	—	—	5	—	—	**25**
Chemistry panel[5]	Local lab	Local lab	SST	5mL	5	—	5	—	5	5	—	—	5	—	—	**25**
Immunogenicity & Virologic assays																
Cellular assays																
ICS	CSR	HVTN Labs	ACD	8.5mL	—	42.5	—	***42.5***	—	—	***42.5***	—	***42.5***	42.5	—	**213**
Ag-specific B-cell phenotyping	CSR	HVTN Labs	ACD	8.5mL	—	***34***	—	—	—	—	34	—	***34***	17	—	**119**
Humoral assays																
Binding Ab Assay	CSR	HVTN Labs	SST	8.5mL	—	***8.5***	—	***8.5***	—	—	***8.5***	—	***8.5***	8.5	—	**43**
Neutralizing Ab Assay	CSR	HVTN Labs	SST	8.5mL	—	8.5	—	***8.5***	—	—	***8.5***	—	***8.5***	8.5	—	**43**
Fc-mediated antibody functions[12]	CSR	HVTN Labs	SST	8.5mL	—	y	—	—	—	—	y	—	y	y	—	
AdC6 and AdC7 Titer																
AdC6 and AdC7 Neutralizing Ab Assay	CSR	Non-HVTN Labs	SST	8.5mL	—	***8.5***	—	***8.5***	—	—	***8.5***	—	—	—	—	**26**
Storage																
PBMC	CSR		ACD	8.5mL	—	59.5	—	42.5	—	—	42.5	—	42.5	68	—	**255**
Serum	CSR		SST	8.5mL	—	25.5	—	17	—	—	17	—	17	17	—	**94**
Visit total					**20**	**187**	**10**	**128**	**20**	**10**	**162**	**10**	**163**	**182**	**0**	**891**
56-Day total					**20**	**207**	**217**	**345**	**148**	**30**	**192**	**172**	**173**	**182**	**0**	
URINE COLLECTION[8]																
Urine dipstick[9]	Local lab	Local lab			X	—	X	—	—	X	—	—	X	—	—	—
Pregnancy Test[6]	Local lab	Local lab			X	X	—	—	X	—	—	X	—	—	—	—

[1] CSR = Central Specimen Repository; HSML-NICD = HIV Sero-Molecular Laboratory-National Institute for Communicable Diseases (Johannesburg, South Africa); UW-VSL = University of Washington Virology Specialty Laboratory (Seattle, Washington, USA)

[2] HVTN Laboratories include: Cape Town HVTN Immunology Laboratory (CHIL, Cape Town, Africa); South African Immunology Laboratory-National Institute for Communicable Diseases (SAIL-NICD, Johannesburg, South Africa); Fred Hutchinson Cancer Research Center (Seattle, Washington, USA); Duke University Medical Center (Durham, North Carolina, USA).

Non-HVTN Laboratories include: Wistar Institute's Vaccine and Immunotherapy Center (Philadelphia, PA).

[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 (post-enrollment).

[6] For a participant who was assigned female sex at birth, pregnancy test must be performed on urine or blood specimens within 24 hours 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.

[7] At an early termination visit for a withdrawn or terminated participant who is not HIV-infected (see Section 9.11), blood should be drawn for HIV diagnostic testing, as shown for visit 10 above. If a participant has a confirmed diagnosis of HIV infection, do not collect blood for HIV diagnostic testing (see Section 9.13).

[8] For participants with confirmed diagnosis of HIV infection, only specimens required for protocol-specified safety laboratory tests, urinalysis, and pregnancy tests will be collected.

[9] And microscopy if needed.

[10] Clinic visit is not required for AESI Health Contact except for conditions specified in Protocol Section 9.5.1.

[11] Specimens indicated for Day 0 (except the pregnancy test) may be obtained within the 14 days prior to vaccination.

[12] Fc-mediated Ab functions may include ADCC, ADNP, ADCP, Fc-binding assays, and ICABA.

y = SST blood collected for the Binding Ab and Neutralizing Ab assays and for storage will also cover specimen needs for the Fc-mediated antibody functions and antibody avidity assays; no separate blood draw is needed

.

Appendix I Procedures at HVTN CRS for Part A

Visit:	01[1]	02	03	04	05	06[6]	07[6]	08[6]
Day:		D0	D14	D28	D168	D364	D728	D1092
Month:		M0	M0.5	M1	M6	M12	M24	M36
Procedure	Scr.	VAC						
Study procedures								
Screening consent (if used)	✓	–	–	–	–	–	–	–
Assessment of understanding	✓	–	–	–	–	–	–	–
Protocol consent	✓	–	–	–	–	–	–	–
Medical history	✓	–	–	–	–	–	–	–
Complete physical exam	✓	–	–	–	✓	–	–	–
Contraception status assessment[2]	✓	✓	✓	✓	–	–	–	–
Behavioral risk assessment[3]	✓	–	–	–	✓	–	–	–
Risk reduction counseling[4]	✓	✓	✓	✓	✓	–	–	–
Confirm eligibility	✓	–	–	–	–	–	–	–
Obtain demographics	✓	–	–	–	–	–	–	–
Randomize	✓	–	–	–	–	–	–	–
Concomitant medications	✓	✓	✓	✓	✓	–	–	–
HIV infection assessment[5]	✓	–	–	–	✓	–	–	–
Abbreviated physical exam	–	✓	✓	✓	–	–	–	–
Intercurrent illness/adverse experience	–	✓	✓	✓	✓	–	–	
Social impact assessment	–	✓	✓	✓	✓	–	–	–
Social impact assessment questionnaire	–	–	–	–	✓	–	–	–
Outside testing and belief questionnaire	–	–	–	–	✓	–	–	–
AESI Health Contact[6]	–	–	–	–	–	✓	–	–
Annual Health Contacts[6]	–	–	–	–	–	–	✓	✓
Specimen collection[7]	✓	✓	✓	✓	✓	–	–	–
Vaccination procedures[8]								
Vaccination[9]	–	✓	–	–	–	–	–	–
Reactogenicity assessments[10]	–	✓	–	–	–	–	–	–
Unblind participant[11]	–	–	–	–	✓	–	–	–

[1] Screening may occur over the course of several contacts/visits up to and including day 0 prior to vaccination.
[2] Contraception status assessment is required only for participants who were assigned female sex at birth and are capable of becoming pregnant. Persons who are confirmed pregnant, pregnancy testing is not required, unless clinically indicated.
[3] Not applicable to HIV-infected participants. Conduct before Risk Reduction Counseling if both occur at same visit.
[4] Includes transmission risk reduction counseling for HIV-infected participants. Conduct after BRA questionnaire if both occur at same visit.
[5] Includes pretest counseling and HIV testing. A subsequent follow-up contact is conducted to provide post-test counseling and to report results to participant. If a participant has a confirmed diagnosis of HIV infection, do not perform HIV infection assessment.
[6] Clinic visits are not required, except that any participant reporting a diagnosis of HIV infection will be asked to come to the clinic so that HIV status can be confirmed. Month 12 is an AESI health contact, Month 24 and 36 are annual health contacts (see Sections 9.5.1 and 9.5.2).
[7] For specimen collection requirements, see Appendix G. For participants with a confirmed diagnosis of HIV infection, specimens listed under "Safety labs" in Appendix G, urinalysis, and urine pregnancy tests will be collected per the protocol schedule.
[8] Not applicable to HIV-infected participants.
[9] 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. See Appendix G.
[10] Reactogenicity assessments performed daily for at least 7 days postvaccination (see Section 9.9).
[11] Unblinding will occur after the Month 6 visit when data are available; timing of unblinding may vary among participants.

Appendix J Procedures at HVTN CRS for Part B

Visit:	01[1]	02	03	04	05	06	07	08	09	10	11[6]	12[6]	13[6]
Day:		D0	D14	D28	D84	D98	D112	D168	D182	D364	D546	D728	D1092
Month:		M0	M0.5	M1	M3	M3.5	M4	M6	M6.5	M12	M18	M24	M36
Procedure	Scr.	VAC 1			VAC 2			VAC 3					
Study procedures													
Screening consent (if used)	✓	–	–	–	–	–	–	–	–	–	–	–	–
Assessment of understanding	✓	–	–	–	–	–	–	–	–	–	–	–	–
Protocol consent	✓	–	–	–	–	–	–	–	–	–	–	–	–
Medical history	✓	–	–	–	–	–	–	–	–	–	–	–	–
Complete physical exam	✓	–	–	–	–	–	–	–	–	✓	–	–	–
Contraception status assessment[2]	✓	✓	✓	✓	✓	✓	✓	✓	✓	–	–	–	–
Behavioral risk assessment[3]	✓	–	–	–	–	–	–	–	–	✓	–	–	–
Risk reduction counseling[4]	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	–	–	–
Confirm eligibility	✓	–	–	–	–	–	–	–	–	–	–	–	–
Obtain demographics	✓	–	–	–	–	–	–	–	–	–	–	–	–
Randomize	✓	–	–	–	–	–	–	–	–	–	–	–	–
Concomitant medications	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	–	–	–
HIV infection assessment[5]	✓	–	–	–	✓	–	–	✓	–	✓	–	–	–
Abbreviated physical exam	–	✓	✓	✓	✓	✓	✓	✓	✓	–	–	–	–
Intercurrent illness/adverse experience	–	✓	✓	✓	✓	✓	✓	✓	✓	✓	–	–	–
Social impact assessment	–	✓	✓	✓	✓	✓	✓	✓	✓	✓	–	–	–
Social impact assessment questionnaire	–	–	–	–	✓	–	–	✓	–	✓	–	–	–
Outside testing and belief questionnaire	–	–	–	–	–	–	–	✓	–	✓	–	–	–
AESI Health Contact[6]	–	–	–	–	–	–	–	–	–	–	✓	–	–
Annual Health Contacts[6]	–	–	–	–	–	–	–	–	–	–	–	✓	✓
Specimen collection[7]	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	–	–	–
Vaccination procedures[8]													
Vaccination[9]	–	✓	–	–	✓	–	–	✓	–	–	–	–	–
Reactogenicity assessments[10]	–	✓	–	–	✓	–	–	✓	–	–	–	–	–
Unblind participant[11]	–	–	–	–	–	–	–	–	–	✓	–	–	–

[1] Screening may occur over the course of several contacts/visits up to and including day 0 prior to vaccination.
[2] Contraception status assessment is required only for participants who were assigned female sex at birth and are capable of becoming pregnant. Persons who are confirmed pregnant, pregnancy testing is not required, unless clinically indicated.
[3] Not applicable to HIV-infected participants. Conduct **before** Risk Reduction Counseling if both occur at same visit.
[4] Includes transmission risk reduction counseling for HIV-infected participants. Conduct **after** BRA questionnaire if both occur at same visit.
[5] Includes pretest counseling and HIV testing. A subsequent follow-up contact is conducted to provide post-test counseling and to report results to participant. If a participant has a confirmed diagnosis of HIV infection, do not perform HIV infection assessment.
[6] Clinic visits are not required, except that any participant reporting a diagnosis of HIV infection will be asked to come to the clinic so that HIV status can be confirmed (see Section 9.5.2). Month 18 is an AESI contact, Month 24 and 36 are annual health contacts (see Sections 9.5.1 and 9.5.2).
[7] For specimen collection requirements, see Appendix H. For participants with a confirmed diagnosis of HIV infection, specimens listed under "Safety labs" in Appendix H, urinalysis, and urine pregnancy tests will be collected per the protocol schedule.
[8] Not applicable to HIV-infected participants.
[9] 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. See Appendix H.
[10] Reactogenicity assessments performed daily for at least 7 days postvaccination (see Section 9.9).
[11] Unblinding will occur after the Month 12 visit when data are available; timing of unblinding may vary among participants.

Appendix K HVTN 139 Visit Windows for Part A

Visit Number	Visit Type	Lower Allowable Window	Lower Target Day	Target Day	Upper Target Day	Upper Allowable Window
01.0	Screening	-56	-		-	-
02.0	**Enrollment**	-	-	0	-	-
03.0	2 Weeks Post-Vaccination	-	-4	14	+4	+7
04.0	Follow-up *Primary Immunogenicity*	-10	-4	28	+7	+14
05.0	Follow-up	-28	-14	168	+14	+28
06.0	AESI Contact	-	-85	364	+85	-
07.0	Health Contact	-	-85	728	+85	-
08.0	Health Contact	-	-85	1092	+85	-

Note: Target dates are relative to Day 0 (Enrollment).

Appendix L HVTN 139 Visit Windows for Part B

Visit Number	Visit Type	Lower Allowable Window	Lower Target Day	Target Day	Upper Target Day	Upper Allowable Window
01.0	Screening	-56	-		-	-
02.0	**Enrollment**	-	-	0	-	-
03.0	2 Weeks Post-Vaccination	-	-4	14	+4	+7
04.0	Follow-up *Primary Immunogenicity*	-10	-4	28	+7	+14
05.0	**Vaccination 2**	-21	-14	84	+14	+21
06.0	2 Weeks Post-Vaccination	-	-4	98	+4	+7
07.0	Follow-up *Primary Immunogenicity*	-10	-4	112	+7	+14
08.0	**Vaccination 3**	-28	-14	168	+14	+28
09.0	2 Weeks Post-Vaccination *Primary Immunogenicity*	-	-4	182	+4	+7
10.0	Follow-up	-28	-14	364	+14	+28
11.0	AESI Contact	-	-84	546	+84	-
12.0	Health Contact	-	-84	728	+84	-
13.0	Health Contact	-	-84	1092	+84	-

Note: Target dates are relative to Day 0 (Enrollment), with the exception of visits 6.0 and 9.0, which are relative to the prior vaccination visit.

Appendix M HVTN low risk guidelines for Southern Africa

The following are intended as guidelines for the investigator to help identify potential vaccine trial participants at "low risk" for HIV infection. These guidelines are based on behaviors within the last 12 months prior to enrollment; however, it may be appropriate to consider a person's behavior over a longer period of time than specified to assess the person's likelihood of maintaining low risk behavior. Some volunteers may not be appropriate for enrollment even if they meet these guidelines. These guidelines should be supplemented and interpreted with local epidemiologic information about HIV prevalence in your area and community networks. The investigator may review the risk level of any volunteer with the site PI and/or the Protocol Safety Review Team.

Assessment of sexual behaviors

Consider whether a volunteer would be appropriate for inclusion if, within 12 months prior to enrollment, the person:

- Abstained from penile/vaginal and penile/anal intercourse, or
- Was in a mutually monogamous relationship with a partner with a known HIV-uninfected status, or
- Had one partner believed to be HIV-uninfected with whom he/she regularly used condoms for penile/vaginal and penile/anal intercourse.

Exclude a volunteer if:

Within the 12 months prior to enrollment: a history of newly acquired syphilis, gonorrhea, chlamydia, trichomoniasis, active HSV lesions, chancroid, pelvic inflammatory disease (PID), genital sores or ulcers, cervicitis, genital warts of the labia minora, vagina, or cervix, or any other symptomatic genital warts.

For South African Volunteers on Pre-exposure prophylaxis (PrEP)

1. PrEP ASSESSMENT

- Reports equal to or greater than six consecutive months of protective PrEP use
- Commits to maintaining protective PrEP use throughout trial
- Participant reports equal to or greater than 70% when asked the following: *"Thinking about the past 4 weeks, what percent of the time were you able to take all your PrEP medications?"*

2. SEXUAL BEHAVIORS

- Persons stably taking PrEP as prescribed above for 6 months or longer are considered low risk of HIV infection, regardless of any sexual behavior that might otherwise be associated with high risk of HIV exposure.

3. NON-SEXUAL BEHAVIORS

In the **last 12 months** did not:

- Inject drugs or other substances without a prescription
- Use cocaine, methamphetamine, or excessive alcohol, which in the investigator's judgment, rendered the participant at greater than low risk for acquiring HIV infection.

The investigator's judgment should consider local epidemiologic information about HIV prevalence in the area and community networks.

Appendix N 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 139 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	

Appendix O Protocol Signature Page

A phase 1 clinical trial to evaluate the safety and immunogenicity of HIV-1 vaccines based on chimpanzee serotypes of adenovirus expressing clade C gp140 and a CH505TF gp120 protein boost in healthy, HIV- uninfected adult participants

I will conduct the study in accordance with the provisions of this protocol and all applicable protocol-related documents. I agree to conduct this study in compliance with United States (U.S.) Health and Human Service regulations (45 CFR 46); applicable U.S. Food and Drug Administration regulations; standards of the International Conference on Harmonization Guideline for Good Clinical Practice (E6(R2)); Institutional Review Board/Ethics Committee determinations; all applicable in-country, state, and local laws and regulations; and other applicable requirements (eg, U.S. National Institutes of Health, Division of AIDS) and institutional policies.

Investigator of Record Name (print) Investigator of Record Signature Date

DAIDS Protocol Number: HVTN 139

DAIDS Protocol Version: Version 1.0

Protocol Date: February 23, 2021