HIV VACCINE
TRIALS NETWORK

PROTOCOL

HVTN 135

A phase 1 clinical trial to evaluate the safety and immunogenicity of the HIV-1 CH505 transmitted/founder gp120 adjuvanted with GLA-SE in healthy, HIV-exposed uninfected infants

DAIDS DOCUMENT ID 38606

A non-IND study

CLINICAL TRIAL SPONSORED BY

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National Institute of Allergy and Infectious Diseases (NIAID)
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Department of Health and Human Services (DHHS)
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1 Ethical considerations

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

- HVTN trials are designed and conducted to enhance the knowledge base necessary to find a preventive vaccine, using methods that are scientifically rigorous and valid, and in accordance with 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 participant mothers routinely on how to reduce HIV transmission risk to their infants.

- Infants enrolled at birth who become HIV infected during the trial will be referred to accredited antiretroviral therapy (ART) treatment sites for their care. If clinical trials are available at the site, infants may be referred for screening. In the event of ART stockouts (supply shortages), ART will be made available until supply issue is resolved. ART will be supplied to the site through a mechanism established by the South African Medical Research Council.

- The HVTN provides training so that the participating site protects the privacy of research participants, and obtains meaningful informed consent. During the study, participants will have their wellbeing monitored, and to the fullest extent possible, their privacy protected. Participants, and/or their parent(s)/guardian(s) may withdraw from the study at any time.

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

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

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

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

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

2 IRB/EC review considerations

US 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 South Africa, 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.

2.1 Minimized risks to participants

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

This protocol minimizes risks to participants by (a) correctly and promptly informing participants and/or their parent(s)/guardian(s) 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; (e) providing HIV risk reduction counseling for the infant; and (e) providing safety monitoring.

2.2 Reasonable risk/benefit balance

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

In all public health research, the risk-benefit ratio may be difficult to assess because the benefits to a healthy participant are not as apparent as they would be

in treatment protocols, where a study participant may be ill and may have exhausted all conventional treatment options. However, this protocol is designed to minimize the risks to participants while maximizing the potential value of the knowledge it is designed to generate.

2.3 Equitable participant selection

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

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

2.4 Appropriate informed consent

45 CFR 46.111 (a) 4 and 5 and 21 CFR 56.111 (a) 4 and 5: Informed consent is sought from each prospective adult subject and the infant subject's parent(s) or guardian(s) 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 study site must comply with the requirements of the DAIDS policy on Enrolling Children (including Adolescents) in Clinical Research, which is available at: https://www.niaid.nih.gov/research/daids-clinical-research-protocol-informed-consent

HIV-infected pregnant women who are 18 years of age and older will provide written informed consent for their own and their infant's study participation before any study-specific procedures are performed. Further, informed consent is assessed throughout the trial (see Section 9.1). The site is provided training in informed consent by the HVTN as part of its entering the HVTN. The HVTN requires a signed consent document for documentation, in addition to chart notes or a consent checklist.

As indicated above, it is generally expected that mothers will provide informed consent for their own and their infant's participation in this study. However, parental consenting requirements at each site will depend on the IRB/EC risk determination described above and all IRB/EC requirements will be followed.

Should the consenting mother of an enrolled infant die or no longer be available for any reason, no further study-specific visits or procedures may be performed until informed consent for continued study participation is obtained from a guardian. The guardian will not replace the mother as a participant but will be

asked to continue bringing the infant for scheduled study visits. In accordance with the DAIDS policy on Enrolling Children (including Adolescents) in Clinical Research (available at the website referenced above), the study site must establish and maintain written procedures describing the standards that will be followed to identify who may serve as guardian for an enrolled infant, reflective of applicable IRB/EC guidance for conduct of human subjects research within the context of available local law, regulation, or government policy.

Children enrolled in this study are considered vulnerable participants per 45 CFR 46 Subpart D. Site IRBs/ECs must consider the potential benefits, risks, and discomforts of the study to children and assess the justification for their inclusion in the study. As part of this assessment, IRBs/ECs must determine the level of risk to children in the categories specified in 45 CFR 46.404-407. Documentation of this determination is required to complete the DAIDS protocol registration process described in Section 13.2 in 45 CFR 46.404-407.

The risk category assigned by the IRB/EC determines the parental informed consent requirements for the study at each site. Per 45 CFR 46.408 (b), the IRB/EC may find that the permission of one parent is sufficient for research to be conducted under 46.404 or 46.405. Where research is covered by 46.406 or 46.407, both parents must give their permission unless one parent is deceased, unknown, incompetent, or not reasonably available, or when only one parent has legal responsibility for the care and custody of the child (as determined locally). IRBs/ECs must document their risk determination and the study site should adapt the signature pages of their site-specific informed consent forms (ICFs) as needed to accommodate the parental consent requirements associated with the IRB/EC determination.

2.5 Adequate safety monitoring

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

This protocol has extensive safety monitoring in place (see Section 11). Safety is monitored daily by HVTN Core and routinely by the HVTN 135 Protocol Safety Review Team (PSRT). In addition, the HVTN Safety Monitoring Board (SMB) periodically reviews study data.

2.6 Protect privacy/confidentiality

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

Privacy refers to an individual's right to be free from unauthorized or unreasonable intrusion into his/her private life and the right to control access to individually identifiable information about him/her. The term "privacy" concerns research participants or potential research participants as individuals whereas the term "confidentiality" is used to refer to the treatment of information about those individuals. This protocol respects the privacy of participants by informing them about who will have access to their personal information and study data (see Appendix A). The privacy of participants is protected by assigning unique identifiers in place of the participant's name on study data and specimens. In 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. The study site participating in the protocol is required to have a standard operating procedure on how the staff members will protect the confidentiality of study participants.

3 Overview

Title

A phase 1 clinical trial to evaluate the safety and immunogenicity of the HIV-1 CH505 transmitted/founder gp120 adjuvanted with GLA-SE in healthy, HIV-exposed uninfected infants

Primary objectives

Primary objective 1

To evaluate the safety and tolerability of HIV-1 CH505 transmitted/founder virus Env gp120 immunogen (CH505TF gp120) adjuvanted with Glucopyranosyl Lipid A - stable emulsion (GLA-SE) in healthy HIV-1 exposed uninfected (HEU) infants

Primary objective 2

To determine whether vaccination with CH505TF gp120 adjuvanted with GLA-SE initiates B-cell lineages potentially capable of generating a broadly neutralizing antibody response

Study products and routes of administration

- **CH505TF gp120**: CH505TF gp120 with the adjuvant GLA-SE. GLA-SE is an oil-in-water stable emulsion (SE) containing the immunological adjuvant Glucopyranosyl Lipid A (GLA). The vaccine will be administered by intramuscular (IM) injection in the thigh at 2 doses of gp120 (20 or 5 mcg) and 2 doses of adjuvant (2.5 mcg or 5 mcg).

- **Placebo**: Sodium Chloride for Injection, 0.9%, administered IM in the thigh at volumes to match the active product.

Table 3-1 Schema

Group	N	Injection schedule in weeks				
		Week 0	**Week 8**	**Week 16**	**Week 32**	**Week 54**
Part A		**Initial Safety**				
1	5	20 mcg CH505TF gp120 +2.5 mcg GLA-SE	20 mcg CH505TF gp120 +2.5 mcg GLA-SE	20 mcg CH505TF gp120 +2.5 mcg GLA-SE	20 mcg CH505TF gp120 +2.5 mcg GLA-SE	20 mcg CH505TF gp120 +2.5 mcg GLA-SE
2	2	Placebo	Placebo	Placebo	Placebo	Placebo
Part B		**Safety Ramp-Up**				
3	2	20 mcg CH505TF gp120 +5 mcg GLA-SE	20 mcg CH505TF gp120 +5 mcg GLA-SE	20 mcg CH505TF gp120 +5 mcg GLA-SE	20 mcg CH505TF gp120 +5 mcg GLA-SE	20 mcg CH505TF gp120 +5 mcg GLA-SE
4	2	Placebo	Placebo	Placebo	Placebo	Placebo
Part C		**Immunogenicity of CH505TF Recombinant Protein**				
5	16	20 mcg CH505TF gp120 +5 mcg GLA-SE	20 mcg CH505TF gp120 +5 mcg GLA-SE	20 mcg CH505TF gp120 +5 mcg GLA-SE	20 mcg CH505TF gp120 +5 mcg GLA-SE	20 mcg CH505TF gp120 +5 mcg GLA-SE
6	3	Placebo	Placebo	Placebo	Placebo	Placebo
7	5	5 mcg CH505TF gp120 +5 mcg GLA-SE	5 mcg CH505TF gp120 +5 mcg GLA-SE	5 mcg CH505TF gp120 +5 mcg GLA-SE	5 mcg CH505TF gp120 +5 mcg GLA-SE	5 mcg CH505TF gp120 +5 mcg GLA-SE
8	3	Placebo	Placebo	Placebo	Placebo	Placebo
Total	**38**	**28 vaccinees, 10 placebos**				

Notes: To ensure safety, enrollment will proceed in stages, see Section 11.3.1.
Groups 3 and 5 (20 mcg CH505TF and 5 mcg GLA-SE) and Groups 2, 4, 6, and 8 (placebo) will be pooled in the ultimate analyses.

Participants

Healthy HIV-1–uninfected infants born to HIV-1–infected mothers through caesarian delivery in South Africa. To quantify maternal HIV antibody response, mothers will also be enrolled in the study but will not receive study product.

Sample size

38 mother-infant pairs

Design

Single center, double-blind, randomized, controlled trial

Duration per participant

24.5 months of scheduled clinic visits

Estimated total study duration

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

Clinical trial sponsor

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

Study product providers

- CH505TF gp120: DAIDS, NIAID, NIH, DHHS (Bethesda, Maryland, USA)
- GLA-SE adjuvant: DAIDS, NIAID, NIH, DHHS (Bethesda, Maryland, USA)

Core operations

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

Statistical and data management center (SDMC)

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

HIV diagnostic laboratory

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

Endpoint assay laboratories

- Fred Hutchinson Cancer Research Center (Seattle, Washington, USA)
- Duke University Medical Center (Durham, North Carolina, USA)
- Duke Human Vaccine Institute (Durham, North Carolina, USA)
- Precision Vaccines Program, Boston Children’s Hospital/Harvard Medical Center (Boston, Massachusetts, USA)

- University of British Columbia (Vancouver, Canada)
- South Africa Immunology Laboratory and National Institute for Communicable Diseases (SAIL-NICD) (Johannesburg, South Africa)

Study site

Perinatal HIV Research Unit, Chris Hani Baragwanath Hospital (Johannesburg, South Africa)

Safety monitoring

Protocol Safety Review Team (PSRT); HVTN Safety Monitoring Board (SMB)

3.1 Protocol Team

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4 Background

4.1 Rationale for trial concept

Despite the success of antiretroviral (ARV) prophylaxis to prevent vertical HIV-1 transmission, more than 180,000 infants acquired HIV-1 in 2017 (4). These infant infections are partly due to suboptimal HIV-1 testing and ARV coverage in areas with high HIV-1 incidence. Yet, even if 90% ARV coverage is achieved for pregnant women known to be infected with HIV-1 globally, it is estimated that more than 100,000 infants will still become infected with HIV-1 annually (5). This high burden is due to several settings in which suppressive antiretroviral therapy may not be achieved or diagnosis of maternal infection may not be timely, including: 1) acute infection of the mother during pregnancy or breastfeeding, 2) late presentation to prenatal care, 3) maternal non-adherence, which can be particularly challenging for the full duration of breastfeeding, 4) other psychosocial factors that are barriers to care, and 5) the development of drug-resistant variants. Thus, there remains a critical need to develop easy-to-implement immune-based prophylaxis strategies to prevent infant HIV-1 acquisition, such as infant HIV-1 immunization.

Maternal ARV-based strategies cannot achieve universal protection of infants against HIV-1

Young women living in areas of high HIV-1 incidence comprise a highly vulnerable population. In fact 30% of new HIV-1 infections in sub-Saharan Africa affect young women aged 15-24 years (6). This population includes young women who are or may become pregnant. Acute infection late in pregnancy and during the postpartum period puts the infant at high risk of HIV acquisition. In Mozambique for example, the rate of postpartum acute HIV-1 acquisition was 3.2/100-person years and up to 4.9/100-person years in 18-19-year-old women (7). Most of these women are not aware of their HIV status if acquired postpartum. Safe and effective, universal infant vaccination is necessary for eliminating postpartum HIV acquisition and for achieving an HIV-1-free generation in areas of high HIV-1 prevalence.

Late ARV initiation results in high rates of mother to child transmission (MTCT) of HIV-1. A South African study reported that women who received < 4 weeks of ARV had a transmission rate of 9.3%, which declined to 5.5% in those with 4-16 weeks of ARV, and further to 0.5% in women who received 16-32 weeks of ARV (8). Up to one-third of new mothers have disengaged from care and discontinued their own ARV therapy by six months postpartum (9). A universal infant HIV-1 vaccine that can be incorporated into the World Health Organization Expanded Program on Immunization (EPI) vaccine schedule is a rational approach towards elimination of the pediatric HIV-1 epidemic.

Induction of broadly neutralizing antibodies is a highly desirable feature for an HIV vaccine

Passive immunization studies in nonhuman primates have provided proof of principle that broadly neutralizing antibodies (bnAbs) can protect from virus exposure (10, 11). However, inducing bnAbs by vaccination has proven challenging. The difficulty of inducing bnAbs constitutes a major obstacle to the development of an efficacious preventive HIV vaccine.

There are several explanations for the difficulties of inducing bnAbs, including the conformational structure of the viral envelope, the antigenic similarity between envelope (Env) conserved epitopes and host antigens, and the requirement for complex B cell maturation pathways (12). About 10-25% of HIV-1–infected individuals develop bnAbs after several years of infection and these antibodies generally exhibit specific characteristics including extensive somatic hypermutation (generally associated with affinity maturation), long heavy chain complementarity determining regions, and/or autoreactivity with host antigens. It has been hypothesized that bnAbs can be induced following priming with a transmitted/founder (TF) Env followed by sequential immunization with different Env immunogens over time to mimic bnAb development in chronically HIV-1–infected individuals (13).

B cell immunogen lineage design is a process to circumvent host immunoregulatory mechanisms to give the bnAb lineages that are typically subdominant in infected patients a survival advantage. This design consists of selecting sequential Env immunogens with high affinity to the B cell receptors (BCRs) of the unmutated common ancestor (UCA) antibody and intermediate ancestor (IA) antibodies that are functional maturation steps leading to the mature bnAb of interest (11). Analysis of the co-evolution of mutations in both the infecting HIV-1 viruses and the resulting BCRs of discrete B cell clones from the time of seroconversion to the development of plasma bnAb induction allows for mapping the pathways that lead to generation of bnAbs. For example, early detection of acute HIV-1 infection in one adult donor (individual CH505) allowed evolutionary development mapping of a bnAb directed against the CD4 binding site (CD4bs) of gp120 (bnAb CH103) (12). Follow up studies in this individual revealed that the same transmitted founder virus also induced the development of a second CD4bs bnAb lineage (bnAb CH235). While CH103 and CH235 evolved though distinct evolutionary paths, CH235 cooperatively selected for resistant viruses that drove the evolutionary development of the CH103 bnAb lineage (14, 15). Such cooperative immunological pressure may be important for inducing rare viral variants that stimulate the maturation of lineages such as CH103. Importantly, despite the diversity of the human antibody repertoire, for many bnAbs there are only a few B cell lineages that can lead to bnAb development and these lineages are the same across individuals (13, 14). Therefore, immunization with an Env lineage that drives bnAb development in one individual is likely generalizable to others. A key question in such designs is whether each immunogen stimulates the early members of these B cell lineages in vaccinees and how much expansion of those populations can be achieved.

This study will address this key question in infants and will be directly compared to similar data obtained in the adult participants in HVTN 115. Preliminary immunogenicity data collected after the third (of five total) immunizations with CH505TF in HVTN115 Part A (see schema Table 4-6) has been analyzed. Remarkably, a high percentage of CD4bs specific antibodies to the vaccine envelope were elicited, as detected by serum binding and B cell assays. Although there were no CD4bs or V2 glycan precursors detected by the specialized bnAb precursor neutralization assays, these data suggest that the right specificities were elicited early on and may be capable of further maturation. If, as hypothesized, the neonatal immune system is more permissive for the rapid development of bnAbs, it may be possible to elicit bnAbs in infants without sequential immunizations with lineage-directed immunogens.

Sequential immunization is a potential approach to achieve neutralization breadth

The Duke CHAVI-ID team described the co-evolution of HIV-1 and a CD4bs bnAb clonal lineage (CH103) in an African HIV-infected individual (CH505) (14). From this analysis, four evolutionarily related Envs were selected as vaccine immunogens (Figure 4-1). The safety and immunogenicity of this 4-valent sequential gp120 Env immunogen (EnvSeq-1), adjuvanted with GLA-SE, will be tested in a phase 1 adult trial (HVTN 115, Part B). Results from preclinical studies have provided support for sequential immunization strategies. Notably, CH505TF gp120 engaged a CD4bs bnAb germline B cell receptor on naïve B cells in CH103 bnAb knock-in mice (15). Moreover, sequential immunization of infant rhesus macaques with CH505 Env immunogens induced plasma neutralizing activity against their respective autologous viruses and recombinant memory B cell-derived monoclonal antibodies capable of neutralizing tier 2 HIV strains (Han, V and Haynes, BF unpublished). As noted above, the critical question is how efficiently infant bnAb precursor B cells are expanded compared with adults. Overall, human and NHP data suggest that in infants, bnAb evolution may be achieved in a shorter time frame than in adults.

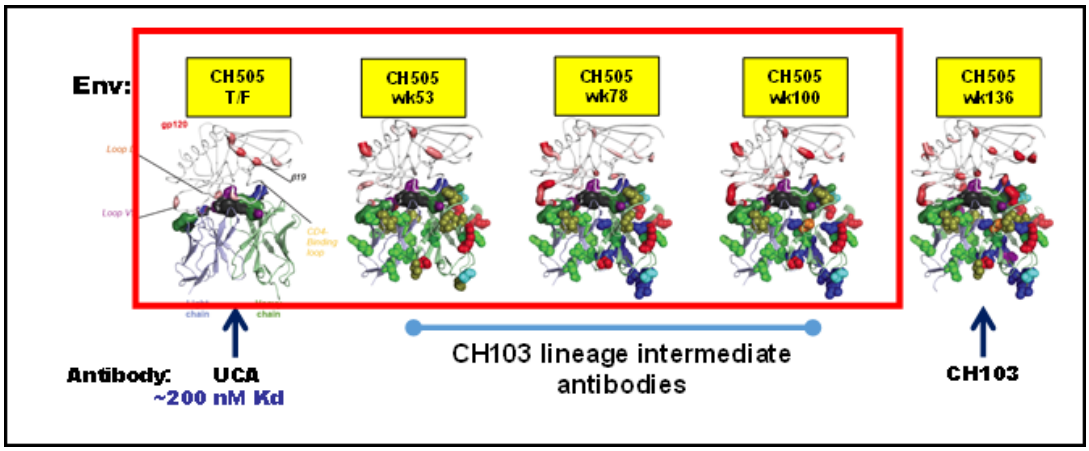

Figure 4-1 CH505 transmitted-founder (TF) and Env variants generated during viral evolution drove affinity maturation of CH103 bnAb lineage. Figure adapted from (14).

Development of broadly neutralizing antibodies in children

Two independent research groups have reported differences in bnAb responses between adults and children. Goo, Overbaugh *et al.* (16) demonstrated that two years after infection, the neutralization breadth in some infants is comparable to the breadth observed in the top 1% of adult neutralizers several years after infection. They also isolated a bnAb from an infected infant that had developed with lower levels of somatic hypermutation and variable gene usage than typically seen in isolated adult bnAbs (17). Similarly, Goulder and colleagues measured neutralizing antibody responses in HIV-1–infected children and found that 70% of slow progressor children, but only 15% of HIV-1 clade C chronically-infected adults, were able to neutralize at least 50% of viruses tested (18). Children also had higher neutralization titers than adults (18). Together, these data suggested that it may be easier to induce bnAbs in children than in adults.

The rationale for assessing HIV vaccines within the first weeks of life is based upon (a) the desire to test whether the unique immune status of neonates is more conducive to HIV vaccination strategies designed to produce broadly neutralizing antibodies and to unequivocally establish the safety of multiple immunizations with a vaccine-adjuvant combination; (b) birth being a reliable point of healthcare contact worldwide such that neonatal immunization is practical and achieves high population penetration in resource poor settings; and (c) the substantial preadolescent window provides a unique opportunity to develop mature immune responses over years (19). If, as hypothesized, infants are capable of more rapidly generating a wider repertoire of high affinity, somatically hypermutated B cell clones when compared to adults given the same complex immunogen, this would substantially increase the prospects for a vaccine to drive a broadly neutralizing antibody response. Furthermore, it would strongly support further testing of lineage-directed vaccines in a neonatal population. The results of the proposed study have important implications for HIV vaccinology that merit careful exploration in this vulnerable population.

Development of broadly neutralizing serum in children infected with HIV is associated with a more diverse antibody repertoire capable of recognizing distinct epitopes when compared to antibodies isolated from mothers infected with the same virus family (20). In addition to increases in the breadth of response, infants vaccinated with similar doses of gp120 vaccines develop higher titer antibodies to the specific V1V2 loop (a response associated with protection in RV144) when compared to adults vaccinated with the same vaccine (21). Thus, the magnitude, breadth, and rate of affinity maturation may be improved in infants as compared to adults.

The mechanisms underpinning the differences between the infant immune and adult immune system are complex and under active investigation, but specific differences in the underlying B cell repertoire have important implications for the development of broadly neutralizing antibodies. It has been known for >50 years that the neonatal immune system is an environment with more permissive peripheral tolerance checkpoints and infant B cells are more polyreactive than adults (22). Over the first four weeks of life, there is a profound reduction in the percentage of transitional B cells, a B cell subset enriched for autoreactivity (23, 24). The number of potentially self-reactive B cells present at birth is important in the context of HIV as many of the bnAbs identified to date cross-react with self-antigens that are recognized by B cells thought to be eliminated by tolerance checkpoints in adults (25, 26). Vaccinating as early as possible maximizes the probability of determining whether these differences in the neonatal immune system are important in the context of HIV vaccination. In addition, breaching peripheral tolerance checkpoints is associated with improved development of HIV-neutralizing titers in experimental systems (27). Our hypothesis is that neonates have a wider repertoire of B cells capable of generating broadly neutralizing antibodies in response to vaccination as compared to adults (28). Our incorporation of powerful systems biology approaches (eg. high resolution flow cytometry, RNASeq, proteomics, metabolomics, multiplex cytokines, etc.), recently optimized for use in small volumes ($\leq$1 mL) of newborn peripheral blood, as tertiary study goals will provide fresh insight into dynamic changes in the infant immune system over the first months of life in relation to vaccine immunogenicity (29). Moreover, use of some of the same systems biology assay platforms (eg, plasma proteomics and metabolomics) in this infant protocol and the adult HVTN 115 study will enable meaningful comparisons to understand the impact of ontogeny on the response to the same adjuvanted HIV vaccine.

In this proof of concept study, we propose to test the safety, tolerability and immunogenicity of the CH505TF gp120 adjuvanted with GLA-SE in healthy HEU newborn infants. We will also test the ability of the vaccine regimen to initiate both CD4bs and V1V2 lineage specific antibodies that have the potential to develop neutralization capacity (ie, bnAb lineage) in an infant population. Although the proposed immunogen (CH505TF gp120) was designed to elicit antibodies against the CD4bs, it contains other regions of the gp120 protein and also elicits bnAbs against the V1V2 region in preclinical models (15). The overarching hypothesis guiding development of HIV vaccines for an infant population is that establishment of both an early and durable broad neutralizing

antibody response could protect against vertical transmission during breastfeeding and, perhaps, horizontal HIV-1 transmission at sexual debut.

4.2 CH505TF gp120

The CH505TF gp120 to be used in this study was derived from the clade C transmitted/founder virus which was isolated from a single acutely HIV-1–infected donor from Malawi (CH505) (14, 30, 31). CH505TF was chosen based on its binding affinity to the CH103 bnAb lineage as shown in Table 4-1. CH505TF binds with high affinity to the unmutated common ancestor (UCA) of the CH103 lineage (30).

The CH505TF gp120 immunogen has an N-terminal deletion to facilitate production by decreasing protein dimer formation and increasing production yield (30). CH505TF is currently being evaluated in combination with GLA-SE in adult participants in HVTN 115.

Table 4-1 Binding of CH103 lineage antibodies to autologous CH505 Envs. Choice of transmitted/founder CH505 Env by binding EC_{50} levels to recombinant sequential CH505 Envs. Data are in EC_{50} binding titers in ELISA. (Red shading highlights the antigens being assessed in HVTN 115 and the antibodies they bind to.)

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

4.3 GLA-SE adjuvant

To compare immunogenicity results between infants immunized with CH505TF gp120 and adults in HVTN 115, this study will use the same adjuvant system.

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 (32, 33). GLA-SE has been tested in over 2500 individuals in phase 1, 2 and 3 trials with a variety of antigens. No significant safety concerns were reported. Previous clinical trial experience with GLA-SE and other antigens in adults is described in Section 4.8.2. Two trials are underway in children evaluating GLA-SE with other vaccines: a protein subunit vacine for schistosomiasis (NCT03799510), and a tuberculosis vaccine (NCT03806699). Our study will further compliment these studies in elucidating the safety and tolerability of this adjuvant in combination with other vaccines in infants. Importantly, the Th1-type immune response elicited is of interest in this population because of potential benefit towards more rapid cellular responses and more rapid bnAb development.

A study in infant rhesus macaques testing HIV-1 Env bivalent clade C gp120 C1086 and gp120 TV1 evaluated various adjuvants including GLA-SE at the dose as proposed in this study. This adjuvant demonstrated good immunogenicity in these infants, which weigh on average 500 g, indicating the immunogenicity of this dose in low-weight NHPs. GLA-SE adjuvanted vaccines has been previously studied in more than 2500 human adults and these GLA-SE adjuvanted vaccines have been demonstrated to be safe (see Table 4-7). Moreover, no major safety signal has been observed in adults who received a GLA-SE adjuvated gp120 vaccine in the Part A of HVTN 115.

4.4 Trial design rationale

An important goal for the vaccine field is to evaluate the concept of inducing broadly neutralizing antibodies against HIV-1. This study complements the work done in adults that evaluates a series of immunogens that, in an NHP model, were found to be capable of recreating the natural evolution of Env that promotes CD4bs specific antibody evolution. We will provide key insight into whether the infant immune milieu is more supportive of the development of several classes of affinity matured, somatically hypermutated bnAbs than the adult immune system, with important biological implications for the development of a generally effective HIV vaccine. In addition, we will also assess whether the promising adjuvant GLA-SE is as safe and tolerable in an infant population as it has been in adult populations.

It is critical to evaluate whether repeated administrations of GLA-SE with a single protein are safe in a neonatal population and do not interfere with required EPI vaccinations (secondary endpoint 1). This study will lay the groundwork for subsequent testing with sequential immunogens (eg, more directed lineage shaping approach, such as in HVTN 115 Part B), which will likely require a larger number of participants. All currently envisioned testing regimens require five (or more) doses of vaccine product. By including five doses with a placebo group powered for a robust consideration of safety, the study will allow for assessment of whether five doses of a GLA-SE adjuvanted vaccine is safe in infants. In

addition, the measurement of EPI vaccine responses after one year of vaccination will provide additional insight on whether GLA-SE causes interference with other important vaccines, as "bystander" interference has been observed with prior neonatal vaccination approaches (34).

The use of a placebo arm does not compromise standard of care for either the mother or the infant as both groups will receive the local standard of care for prevention of HIV transmission through breastfeeding.

4.4.1 Study Population

HIV exposed infants represent the population that may potentially benefit from this vaccine approach. Our overall objective is to evaluate interventions that substantially reduce the risk of HIV-1 transmission through breastmilk, hence the use of this population of HIV-exposed, uninfected infants.

4.4.2 Cord Blood

To minimize blood draws and to optimize our understanding of cellular responses, we will be using cord blood to evaluate baseline immunogenicity. Without the use of cord blood, some of the baseline immunologic studies we are proposing would require volumes of blood that would be unsafe to draw directly from neonates. For logistical reasons, cord blood can only reliably be obtained during planned Caesarian sections and planned Caesarian sections occur more frequently in this population. We will only enroll women who will require a planned Caesarian section, as determined by their obstetrical care provider.

4.4.3 Immunization number

Several interlinked considerations have informed the design of our study, utilizing five immunizations of CH505TF gp120 starting at birth, with the primary immunogenicity analysis at one year. These include: 1) to begin vaccination at birth to engage the unique qualities of the neonatal immune system, 2) the need to test for infant antibody responses at a timepoint where maternal antibodies have waned, and 3) to compare the infant immune response to the adult immune response in HVTN 115.

4.4.4 Dose (amount and number)

The intention of this study is to evaluate the safety and immunogenicity of CH505TF gp120 adjuvanted with GLA-SE in infants and to compare these data to the findings of the adult HVTN 115 study using the same adjuvanted vaccine formulation. In this study, HEU infants will be enrolled within 5 days of birth to receive vaccination with the CH505TF gp120 immunogen adjuvanted with GLA-SE or placebo. The highest GLA-SE dose (5 mcg) chosen for this study is supported by data coming from a nonhuman primate study that demonstrated the immunogenicity of GLA-SE in infant rhesus macaques (35). It is important to note that this is half the dose of GLA-SE administered to adults in the HVTN 115

study. This is consistent with the approach for vaccines licensed for use in pediatric populations such as Hepatitis A and Hepatitis B, both of which use half the adult dose in children. We aim to do a dose escalation for the adjuvant, starting with a lower 2.5 mcg GLA-SE dose in a small number of infants, use a planned enrollment hold to review safety data from these infants, and then increase to the 5 mcg GLA-SE dose with an additional enrollment hold for PSRT review of safety data.

There are HIV-specific concerns regarding somatic hypermutation, a necessary but not sufficient element for driving a bnAb response, that support the 5 mcg dose. In preclinical studies of other adjuvants (cholera toxin A1-DD and delta-inulin), increasing the dose of adjuvant is clearly associated with increasing somatic hypermutation (36, 37). Insufficient TLR4 stimulation (the target of GLA-SE) has been demonstrated to mediate the poor affinity maturation and lack of germinal center responses observed with formalin-inactivated RSV vaccines (38). In addition, pre-clinical data would suggest that there is likely to be less reactogenicity in infants to the GLA-SE adjuvant. In vitro studies have demonstrated that both neonatal and infant whole blood stimulated with GLA produces reduced amounts of the pyrogens TNF, IL-12 (p40), and IFN-γ when compared to adult blood (39). These inflammatory cytokines are likely mediators of reactogenicity (40).

The immunogen dose is the lowest dose (20 mcg) of CH505TF gp120 evaluated in HVTN 115 Part A. In HVTN 115 Part A, there were no statistically significant differences in magnitude of elicited antibodies or in response rates between the 20 mcg, 100 mcg, and 400 mcg dosing groups. In the adult cohort, no suppressive effect was seen with higher protein doses in HVTN 115 Part A; however, consistent with other vaccines used in both adult and infant populations, we have elected to use the lower 20 mcg dose to reduce the risk of high-dose suppression. It is possible that we may observe interference from maternal polyclonal antibodies vertically transferred to the infant; given the proven efficacy of EPI vaccines in this population where similar maternal antibody interference could occur, we believe the dose of 20 mcg offers the best balance between safety and immunogenicity.

In addition to the 20 mcg dose of CH505TF gp120, we propose to include a 5 mcg arm to further explore immunogenicity of CH505TF gp120 recombinant protein. This choice is supported both by preclinical studies of CH505TF gp120 and by the results of HVTN 115 Part A. As described below in Section 4.7.3, we performed a dose ranging study in rhesus macaques. After the second and third immunizations, neutralization titers were similar in all dose groups (5 to 600 mcg/dose; Figure 4-2).

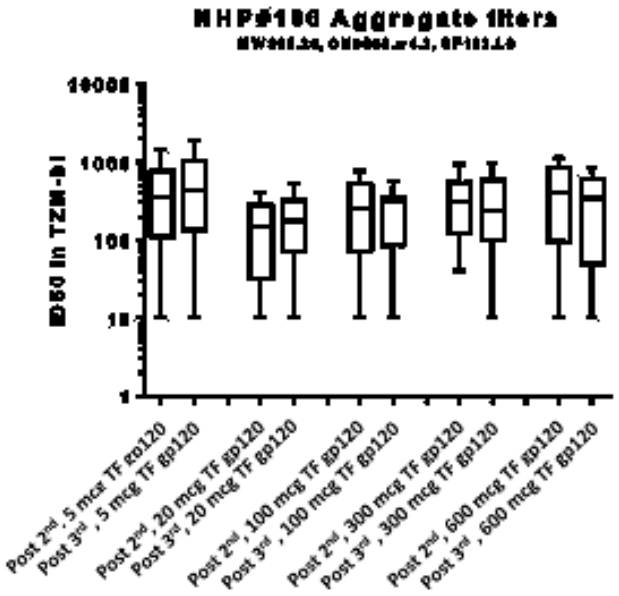

Figure 4-2 Neutralization activity elicited by CH505TF gp120 immunization against three isolates in the TZM-bl assay. There are no statistically significant differences between groups.

In HVTN 115 Part A, the frequency of antigen-specific B cells in peripheral blood after the fifth dose of vaccine was similar in all groups, with a slightly higher frequency and response rate observed in the higher dose groups compared with the 20 mcg/dose group. In this pediatric study, the selection of lower doses is consistent with vaccine strategies for other pathogens where lower doses are often used. While this study will not be powered for a formal comparison between these arms, previous pediatric vaccine studies have demonstrated that immune responses induced by a lower dose of protein were comparable to or greater than those seen with the higher dose. In the malaria RTS,S vaccine regimen, a delayed fractional immunogen dose equal to 20 mcg induced higher antibody avidity and antibody somatic hypermutation as compared to the higher dose (41). The inclusion of the 5 mcg dose in this study will permit a comparison of antibody avidity with the 20 mcg dose in infants and with all doses (20, 100 and 400 mcg) in adult immune responses from the HVTN 115 study.

The inclusion of five doses of a single product with a placebo arm will allow for directly assessing safety and testing whether the neonatal immune system has characteristics favorable to lineage-directed vaccination strategies.

4.4.5 Schedule

In this study, the CH505TF gp120 vaccine will be given at 0, 8, 16, 32, and 54 weeks. The first dose will be given within the first five days of life and, to avoid potential confounding of reactogenicity assessments, subsequent boosts will be given at least 7 days after scheduled EPI vaccinations (see Table 4-2). If an infant is found to have delayed EPI vaccinations, those vaccines will be administered by

the site as soon as possible, followed by the next scheduled study product administration no sooner than 7 days later. The boost at week 54 is proposed to guide affinity maturation and the potential development of bnAb B cell lineages.

This schedule is informed by the results of the PACTG 230 study (see Section 4.8.3). The PACTG 230 study immunized infants at 0, 4, 12, 20 weeks (extended schedule) and at 0, 2, 8 and 20 weeks (accelerated schedule) –both with high titers of antibodies that were similar at weeks 24 and 52 (42). A recent infant NHP immunization study comparing three accelerated (0, 3, and 6 weeks) dosing Env arms (protein only, MVA/protein prime boost, combination MVA/protein) vs. a fourth extended dosing (0, 6, 12 and 32 weeks) Env regimen (combination MVA/protein) demonstrated highest avidity antibody in the extended dosing arm (43). In vaccine studies with adult macaques, higher avidity anti-Env antibodies have been correlated with protection against rectal and vaginal simian-human immunodeficiency virus (SHIV) or simian immunodeficiency virus (SIV) challenge (44-47).

Table 4-2 Relationship of HVTN 135 Study Product Administration Schedule to National EPI schedule in South Africa

Infant Age	Vaccine	Proposed Schedule in HVTN 135
Birth	Oral Polio (OPV) Tuberculosis (BCG)	
Day 5		Dose 1
Week 6	Oral Polio (OPV) Rotavirus (RV) Diphtheria-tetanus-acellular pertussis-injectable polio- Haemophilus influenza B Hepatitis B (DTaP-IPV-Hib-Hep B) Pneumococcal Conjugate Vaccine (PCV)	
Week 8		Dose 2
Week 10	Diphtheria-tetanus-acellular pertussis-injectable polio- Haemophilus influenza B Hepatitis B (DTaP-IPV-Hib-Hep B)	
Week 14	Rotavirus (RTV) Diphtheria-tetanus-acellular pertussis-injectable polio- Haemophilus influenza B Hepatitis B (DTaP-IPV-Hib-Hep B) Pneumococcal Conjugate (PCV)	
Week 16		Dose 3
Week 24	Measles Vaccine	
Week 32		Dose 4
Week 36	Pneumococcal Conjugate (PCV)	
Week 52	Measles Vaccine	
Week 54		Dose 5

In addition, there are several important scientific questions favoring keeping the schedule the same as the adult schedule in HVTN 115 Part A. As described above, a high degree of somatic hypermutation is thought to be required for the development of most, but not all (17), bnAbs. While there have been no detailed kinetic studies (to our knowledge) of how repeated vaccinations in neonates

affects the rate of somatic hypermutation, a higher degree of somatic hypermutation with five vaccinations as compared to two vaccinations was demonstrated in a nonhuman primate model (48). In humans there are even fewer data. Although direct interpretation is difficult due to the long lag between immunizations, data from RV144/305 strongly suggest that a fifth vaccination leads to further somatic hypermutation (49). Data from this study will be compared to data derived from the adult population (HVTN 115) to assess if the quality and kinetics of vaccine-induced somatic hypermutation differ in neonatal vs adult B cells.

4.4.6 Choice of control

Sodium Chloride for Injection, 0.9% will be used as placebo.

Inclusion of placebo groups in this study is deemed necessary to maintain blinding when evaluating clinical events and their relationship to study vaccine. In addition, comparisons of safety and immunogenicity data between vaccine and placebo recipients could provide useful descriptive results despite the small sample size.

4.5 Plans for future product development and testing

A goal of iterative human clinical trials to learn how to induce broadly neutralizing antibodies. This study will allow us to compare infant and adult immune responses to CH505TF gp120 and initiate the first steps in the CH103 bnAb lineage. Considerable data have been reported that bnAb precursors are rare and subdominant and, in some cases, are made so by tolerance controls of bnAb precursors in bone marrow (12, 50, 51). Thus, this trial represents the first in a potential series of iterative phase 1 clinical studies to begin learning how to induce bnAbs in infant vaccinees. Importantly, the match of the immunogens and adjuvants with Part A of the adult trial (HVTN 115) will address the question of whether infants respond differently than adults. Next steps following this trial will be informed by the results of the lineage based design strategy being tested in adults through HVTN 115 as well as several upcoming adult trials testing next generation immunogens which may stimulate bnAb lineages.

4.6 Preclinical safety studies

Table 4-3 Summary of preclinical safety studies

Study number	Product	Type of study	Animal	N per group	Dose groups	Route	Schedule
1726-031	Stable CH505TF	Toxicity	New Zealand White Rabbits	10 M, 10 F	Group 1: saline control Group 2: CH505TF + GLA-SE Group 3: DNA + CH505TF + GLA-SE Group 4: GLA-SE	IM	Days 1, 15, 29, 43, 57, 71, 85

4.6.1 Toxicology study of CH505TF gp120 adjuvanted with GLA-SE in rabbits

A toxicity study with Stable CH505TF gp120 in New Zealand White (NZW) rabbits was conducted in compliance with Good Laboratory Practices (GLP). Seven biweekly IM injections of 400 mcg CH505TF gp120 with 20 mcg GLA-SE adjuvant or 20 mcg GLA-SE alone, to NZW rabbits for 13 weeks were 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. A more detailed description of the toxicity study can be found in the Investigator's Brochure (IB).

4.7 Preclinical immunogenicity studies

Table 4-4 Summary of preclinical immunogenicity studies

Study number	Product	Animal	N per group	Regimen groups	Route	Schedule (weeks)	Assay
501	CH505TF gp120 Env with GLA-SE	Guinea pigs	4	1 mcg CH505TF only 1 mcg CH505TF, 5 mcg GLA-SE 1 mcg CH505TF, 10 mcg GLA-SE 1 mcg CH505TF, 25 mcg GLA-SE 5 mcg CH505TF only 5 mcg CH505TF, 5 mcg GLA-SE 5 mcg CH505TF, 10 mcg GLA-SE 5 mcg CH505TF, 25 mcg GLA-SE 50 mcg CH505TF only 50 mcg CH505TF, 5 mcg GLA-SE 50 mcg CH505TF, 10 mcg GLA-SE 50 mcg CH505TF, 25 mcg GLA-SE	IM	0, 3, 6, 9, 12	Ab binding
NHP 79	EnvSeq-1 gp120 Env with GLA-SE	Rhesus Macaques	4	100 mcg CH505TF only 100 mcg CH505 sequential Envs 100 mcg CH505 additive Envs	IM	0, 6, 12, 19, 24, 57	nAb, binding Ab, blocking Ab
NHP 106	CH505TF gp120 Env with GLA-SE	Rhesus Macaques	4	5 mcg CH505TF Env 20 mcg CH505TF Env 100 mcg CH505TF Env 300 mcg CH505TF Env 600 mcg CH505TF Env	IM	0, 4, 12, 29, 37	nAb, binding Ab, differential Ab, blocking Ab

The CH505TF gp120 used in the initial animal studies (guinea pig 501 and NHP 79) was generated in transiently-transfected 293 cells. The 293-produced CH505TF gp120 has a different glycosylation profile than that of the CHO-produced protein used for the rabbit toxicology study, mouse immunogenicity, the nonhuman primate (NHP) dose response study (NHP 106).

4.7.1 Immunogenicity of CH505-derived gp120 envelopes with GLA-SE in guinea pigs, study # 501

In a guinea pig study, the GLA-SE adjuvant increased binding antibody magnitude to gp120 at all doses over the no adjuvant alone group (Barton Haynes, personal communication; see Figure 4-3).

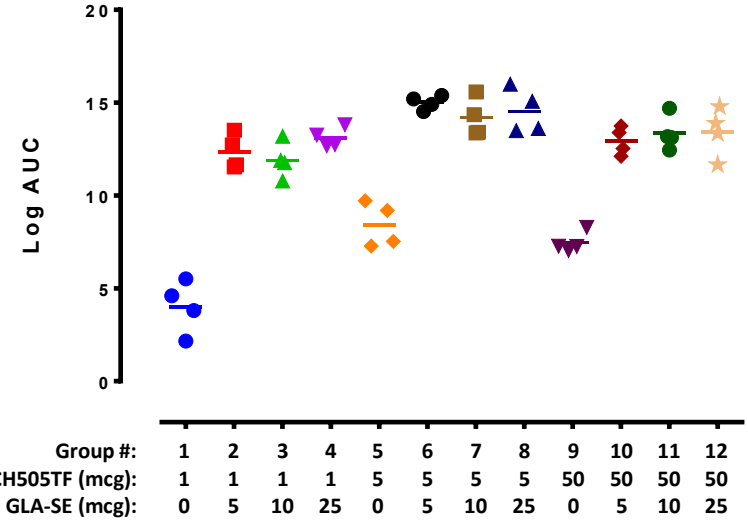

Figure 4-3 GLA-SE enhanced immunogenicity of CH505TF gp120 protein in guinea pigs. Binding antibodies from guinea pigs immunized with varying doses of CH505TF gp120 and varying amounts of GLA-SE were measured by enzyme-linked immunosorbent assay (ELISA). The data were used to calculate the log-transformed area under the curve (AUC) as shown on the y-axis. CH505TF gp120 was immunogenic at all doses. Groups 1, 5 and 9 received no GLA-SE and had significantly less anti-gp120 antibodies than the groups with 5 mcg (Groups 2, 6 and 10), 10 mcg (Groups 3, 7, and 11) or 25 mcg (Groups 4, 8, and 12) of GLA-SE. Moreover, there was no difference between the three GLA-SE doses (statistical analysis not shown).

4.7.2 Immunogenicity of CH505-derived gp120 Envelopes, study NHP 79

The EnvSeq-1 immunogens consist of four gp120 proteins derived from CH505: CH505TF, CH505w53, CH505w78, and CH505w100. These proteins were administered to 3 groups of four rhesus macaques (NHP 79) with the GLA-SE adjuvant: 1) CH505TF Env gp120 alone; 2) the Envs given in a sequential regimen (CH505TF, CH505w53, CH505w78, and CH505w100); and 3) 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). These Envs were research grade products made by transient transfection of 293 cells and have different glycosylation profiles than the CHO-produced CH505TF gp120s to be used in this clinical trial. This study demonstrates the proof of concept of using sequential Env variants in NHPs; the first step of this process using CH505TF is what will be tested in this clinical protocol. A follow on study employing the EnvSeq-1 immunogens administered in additive or sequential combinations may be a next step and will be informed by results from the HVTN 115 Part B trial which will test this approach in adults.

The CH505 gp120 Env derivatives (including CH505TF) were found to be immunogenic. An important readout in this study was the development of antibodies capable of binding a specific epitope on the CD4bs of the envelope protein. A defining feature of the CH103-like bnAbs is the capacity to bind wild type CH505 Env gp120 but not to CH505 Env with a deletion of isoleucine at aa position 371 (CH505 Env IΔ371 gp120). The data from the NHP 79 study suggest that the UCAs of bnAb lineages could be expanded by vaccination, but that they

did not evolve into bnAbs. In this study of 12 monkeys, 10/12 monkeys had differential binding antibodies isolated but overall the Env differential binding antibodies were subdominant and comprised only about 13% of the total activity of the antibody response, which is consistent with the low rate of bnAb development in HIV-infected adults (52) (Table 4-5).

Table 4-5 Frequency of antibodies isolated from CH505 envelope-vaccinated macaques. CH505 differential-binding antibodies (CH505 TF gp120+, CH505 TF gp120 Δ371I+/− (greater than or equal to threefold vs. Wild Type)) were referred to as CH505 differentials. Adapted from Williams et al. (52).

		All antibody lineages isolated				Unique clonal lineages			
		Total Abs	CH505 Env +	CH505 differentials		Unique	CH505 Env +	CH505 differentials	
Vaccine Groups	Animal ID			Count	% of total	lineages		Count	% of total
Group 1: CH505TF alone	5346	15	13	4	27%	13	11	2	15%
	5356	2	1	0	0%	2	1	0	0%
	5360	11	9	0	0%	10	8	0	0%
	5361	78	74	9	12%	68	64	7	10%
Group 4: EnvSeq1 given sequentially	5362	2	2	1	50%	2	2	1	50%
	5363	14	14	1	7%	14	14	1	7%
	5551	11	10	1	9%	11	10	1	9%
	5553	35	24	3	9%	34	23	3	9%
Group 5: EnvSeq1 given additively	5554	23	19	3	13%	21	17	3	14%
	5556	49	39	9	18%	43	33	7	16%
	5558	28	23	4	14%	25	21	4	16%
	5560	10	9	1	10%	10	9	1	10%
Summary		278	237	36	13%	253	213	30	12%

4.7.3 Immunogenicity of CH505TF gp120, study NHP 106

A dose ranging study was performed in rhesus macaques using 5 to 600 mcg of Env CH505TF gp120 (produced by stably transfected CHO-DG44 cells) along with 25 mcg GLA-SE with each dose administered 5 times (Barton Haynes, personal communication). Immunogenicity was demonstrated at all dose levels and no adverse reactions were observed. Env binding antibody (Figure 4-4) and CD4bs antibody (Figure 4-5) levels were determined and the CH505 TF gp120 was found to be immunogenic after 2 immunizations. All dose levels gave similar binding responses. When we assessed the frequency of memory B cells that bind antigen probes in a CD4bs differential manner (called differential binding memory B cells), all animals in the 20, 100, 300, and 600 mcg protein dose groups demonstrated differential binding after 3 injections (Figure 4-6). In this group of adult macaques, the frequency of differential binding B cells in the 5 mcg dose group was lower than that seen in the other groups. The inclusion of the 5 mcg dose in this study will permit evaluation of both antibody levels and B cell frequencies in human infants, enabling us to determine if the permissive environment of the infant immune system results in a higher frequency of differential binding B cells in a dose sparing manner.

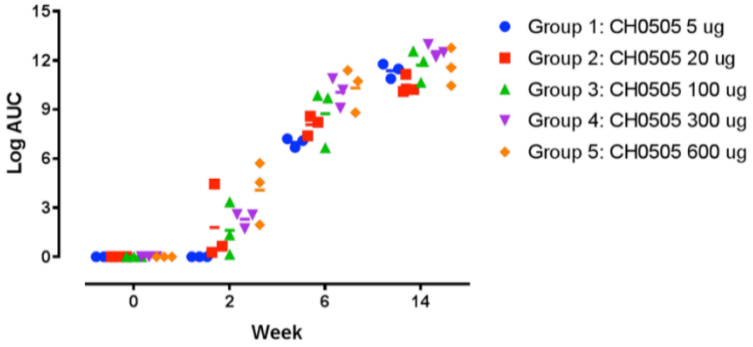

Figure 4-4 Binding of Immunized NHP 106 Plasma to CH505TF gp120. Each data point represents a single animal. Animals were immunized with the indicated dose at weeks 0, 4 and 12. X-axis shows bleed time. Bleed 0 was performed before immunization and subsequent bleeds were performed 2 weeks after each respective immunization. No animals had binding antibodies prior to immunization; binding was detected in all animals after the second immunization. After the third immunization, binding antibodies were similar among all groups. While all doses gave a similar binding response, the higher doses of 300 and 600 mcg gave earlier responses and trended to higher responses compared to the lower doses. No additional rise in antibody levels was observed after the fourth or fifth immunizations (data not shown).

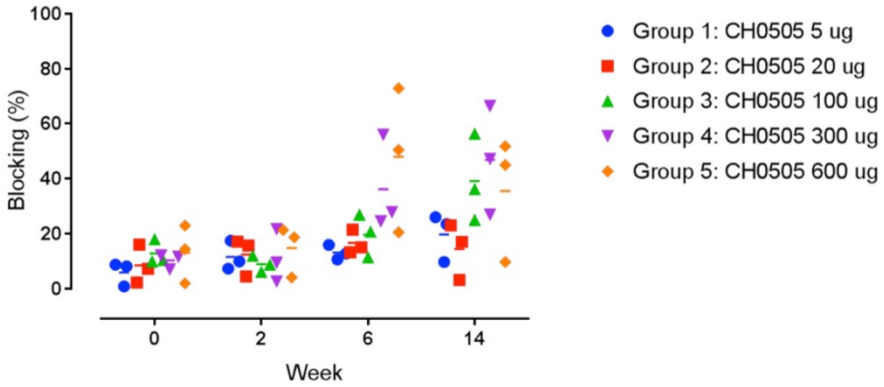

Figure 4-5 Inhibition of CD4 binding by NHP 106 plasma after immunization with 5 different doses of CH505TF gp120. Each data point represents a single animal. Animals were immunized with the indicated dose at weeks 0, 4 and 12. Note that the x-axis shows bleed time. Bleed 0 was performed before immunization and subsequent bleeds were performed 2 weeks after each respective immunization. The ability of plasma antibody to block the binding of CD4bs mAb CH106 to heterologous B.63521 gp120 was quantified by ELISA. Blocking antibodies were detectable in the high dose groups after the second immunization and increased in Group 3 after the third immunization.

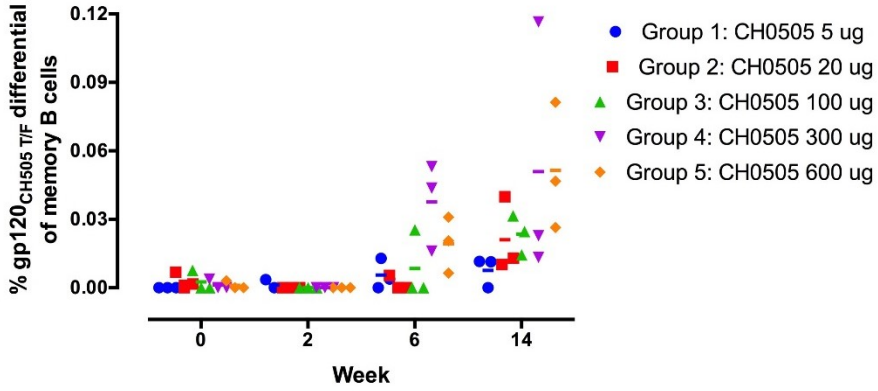

Figure 4-6 Percentage of differential binding memory B cells. Peripheral blood mononuclear cells (PBMC) from immunized macaques were assayed to determine the frequency of memory B cells that bind to CH505TF gp120 but not to CH505TF gp120 ΔI371 that disrupts the CD4 BS. Each data point represents a single animal. Animals were immunized at the indicated dose at weeks 0, 4 and 12. Note that the x-axis shows bleed time. Bleed 0 was performed before immunization and subsequent bleeds were performed 2 weeks after each respective immunization. A pattern of increased differential binding memory B cell induction with increasing vaccine dose is noted starting after two immunizations and increasing after a third immunization.

4.8 Clinical studies

4.8.1 Clinical studies of CH505TF gp120 with GLA-SE adjuvant in adults, HVTN 115

CH505TF gp120 is being evaluated in HVTN 115, the first clinical trial designed to use a B-cell lineage approach to elicit CD4bs bnAbs. In Part A, the dose ranging portion of HVTN 115, the CH505TF gp120 was administered at months 0, 2, 4, 8, and 12 at doses of 20, 100, and 400 mcg with 10 mcg of GLA-SE (Table 4-6).

Table 4-6 Schema for HVTN 115 Part A

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

Based on safety and immunogenicity data from Part A, HVTN 115 Part B will administer Env gp120 immunogens that induced a CD4bs bnAb clonal lineage in an HIV-infected individual in both sequential and additive strategies at a dose of 400mcg. The primary immune signal is differential binding antibodies between CH505TF Env gp120 (wild type) and CH505 Env IΔ371 gp120 (mutant): a differential binding ratio of wild-type/mutant ≥ 2.5 is characteristic of a CD4bs specificity.

HVTN 115 Part A reached full enrollment with 42 participants in May 2018. The last participant received their final vaccination on May 7, 2019. As of August 20, 2020, all scheduled study visits have been completed and the study remains blinded from a safety perspective. Most participants reported none or mild local signs and symptoms after vaccine administrations. There have been 2 cases of severe (grade 3) erythema/induration, which resolved within days. One of those participants experienced moderate injection site cellulitis, which resolved with oral antibiotics. Most participants reported none or mild systemic reactogenicity signs or symptoms after vaccinations. One case of severe (grade 3) pain at the injection site and 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.

Preliminary analysis of the immunogenicity data from Part A suggests that there were no statistically significant differences between dose groups for magnitude and response rates for antibodies to CH505, CD4 binding site, or B-cell responses between the treatment groups. For this reason, the lower doses have been chosen for this study to maximize safety and immunogenicity in human infants.

4.8.2 Clinical studies of the GLA-SE adjuvant and protein combinations in adults

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 (32, 33). Although the CH505TF gp120 protein in combination with GLA-SE has not been evaluated in humans to date, as summarized in Section 4.8.1, the Stable CH505TF gp120 is under evaluation in HVTN 115. In addition, there is considerable clinical trial experience with the GLA-SE adjuvant in combination with other antigens. These data are summarized below and in Table 4-7 and Table 4-8. Over 2500 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 IB for additional details of the clinical trials performed with GLA-SE.

Five studies have included the 10 mcg dose of the GLA-SE adjuvant, a higher dose than proposed for this study. The first was an open-label phase 1 clinical trial conducted in Brazil with a *Schistosoma mansoni* antigen (Sm14) (53). 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) (53). 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 vaccine. Humoral and CD4+ T cell responses to Sm14 were reported (53).

The second clinical trial using the 10 mcg dose of GLA-SE is a recently completed phase 1, 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. This study is complete. 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 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. 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 (54).

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 (55). No other safety concerns were reported. Immune responses were F protein dose-dependent, and the adjuvant enhanced both humoral and cellular immune responses (55). 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 (56).

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 (57). 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 (57). The unadjuvanted LEISH-F3 was essentially non-immunogenic while antibody and cytokine responses were noted in vaccinees in both dose groups of GLA-SE (57).

GLA-SE at a 5 mcg dose has also been tested together with protein antigens in several other clinical trials which are included in Table 4-7 and Table 4-8.

Table 4-7 Completed clinical trials using IDRI GLA-SE adjuvant formulations in combination with other vaccines (as of March 20, 2018)

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
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
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
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
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
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 4-8 Ongoing clinical trials using IDRI GLA-SE adjuvant formulations in combination with other vaccines (as of March 20, 2018)

Sponsor/Partner	Disease Area/Antigen	GLA-SE Dose	# Receiving GLA-SE	Total # in Study
IDRI NCT03302897	Leprosy (LEP-F1)	5 mcg	24	24
NIH/NIAID/DMID NCT02508376	TB (ID93)	5 mcg	20	70
University Hospital Tuebingen NCT02647489	Malaria (PAMVAC)	5 mcg	21	63
Institut National de la Santé Et de la Recherche Médicale NCT02658253	Malaria (PRIMVAC)	2.5 mcg	29	68
Immune Design NCT02035657	Merkel cell carcinoma	5 mcg	9	9
Immune Design NCT02501473	Non-Hodgkin's lymphoma	5 mcg 10 mcg 20 mcg	3 3 4	10
Immune Design NCT02180698	Sarcoma	5 mcg 10 mcg 20 mcg	4 4 4	12
Immune Design NCT02387125	Cancer (CMB305, G305)	Not Available		
Immune Design NCT02609984	Cancer (CMB305)	Not Available		
Immune Design NCT02320305	Melanoma (MART1)	Not Available		
HVTN 115 NCT03220724	HIV Vaccine	10 mcg	116	132

4.8.3 Clinical studies with related HIV-1 Env gp120 immunogens in infants (PACTG 230 and PACTG 326)

The adult RV144 pox prime-rgp120 protein boost trial was the first HIV-1 vaccine study to show some efficacy against HIV-1 acquisition. A vaccine efficacy of 31% was achieved in a heterosexual adult Thai population at three years post vaccination (58) and up to 60% protection was observed one-year post vaccination (58, 59). The subsequent immune correlate analysis showed that an IgG response, IgG3 in particular, against the variable loops 1 and 2 (V1V2) predicted decreased HIV-1 acquisition risk (60-63) whereas an Env-specific IgA response to specific HIV epitopes was associated with a lack of vaccine efficacy (64). Moreover, an IgG response against the variable loop 3 (V3) region was also associated with protection in the setting of low levels of vaccine-elicited responses (62).

Samples from two pediatric trials (PACTG 230 and PACTG 326) were then evaluated to determine immune responses in infants (see Table 4-9). In PACTG 230, infants were vaccinated between 0-20 weeks of age with four doses of Chiron rgp120 (SF2 strain) with MF59 (n=74), VaxGen rgp120 (MN strain) with aluminum hydroxide (alum, n=80), or adjuvant only placebo (MF59 or alum) (n=30) (65). In PACTG 326, infants received four doses of ALVAC-HIV alone (n= 12), ALVAC-HIV with AIDSVAX B/B with the adjuvant alum (n=10) or placebo (n=12; n=8 saline & n=3 alum/saline) between 0-12 weeks of age (66). Infants developed robust Env-specific antibody responses to these first-generation HIV-1 Env vaccines. At one year of age, most maternally-acquired antibodies had waned, and vaccine Envelope-specific IgG responses were significantly higher in vaccinees compared to placebo recipients (21). Importantly, infants receiving the Chiron vaccine had higher and more durable IgG responses than adult vaccinees in the RV144 vaccine trial, with vaccine-elicited IgG responses still detectable in 56% of infants at two years of age. Remarkably, at peak immunogenicity, the concentration of anti-V1V2 IgG, a response associated with reduced risk of HIV acquisition in the RV144 adult vaccine trial, was 22-fold higher in infants who received the Chiron rgp120/MF59 vaccine/adjuvant combination than in RV144 vaccinees. V1V2-specific IgG3 responses, the subclass of the IgG response that best associated with protection in the RV144 study, were detected in 43% of the vaccinated infants at 24 weeks after vaccination. Finally, vaccine-elicited IgA responses rarely detected in the vaccinated infants. To determine if the difference in infant and RV144 adult responses was due to distinct vaccine regimens, the infant vaccine-elicited antibody responses were then compared to that of adults immunized with the same vaccine regimens (67). At peak immunogenicity, no difference was observed in the magnitude of gp120 or V1V2-specific IgG between adults and infants immunized with the Alum adjuvanted VaxGen vaccines; however, infants who received the MF59 adjuvanted Chiron vaccine had more robust responses than adults immunized with the same vaccine. Overall, these studies have multiple implications for the proposed clinical trial: 1) they demonstrated that infant vaccination can elicit high levels and durable Env-specific antibodies that have been associated with protection in vaccinated adults; 2) they indicate possible differences in how adjuvants modulate immune

responses in adults and infants (68-71); 3) they also indicate that presence of maternal anti-HIV-1 responses are not likely impact the immunogenicity of the vaccine. Ongoing studies will determine if the higher levels of antigen-specific antibodies generated in infant immunization have antiviral activity comparable to those elicited in adults.

Table 4-9 Previous pediatric HIV vaccine trials including gp120 immunogen

Study Name	Vaccine (dose)/Adjuvant	Schedule	Population	Related Serious Adverse Events	Reference
PACTG 218	Chiron rgp120 (15 or 50 mcg)) Microgene sys rgp160 (40 or 320 mcg) Genentech rgp120 (75 or 300 mcg) Alum/MF59	0, 1, 2, 3, 4, 6 months	HIV-infected children enrolled between ages 1 month to 18 years/n=79/USA	None	(72)
PACTG 230	VaxGen rgp120/Alum Chiron rgp120/MF59	0, 4, 12, 20 weeks OR 0, 2, 8, 20 weeks	HIV-exposed children enrolled at birth /n=183/USA	None	(65)
PACTG 326	Part 1:ALVAC vCP205 Part 2: ALVAC vCP1452 +/- AIDSVAX B/B (200 mcg total)/Alum	0, 4, 8, 12 weeks	HIV-exposed children enrolled at birth/n=28/USA HIV-exposed children enrolled at birth/n=30/USA	None	(66, 73)

4.9 Potential risks of study products and administration

Table 4-10 includes general risks of vaccine administration along with risks known from prior clinical studies of CH505TF gp120 products and similar envelope proteins with GLA-SE adjuvant.

Table 4-10 Summary of potential risks of study products and administration

Common	• Mild to moderate injection site pain, tenderness, erythema, or swelling/induration/edema • A vaccine-induced positive HIV antibody test result
Less common	• Severe injection site pain or tenderness • Fever, chills, flu-like syndrome, arthralgia, rash, decreased appetite • 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

5 Objectives and endpoints

5.1 Primary objectives and endpoints

Primary objective 1:

To evaluate the safety and tolerability of HIV-1 CH505 transmitted/founder virus Env gp120 immunogen (CH505TF gp120) adjuvanted with Glucopyranosyl Lipid A - stable emulsion (GLA-SE) in healthy HIV-1 exposed uninfected (HEU) infants

Primary endpoint 1:

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

Primary objective 2:

To determine whether vaccination with CH505TF gp120 adjuvanted with GLA-SE initiates B-cell lineages potentially capable of generating a broadly neutralizing antibody response

Primary endpoints 2:

Magnitude of HIV-1 Env gp120, CD4 binding site and V1V2-specific serum IgG binding antibodies, as assessed by BAMA two weeks after the 5th vaccination

Quantification and phenotypic characterization of peripheral B cells capable of binding HIV-1 Env gp120, the CD4 binding site (including differential binding to the IΔ371 mutant) and the V1V2 binding site, as assessed by flow cytometry two weeks after the 3rd and 5th vaccinations

5.2 Secondary objectives and endpoints

Secondary objective 1:

To evaluate the effect of vaccination with CH505TF gp120 adjuvanted with GLA-SE on antibody responses to the South African Expanded Program on Immunization (EPI) schedule vaccinations

Secondary endpoint 1:

EPI vaccine-specific antibody responses, as assessed by Pediatric Vaccine Multiplex Assay (PVMA) 2 weeks after the 5th vaccination

Secondary objective 2:

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

Secondary endpoint 2:

Magnitude and breadth of serum neutralization of vaccine-matched viral isolates, and viruses engineered to detect precursors of CD4 binding site and V1V2 antibodies 2 weeks after the 5th vaccination.

Secondary objective 3:

To measure Fc-mediated antibody effector functions

Secondary endpoints 3:

Response rate and magnitude of vaccine-elicited serum binding antibodies to FcR proteins, as assessed by BAMA 2 weeks after the 5th vaccination

Response rate and magnitude of serum Antibody-dependent cellular cytotoxicity (ADCC), as assessed by flow cytometry and/or luciferase assays 2 weeks after the 5th vaccination

Response rate and magnitude of serum Antibody-dependent cellular phagocytosis (ADCP), as assessed by flow cytometry 2 weeks after the 5th vaccination

5.3 Exploratory objectives

Exploratory objective 1:

To compare antibody and B cell responses generated in infants in response to CH505TF gp120 adjuvanted with GLA-SE with adults vaccinated with the same immunogen and same adjuvant on the same schedule (HVTN 115)

Exploratory objective 2:

To characterize B cell lineages and evaluate the amount of B-cell receptor somatic hypermutation

Exploratory objective 3:

To evaluate effects of maternal antibody in cord blood, serum/plasma, and breast milk on the humoral response of infants

Exploratory objective 4:

To evaluate additional vaccine-induced antibody effector functions such as Antibody-dependent neutrophil phagocytosis (ADNP) and/or Antibody dependent complement deposition (ADCD)

Exploratory objective 5:

To measure the kinetics and maturation of the polyclonal antibody response with biophysical measurements (eg, BioLayer Interferometry, Surface Plasmon Resonance)

Exploratory objective 6:

Evaluation of breast milk and fecal microbiome at birth and over time to explore the effect of the microbiome on the development of the immune system and on vaccine immunogenicity

Exploratory objective 7:

To use systems biology approaches (eg, high resolution flow cytometry. transcriptomics, proteomics, metabolomics and/or plasma cytokine/chemokine assessment) to determine whether the pre- and/or postvaccination systemic immune milieu is associated with vaccine immunogenicity.

Exploratory objective 8:

To further evaluate immunogenicity, additional assays may be performed based on the HVTN Lab Assay Algorithm

Exploratory objective 9:

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

6 Statistical considerations

6.1 Accrual and sample size calculations

Recruitment will target enrolling 38 healthy, HIV-uninfected infant participants born by Caesarian section to HIV-infected mothers (HIV exposed, uninfected infants). Although this study is proposed to be a direct comparison with HVTN 115, there are important differences in the populations that justify the inclusion of a larger placebo group. Specifically, we anticipate a higher rate of background severe adverse events in this population, as previous vaccine studies have determined a rate of 18% SAE in the placebo arm (74). This is markedly different from the background rate of severe adverse events in typical HVTN adult studies (~1-2%), supported by emerging safety data from the HVTN 115 study in adults (see Section 4.8.1). Therefore, we propose including a larger placebo group to ensure capacity for determining a safety signal in this vulnerable population.

There are both technical and logistical considerations that favor enrolling infants delivered via Caesarian section. From a technical standpoint, assessment of the evolution of antibodies requires an understanding of the baseline nature of B cells prior to vaccination. Standard assessment of antigen-specific B cell lineages requires several milliliters of blood, a volume that is unsafe in neonates. We intend to protect the participants of the study by performing baseline cellular assays on cord blood that would otherwise be discarded. For logistical reasons related to the study site, cord blood can only be collected during Caesarian sections.

Since enrollment of mother-infant pairs is concurrent with infants receiving the first study vaccination, all participants will be included in the safety analysis. 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. While the site staff report a historical rate of 10% missingness in infant studies conducted at the site, immunogenicity data from previous phase 1 and 1 phase 2a HVTN trials indicate that 25% is a reasonable estimate for the rate of missing data for the later timepoint (1 year). For this reason, the sample size calculations below account for 25% of enrolled participants having missing data for the primary immunogenicity endpoint.

6.1.1 Sample size calculations for safety

The goal of the safety evaluation for this study is to identify safety concerns associated with product administration. The ability of the study to identify SAEs can be expressed by the true event rate above which at least 1 event would likely be observed and the true event rate below which no events would likely be observed. Specifically, in the pooled Groups 3 and 5 (20 mcg CH505TF gp120 + 5 mcg GLA-SE vaccine arms) of the study (n = 18), there is 90% chance of

observing at least one event, if the true rate is 12% or more, and there is a 90% chance of observing no events, if the true rate is 0.58% or less. In the pooled placebo group of the study (n = 10), there is 90% chance of observing at least one event, if the true rate is 20% or more. There is a 90% chance of observing no events, if the true rate is 1% or less.

As a reference, in HVTN vaccine trials in Southern Africa from April 2008 through March 2018, about 1.7% of adult participants who received placebos experienced an SAE. Based on recent infant clinical trial studies including infants at the PHRU site, approximately 18% of infant participants who received placebos experienced an SAE (54).

Binomial probabilities of observing 0, 1 or more, and 2 or more events among arms of size 18 and 10 are presented in Table 6-1 for a range of possible true adverse event rates. These calculations provide a more complete picture of the sensitivity of this study design to identify potential safety problems with the vaccine.

Table 6-1 Probability (Pr) of observing 0 events, 1 or more events, and 2 or more events, among arms of size 18 and 10, for different true event rates

True event rate (%)	Pr(0/18)	Pr(1+/18)	Pr(2+/18)	Pr(0/10)	Pr(1+/10)	Pr(2+/10)
1	83.5	16.5	1.4	90.4	9.6	0.4
5	39.7	60.3	22.6	59.9	40.1	8.6
10	15	85	55	34.9	65.1	26.4
20	1.8	98.2	90.1	10.7	89.3	62.4
30	0.2	99.8	98.6	2.8	97.2	85.1
40	0	100	99.9	0.6	99.4	95.4
50	0	100	100	0.1	99.9	98.9
60	0	100	100	0	100	99.8
70	0	100	100	0	100	100
80	0	100	100	0	100	100
90	0	100	100	0	100	100

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 an observed rate. Calculations are done using the score test method (75). If none of the 18 participants in Groups 3 and 5 experience a safety event, the 95% 2-sided upper confidence bound for the true rate of such events is 18%.

Table 6-2 Two-sided 95% confidence intervals based on observing a particular rate of safety endpoints for arms of size 18 and 10

Observed event rate	95% Confidence interval (%)
0/18	[0, 17.6]
1/18	[1, 25.8]
2/18	[3.1, 32.8]
3/18	[5.8, 39.2]
4/18	[9, 45.2]
5/18	[12.5, 50.9]
6/18	[16.3, 56.3]
7/18	[20.3, 61.4]
8/18	[24.6, 66.3]
9/18	[29, 71]
10/18	[33.7, 75.4]
11/18	[38.6, 79.7]
12/18	[43.7, 83.7]
0/10	[0, 27.8]
1/10	[1.8, 40.4]
2/10	[5.7, 51]
3/10	[10.8, 60.3]
4/10	[16.8, 68.7]
5/10	[23.7, 76.3]
6/10	[31.3, 83.2]

6.1.2 Sample size calculations for immunogenicity

To address binding antibody endpoints, the analysis will descriptively summarize binding response positivity call rates to test superiority of IgG binding Ab responses for CH103-like CD4bs and V1V2 for comparisons versus zero using binomial exact tests with 5% type-I error rate each. Note that if in HIV-infected women, maternal antibodies that differentially bind to the CD4bs occur at a rate of 51% [estimated from n = 14 CAPRISA 002 women infected 2-3 years (76) and are transmitted to the infants, comparisons at early timepoints may be confounded by background positivity. Therefore, we have chosen the 2 weeks post 5th vaccination timepoint (~ one year) for the power calculation. By this time, both transplacental and breastmilk-associated antibodies (breast-feeding typically ceases at 6 months of age in the South Africa setting) are expected to have waned to below detectable levels, obviating the requirement to increase the placebo recipient sample size for a comparison with this high background rate. The following calculations assume a 25% rate of missing data to accommodate missingness at the 1-year timepoint.

The main goals of this trial regarding immunogenicity outcomes involve a preliminary estimation of rates of "response" (defined for Primary Endpoint 2 as initiation of either the CD4bs or the V1V2 B cell lineage) based on data from immune 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 rate of responses in the vaccinees is shown in Table 6-3. Calculations are done using the score test method (75). The n = 13 assumes a 25% rate of 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 = 13)

No. of responses	Observed response rate (%)	95% Confidence interval
1/13	7.7	[1.4, 33.3]
2/13	15.4	[4.3, 42.2]
3/13	23.1	[8.2, 50.3]
4/13	30.8	[12.7, 57.6]
5/13	38.5	[17.7, 64.5]
6/13	46.2	[23.2, 70.9]
7/13	53.8	[29.1, 76.8]
8/13	61.5	[35.5, 82.3]
9/13	69.2	[42.4, 87.3]
10/13	76.9	[49.7, 91.8]
11/13	84.6	[57.8, 95.7]
12/13	92.3	[66.7, 98.6]
13/13	100	[77.2, 100]

As a reference, the range of the lineage initiate response rates seen in adult participants in HVTN 115, Part A was 30%-40%, varying by dose. The sample size of n = 13 yields greater than 90% power to detect a response if the true rate is 30% or greater.

Group 7 is not powered for a formal immunogenicity analysis but is instead intended to provide data for a cursory investigation of immunogenicity of a variant regimen with a lower protein dose: (5 mcg instead of 20 mcg of protein). After an expected loss of 25% of immunogenicity data, the sample sizes of n = 4 for this group is sufficient to detect an extreme loss of immunogenicity due to the lower dose. If 0 of 4 participants in this group is a responder, the upper end of the 95% confidence interval for the true response rate for that regimen is 49%.

As shown in Table 6-4, there is low power for a formal comparison of immunogenicity response rates between the Group 3 and 5 pooled vaccine arms of size n = 13 versus a pooled placebo arm of size n = 8 after accounting for missingness. The sizes of differences that the trial is powered to detect are around 70%. These calculations use a Fisher's exact 2-sided test with a Type I error rate of 0.05. There is insufficient power for a formal comparison across the vaccine regimens or between Group 7 and the pooled placebo group.

Table 6-4 Power for comparison of response rates between 2 pooled arms (n1 = 8, n2 = 13)

	Minimum true response rate in Arm 2 in order to detect a difference	
True response rate Arm 1 (%)	80% power	90% power
0	64	73
10	79	87
20	89	95
30	96	-

6.1.3 Considerations for comparison of immunogenicity to adults in HVTN 115

To address Exploratory Objective 1 (comparison to adults), the analysis will descriptively summarize binding response positivity call rates, and test superiority of the frequency of differential memory B cell binding (to wild type vs mutant IΔ371 CH505) and of the magnitude and breadth of the IgG binding Ab response to a panel of gp120 proteins using a two-sided Wilcoxon rank sum test with 5% type-I error rate per comparison. This exploratory objective involves a comparison across populations (infants versus adults, in different demographic settings). Since this factor is necessarily non-randomizable, these comparisons are of the "observational" category, in the sense that they do not share the statistical benefits of the randomized controlled clinical trial design. Specifically, while the "study" factor differentiates adults from infants, these cohorts differ in many other respects. Therefore, the interpretation of any differences observed will require more careful consideration of the potential confounding effects of measured and unmeasured differences across the populations besides the age of the vaccine recipient.

Immunogenicity in the HVTN 115 Part B vaccine recipients (n = 20 per group, n = 60 total), pooled with the corresponding dose group from Part A (n = 12) will be compared to immunogenicity in the HVTN 135 vaccine recipients. After an expected 15% of immunogenicity data missingness in HVTN 115 at the immunogenicity timepoint post 5th vaccination (at one year), sample sizes for comparisons across these study populations will be approximately n = 13 infants vs n = 27 (one HVTN 115 Part B arm, and one HVTN 115 Part A arm) or n = 61 (all 3 Part B vaccine arms, and 1 Part A arm). Comparisons are well-powered under this scenario for both binary responses and for comparisons of continuous responses (eg measuring antibody binding magnitudes). If the true response rate in adults is 30%, there is 80% power to detect a difference vs infant response rates if the true rate in infants is 82% or greater when comparing to n = 27 adults, and there is 80% power to detect a difference vs n = 61 adults when the true rate in infants exceeds 77%.

For continuous endpoints, there is 80% power to detect a difference across adult vs infant groups of 1.16 standard deviations (SDs) when considering n = 27 adults, and 1.04 SDs when considering n = 61 adults. As a point of reference,

using the mean and SD values from the HVTN 088 trial, the difference of 1.04 SD translates to a difference of 0.38 units of log10 binding magnitude; if one of the comparison groups has the same mean as was seen among non-negative values among vaccine recipients in the HVTN 088 trial (4.05 units), then after incorporating the expected 6% zero-valued (negative responder) measures, this corresponds to observing an increase or decrease in the overall mean from 3.78 in group 1 to 4.16 in group 2. These calculations assume a 15% loss-to-follow-up rate in HVTN 115 and a 25% rate in HVTN 135 and the (94%) response rate observed in the HVTN 088 vaccine recipients. The same approach will be used to test superiority of the magnitude of the IgG binding Ab response to each individual gp120 antigen in the panel.

6.2 Randomization

A participant's randomization assignment will be computer generated and provided to the HVTN clinical research site (CRS) pharmacist through a Web-based randomization system. At the 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 Manual of operations [MOP]).

6.3 Blinding

Participants and site staff (except for site pharmacists) will be blinded as to participant treatment arm assignments (eg, vaccine or placebo). 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 to ensure proper trial conduct. Any discussion of study product assignment between pharmacy staff and any other HVTN CRS staff is prohibited. The HVTN SMB members also are unblinded to treatment assignment 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 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 of mother-infant pairs is concurrent with the infant receiving the first vaccination, all infants 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 and Wilcoxon rank sum 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 study by treatment arm and visit. Each box plot will show the first quartile, the median, and the third quartile. Outliers (values outside the box plot) will also be plotted. If appropriate, horizontal lines representing boundaries for abnormal values will be plotted.

For each local laboratory measure, summary statistics will be presented by treatment arm and timepoint, as well as changes from baseline for postenrollment values. In addition, the number (percentage) of participants with local laboratory values recorded as meeting Grade 1 AE criteria or above as specified in the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (see Section 9.7) 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, from specimens collected outside of the visit window, or from HIV-infected participants postinfection are excluded.

Since the exact date of HIV infection is unknown, any assay data from blood draws 4 weeks prior to an infected participant's last seronegative sample and thereafter may be excluded. If an HIV-infected participant does not have a seronegative sample postenrollment, then all data from that participant may be excluded from the analysis.

Discrete categorical assay endpoints (eg, response rates) will be analyzed by tabulating the frequency of positive response for each assay by antigen and treatment arm at each timepoint for which an assessment is performed. Crude response rates will be presented with their corresponding 95% confidence interval estimates calculated using the score test method (75). Because of the small numbers of control participants in each group, no adjustment will be made to the vaccine arm estimates for the false positive rates in the control arms. Barnard or Fisher's exact tests, as specified in the SAP, will be used to compare the response rates across treatment groups, with a significant difference declared if the 2-sided p-value is ≤ 0.05; however the primary analysis will prioritize the one-sample comparison of those response rates to zero due to the power considerations discussed above. In general Barnard's is preferred since under most circumstances it is more powerful than Fisher's (77).

In addition to response rate estimates for each timepoint, the probability of observing at least 1 positive response by a given timepoint and the probability of observing more than 1 positive response by a given timepoint will be estimated, with corresponding confidence intervals, for each vaccine arm using maximum likelihood-based methods (78).

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

Some immunologic assays have underlying continuous or count-type readout that are dichotomized into responder/nonresponder categories (eg, nAb multiplex assay positivity). If treatment arm differences for these assays are best summarized by a mixture model, then Lachenbruch's test statistic (79) will be used to evaluate the composite null hypothesis of equal response rates in the 2 arms and equal response distributions among responders in the 2 such arms. This test statistic equals the square of a binomial Z-statistic for comparing the response rates plus the square of a Wilcoxon statistic for comparing the response distributions in the subgroup of responders. A permutation procedure is used to obtain a 2-sided p-value. For estimation, differences in response rates between arms will be estimated using the methods described above, and in the subgroup of positive responders, differences in location parameters between arms will be estimated using the methods described above.

Based upon previous HVTN trials as well as experience conducting infant clinical trials in the study population, missing up to 25% of immunogenicity results for a specific assay is anticipated due to study participants terminating from the study early, problems in shipping specimens, or low cell viability of processed peripheral blood mononuclear cells (PBMCs). To achieve unbiased statistical estimation and inferences with standard methods applied in a complete-case manner (only including participants with observed data in the analysis), missing data need to be missing completely at random (MCAR). Following the most commonly used definition, MCAR assumes that the probability of an observation being missing does not depend on any participant characteristics (observed or unobserved). When missing data are minimal (specifically if no more than 20% of participants are missing any values), then standard complete-case methods will be used, because violations of the MCAR assumption will have little impact on the estimates and hypothesis tests.

If a substantial amount of immunogenicity data is missing for an endpoint (at least 1 value missing from more than 20% of participants), then using the methods that require the MCAR assumption may give misleading results. In this situation, analyses of the immunogenicity endpoints at a specific timepoint will be performed using parametric generalized linear models fit by maximum likelihood. These methods provide unbiased estimation and inferences under the parametric modeling assumptions and the assumption that the missing data are missing at random (MAR). MAR assumes that the probability of an observation being missing may depend upon the observed responses and upon observed covariates, but not upon any unobserved factors. Generalized linear models for response rates will use a binomial error distribution and for quantitative endpoints, a normal error distribution. For assessing repeated immunogenicity measurement, linear mixed effects models will be used. If the immunological outcomes are left- and/or right- censored, then the linear mixed effects models of Hughes (80) will be used, because they accommodate the censoring. In addition, secondary analyses of repeated immunogenicity measurements may be done using weighted GEE (81) methods, which are valid under MAR. All the models described above in this paragraph will include as covariates all available baseline predictors of the missing outcomes.

Some “resource-intensive” immunogenicity endpoints are only measured in subset of participants, eg, immunogenicity endpoints based on mucosal samples. For such endpoints, exploratory analyses will be conducted to assess the correlation of participant characteristics measured in (nearly) all participants with the resource-intensive endpoints. For example, if the same assay is performed on blood and mucosal samples, then a scatterplot and Spearman rank correlation coefficient (r) will be used to assess the correlation of responses. If at least moderate correlations exist (eg, $r \geq 0.3$), then the semiparametric efficient analysis method of Rotnitzky and Robins (82) will be used (described in Gilbert, Sato et al. for application to vaccine studies (83)) to estimate the mean of the resource-intensive endpoint for each group and to compare means between groups.

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 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 (84). 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, antibody binding magnitudes) 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 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 of neutralizing antibodies is defined as the average of the log10 nAb titer over the panel of isolates, where titers that are below the limit-of-detection are set to half of that limit. Response

breadth of binding antibodies is similarly defined as the average of the response over the panel of antigens. Details of the 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 and 6.4.5.2. Interim blinded safety and immunogenicity data should not be shared outside of the SMB, 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 for review by the SMB. Ad hoc safety reports may also be prepared for SMB review at the request of the 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 within any study Part (A, B, or C) have completed the corresponding primary immunogenicity visits 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 within any study Part have completed the corresponding immunogenicity visit and data are available for analysis from at least 80% of these participants. The Laboratory Program 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 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

This study will be conducted among 38 healthy infants born to HIV-infected mothers in South Africa. Infants will be enrolled in pairs with their mothers, although only infants will receive study product. Mothers will be HIV-infected (seropositive) adults on cART (combination anti-retroviral therapy) who are clinically stable and who comprehend the purpose of the study and have provided written informed consent for their and their infant's participation in the study. Mother-infant pairs will be selected for the study according to the criteria in Sections 7.1, 7.2, 7.3, and 7.4. 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 mother-infant pair's overall fitness for trial participation. Some mother-infant pairs 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 mother-infant pairs may be poor candidates for retention.

Due to the logistical requirements that require identification of mothers during pregnancy but an inability to fully assess inclusion criteria for infants until birth the screening periods will be different for the infants and their mothers. All inclusion and exclusion criteria **for the infant** must be within 5 days prior to enrollment. To allow for identification of women during pregnancy, the screening time-frame will be different. All inclusion and exclusion criteria for the mother must be assessed within 273 days (9 months) of enrollment, unless otherwise specified in the eligibility criteria below.

7.1 Inclusion criteria: Mother

General and Demographic Criteria

1. **Mother's age** is at least 18 years, and willing and able to provide written informed consent for her and her infant's participation in this study.

2. **Mother is in the second or third trimester of singleton pregnancy,** as determined by a clinical exam, or sonography and reported menstrual history.

3. **Mother agrees to donate umbilical cord blood.**

4. **Mother has a planned Caesarian Section** at Chris Hani Baragwanath Academic Hospital, Soweto and plans to remain in the area after delivery.

5. **Mother is determined by the site investigator to be in good overall health** at the time of delivery based on medical history, and physical exam.

6. **Mother has a documented CD4 count > 350 cells/microliter** during her pregnancy.

7. **Mother has documented SARS CoV-2 negative PCR test within 2 days before delivery to 5 days after delivery**

8. **Mother has access to the participating HVTN CRS** and willingness to be followed for the planned duration of the study.

9. **Assessment of understanding**: Mother demonstrates understanding of this study; completes a questionnaire prior to delivery with verbal demonstration of understanding of all questionnaire items answered incorrectly.

10. **Mother agrees not to enroll either herself or her infant in another research study** for the duration of the trial without prior approval of the HVTN 135 PSRT.

11. **Mother has confirmed HIV-1 infection** documented by medical records at any time during or prior to screening, and confirmed by the HVTN CRS by serology.

12. **Mother has been on cART for at least sixteen weeks prior to delivery** and intends to continue with cART for the duration of breastfeeding.

13. **Mother has a viral load of less than 400 copies/mL** between two weeks before and 5 days after delivery**.**

7.2 Exclusion criteria: Mother

1. **Any WHO Grade IV illness within one year prior to study enrollment** as determined by the history and physical examination and review of the medical record (if available). These include HIV wasting syndrome, PJP Pneumonia, Cerebral Toxoplasmosis, extrapulmonary Cryptococcosis, Progressive Multifocal Leukoencephalopathy, any disseminated endemic mycosis (histoplasmosis), candidiasis of the esophagus, trachea, bronchi or lung, disseminated atypical mycobacteria, non-typhoid Salmonella septicemia, extrapulmonary tuberculosis, lymphoma, Kaposi's sarcoma.

2. **Prior participation in any HIV-1 vaccine or anti-HIV antibody-mediated prevention trial.**

3. **Receipt of any investigational agent during this pregnancy.**

4. **Receipt of blood products, immunoglobulin, or immunomodulating therapy within 45 days prior to delivery of the placenta.**

5. **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.

6. **Any condition that places the newborn at higher risk of early-onset sepsis,** such as concern for active maternal infection at delivery as determined by local site investigators (eg, fever).

7. **Detectable Hepatitis B surface antigen.**

7.3 Inclusion criteria: Infant

1. **Born via Caesarean section to an HIV-1–infected woman** who meets all maternal inclusion/exclusion criteria listed above.

2. Estimated **gestational age at birth is at least 37 weeks**.

   Note: If gestational age at birth is not documented in the infant's available birth records, study staff may assess gestational age at the earliest possible opportunity during the screening period and use this assessment for purposes of eligibility determination.

3. **Weight at birth** is at least 2.5 kg.

4. **Has initiated antiretroviral prophylaxis** consistent with current site-specific standard of care.

5. **Hemoglobin** >14.0 g/dL.

6. **White Blood Cell Count** ≥ 7000 cells/mm^3

7. **Platelets** > 100,000 cells/mm^3

8. **Alanine aminotransferase (ALT)** <1.25 times upper limit of age adjusted normal.

9. **Creatinine <** 1.1 times upper limit of age adjusted normal.

10. **Negative HIV-1** nucleic acid test on specimen drawn within 72 hours of birth.

11. **Written informed consent provided by mother.**

12. **Age is equal to or less than five days.**

7.4 Exclusion criteria: Infant

1. Any clinically significant **congenital anomaly/birth defect**.

2. **Documented or suspected serious medical illness**, infection, clinically significant finding from physical examination or immediate life-threatening condition, including requirement for ongoing supplemental oxygen, as judged by the examining clinician.

3. **Receipt of or anticipated need for blood products**, immunoglobulin, or immunosuppressive therapy. This includes infants who require Hepatitis B Immunoglobulin (HBIG) but does not require exclusion of infants who receive Hepatitis B vaccine in the newborn period.

4. **Receipt of any other investigational product**.

7.5 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.

7.5.1 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

- Prevaccination abnormal vital signs or clinical symptoms that may mask assessment of vaccine reaction

- Child becomes moderately or severely underweight as defined by the DAIDS AE Grading Table for Underweight < 2 years of age.

- If an infant has delayed EPI immunizations at the time of a scheduled study vaccine visit, the EPI immunizations will be provided to the infant at least 7 days prior to administration of the study vaccination. The only exception is the initial vaccination.

Vaccinations should not be administered outside the visit window, as specified in Appendix I.

7.5.2 Participant departure from vaccination schedule

Every effort should be made to follow the vaccination schedule per the protocol. If an infant misses a vaccination and the visit window period for the vaccination has passed, that vaccination cannot be given. The mother-infant pair should be asked to continue study visits. The infant should resume the vaccination schedule

with the next vaccination unless there are circumstances that require further delay or permanent discontinuation of vaccination (see Sections 7.5.3 and 7.5.4).

7.5.3 Discontinuing vaccination for a participant

Under certain circumstances, an individual infant's vaccinations will be permanently discontinued. Specific events that will result in stopping an individual'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 PSRT)

- Clinically significant condition (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:
  - 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 except for fever (temporal artery temperature), vomiting, 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
  - Any type 1 hypersensitivity reaction considered to be related to study vaccination.

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

The mothers of infants 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 135 Study Specific procedures [SSP]).

Infants diagnosed with HIV infection during the study should be encouraged to participate in follow-up visits for sole purposes of safety monitoring as indicated in Section 9.10.

7.5.4 Participant termination from the study

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

- Infant's mother/guardian voluntarily withdraws consent to participate,
- Mother-infant pair relocates and remote follow-up is not possible,
- HVTN CRS determines that the mother-infant pair is lost to follow-up, or
- Investigator decides, in consultation with Protocol Team leadership, to terminate participation (eg, if infant's mother exhibits inappropriate behavior toward clinic staff).
- Any condition where termination from the study is required by applicable regulations.

8 Study product preparation and administration

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

8.1 Vaccine regimen

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

Part A

Group 1

Treatment 1 (T1): 20 mcg Stable CH505TF gp120 admixed with 2.5 mcg GLA-SE, to be administered as a 0.25 mL intramuscular (IM) injection into either thigh at Weeks 0, 8, 16, 32, and 54.

Group 2

Control 2 (C2): Placebo for 20 mcg Stable CH505TF gp120 + 2.5 mcg GLA-SE (Sodium Chloride for Injection, 0.9%) to be administered as a 0.25 mL IM injection, into either thigh at Weeks 0, 8, 16, 32, and 54.

Part B

Group 3

Treatment 3 (T3): 20 mcg Stable CH505TF gp120 admixed with 5 mcg GLA-SE, to be administered as a 0.5 mL IM injection into either thigh at Weeks 0, 8, 16, 32, and 54.

Group 4

Control 4 (C4): Placebo for 20 mcg Stable CH505TF gp120 + 5 mcg GLA-SE (Sodium Chloride for Injection, 0.9%) to be administered as a 0.5 mL IM injection, into either thigh at Weeks 0, 8, 16, 32, and 54.

Part C

Group 5

Treatment 5 (T5): 20 mcg Stable CH505TF gp120 admixed with 5 mcg GLA-SE, to be administered as a 0.5 mL IM injection into either thigh at Weeks 0, 8, 16, 32, and 54.

Group 6

Control 6 (C6): Placebo for 20 mcg Stable CH505TFgp120 + 5 mcg GLA-SE (Sodium Chloride for Injection, 0.9%) to be administered as a 0.5 mL IM injection, into either thigh at Weeks 0, 8, 16, 32, and 54.

Group 7

Treatment 7 (T7): 5 mcg Stable CH505TF gp120 admixed with 5 mcg GLA-SE, to be administered as a 0.5 mL IM injection into either thigh at Weeks 0, 8, 16, 32, and 54.

Group 8

Control 8 (C8): Placebo for 5 mcg Stable CH505TF gp120 + 5 mcg GLA-SE (Sodium Chloride for Injection, 0.9%) to be administered as a 0.5 mL IM injection, into either thigh at Weeks 0, 8, 16, 32, and 54.

8.2 Study product formulation

8.2.1 Stable CH505TF gp120; labeled as CH505TF gp120

The Stable CH505TF gp120 will be provided at a concentration of 0.8 mg/mL of protein in phosphate-buffered saline per vial. Each sterile, single use vial contains 0.75 mL of study product. The study product should be stored frozen at ≤ -65°C. When thawed, the Stable CH505TF gp120 will be clear, and colorless to slightly yellow, liquid. The study product is described in further detail in the IB.

8.2.2 GLA-SE (Glucopyranosyl Lipid A-Stable Emulsion); labeled as AP 10-201

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

8.2.3 Placebo for Stable CH505TF gp120 + GLA-SE

Sodium Chloride for Injection, 0.9% will be used as the placebo. It must be stored in accordance with manufacturer's recommendation.

8.3 Preparation of study products

8.3.1 Group 1 (T1): 20 mcg Stable CH505TF gp120 + 2.5 mcg GLA-SE

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

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

Using aseptic technique, the pharmacist will add 0.2 mL of Stable CH505TF gp120 and 0.8 mL of Sodium Chloride for injection, 0.9% to an empty sterile vial. Mix the contents of this vial thoroughly using a vortex machine at high speed for 3 seconds. The final concentration of Stable CH505TF gp120 is 160 mcg/mL.

Next, using aseptic technique, withdraw 0.4 mL of the diluted 160 mcg/mL Stable CH505TF gp120 admixture and 0.4 mL of GLA-SE (20 mcg/mL) and inject it into an empty sterile vial. Mix thoroughly by gently inverting 10 times, yielding a concentration of 80 mcg/mL Stable CH505TF gp120 and 10 mcg/mL GLA-SE.

Finally, using aseptic technique, withdraw 0.25 mL from the vial containing 80 mcg/mL Stable CH505TF gp120 and 10 mcg/mL GLA-SE, using a 1 mL size syringe. Remove the needle for preparation and attach the needle for administration to the prepared study product in syringe before dispensing.

The prepared syringe for administration must be covered with an overlay and then labeled as “Stable CH505TF gp120 + GLA-SE or Placebo”. The syringe must also be labeled for IM administration into the thigh, with an expiration date and time of 8 hours following the last mixing procedure.

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

8.3.2 Group 2 (C2): Placebo for 20 mcg Stable CH505TF gp120 + 2.5 mcg GLA-SE

The pharmacist using aseptic technique will withdraw 0.25 mL of Sodium Chloride for Injection, 0.9% into a 1 mL size syringe, remove the needle for preparation and attach the needle for administration to the prepared study product in syringe before dispensing.

The final syringe for administration must be covered with an overlay and then labeled as “Stable CH505TF gp120 + GLA-SE or Placebo”. The syringe must also be labeled for IM administration into thigh, with an expiration date and time of 8 hours following the last mixing procedure.

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

8.3.3 Group 3 (T3): 20 mcg Stable CH505TF gp120 + 5 mcg GLA-SE

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

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

Using aseptic technique, the pharmacist will add 0.1 mL of Stable CH505TF gp120 and 0.9 mL of Sodium Chloride for Injection, 0.9% to an empty sterile vial. Mix the contents of this vial thoroughly using a vortex machine at high speed for 3 seconds. The final concentration of Stable CH505TF gp120 is 80 mcg/mL.

Next, using aseptic technique, withdraw 0.4 mL of the diluted 80 mcg/ml Stable CH505TF gp120 admixture and 0.4 mL of GLA-SE (20 mcg/mL) and inject it into an empty sterile vial. Mix thoroughly by gently inverting 10 times, yielding a concentration of 40 mcg/mL Stable CH505TF gp120 and 10 mcg/mL GLA-SE.

Finally, using aseptic technique, withdraw 0.5 mL from the vial containing 40 mcg/mL Stable CH505TF gp120 and 10 mcg/mL GLA-SE, using a 1 mL size syringe. Remove the needle for preparation and attach the needle for administration to the prepared study product in syringe before dispensing.

The prepared syringe for administration must be covered with an overlay and then labeled as “Stable CH505TF gp120 + GLA-SE or Placebo”. The syringe must also be labeled for IM administration into the thigh, with an expiration date and time of 8 hours following the last mixing procedure.

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

8.3.4 Group 4 (C4): Placebo for 20 mcg Stable CH505TF gp120 + 5 mcg GLA-SE

The pharmacist using aseptic technique will withdraw 0.5 mL of Sodium Chloride for Injection, 0.9% into a 1 mL size syringe, remove the needle for preparation and attach the needle for administration to the prepared study product in syringe before dispensing.

The final syringe for administration must be covered with an overlay and then labeled as “Stable CH505TF gp120 + GLA-SE or Placebo”. The syringe must

also be labeled for IM administration into the 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.

8.3.5 Group 5 (T5): 20 mcg Stable CH505TF gp120 + 5 mcg GLA-SE

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

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

Using aseptic technique, the pharmacist will add 0.1 mL of Stable CH505TF gp120 and 0.9 mL of Sodium Chloride for Injection, 0.9% to an empty sterile vial. Mix the contents of this vial thoroughly using a vortex machine at high speed for 3 seconds. The final concentration of Stable CH505TF gp120 is 80 mcg/mL.

Next, using aseptic technique, withdraw 0.4 mL of the diluted 80 mcg/mL Stable CH505TF gp120 admixture and 0.4 mL of GLA-SE and inject it into an empty sterile vial. Mix thoroughly by gently inverting 10 times, yielding a concentration of 40 mcg/mL Stable CH505TF gp120 and 10 mcg/mL GLA-SE.

Finally, using aseptic technique, withdraw 0.5 mL from the vial containing 40 mcg/mL Stable CH505TF gp120 and 10 mcg/mL GLA-SE, using a 1 mL size syringe. Remove the needle for preparation and attach the needle for administration to the prepared study product in syringe before dispensing.

The prepared syringe for administration must be covered with an overlay and then labeled as “Stable CH505TF gp120 + GLA-SE or Placebo”. The syringe must also be labeled for IM administration into the thigh, with an expiration date and time of 8 hours following the last mixing procedure.

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

8.3.6 Group 6 (C6): Placebo for 20 mcg Stable CH505TF gp120 + 5 mcg GLA-SE

The pharmacist using aseptic technique will withdraw 0.5 mL of Sodium Chloride for Injection, 0.9% into a 1 mL size syringe, remove the needle for preparation and attach the needle for administration to the prepared study product in syringe before dispensing.

The final syringe for administration must be covered with an overlay and then labeled as "Stable CH505TF gp120 + GLA-SE or Placebo". The syringe must also be labeled for IM administration into thigh, with an expiration date and time of 8 hours following the last mixing procedure.

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

8.3.7 Group 7 (T7): 5 mcg Stable CH505TF gp120 + 5 mcg GLA-SE

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

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

Using aseptic technique, the pharmacist will add 0.1 mL of Stable CH505TF gp120 and 3.9 mL of Sodium Chloride for Injection, 0.9% to an empty sterile vial. Mix the contents of this vial thoroughly using a vortex machine at high speed for 3 seconds. The final concentration of Stable CH505TF gp120 is 20 mcg/mL.

Next, using aseptic technique, withdraw 0.4 mL of the diluted 20 mcg/mL Stable CH505TF gp120 admixture and 0.4 mL of GLA-SE (20 mcg/mL) and inject it into an empty sterile vial. Mix thoroughly by gently inverting 10 times, yielding a concentration of 10 mcg/mL Stable CH505TF gp120 and 10 mcg/mL GLA-SE.

Finally, using aseptic technique, withdraw 0.5 mL from the vial containing 10 mcg/mL Stable CH505TF gp120 and 10 mcg/mL GLA-SE, using a 1 mL size syringe. Remove the needle for preparation and attach the needle for administration to the prepared study product in syringe before dispensing.

The prepared syringe for administration must be covered with an overlay and then labeled as "Stable CH505TF gp120 + GLA-SE or Placebo". The syringe must also be labeled for IM administration into the 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.

8.3.8 Group 8 (C8): Placebo for 5 mcg Stable CH505TF gp120 + 5 mcg GLA-SE

The pharmacist using aseptic technique will withdraw 0.5 mL of Sodium Chloride for Injection, 0.9% into a 1 mL size syringe, remove the needle for preparation

and attach the needle for administration to the prepared study product in syringe before dispensing.

The final syringe for administration must be covered with an overlay and then labeled as “Stable CH505TF gp120 + GLA-SE or Placebo”. The syringe must also be labeled for IM administration into thigh, with an expiration date and time of 8 hours following the last mixing procedure.

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

8.4 Administration

All injections should be administered IM in the thigh of either leg as listed in Section 8.1.

The prepared study product in the syringe should be rolled gently prior to administration.

The prepared study product in the syringe is to be stored between 4° C and 25° C and must be administered as soon as possible and before the 8-hour expiration.

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

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

8.5 Acquisition of study products

Stable CH505TF gp120 is manufactured by Berkshire Sterile Manufacturing and GLA-SE is manufactured by IDRI. Stable CH505TF gp120 and GLA-SE will be provided by DAIDS. Sodium Chloride for Injection, 0.9% will not be provided through the protocol and must be obtained by the site.

Once an HVTN CRS is protocol registered, the pharmacist can obtain study products from the NIAID Clinical Research Products Management Center (CRPMC) by following the ordering procedures 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 must also record the lot # and manufacturer of the Sodium Chloride for Injection, 0.9% used to prepare the participant's study product injection. 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 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 F and Appendix G.

9.1 Informed consent

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

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

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

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

Mothers will provide written informed consent for their own and their infant’s study participation before any study-specific procedures are performed. The informed consent process will be conducted during pregnancy. The study will be discussed again with the mother after delivery and her prior consent decision will be confirmed at that time. If the mother changes her mind and withdraws her consent at that time, the mother-infant pair will not be enrolled in the study and no further study-specific procedures will be performed.

Due to the need to draw samples prior to enrollment (with the first vaccination), the informed consent will specifically address the need to collect biospecimens including peripheral and cord blood prior to full enrollment. The protocol-specific

consent process will specify that if the mother-infant pair is never enrolled in the study, any biospecimens will be destroyed.

Should the consenting mother of an enrolled infant die or no longer be available for any reason, no further study-specific visits or procedures may be performed until informed consent for continued study participation is obtained from a guardian. In accordance with the DAIDS policy on Enrolling Children (including Adolescents) in Clinical Research (available at the website referenced in Section 14), the CRS must establish and maintain written procedures describing the standards that will be followed to identify who may serve as guardian for an enrolled infant, reflective of applicable IRB/EC guidance for conduct of human subjects research within the context of available local law, regulation, or government policy.

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

The 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, Good Clinical Practice: Consolidated Guidance 4.8.

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

The sample informed consent form(s) include instructions for developing specific content. Regarding protocol registration, the site should follow procedures outlined in the current version of the DAIDS Protocol Registration Manual.

9.1.1 Assessment of Understanding

Study staff are responsible for ensuring that the mother fully understands the study requirements for both her and her infant before enrollment. This process involves reviewing the informed consent form, allowing time to reflect on the procedures and issues presented, and answering all questions completely.

An Assessment of Understanding is used to document the mother's understanding of key concepts in this HIV vaccine trial. The mother must complete the Assessment of Understanding before enrollment. Staff may provide assistance in reading and understanding the questions and responses, if necessary. The mother 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 consent document be signed 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 **for the infant** must be assessed within 5 days prior to enrollment. To allow for identification of women during pregnancy, the screening time-frame will be different for the mothers. All inclusion and exclusion criteria **for the mother** must be assessed within 273 days (9 months) of enrollment, unless otherwise specified in the eligibility criteria (or below in this section). Enrollment for the mother-infant pair is simultaneous with the infant's first vaccination

9.2.1 Pre-enrollment procedures, Mother

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

- Medical history, documented in the case history record
- 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 mother is taking, including antiretroviral regimen, prescription and nonprescription drugs, vitamins, topical products, alternative/complementary medicines (eg, herbal and health food supplements), recreational drugs, vaccinations, and allergy shots
- Risk reduction counseling
- Assessment of Understanding
- Specimen collection

- Laboratory tests, including:
  - CD4 count, if not available from medical records
  - SARS-CoV-2 PCR test, if not available from medical records
  - Hepatitis B surface antigen (HBsAg), if not available from medical records during pregnancy
  - HIV enzyme immunoassay (EIA) test
  - HIV polymerase chain reaction (PCR) Viral Load
- Obtaining of volunteer demographics in compliance with the NIH Policy on Reporting Race and Ethnicity Data: Subjects in Clinical Research, Aug. 8, 2001 (available at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-01-053.html)

9.2.2 Pre-enrollment procedures, Infant

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

- Medical history
- Complete physical examination, see HVTN 135 SSP for details
- Assessment of concomitant medications the infant is taking, including prescription and nonprescription drugs, antiretroviral prophylaxis regimen, vitamins, topical products, alternative/complementary medicines (eg, herbal and health food supplements), vaccinations
- Specimen collection
- Laboratory tests, including:
  - Complete blood count (CBC)
  - Chemistry panel (ALT, Creatinine)
  - Screening HIV Test
- Obtaining of volunteer demographics in compliance with the NIH Policy on Reporting Race and Ethnicity Data: Subjects in Clinical Research, Aug. 8, 2001 (available at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-01-053.html)

9.3 Enrollment and vaccination visits, Infant

Once the mother has provided written informed consent for their own and their infant's study participation and the mother-infant pair has been found to meet all

eligibility criteria (see Sections 7.1, 7.2, 7.3, and 7.4), the HVTN CRS requests the randomization assignment via a Web-based randomization system. Enrollment for the mother-infant pair is simultaneous with the infant's first vaccination and must be done within five days of life. In general, the time interval between randomization and enrollment should not exceed 4 working days.

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

- Abbreviated physical examination, including weight, vital signs, and a symptom-directed evaluation by history and/or appropriate physical exam based on the mother's reported symptoms for her infant (see HVTN 135 SSP);
- Feeding History;
- Assessment of baseline reactogenicity parameters;
- Assessment of concomitant medications (as described in Section 9.2);
- Assessment of any new or unresolved AEs/intercurrent illnesses; and

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

Immediately following vaccination, the participant remains in the clinic for observation and ongoing monitoring for a minimum of 60 minutes. An initial reactogenicity assessment is made at a target of 30 minutes after injection, with an acceptable range of 25-60 minutes. Before leaving the clinic, the mother is given the Participant Diary and is instructed on how to complete it. The site will make arrangements to be in contact with the mother during the reactogenicity period (as described in Section 9.7).

The following procedures will be performed **as specified in Appendix D**:

- Blood collection performed **prior** to vaccination
- Stool specimen collection, optional, performed **prior to or following** vaccination

9.4 Follow-up visits, Infants

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

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

- Assessment of new or unresolved AEs/intercurrent illnesses.
- Blood collection
- Feeding History

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

- Laboratory tests, including:
  - Complete blood count (CBC)
  - Chemistry panel (ALT, Creatinine)
- 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
- Abbreviated physical examination including weight, vital signs, and a symptom-directed evaluation by history and/or appropriate physical exam based on the mother's reported symptoms for her infant
- Complete physical examination, see HVTN 135 SSP for details
- Stool specimen collection, optional

9.5 Enrollment and Follow-up Visits, Mother

The following procedures will be performed at every visit:

- Risk reduction counseling
- Social Impact Assessment
- Assessment of new or continuing concomitant medications (as described in Section 9.2.1)

The following procedures will be performed **at scheduled follow-up visits** as specified in Appendix E and Appendix G:

- Blood collection
- Breast milk collection, optional
- Stool collection, optional
- Outside testing of baby questionnaire

- Belief questionnaire

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.

The current RSA standard of care requires close monitoring for the development of HIV infection in infants born to HIV-infected women. To minimize phlebotomy in this vulnerable population, infants will receive HIV testing through well baby clinics according to the local standard of care. Results from well-baby clinic testing will be obtained by CRS staff.

Since the RSA standard of care requires antibody testing at month 18 for all infants regardless of risk, all babies will have HIV testing performed using the HVTN HIV testing algorithm at month 17 so the results can be provided to the babies' pediatrician at the 18-month timepoint. Any testing required after month 18 should be conducted through the CRS using the HVTN HIV testing algorithm until there is no evidence of Vaccine Induced Seropositivity (VISP), see Section 9.6.1.

Additionally, to further enhance surveillance, infants of mothers who have a viral load of ≥ 400 copies/mL after initial enrollment will receive additional monitoring, including HIV testing (see HVTN 135 SSP). Mothers who have a viral load ≥ 400 copies/mL will also have a reflex testing performed to look for drug resistant viral variants and offered counseling and options for alternative ARV therapy if necessary.

Mothers will be counseled routinely during the trial on the prevention of HIV infection in their baby and on the potential negative social impacts of testing antibody positive due to VISP (see HVTN 135 SSP). They will also be counseled on the risks of HIV antibody testing outside of the HVTN CRSs after 18 months of age and will be discouraged from doing so during study participation and/or during any period of VISP.

Study staff will take particular care to inform mothers of the potential for routine or emergency HIV testing being offered or performed outside the study CRS at emergency rooms, clinics, and medical offices after 18 months of age. CRS staff should also inform mothers of the need to maintain study blinding by getting HIV testing only at the study CRS. CRS staff should provide mothers with CRS contact information and should encourage mothers to ask medical providers to contact the CRS. The CRS can verify that the infant is in an HIV vaccine clinical trial and should only be tested at the study CRS.

Infants identified as being HIV-infected during screening are not enrolled. Potential and enrolled infants identified as being HIV-infected will be urgently referred for medical treatment, counseling, and management of the HIV infection

as per local standard of care. Infants who are found to be HIV-infected after enrollment will not receive any additional study product. The mothers will be encouraged to maintain their infants' participation in follow-up visits 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, also known as vaccine-induced seropositivity (VISP), 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:

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

- The current RSA standard of care for infants (less than 18 months of age) born to HIV-infected women is capable of distinguishing between infection and antibody positivity as it relies on PCR-based testing to confirm HIV infection.

- Beginning at age 17 months, or earlier if indicated as above, HIV testing will be performed via the CRS according to the HVTN HIV testing algorithm (see HVTN 135 SSP), which is able to distinguish vaccine-induced antibody responses from actual HIV infections.

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

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

9.6.2 VISP registry

Experimental HIV vaccines may induce antibody production to HIV antigens, producing reactive results on commercially available HIV test kits. This is called "vaccine-induced seropositivity" (VISP). 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 the mother requests that her baby's name be removed.

9.7 Assessments of reactogenicity

For all infant participants, reactogenicity assessments are performed before and 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. Mothers (or caregivers) are instructed to record their infant's symptoms using a Participant Diary. Contacts between the mother and the site staff should take place at least once between 1-3 days postvaccination. In general, a mother who reports any infant postvaccination reaction greater than mild should have their infant seen by a CRS clinician, or a clinician at a local health facility within 24 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: 25-60 minutes after vaccination	HVTN CRS clinician
	Between early assessment and 11:59pm day 0	HVTN CRS clinician or mother/caregiver
1-7[b]	Between 12:00am and 11:59pm on the respective day	HVTN CRS clinician or mother/caregiver

[a] Day of vaccination

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

9.7.1 Assessment of systemic and local symptoms

Systemic symptoms include fever, sleepiness/lethargy, rash, vomiting, anorexia, seizure. Local symptoms include pain and/or tenderness, erythema/redness, and induration/swelling at the injection site. The daily maximum severity reached for each symptom during the assessment period is reported.

Body temperature is measured by temporal artery thermometry (TAT). This includes temperatures taken in the clinic, as well as temperatures taken by mothers (or caregivers) during the reactogenicity period. Refer to the HVTN 135 SSP for details.

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

9.7.2 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 greater than 2.5 cm are followed daily; otherwise, the frequency of follow-up is based on clinician judgment. See HVTN 135 SSP for detail.

9.8 Visit windows and missed visits

Visit windows are included in Appendix I and Appendix J. The procedures for documenting missed visits and out of window visits are described in HVTN 135 SSP. If the missed visit is one that required safety assessments or local safety labs, HVTN CRS staff should attempt to bring the mother-infant pair 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 Sections 7.5.2 and 7.5.3 for resolution.

9.9 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 CBC and chemistry panel), and social impact assessment. If the infant is older than 18 months, an HIV test should also be performed. Prior to 18 months of age, the mother's antibodies may impact the result of VISP testing so VISP testing should be deferred until after 18 months. For infants who have a confirmed diagnosis of HIV infection, referrals for care should be provided.

9.10 Procedures for Infants Identified as HIV infected during the study

If HIV infection is confirmed, no additional study product will be administered. All infants identified with HIV infection will be actively referred to non-study sources of HIV care and treatment. Mothers will be encouraged to continue with all scheduled study visits. Follow-up duration for infants diagnosed with HIV infection may be adjusted in consultation with the CRS investigator and the HVTN 135 PSRT (eg, to avoid interference with infant's initiation of HIV treatment). At postinfection follow-up visits, only specimens required for protocol-specified safety laboratory tests, will be collected (see Appendix D).

10 Laboratory

10.1 HVTN CRS laboratory procedures

The Site Lab Instructions and HVTN 135 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 D and Appendix E. For tests performed locally, the local lab may assign appropriate tube types.

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

Of note, all assays described below are performed as research assays to evaluate the ability of the vaccine to induce immune responses in the context of the participants' genetic background 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 D and Appendix E. Not shown is any additional blood volume that would be required if a safety lab needs to be repeated. The additional blood volume would likely be minimal. The cumulative blood draw volume will not exceed 5% of total blood volume in any 30-day period for each infant participant and will not exceed 500mL in any 56-day (8-week) period for each adult participant. Additionally, the maximum daily blood draw volume will not exceed 2.5% of total blood volume for each infant participant. The blood volume limits are conservative, designed to minimize participant harm, and are within the general consensus of the pediatric research community both in Africa and the US (85).

10.3 Primary immunogenicity timepoints

The primary immunogenicity timepoint in this study occurs 2 weeks after third and fifth vaccinations for cellular assays, and 2 weeks after the fifth vaccination for humoral assays. Endpoint assays are performed on specimens collected from participants at the primary immunogenicity timepoint and may be performed on samples collected from participants at other timepoints; the schedule is shown in Appendix D.

10.4 Endpoint assays: cellular

10.4.1 Env-specific B cell phenotyping

HIV-1 Env-specific B cells and plasmablasts induced by vaccination will be identified and characterized using fluorescently-labeled recombinant Env proteins (including CH505) in combination with a flow cytometry antibody panel. In particular, 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.

10.4.2 B-cell lineage and repertoire analysis

Single memory B cells or plasmablasts isolated from PBMCs may be single-cell or bulk sorted. For memory B cells, Env reactive memory B cells will be sorted using CH505 Env hooks, and then single-cell sorted into individual wells of 96 well plates. VH and VL genes will be amplified and cloned into an IgG1 backbone and tested for Env binding and HIV neutralization. In some cases, memory B cells will be cultured in limiting dilution cultures and screened for binding and neutralization before PCR and VH and VL rescue. For plasmablasts, VH and VL genes will be amplified and cloned into an IgG1 backbone for expression and determination of binding and neutralization. Finally, to evaluate levels of BCR somatic hypermutation next generation sequencing for all VH and VL families may be performed on memory B cells or plasmablasts for VH and VL genes for either single-cell or bulk sorted Env-specific B cells.

10.5 Endpoint assays: humoral

10.5.1 Binding antibody multiplex assay (BAMA)

HIV-1 Env binding IgG antibodies to the vaccine immunogens (EnvSeq-1 gp120 TF) will be assessed on serum/plasma samples from study participants taken at the primary immunogenicity timepoint and, due to the contributions of maternal antibody at birth, will be compared to matched samples from placebo controls. Conformational epitopes (eg, CD4BS specificities using differential binding to EnvSeq-1 gp120 TF compared to CH505 IΔ371 protein and sCD4 blocking) will be determined. Specimens from other timepoints as well as other HIV antigens (ie, other conformational epitopes and linear epitopes), IgA and antibody subclasses (IgG1, IgG2, IgG3, IgG4) may also be assayed based on the results of the initial assay. Cord blood, serum and/or breast milk samples may be tested to evaluate the effects of maternal antibodies.

10.5.2 Pediatric Vaccine Multiplex Assay (PVMA)

The PVMA will assess serum samples for interaction (ie, additivity, antagonism or synergy) of the candidate vaccine with standard infant vaccines. The PVMA simultaneously measures antibodies against hepatitis B, *Haemophilus influenzae*

type B, diphtheria, tetanus, pertussis, rubella and respiratory syncytial virus. Polio antigens will also be assessed. The assay developed by Itell, Permar and Fouda et al. is a reliable, high-throughput technique that requires minimal sample volume and measures multiple antibody concentrations (86). Serum samples will be tested at the primary immunological timepoint after the 5th vaccination.

10.5.3 Neutralizing antibody assay

HIV-1–specific nAb assays will be performed on serum samples from study participants taken at the primary immunogenicity timepoint(s). Specimens from other timepoints may also be analyzed at the discretion of the HVTN Laboratory Program, which may be contingent on the results of the primary immunogenicity timepoints. The TZM-bl assay will test neutralization of the vaccine strain (CH505TF) and a single highly neutralization-sensitive autologous Tier 1 virus as a positive control (CH505.w4.3). The global panel and/or clade-specific panels may be used to assess heterologous Tier 2 neutralization. (87, 88). Additional assays will be performed to detect early precursors of CH103, CH235 and CH01 bnAb lineages.

10.5.4 Antibody avidity

Antibody avidity may be measured utilizing the binding antibody multiplex assay with the addition of a dissociation step to calculate the antibody avidity index (BAMA-AI) and will be assessed on serum samples from study participants taken at the primary immunogenicity timepoints and due to the contributions of maternal antibody at birth, will be compared to age-matched samples from placebo controls. Biolayer Interferometry (BLI) and/or Surface Plasmon Resonance (SPR) technologies may also be utilized to define antibody avidity.

10.5.5 FcγR binding assay

The Fc-FcγR complex plays a critical role in eliciting cytotoxicity by mediating antibody Fc effector functions (such as ADCC and ADCP). Vaccine elicited 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. Serum samples from study participants will be taken at the primary immunogenicity timepoint. Specimens from other timepoints may also be assayed based on the results of the initial assay.

10.5.6 Antibody-dependent cellular cytotoxicity (ADCC)

ADCC activity may be assessed using serum samples from study participants taken at a primary immunogenicity timepoint. Specimens from other timepoints may also be analyzed at the discretion of the HVTN Laboratory Program, which may be contingent on the results of the primary immunogenicity timepoint. For the Granzyme B flow-based cytotoxicity assay (89), participant sera are incubated with effector cells and gp120-coated 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 (90), participant sera are incubated with IMC-infected cells and percent killing is measured using ViviRen luminescence.

10.5.7 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 vaccine recipients at a primary immunogenicity timepoint and compared to age-matched samples from placebo controls. 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 (91, 92). An array of antigens or viruses in addition to specimens from other timepoints may also be analyzed at the discretion of the HVTN Laboratory Program, which may be contingent on the results of the primary immunogenicity timepoint.

10.5.8 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 age-matched samples from 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 (93). An array of antigens or viruses in addition to specimens from other timepoints may also be analyzed at the discretion of the HVTN Laboratory Program, which may be contingent on the results of the primary immunogenicity timepoint.

10.5.9 Antibody dependent complement deposition (ADCD)

To assess the ability of vaccine-elicited antibodies to bind complement, complement activation may be measured using serum samples from vaccine recipients and compared with placebo controls at a primary immunogenicity timepoint (94, 95). Additional specimens from other timepoints may also be analyzed at the discretion of the HVTN Laboratory Program, which may be contingent on the results of the primary immunogenicity timepoint.

10.6 Microbiome analysis

Aliquots of breast milk and swabs of stool and/or bulk stool samples will be shipped to a central laboratory. Samples will be subjected to culture to obtain microbial samples. These samples will be cryopreserved and/or processed to yield a homogeneous flora lysate of each culture type to study their impact on immunity. These lysates are then available to use to assess antibody binding by Western blot, ELISA, or other methods. In addition, DNA will be extracted from

samples and subjected to quality control qPCR assays to measure total microbial load and assess for PCR inhibitors. Broad-range 16S rRNA PCR and/or metagenomics will be performed. Microbial community composition will be assessed using taxonomic assignment of reads to a custom reference set of gene sequences from the human gut. Finally, bulk stool samples may also have antibodies extracted for analysis of the presence of mucosal antibodies elicited by the vaccine.

10.7 Systems Biology

In vitro (cord blood) and in vivo (infant peripheral blood) samples will be assessed for correlates of vaccine safety and immunogenicity. In vitro studies will include stimulation of cryopreserved cord blood mononuclear cells (CBMC) and infant cord blood under 4 conditions: control, adjuvant (GLA-SE), antigen (gp120) and adjuvant/antigen combined. Samples will be fractionated and cryopreserved for downstream analysis of analytes. In vitro and in vivo samples will be used for plasma- and cell-based OMIC assays which may include RNASeq, high resolution flow cytometry, leukocyte epigenetics, plasma cytokines/chemokines, proteomics, and metabolomics in relation to vaccine immunogenicity. To identify molecular signatures that correlate with the immunogenicity of adjuvanted HIV vaccine, *in silico* analyses will employ programs such as *NetworkAnalyst, DIABLO* and related bioinformatic tools.

10.8 Lab assay algorithm

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

10.9 Exploratory studies

Maternal and infant 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.

10.10 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 CRS.

As part of consenting for the study, participants document their initial decision to allow or not allow their or their infant's 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 or their infant's 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.

10.11 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 135 PSRT

The HVTN 135 PSRT is composed of the following members:

- DAIDS medical officer representative
- Protocol chairs and cochairs
- Protocol Team leader
- Core medical monitor
- Clinical safety specialist
- Regional medical liaison

The clinician members of HVTN 135 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 135 PSRT meetings.

11.1.2 HVTN SMB

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

The SMB reviews safety data, unblinded as to treatment arm, approximately every 4 months. The reviews consist of evaluation of cumulative reactogenicity events, AE, laboratory safety data, and individual reports of adverse events requiring expedited reporting to DAIDS. The SMB conducts additional special reviews at the request of the HVTN 135 PSRT.

The study site 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 135 PSRT and HVTN SMB (see Sections 11.1.1 and 11.1.2);

11.1.4 HVTN Core roles and responsibilities in safety monitoring

- Daily monitoring of clinical data for events that meet the safety pause and HVTN 135 PSRT AE review criteria (see Section 11.4);
- Notifying HVTN CRSs and other groups when safety pauses or planned holds are instituted and lifted (see Section 11.4);
- Querying HVTN CRSs for additional information regarding reported clinical data; and
- Providing support to the HVTN 135 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, local lab results, and concomitant medications) before the end of the next business day, excluding federal or bank holidays. 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). **AEs will be collected for infants only**. 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 as shown below in Table 11-1, rows for pain/tenderness at the injection site and sleepiness/lethargy were extracted from IMPAACT 2004. The grading of creatinine is a required modification to the DAIDS table dictated by the inappropriateness of using a "baseline" creatinine in an infant as the reference range as this analyte fluctuates greatly throughout the first year of life.

Table 11-1 Study-Specific Severity Grading Table

	Grade 1 Mild	**Grade 2 Moderate**	**Grade 3 Severe**	**Grade 4 Potentially Life-Threatening**
Pain/tenderness at injection site	Mild reaction to light touch with no or minimal limitation of movement of limb	Persistent crying (up to one hour) with no or light touch or significant limitation of movement of limb	Persistent crying for more than one hour or interference with infant's ability to sleep or eat	Inconsolable crying for more than two hours
Sleepiness/lethargy	Transiently lethargic but otherwise normal routine	More sleeping than usual, not on normal routine without alternative explanation	Refuses to play/smile with parent/caregiver or somnolent, needs to be stimulated to take feedings	Non-responsive, unable to rouse
Creatinine, High	1.1 to 1.3 x ULN	> 1.3 to 1.8 x ULN	> 1.8 to < 3.5 x ULN	≥ 3.5 x ULN
Platelets, Decreased (cells/mm^3) ≤ 5 days of age	75,000 to < 100,000	50,000 to < 75,000	25,000 to < 50,000	<25,000
Platelets, Decreased (cells/mm^3) > 5 days of age	100,000 to < 125,000	50,000 to <100,000	25,000 to < 50,000	<25,000

Additionally, to report weight loss in an infant as an AE, the "Underweight" row on the DAIDS Grading Table should be used. Weight loss should **not** be graded using the "Unintentional Weight Loss" row.

All AEs are reported to the SDMC on the appropriate CRF. Clinic staff should evaluate every AE to determine if (1) the AE meets the requirements for expedited reporting (Section 11.2.3), (2) if the AE meets the criteria for a safety pause/prompt AE review (Section 11.3), 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 H.

The study site is expected to notify HVTN clinical safety staff of any serious safety concern requiring their attention (Table 11-3). Telephone numbers and email addresses are found on the protocol home page on the HVTN Members' site (https://members.hvtn.org/protocols/hvtn135). 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 11-3). 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 SAE Reporting Category will be used for this study.

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:

- CH505TF gp120 with GLA-SE or Placebo

The NIAID/DAIDS will report all unexpected SAEs related to the study products observed in this clinical trial to the South African Healthcare 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 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 Evaluations for moving from Part A to Part B and Part B to Part C

To ensure safety, enrollment will proceed in stages. In addition to monitoring participant safety throughout the study period, the HVTN 135 PSRT will review safety data as shown in Table 11-2.

There are two planned enrollment holds and one planned safety review. An enrollment hold prevents advancement of the study to the next part but does not prevent participants from receiving further vaccinations. Part B cannot enroll until the safety data following the first vaccination in all seven infants in Part A has been reviewed (Enrollment Hold #1). Part C cannot begin enrollment until all safety data following the third vaccination in Part B has been reviewed (enrollment hold #2).

There is also a planned safety review to occur after the final infant in part B (the fourth infant) has receives the first vaccination. During this safety review, no infants in Part B will receive further vaccinations until the PSRT has reviewed all cumulative safety information.

Table 11-2 Summary of planned enrollment holds and safety reviews

Planned Holds and Reviews	Timepoint/Data Reviewed	Actions
Planned Safety Hold #1 **Enrollment in Parts B and C are on hold.**	Begins after the 7th participant receives first vaccination in Part A. Review of all cumulative safety data available for the seven participants in Part A (Groups 1 and 2) up to and including the 2-week visit after the first vaccination.	The PSRT will make a decision based on this safety data regarding the appropriateness of beginning enrollment in Part B from a safety perspective.
Planned Safety Review in Part B **All vaccinations in Part B are on hold.** **Enrollment in Part C is on hold.**	Begins after the 4th participant in Part B (Groups 3 and 4) receives their first vaccination. Review of all cumulative safety data available for: • all participants in Part A. • all four participants in Part B (Groups 3 and 4) up to and including the 2-week visit after the first vaccination.	The PRST will make a decision based on this safety data regarding the appropriateness of continuing Part B vaccinations from a safety perspective.
Planned Safety Hold #2 **Enrollment in Part C is on hold.**	Begins after the 4th participant in Part B (Groups 3 and 4) receives their third vaccination. Review of all cumulative safety data available for: • all participants in Part A. • the four participants in Part B (Groups 3 and 4) up to and including the 2-week visit after the third vaccination.	The PSRT will make a decision based on this safety data regarding the appropriateness of beginning enrollment in Part C from a safety perspective.

11.4 Safety pause and prompt PSRT AE review

When a trial is placed on safety pause, all enrollment and vaccination with the product related to the event that triggered the pause will be held until further notice. The AEs that will lead to a safety pause or prompt HVTN 135 PSRT AE review are summarized in Table 11-3. 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 135 PSRT, participant safety may be threatened. Criteria for an individual participant's departure from the schedule of vaccinations are listed in Section 7.5.

Table 11-3 AE notification and safety pause/AE review rules

Event and relationship to study products	Severity	HVTN CRS action [a]	HVTN Core action
SAE, related	Any Grade	Phone immediately, email and submit forms immediately	Immediate pause
SAE, not related	Grade 5	Phone immediately, email and submit forms immediately	Immediate PSRT notification
AE, related	Grade 4 or 3	Email and submit forms immediately	Immediate PSRT notification and prompt PSRT AE review to consider pause
25% or more infants (four or more infants if fewer than 20 have been enrolled) with a grade 3 or higher reactogenicity signs and symptoms within the first full 3 days after study product administration	Grade 4 or 3	Phone immediately, 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/hvtn135)

For all immediate safety pauses, HVTN Core notifies the HVTN 135 PSRT, HVTN Regulatory Affairs, DAIDS Pharmaceutical Affairs Branch (PAB), DAIDS Regulatory Affairs Branch (RAB), DAIDS Safety and Pharmacovigilance Team (SPT), and the participating HVTN CRS. When an immediate safety pause is triggered, HVTN Core notifies the SMB, and the SMB will be convened as soon as possible to review the event.

Once an immediate safety pause is triggered and the trial is paused, the HVTN 135 PSRT and the SMB review safety data and decide whether the pause can be lifted or permanent discontinuation of vaccination is appropriate. HVTN Core notifies the participating HVTN CRS, HVTN Regulatory Affairs, DAIDS PAB, DAIDS RAB, and DAIDS SPT of the decision regarding resumption or discontinuation of study vaccinations. Based on the HVTN 135 PSRT assessment, DAIDS RAB notifies the SAHPRA as needed.

If an immediate HVTN 135 PSRT notification or prompt HVTN 135 PSRT AE review is triggered, HVTN Core notifies the HVTN 135 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 135 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 135 PSRT (see Section 11.5.2).

11.5 Review of cumulative safety data

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

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

11.5.1 Daily review

Blinded daily safety reviews are routinely conducted by HVTN Core for events requiring expedited reporting to DAIDS, and events that meet safety pause criteria or prompt HVTN 135 PSRT AE review criteria.

11.5.2 Weekly review

During the injection phase of the trial, the HVTN 135 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 135 PSRT. HVTN Core reviews reports of clinical and laboratory AEs. Events identified during the review that are considered questionable, inconsistent, or unexplained are referred to the HVTN CRS clinic coordinator for verification.

11.6 Study termination

This study may be terminated early by the determination of the HVTN 135 PSRT, a pertinent national regulatory authority, SAHPRA, NIH, Office for Human Research Protections (OHRP), or study product developer(s). In addition, the conduct of this study at the HVTN CRS may be terminated by the determination of the IRB/EC and any applicable RE.

12 Protocol conduct

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

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

12.1 Social impacts

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

In addition, the study site may participate in the VISP registry established by the HVTN. This registry allows trained staff to verify that an individual has received an HIV vaccine and therefore has the potential for VISP. The registry also supports poststudy HIV counseling and testing services for HIV vaccine recipients (see Section 9.6.2).

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

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

12.2 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 135 are described below.

Protocol history and modifications

Date: December 18, 2020

Protocol version: 3.0
Protocol modification: Full protocol amendment 2

Item 1 Revised in Section 3, *Overview*, Section 4, *Background*, Section 6, *Statistical considerations*, Section 8, *Study product preparation and administration* and Appendix A, *Sample informed consent form*: primary immunogenicity dose group, CH505TF gp120 dose for Groups 3 and 7 and group size for Groups 5 and 7

Item 2 Updated in Section 4.8.1, *Clinical studies of CH505TF gp120 with GLA-SE adjuvant in adults, HVTN 115* and Section 6.1, *Accrual and sample size calculations*: safety data from HVTN 115 Part A

Item 3 Updated per Letter of Amendment 1 version 2.0, dated September 18, 2020

Item 4 Updated Section 14, *Documents references* and Appendix F*, Procedures at HVTN CRS :* web address and footnote cross-reference

Item 5 Updated Title page and Section 13, *Version history*: date, version number and contents of this amendment

Date: September 18, 2020

Protocol version: Version 2.0
Protocol modification: Letter of Amendment 1

Item 1 Added in Section 7.1, *Inclusion criteria: Mother*, Section 9.2.1*, Pre-enrollment procedures, Mother*, Appendix A, *Sample informed consent form,* Appendix C, *Table of procedures (for sample informed consent form),* Appendix E*, Laboratory procedures: Mother*: SARS-CoV-2 testing

Item 2 Updated in Section 3.1, *Protocol Team:* Membership

Item 3 Updated in Appendix A, *Sample informed consent form* and in Appendix B*, Sample consent form for use of samples and information in other studies:* contact information for South African Healthcare Products Regulatory Authority (SAHPRA)

Date: January 24, 2020

Protocol version: 2.0
Protocol modification: Full protocol amendment 1

Item 1 Revised in Section 9.3, *Enrollment and vaccination visits, infant* and Appendix A, *Sample informed consent form*: time infant participant will be observed in clinic post administration of study product

Item 2 Revised in Section 8.3, *Preparation of study products*: syringe labelling

Item 3 Added in Section 8.5, *Acquisition of study products*: manufacturer of study products

Item 4 Revised in Appendix A, *Sample informed consent form*: text changes throughout to improve clarity

Item 5 Updated in Appendix A, *Sample informed consent form*, item 4: number of participants in HVTN 115 who received vaccine

Item 6 Updated in Appendix A, *Sample Informed Consent Form* and Appendix B, *Sample informed consent for the use of samples and information in other studies*: SAHPRA contact information

Item 7 Updated in Section 3.1, *Overview*: protocol team members and Statistical and data management center (SDMC)

Item 8 Corrected in Section 7.5.3*, Discontinuing vaccination for a participant*: method of body temperature measurement

Item 9 Added in Section 9.7.1, *Assessment of systemic and local symptoms*: erythema/redness as local symptom examples

Item 10 Deleted in Section 9.6, *HIV counseling and testing*: reference to HVTN HIV testing algorithm

Item 11 Added in Section 10.5.6, *Antibody-dependent cellular cytotoxicity (ADCC)* and Section 16, *Literature cited*: references to assays described

Item 12 Updated in Appendices D, *Laboratory procedures: Infant*, and Appendix E*, Laboratory procedures: Mother*: cord blood volume, tube types, test location, and footnotes

Item 13 Corrected in Appendix I, *Infant visit windows* and Appendix J, *Mother visit windows*: target day for visits 9 and 10

Item 14 Added to Title page: “A non-IND study”

Item 15 Added to Section 8.4, *Administration*: storage temperature for product in syringe

Item 16 Updated in Section 14, *Document references*: URL for ICH

Item 17 Updated Section 13, *Version history*: added amendment 1

Date: July 30, 2019

Protocol version: 1.0
Protocol modification:

Original Protocol

14 Document references (other than literature citations)

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

- Assessment of Understanding. Accessible through the HVTN protocol-specific website.

- DAIDS Policy on Enrolling Children (including Adolescents) in Clinical Research: Clinical Research Site Requirements Available at https://www.niaid.nih.gov/sites/default/files/enrollingchildrenrequirements.pdf

- 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 135 Special Instructions. Accessible through the HVTN protocol-specific website.

- HVTN 135 Study Specific Procedures. Accessible through the HVTN protocol-specific website.

- HVTN 135 Site Lab 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

- Lab assay algorithm (available upon request).

- International Council on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) E6, Guideline for Good Clinical Practice: Section 4.8, Informed consent of trial subjects. Available at https://www.ich.org/page/efficacy-guidelines

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

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

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

- Requirements for Source Documentation in DAIDS Funded and/or Sponsored Clinical Trials. Available at https://www.niaid.nih.gov/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. Available at https://www.hhs.gov/ohrp/regulations-and-policy/regulations/45-cfr-46/index.html

- New EPI Vaccines Guidelines, National Department of Health, Republic of South Africa. Available at https://ndoh.dhmis.org/owncloud/index.php/s/R5cmdp0gY4Fa43Z?path=%2FChidrens%20Vaccines

- 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

Ab	antibody
Ad	adenovirus
ADCC	Antibody-dependent cellular cytotoxicity
ADCD	Antibody dependent complement deposition
ADCP	Antibody-dependent cellular phagocytosis
ADNP	Antibody-dependent neutrophil phagocytosis
AE	adverse event
AESI	AE of special interest
ALT	alanine aminotransferase
ANOVA	analysis of variance
ART	antiretroviral therapy
ARV	antiretroviral
AST	aspartate aminotransferase
AUC	area under the curve
AUC-MB	area-under-the-magnitude-breadth
AVEG	AIDS Vaccine Evaluation Group
β-HCG	beta human chorionic gonadotropin
BAMA	Binding antibody multiplex assay
BCR	B cell receptor
BMI	body mass index
bnAb	broadly neutralizing antibody
CAB	Community Advisory Board
cART	combination anti-retroviral therapy
CBC	complete blood count
CBMC	Cord Blood Mononuclear Cells
CD4bs	CD4 binding site
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
EIA	enzyme immunoassay
ELISA	enzyme-linked immunosorbent assay
ELISpot	enzyme-linked immunospot
Env	HIV envelope protein
EPI	Expanded Program on Immunization
FPR	false positive rate
Fred Hutch	Fred Hutchinson Cancer Research Center
GCP	Good Clinical Practice
GEE	generalized estimating equation
GLA-SE	Glucopyranosyl Lipid A - stable emulsion
GLP	Good Laboratory Practices
GPP	Good Participatory Practices
HAART	highly active antiretroviral therapy
HBsAg	hepatitis B surface antigen
HCV	hepatitis C virus
HEU	HIV-1 exposed uninfected
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HSML-NICD	HIV Sero-Molecular Laboratory, National Institute for Communicable Diseases
HVTN	HIV Vaccine Trials Network
IA	intermediate ancestor
IB	Investigator's Brochure
IBC	Institutional Biosafety Committee
ICF	informed consent form
ICH	International Council on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICS	intracellular cytokine staining
IFN-γ	interferon gamma
IM	intramuscular
IND	Investigational New Drug
IRB	Institutional Review Board
IUD	intrauterine device
LTFU	loss to follow-up

mAb	monoclonal antibody
MAR	missing at random
MCAR	missing completely at random
MIMOSA	Mixture Models for Single-cell Assays
MMR	measles, mumps, and rubella
MOP	Manual of Operations
MTCT	mother to child transmission
nAb	neutralizing antibody
NHP	nonhuman primate
NHP	nonhuman primate
NIAID	National Institute of Allergy and Infectious Diseases (US NIH)
NICD	National Institute for Communicable Diseases (Johannesburg, South Africa)
NIH	US National Institutes of Health
NZW	New Zealand White
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
PI	Principal Investigator
PSRT	Protocol Safety Review Team
PTE	potential T -cell epitope
PVMA	Pediatric Vaccine Multiplex Assay
RAB	DAIDS Regulatory Affairs Branch
RAC	NIH Recombinant DNA Advisory Committee
RE	regulatory entity
RSC	DAIDS Regulatory Support Center
SAE	serious adverse event
SAHPRA	South African Healthcare Products Regulatory Authority
SAIL-NICD	South Africa Immunology Laboratory and National Institute for Communicable Diseases
SAP	Statistical Analysis Plan
SCHARP	Statistical Center for HIV/AIDS Research and Prevention
SD	standard deviation
SDMC	statistical and data management center
SFC	spot-forming cell

SFU	spot-forming unit
SHIV	simian-human immunodeficiency virus
SIV	simian immunodeficiency virus
SMB	Safety Monitoring Board
SPT	DAIDS Safety and Pharmacovigilance Team
TB	tuberculosis
TF	transmitted/founder
UCA	unmutated common ancestor
UW-VSL	University of Washington Virology Specialty Laboratory
VISP	Vaccine induced seropositivity
VRC	Vaccine Research Center (NIAID)

16 Literature cited

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Appendix A Sample informed consent form

Title: A phase 1 clinical trial to evaluate the safety and immunogenicity of the HIV-1 CH505 transmitted/founder gp120 adjuvanted with GLA-SE in healthy, HIV-exposed uninfected infants

HVTN protocol number: HVTN 135

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

These are some of the things you should know about this study:

<table>
<tr>
<td>The HIV Vaccine Trials Network (HVTN) and PHRU are doing a study to test an HIV vaccine in babies. The study vaccine contains a protein called CH505TF gp120 and an adjuvant called GLA-SE. An adjuvant is a substance added to a vaccine to increase the body's immune response. We want to see if the vaccine is safe to give to babies and does not make them too uncomfortable. We also want to see if babies' immune systems respond to the study vaccine and in the same way that adults respond to the study vaccine.</td>
<td>• About 38 uninfected babies and their HIV-infected mothers (aged 18 and older) will be enrolled at one site, the PHRU clinic
• About 14 visits over 2 ½ years
• Babies will get 1 injection into the upper thigh at 5 separate visits over about 1 ½ years
• Babies will get either the study vaccine or placebo (a substance that does not contain vaccine)
• That is a 3-in-4 chance of your baby getting the study vaccine</td>
</tr>
<tr>
<td colspan="2">This study is divided into three parts: Part A, B and C. Parts A and B both include two groups and Part C contains 4 groups. 7 babies will take part in Part A of this study. After all 7 babies have joined the study and received 1 vaccination, we will decide whether or not to do Part B
4 babies will join Part B. After the 4 babies in part B each receive 1 vaccine, we will determine if it is safe for these babies to continue receiving additional vaccines. After the 4 babies in Part B have all received 3 vaccinations, we will decide whether or not to do Part C.
If we decide that the study vaccine is safe to give to babies in Part B, then we will do Part C. If we do Part C, 27 more babies will join.</td>
</tr>
<tr>
<td colspan="2">If you choose to join the study with your baby, here are some things that will happen:
• We will request your medical records, including your HIV status
• Take blood from you and your baby, including using blood from the umbilical cord that would otherwise be discarded
• Physical exams of you and your baby
• Test you for Hepatitis B, SARS-CoV-2 (if not in your medical records), your viral load, CD4 and T cell counts
• Test your baby for HIV
• Ask about how much breastmilk, formula or solid food your baby is eating</td>
</tr>
<tr>
<td colspan="2">There is no direct benefit to you or your baby from being in this study. Whether or not you and your baby join this study is up to you. You and your baby do not have to take part in this study. If you and your baby do not join this study, this will not affect the care that you and your baby receive at this clinic.</td>
</tr>
<tr>
<td colspan="2">Some general risks of vaccines include fever, chills, rash, aches and pains, nausea, headache, dizziness, and feeling tired. Vaccines can also cause pain, redness, swelling, or itching where your baby receives the injection. There may be other side effects that we don't yet know about, even serious ones.</td>
</tr>
</table>

The rest of this form provides a more complete description of this study.

About the study

The HIV Vaccine Trials Network (HVTN) and PHRU are doing a study to test an HIV vaccine. HIV is the virus that causes AIDS.

About 38 babies and their mothers (aged 18 and older) will take part in this study at one site, the PHRU clinic. The researcher in charge of this study at this clinic is Dr. Avy Violari. The United States (US) National Institutes of Health (NIH) is paying for the study.

This study is divided into three parts: Part A, B and C. Parts A and B are divided into two groups. Seven babies will take part in Part A of this study. After all seven babies have joined the study and received one vaccination, we will decide whether or not to do Part B. We will be looking to see if the study vaccine is safe to give to babies in Part A. If we decide that the study vaccine is safe to give to babies in Part A, we will do Part B. Four babies will join Part B. After all four babies in Part B receive their first vaccination, we will decide whether to continue their vaccinations. Again, we will be looking to see if the study vaccine is safe to give to babies in Part B. After all four babies in Part B receive their third vaccination, we will decide whether or not to do Part C. If we decide that the study vaccine is safe to give to babies in Part B, then we will do Part C. Part C is divided into 4 groups. If we do Part C, 27 more babies will join.

If you decide to join the study, you may be in any of these groups depending on when you joined the study.

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

   - Is the study vaccine safe to give to babies?
   - Are babies able to take the study vaccine without becoming too uncomfortable?
   - How do babies' immune systems respond to the study vaccine? (Your immune system protects you from disease.) Do babies respond in the same way that adults respond to the study vaccine?
   - How does getting the study vaccine effect babies' response to other vaccines they may get early in life?

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

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

3. **We do not know if the study vaccine will decrease, increase, or not change babies' risk of getting HIV if they 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.

Studies of similar products involving thousands of adults were found to be safe and did not affect the risk of getting HIV.

Several studies have tested whether HIV vaccines can reduce the risk of getting HIV from another person. In some studies, people who got the vaccine seemed to have the same risk of getting HIV as people who did not get the vaccine. In one study, people who got the vaccine seemed to have a lower risk of getting HIV than people who did not get the vaccine. In studies with a different vaccine, some people who got the vaccine had a higher risk of getting HIV than people who did not get the vaccine. This study differs from the studies in which people who got the vaccine had a higher or lower risk of getting HIV. The study staff can tell you about the differences.

We do not know whether the vaccine in this study will affect babies' risk of getting HIV. It's very important for mothers to continue to take their ARVs to minimize the risk of HIV transmission through breast milk. We will tell you how you can reduce the risk of giving your baby HIV.

4. **This study vaccine is experimental.**

The study vaccine is called CH505TF gp120. From here on, we will call it CH505 or the study vaccine. It is an experimental HIV vaccine. That means we do not know if the study vaccine will be safe to use in babies, or if it will work to prevent HIV infection. This study vaccine is used only in research studies.

CH505 was developed by Duke Human Vaccine Institute and National Institute of Allergy & Infectious Disease (NIAID). The study vaccine has man-made pieces of protein that look like part of the protein found in HIV. A baby's immune system might learn to recognize these proteins and prepare itself to fight HIV. This is called an immune response.

The study vaccine has been given to about 36 adults in another study that is ongoing. In the ongoing study in adults, the study vaccine appears safe and people have been able to take it without becoming too uncomfortable. The study vaccine has not been given to babies before. In animal studies, the study vaccine appears safe. Even if something looks like it is safe or works in animals, it may not be true for people.

The 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 developed by the Infectious Disease Research Institute (IDRI).

General risks of vaccines:

All vaccines can cause fever, chills, rash, aches and pains, nausea, headache, dizziness, fussiness, 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 your baby has ever had a bad reaction to any injection or vaccine.

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

Risks of the study vaccine:

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

The study vaccine has not been given to babies before, but has been given to adults in other studies. The most common complaints were pain or tenderness where they got the injection. In those studies, two people had reddening and hardening of their skin where they got the injection, but it went away in a few days. One of those people had a skin infection where they got the injection. It did not affect that person's daily routine. That person took some medicine and it got better within 5 days. Two other people in those studies had bad headaches. Let the study staff know if your baby is fussier or is crying more than usual.

The adjuvant, GLA-SE, has been tested in about 2,500 adults with vaccines for other diseases. The most common complaints were pain and tenderness at the injection site and feeling tired. GLA-SE is currently being tested in children and has not yet been tested in babies. We do not know how the babies will react to the study vaccine or adjuvant.

Joining the study

5. It is completely up to you whether or not to join the study with your baby.

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 and your baby leave it after you have joined, you and your baby's other care at this clinic and the benefits or rights you would normally have will not be affected.

If you and your baby join this study, your baby may not be allowed to join other HIV vaccine or HIV prevention studies now or in the future. You and your baby

cannot be in this study while you are in another study where either of you get a study product. Being in more than one study may not be safe.

Also during the study, your baby should not donate blood or tissue.

If you choose not to join this study with your baby, you or your baby may be able to join another study.

6. **If you want to join the study with your baby, we will screen you and your baby to see if you are eligible.**

Screening involves a physical exam and a health history from both you and your baby. A physical exam may include, but is not limited to:

- Checking your and your baby's weight, temperature and blood pressure
- Looking in your and your baby's mouth and throat
- Listening to your and your baby's heart and lungs
- Feeling your and your baby's abdomen (stomach and liver)
- Doing a genital exam on your baby (which includes touching that area on the outside)

We will also do blood tests on both you and your baby. We will draw blood from your vein using a needle for these tests. After the baby is delivered, we will take blood samples from the part of the umbilical cord that is discarded. We will test your baby for HIV. The tests on your and your baby's blood tell us about some aspects of you and your baby's health, such as how healthy each of your kidneys, liver, and immune systems are. We may also test you for Hepatitis B and SARS-CoV-2, test your viral load, CD4 and T cell counts. We will ask you about medications you and your baby are taking, including your antiretroviral medications.

We will ask your doctor for your or your baby's medical records, including your HIV status so that we can reduce the amount of tests that we need to do for the study.

We will review the screening results with you. The screening results may show you and your baby are not eligible to join the study, even if you want to. If you and your baby are not eligible to join the study, any samples that have been taken will be destroyed.

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

7. **If we find that you or your baby 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.

Being in the study

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

8. **You and your baby will come to the clinic for scheduled visits about [#] times over about two years.**

Site: 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 and your baby may have to come for more visits if either of you have a lab or health issue.

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

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

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

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

10. **We will give your baby either the study vaccine or a placebo.**

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

    Your baby has about a 3-in-4 chance of getting the study vaccine. *Site: Modify the randomization metaphor in the next sentence as appropriate to your local culture*. Whether your baby gets the study vaccine or the placebo is completely random, like flipping a coin.

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

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

11. We will give your baby the study products on a schedule.

All babies will get one injection into the upper thigh at each of 5 separate visits.

Your baby will be in one of three parts: Part A, B or C. Parts A and B are divided into two groups. Seven babies will take part in Part A of this study. Five of the seven babies will get the study vaccine and the lower dose of the adjuvant. The other two babies will get a placebo. After all seven babies have joined the study and received one vaccination, we will decide whether or not to do Part B. We will be looking to see if the study vaccine is safe to give to babies in Part A. If we decide that the study vaccine is safe to give to babies in Part A, we will do Part B.

If we decide to do Part B, four more babies will join. Two of the four babies will get a dose of the study vaccine and a higher dose of the adjuvant. The other two babies will get a placebo. After all four babies in Part B receive their first vaccination, we will decide whether to continue their vaccinations. Again, we will be looking to see if the study vaccine is safe to give to babies in Part B. After all four babies in Part B have received three vaccinations, we will decide whether or not to open Part C. Again, we will be looking to see if the study vaccine is safe to give to babies in Part B. If we decide that the study vaccine is safe to give to babies in Part B, then we will do Part C.

Part C is divided into 4 groups. If we decide to do Part C, 27 more babies will join. In Part C, 16 babies will get the study vaccine and the higher dose of the adjuvant. Also in Part C, five babies will get the lower dose of the study vaccine and the higher dose of the adjuvant. Six babies in Part C will get a placebo.

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

Group	# of babies	Dose (protein/ adjuvant)	Injection schedule in months				
			0	2	4	7 ½	12 ½
Part A							
1	5	Study vaccine 20 mcg /Low adjuvant 2.5 mcg	Study Vaccine	Study Vaccine	Study Vaccine	Study Vaccine	Study Vaccine
2	2		Placebo	Placebo	Placebo	Placebo	Placebo
Part B							
3	2	Study vaccine 20 mcg /High adjuvant 5 mcg	Study Vaccine	Study Vaccine	Study Vaccine	Study Vaccine	Study Vaccine
4	2		Placebo	Placebo	Placebo	Placebo	Placebo
Part C							
5	16	Study vaccine 20 mcg / High adjuvant 5 mcg	Study Vaccine	Study Vaccine	Study Vaccine	Study Vaccine	Study Vaccine
6	3		Placebo	Placebo	Placebo	Placebo	Placebo
7	5	Low study vaccine 5 mcg /High adjuvant 5 mcg	Study Vaccine	Study Vaccine	Study Vaccine	Study Vaccine	Study Vaccine
8	3		Placebo	Placebo	Placebo	Placebo	Placebo
38 babies total (28 get vaccine and 10 get placebo)							

You and your baby 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 your baby is feeling and if they 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 your baby is doing. Contact the clinic staff if you have any issues or concerns after your baby gets an injection. If they have a problem, we will continue to check on it until it goes away.

If something happens to you and you are unable to take your baby to the study visits, a caregiver can bring your baby instead.

12. In addition to giving your baby the study products, over the course of the trial, we will:

- Do HIV testing of your baby, as well as counseling you on how you can avoid giving HIV to your baby

- Do physical exams of your baby

- Ask questions about you and your baby's health, including medications each of you may be taking, and how much breastmilk, formula, or solid food your baby is eating

- Ask questions about any personal problems or benefits you and your baby may have from being in the study

- Take blood samples from both you and your baby by drawing blood from the vein using a needle to do various tests, including checking your viral load. If during the study your viral load goes above 400 copies per milliliter we will check to see if this is due to drug resistance. If this happens, we or your doctor may have to change your ART medication.

When we take blood, the amount will depend on the lab tests we need to do and the baby's weight. For your baby, it will be some amount between 2 mL and 27 mL (1/2 of a teaspoon to just less than 3 tablespoons). For you, it will be some amount between 10 mL and 40 mL (2 teaspoons to 4 tablespoons). Your and your baby's 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 C, Table of procedures (for informed consent form) in this section. You may also distribute it as a separate sheet to your participants if that is helpful.

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 you or your baby's health, we will tell you.

13. We would also like to collect stool samples and breast milk samples.

We would like to collect a few small samples of your and your baby's stool to look at the bacteria living in each of your stomachs. We would also like to collect two samples of your breast milk to look at the antibodies in it. We want to learn more about how a mother's immune system affects her baby's immune system.

We would collect a total of eight stool samples from your baby at different visits, but we would collect only one stool sample from you. You and your baby can give stool samples without having any extra clinic visits. You can see the table above to find out which visits we would collect stool from you and your baby.

We would collect your breast milk at screening and at the 4 ½ month visit.

If you agree, we will give you more information about how we will collect these samples. You do not have to give any of these samples if you choose. Or you can choose to give all of these samples and change your mind later. At the end of this consent form, we will ask if you allow us to collect these samples.

14. We will counsel you about protecting your baby from HIV.

We will talk with you about ways to keep your risk of giving your baby HIV low.

15. The HVTN will test your and your baby's samples for this study.

We will send your and your baby's samples (without your names) to labs approved by the HVTN for this study, which are located in the United States and South Africa. In rare cases, some of your and your baby's 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 and your baby's samples. Your and your baby's genes are passed to each of you from your birth parents. They affect how you and your baby looks and how your and your baby's 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 and your baby's genes, not all of your and your baby's genes (your genomes). The researchers will study only the genes related to the immune system and HIV and those that affect how people get HIV.

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

These tests done on your and your baby's samples are for research purposes, not to check your and your baby's 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 and your baby's samples are no longer needed for this study, the HVTN will continue to store them.

16. We will do our best to protect your and your baby's private information.

Your and your baby's study records and samples will be kept in a secure location. We will label all of your and your baby's samples and most of the records with a code number, not your or your baby's name or other personal information. However, it is possible to identify you or your baby, if necessary. We will not share your or your baby's name with the lab that does the tests on the samples, or with anyone else who does not need to know your or your baby's 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 baby's name with the HVTN in case you or your baby needs proof in the future that your baby participated in an HIV vaccine study. The HVTN will keep your baby's name in a secure file with these items:

- The name of the study
- Your baby's age or date of birth
- Your baby's study ID number
- What study product they received

There are no HIV test results kept in this file. The HVTN will not share any information that could identify your baby without your agreement. The HVTN will remove your baby's name from the file if you do not want it there. Once your baby is no longer a minor (at least 18 years old), they also can ask the HVTN to remove their name from the file if they do not want it there.

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

- The US National Institutes of Health (NIH) and its study monitors,
- Any regulatory agency that reviews clinical trials,
- The University of the Witwatersrand Human Research Ethics Committee,
- South African Health Products Regulatory Authority (SAHPRA),
- Duke Human Vaccine Institute (DHVI) and people who work for them,
- The Infectious Disease Research Institute (IDRI) and people who work for them,
- The HVTN and people who work for them,
- The Safety Monitoring Board (SMB) and
- The US Office for Human Research Protections (OHRP).

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]
- COVID-19: At this clinic, we have to report COVID-19 test results to the National Institute of Communicable Diseases (NICD) and the national and provincial Department of Health (DoH) which includes your age and gender. The DoH may also require that we share additional demographic information about your COVID-19 test results to support contact tracing.
- [Item 3]

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

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

17. We will stop your baby's injections if they get HIV.

We will also take fewer samples, and we will help you get care and support for your baby. We may ask you to keep your baby in the study to complete other study procedures. We will encourage you to keep your baby in the study if you choose. We will discuss your baby's study options with you. We will counsel you about your baby having HIV. Your baby's HIV care will be free of charge and will follow the standard of care in South Africa. This clinic is accredited by the Department of Health which means that if your baby gets HIV, your baby can receive ART and laboratory monitoring at this clinic.

18. We may take you and your baby out of the study at any time.

We may take you and your baby out of the study if:

- you do not follow instructions,
- we think that staying in the study might harm you or your baby,
- you enroll your baby in a different research study where they get another study product, or

- the study is stopped for any reason.

Other Risks

19. 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 your baby got the injection. Taking blood can cause a low blood cell count (anemia), making you or your baby feel tired.

Personal problems/discrimination/testing HIV antibody positive:

About 10 to 20% of people who join HVTN studies report personal problems or discrimination because of joining an HIV vaccine study. Family or friends may worry, get upset or angry, and treat you or your baby unfairly as a result. Rarely, a person has lost a job because the study took too much time away from work.

The body makes antibodies to fight or prevent infection. As you know, HIV-infected moms transfer antibodies to their babies which disappear over time. That is why we do special tests to find out whether your baby is HIV-infected or not. Most vaccines cause the body to make antibodies as a way of preventing infection. Your baby's body may make antibodies to HIV because they received an HIV study vaccine. The study vaccine may also cause your baby to test positive on some types of HIV antibody tests, even if they do not have HIV. This is called vaccine-induced seropositivity (VISP). VISP means that after your baby gets the study vaccine, a routine HIV test done outside this clinic may say they have HIV, even if they don't. For this reason, **you should plan to get your baby HIV tested only at this clinic after your baby is 18 months old until it is confirmed they do not have VISP.** Our tests can tell the difference between true HIV infection and a positive result that is caused by the study vaccine.

If your baby has a positive test result caused by the study vaccine at any time, we can arrange free HIV testing for as long as your baby needs it. If this happens, we do not know how long your baby will test positive due to the study vaccine. If your baby receives a positive HIV test result and we determine it is because they have HIV, we will provide follow-up care for your baby.

It is unlikely, but your baby could test negative at the end of the study and VISP some time later, even though they 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 your baby has HIV even if they do not, they could face discrimination and other problems. For example, in some countries, they could be denied medical or dental care, employment, insurance, a visa, or entry into the military. If your baby has a positive HIV antibody test caused by the study vaccine, they will not be able to donate blood or organs. Your family and friends may treat them differently. We will give you a brochure that tells you more about testing HIV positive because of an HIV vaccine, and how your baby can avoid some of these problems.

Risks of embarrassment/anxiety/emotional stress:

You may feel embarrassed when we ask about your HIV status and ARV usage. Also, waiting for your baby's HIV test results or other health test results could make you feel anxious. You could feel worried if the test results show that your baby has HIV or if tests show that you have SARS-CoV-2. If you feel embarrassed or anxious, please tell us and we will try to help you.

Risks of the nasal swab/throat swab procedure:

If we test you for SARS-CoV-2, we will either use a nasal swab or throat swab to collect the specimen. The feeling of having a small, soft-tipped swab inserted into your nostril and twirled around may be a little uncomfortable, but it should not be painful. There is a small chance there could be some bleeding, but this is unlikely. Similarly, the feeling of having a small throat swab inserted into your throat may cause gagging and feel a little uncomfortable, but it should not be painful.

Risks of disclosure of your personal information:

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

Risks of genetic testing:

There may be some risks from tests of your or your baby's genes. If others found out the results of these tests, they could treat you or your baby badly or unfairly. However, this is almost impossible because the results will not be given to the study staff or to you, and will not be in your or your baby's study records. It would be illegal under South African law to share your or your baby's personal information.

Unknown risks:

To prevent HIV transmission through breastfeeding, you should take your ART every day. In this study we are looking to see whether this vaccine approach, may in the future, protect against breast milk transmission. We do not know if the study vaccine will assist in the prevention of breast milk transmission. This vaccine regimen aims to encourage the baby's immune system to produce potent antibodies. We do not know whether we will achieve this. We also do not know whether this study vaccine will prevent HIV infection later on in life. Should your baby get infected through breast milk, we do not know whether this study vaccine will influence the way your baby responds to HIV infection. Your baby should go onto ART if they become HIV infected. There are many ways for your baby to prevent HIV infection when they get older.

Site: Any change to the following text requires approval from HVTN Regulatory Affairs at vtn.core.reg@hvtn.org.

We do not know if getting these study vaccines will affect how your child will respond to any future approved HIV vaccine. It could be that a future HIV vaccine may not work as well for your child because he or she got the study vaccines. Currently, no HIV vaccine has been approved for use. We also do not know whether getting the study vaccine may affect the way your baby responds to other vaccines they may get early in life. This study may help answer that question.

Benefits

20. The study may not benefit you.

We do not expect the study vaccine to benefit your baby 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 your baby avoid getting HIV. The lab tests and physical exams that you get while in this study may result in earlier detection and treatment of diseases you are not aware of.

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

Your rights and responsibilities

21. If you and your baby join the study, you both have rights and responsibilities.

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

Leaving the study

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

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

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

Injuries

Site: 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.

23. **If you or your baby get sick or injured during the study, contact us immediately.**

You and your baby's health is important to us. *(Site: 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 and your baby get care elsewhere.

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

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. In this situation, some of the study product providers have agreed to pay medical costs for study-related injuries that are determined to be caused by their own study products.

The HVTN has limited funds to pay medical costs that it determines are reasonable. *(Site: 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 or your baby 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 or your baby's injury is study related. If you wish, the HVTN will ask independent experts to review the decision. You always have the right to use the court system if you are not satisfied.

Questions

24. If you have questions or problems at any time during you and your baby's 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 your baby has 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 or your baby's rights as a research participant, or problems or concerns about how you and your baby 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
Department of Health
Private Bag X828
PRETORIA
0001

Tel: (012) 842 7629/26
e-mail: Boitumelo.Semete @sahpra.org.za

Your permissions and signature

25. In Section 13 of this form, we told you about optional additional samples that we would like to collect from you and your baby. Please write your initials or make your mark in the box next to the options you choose. Whatever you choose, the clinic keeps track of your decision. You can change your mind after signing this form.

☐ I agree to provide stool samples from me and my baby.

☐ I do not agree to provide stool samples from me and my baby.

AND

☐ I agree to provide breast milk samples.

☐ I do not agree to provide breast milk samples.

26. If you agree to join this study with your baby, 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 and your baby 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 with your baby.

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

Mother/Guardian's name (print)	Mother/Guardian'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 consent form for use of samples and information in other studies

Title: A phase 1 clinical trial to evaluate the safety and immunogenicity of the HIV-1 CH505 transmitted/founder gp120 adjuvanted with GLA-SE in healthy, HIV-exposed uninfected infants

HVTN protocol number: HVTN 135

Site: [Insert site name]

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 and your baby's 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.

Key Information

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

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

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

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

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

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 or your baby's 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 and your baby's 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 and your baby's 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 or my baby's samples?

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

5. Will we benefit from allowing our 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 or your baby's medical record. The studies are only being done for research purposes.

6. Will the HVTN sell our samples and information?

No, but the HVTN may share your or your baby's samples with HVTN or other researchers. Once we share your or your baby's samples and information, we may not be able to get them back.

7. How do other researchers get our samples and information?

When a researcher wants to use your or your baby's 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 or your baby's 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 HVTN keeps a key to the code but will not share this key with other researchers or with anyone else who does not need to know your or your baby's name. Your and your baby's names will not be part of the information. However, some information that we share may be personal, such as your and your baby's race, ethnicity, gender, health information from the study, and HIV status. We may share information

about the study product your baby received and how your baby's body responded to the study product.

9. What kind of studies might be done with my or my baby's 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 or your baby's samples.

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

If you agree, your or your baby's samples could also be used for genome wide studies. In these studies, researchers will look at all of your or your baby's 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 and your baby's name and other personal information will not be included. Usually, no one would be able to look at your or your baby's genome and link it to you or your baby as a person. However, if another database exists that also has information on your or your baby's genome and your or your baby's name, someone might be able to compare the databases and identify you or your baby. 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 or your baby's samples could show you or your baby 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 or your baby's study records and are not given to you.

11. Who will have access to my and my baby's information in studies using our extra samples?

People who may see your or your baby's information are:

- Researchers who use your or your baby's extra samples and information for other research
- Government agencies that fund or monitor the research using your or your baby's 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 and your baby's information. The results of any new studies that use your or your baby's extra samples and information may be published. No publication will use your or your baby's name or identify you or your baby personally.

Questions

12. If you have questions or problems about allowing your or your baby's samples and information to be used in other studies, use the following important contacts.

If you have questions about the use of your or your baby's 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 or your baby may have been harmed because of studies using your or your baby's samples or information, contact
[name or title and telephone number of the investigator or other study staff].

If you have questions about your or your baby's 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.

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
Department of Health
Private Bag X828

PRETORIA
0001

Tel: (012) 842 7629/26
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 or your baby's samples and information can be used. You can change your mind after signing this form.

☐ I allow my and my baby's 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 and my baby's cells growing over time.

OR

☐ I agree to the option above *and* also to allow my and my baby's extra samples and information to be used in genome wide studies.

OR

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

Mother/ Guardian's name (print)	Mother/ Guardian'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 Table of procedures (for sample informed consent form)

			Time after first injection visit											
Procedures for Infant	**Screening visit(s)**	**First injection visit**	**1 day**	**2 weeks**	**2 months**	**2 ½ months**	**4 months**	**4 ½ months**	**8 months**	**8½ months**	**12 ½ months**	**13 months**	**17 months**	**2 years + 2 weeks**
Injection		√			√		√		√		√			
Medical history	√													
Complete physical	√													√
Brief physical		√	√	√	√	√	√	√	√	√	√	√	√	
Blood drawn	√	√	√	√		√		√		√	√	√	√	√
Cord blood collection	√													
Stool collection		√		√	√		√		√		√	√		√

			Time after first visit											
Procedures for Mother	**Screening visit(s)**	**First visit**	**1 day**	**2 weeks**	**2 months**	**2 ½ months**	**4 months**	**4 ½ months**	**8 months**	**8½ months**	**12 ½ months**	**13 months**	**17 months**	**2 years + 2 weeks**
Medical history	√													
Complete Physical	√													
Questionnaire					√				√					√
Blood drawn	√	√				√		√		√		√		
Nasal or throat swab*	√													
Breastmilk Collection		√						√						
Stool collection		√												

*Swab for SARS-CoV-2 testing will only be collected if testing results are not already available in your medical records.

Not shown in this table is a time after all study participants have completed their last scheduled visit when you can find out what products your baby received.

Appendix D Laboratory procedures: Infant

Procedure	Ship to[1]	Assay location[2]	Tube[4]	Tube size (vol. capacity)[4]	Visit: 1 Day: Week: Month:	2 D0 W0 M0 VAC1 CH505TFpg120 +GLA-SE or Placebo	3 D1	4 D14 W2 M0.5	5 D56 W8 M2 VAC2 CH505TFpg120 +GLA-SE or Placebo	6 D70 W10 M2.5	7 D112 W16 M4 VAC3 CH505TFpg120 +GLA-SE or Placebo	8 D126 W18 M4.5	9 D224 W32 M8 VAC4 CH505TFpg120 +GLA-SE or Placebo	10 D238 W34 M8.5	11 D379 W54 M12.5 VAC5 CH505TFpg120 +GLA-SE or Placebo	12 D393 W56 M13	13 D516 W74 M17	14 D743 W106 M24.5	Total
BLOOD COLLECTION																			
Diagnostics																			
Screening HIV Test	Local lab	Local lab	EDTA	1mL	1	—	—	—	—	—	—	—	—	—	—	—	—	—	1.0
HIV diagnostics[8,12]	HSML-NICD	HSML-NICD	EDTA	5mL	—	—	—	—	—	—	—	—	—	—	—	—	5	10	15.0
Safety labs[9]																			
CBC/Diff	Local lab	Local lab	EDTA	0.5mL	0.5	—	—	0.5	—	0.5	—	0.5	—	0.5	—	0.5	—	0.5	3.5
Chemistry panel[5]	Local lab	Local lab	SST	0.8mL	0.8	—	—	0.8	—	0.8	—	0.8	—	0.8	—	0.8	—	0.8	5.6
Immunogenicity assays																			
Humoral assays																			
Pediatric Vaccine Multiplex Assay	BARC	HVTN Labs	SST	2mL	—	—	—	—	—	—	—	—	—	2	—	***2***	—	2	6.0
Binding Ab	BARC	HVTN Labs	SST	2mL	—	—	—	—	—	—	—	—	—	2	2	***2***	—	2	8.0
HIV-1 neutralizing Ab	BARC	HVTN Labs	SST	2mL	—	—	—	—	—	—	—	—	—	2	—	***2***	—	2	6.0
Ab Effector Function Assays[10]	BARC	HVTN Labs	SST	2mL	—	—	—	—	—	—	—	—	—	—	—	***2***	—	2	4.0
Ab Avidity	BARC	HVTN Labs	SST	2mL	—	—	—	—	—	—	—	—	—	y	y	***y***	—	y	0.0
Cellular assays																			
Ag-Specific B-Cell Phenotyping	BARC	HVTN Labs	NaHep	2mL	—	—	—	4	—	6	—	***6***	—	4	—	***6***	—	6	32.0
B-cell lineage	BARC	HVTN Labs	NaHep	2mL	—	—	—	—	—	—	—	z	—	—	—	z	—	—	0.0
Systems Biology Assays - Venous Blood	BARC	Non HVTN Labs	TBD	TBD	2[11]	—	2	1	—	1	—	1	—	1	—	1	—	1	8.0
In Vitro Vaccine Modeling - Cord Blood	BARC	Non HVTN Labs	TBD	TBD	20[6]	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Specimen storage																			
Serum - Cord Blood	BARC		SST	8.5mL	8.5[6]	—	—	—	—	—	—	—	—	—	—	—	—	—	
Plasma - Cord Blood	BARC		NaHep	10mL	v														
CBMC	BARC		NaHep	10mL	60[6]	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
Serum - Venous Blood	BARC		SST	2mL	—	2	—	—	—	—	—	—	—	—	4	—	—	—	6.0
Plasma - Venous Blood	BARC		NaHep	2mL	—	—	—	w	—	w	—	w	—	w	—	w	—	w	
Visit total					**3.3**	**2.0**	**2**	**6.3**	**0**	**8.3**	**0**	**8.3**	**0**	**12.3**	**6**	**16.3**	**5**	**26.3**	**96.1**
30-Day total[7]					**4.3**	**5.3**	**7.3**	**13.6**	**0.0**	**8.3**	**0**	**8.3**	**0**	**12.3**	**6**	**22.3**	**5**	**26.3**	
STOOL COLLECTION (OPTIONAL)																			
Stool	BARC	HVTN Labs			—	X	—	X	X	—	X	—	X	—	X	X	—	X	

[1]BARC = Bio Analytical Research Corporation South Africa (Pty) Ltd (Johannesburg, South Africa); HSML-NICD = HIV Sero-Molecular Laboratory–National Institute for Communicable Diseases (Johannesburg, South Africa)

[2]HVTN Laboratories include: Fred Hutchinson Cancer Research Center (Seattle, Washington, USA); Duke University Medical Center (Durham, North Carolina, USA); Duke Human Vaccine Institute (Durham, North Carolina, USA); South African Immunology Laboratory-National Institute for Communicable Diseases (SAIL-NICD, Johannesburg, South Africa)
Non HVTN Laboratories include: Precision Vaccines Program, Boston Children's Hospital/Harvard Medical Center (Boston, Massachusetts, USA); University of British Columbia (Vancouver, Canada)

[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. Use of alternate blood collection tubes for immunogenicity or storage specimens must be approved by the HVTN laboratory program.

[5]Chemistry panels are defined in section 9.2.2 (pre-enrollment) and section 9.4 (postenrollment).

[6]Speicmens for In Vitro Vaccine Modeling - Cord Blood, and Serum - Cord Blood and CBMC specimen storage will be collected from umbilical cord blood at delivery.

[7]The 30-day total blood volume does not include up to 1mL collection for HIV diagnostics following local standard of care guidelines and any additional HIV testing based on mother's viral load. However, the 30-day blood draw limit of 5% of total blood volume is not exceeded at any visit by the collection of blood for HIV diagnostic testing.

[8]At an early termination visit for a withdrawn or terminated infant who is not HIV-infected (see Section 9.9), blood should be drawn for HIV diagnostic testing. Refer to the HVTN 135 SSP for more information. If the infant has a confirmed diagnosis of HIV infection, do not collect blood for HIV diagnostic testing (see Section 9.10).

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

[10]Ab Effector Function Assays may include FcγR binding assay, ADCC, ADCP, ADNP and ADCD.
[11]Specimen for System Biology Assays - Venous Blood will be collected within the first 24 hours after birth, prior to vaccination.
[12]Additional HIV diagnostic testing may be performed based on mother's viral load.
v=NaHep plasma - Cord Blood aliquots will be harvested for storage during CBMC processing; no separate blood draw is needed.
w=NaHep plasma - Venous Blood aliquots will be harvested for storage during PBMC processing; no separate blood draw is needed.
y=SST collected for Binding Ab assay will also cover specimen needs for Ab avidity assay; no separate blood draw is needed.
z=NaHep collected for Ag-Specific B-Cell Phenotyping will also cover specimen needs for B-cell lineage; no separate blood draw is needed.

Appendix E Laboratory procedures: Mother

Procedure	Ship to[1]	Assay location[2]	Tube[4]	Tube size (vol. capacity)[4]	Visit: 1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
					Day: Screening visit[3]	D0	D1	D14	D56	D70	D112	D126	D224	D238	D379	D393	D516	D743	
					Week:	W0		W2	W8	W10	W16	W18	W32	W34	W54	W56	W74	W106	
					Month:	M0		M0.5	M2	M2.5	M4	M4.5	M8	M8.5	M12.5	M13	M17	M24.5	
BLOOD COLLECTION																			
Screening/Diagnostic																			
Screening HIV EIA	Local lab	Local lab	SST	5mL	5	—	—	—	—	—	—	—	—	—	—	—	—	—	
CD4+T Cell Count	Local lab	Local lab	EDTA	5mL	5[5]	—	—	—	—	—	—	—	—	—	—	—	—	—	0
HBsAg	Local lab	Local lab	SST	5mL	5[5]	—	—	—	—	—	—	—	—	—	—	—	—	—	0
HIV PCR Viral Load	Local lab	Local lab	EDTA	10mL	10	—	—	—	—	10	—	10	—	10	—	10	—	—	40
HIV Genotypic Antiretroviral Resistance Testing	Local lab	Local lab	EDTA	10mL	—	—	—	—	—	y	—	y	—	y	—	y	—	—	0
ARV detection by dried blood spots	BARC	HVTN Labs	EDTA	2mL	—	—	—	—	—	2	—	2	—	2	—	2	—	—	8
Immunogenicity assays																			
Humoral assays																			
Binding Ab	BARC	HVTN Labs	SST	8.5mL	—	8.5	—	—	—	—	—	—	—	8.5	—	8.5	—	—	26
Systems Biology Assays	BARC	Non HVTN Labs	TBD	TBD	2[6]	—	—	—	—	—	—	—	—	—	—	—	—	—	0
Visit total					**27**	**8.5**	**0**	**0**	**0**	**12**	**0**	**12**	**0**	**20.5**	**0**	**20.5**	**0**	**0**	**101**
56-Day total					**27**	**35.5**	**35.5**	**35.5**	**35.5**	**12**	**12**	**24**	**24**	**20.5**	**0**	**20.5**	**0**	**0**	
NASOPHARYNGEAL/OROPHARYNGEAL SWAB COLLECTION[7]																			
SARS-CoV-2 RNA PCR	Local lab	Local Lab			X[5]														
BREASTMILK COLLECTION (OPTIONAL)																			
Breastmilk	BARC	HVTN Labs			—	X[8]	—	—	—	—	—	X	—	—	—	—	—	—	
STOOL COLLECTION (OPTIONAL)																			
Stool	BARC	HVTN Labs			—	X	—	—	—	—	—	—	—	—	—	—	—	—	

[1]BARC = Bio Analytical Research Corporation South Africa (Pty) Ltd (Johannesburg, South Africa); HSML-NICD = HIV Sero-Molecular Laboratory–National Institute for Communicable Diseases (Johannesburg, South Africa)

[2]HVTN Laboratories include: Fred Hutchinson Cancer Research Center (Seattle, Washington, USA); Duke University Medical Center (Durham, North Carolina, USA).
Non HVTN Laboratories include: Precision Vaccines Program, Boston Children's Hospital/Harvard Medical Center (Boston, Massachusetts, USA); University of British Columbia (Vancouver, Canada)

[3]Screening may occur over the course of several contacts/visits up to and including D0, prior to infant vaccination.

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

[5]Collect specimens for specified testing only if results are not available through medical records. See protocol section 9.2 for more information.

[6]Specimen for System Biology Assays will be collected within the first 24 hours after birth.

[7] Local labs may assign appropriate alternative specimen type for SARS-CoV-2 testing.

[8]Optional breastmilk collection will be obtained within 7 days after delivery but no sooner than 48 hours after delivery.

y=EDTA blood for HIV Genotypic Antiretroviral Resistance Testing - Reflex assay will only be collected if HIV PCR viral load is ≥400 copies/mL at specified visits. Refer to the HIV Testing SSP for additional information.

Appendix F Procedures at HVTN CRS: Infant

Visit:	01[1]	02	03	04	05	06	07	08	09	10	11	12	13	14	Post
Day:		D0	D1	D14	D56	D70	D112	D126	D224	D238	D379	D393	D516	D743	
Week		W0		W2	W8	W10	W16	W18	W32	W34	W54	W56	W74	W106	
Month:		M0		M0.5	M2	M2.5	M4	M4.5	M8	M8.5	M12.5	M13	M17	M24.5	
Procedure	Scr.	**VAC1**			**VAC2**		**VAC3**		**VAC4**		**VAC5**				
Study procedures															
Medical history	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Feeding history		X	X	X	X	X	X	X	X	X	X	X	X	X	—
Complete physical exam	X	—	—	—	—	—	—	—	—	—	—	—	—	X	—
Abbreviated physical exam	—	X	X	X	X	X	X	X	X	X	X	X	X	—	—
Obtain demographics (for infant)	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Randomize	—	X	—	—	—	—	—	—	—	—	—	—	—	—	—
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	—
Intercurrent illness/adverse experience	—	X	X	X	X	X	X	X	X	X	X	X	X	X	—
HIV assessment	X	—	—	—	—	—	—	—	—	—	—	—	X	X	—
Confirm HIV assessment results provided to mother	—	X	—	—	—	—	—	—	—	—	—	—	—	X	X
Vaccination procedures															
Vaccination	—	X	—	—	X	—	X	—	X	—	X	—	–	—	—
Reactogenicity assessments[2]	—	X	—	—	X	—	X	—	X	—	X	—	–	—	—
Specimen collection[3]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	—
Poststudy															
Unblind participant	—	—	—	—	—	—	—	—	—	—	—	—	—	—	X

[1] Screening may occur over the course of several contacts/visits up to and including day 0 prior to vaccination.
[2] Reactogenicity assessments performed daily for at least 3 days postvaccination (see Section 9.7).
[3] For specimen collection requirements, see Appendix D, Laboratory procedures- Infant.

Appendix G Procedures at HVTN CRS: Mother

Visit:	01[1]	02	03	04	05	06	07	08	09	10	11	12	13	14	Post
Day:		D0	D1	D14	D56	D70	D112	D126	D224	D238	D379	D393	D516	D743	
Week:		W0		W2	W8	W10	W16	W18	W32	W34	W54	W56	W74	W106	
Month:		M0		M0.5	M2	M2.5	M4	M4.5	M8	M8.5	M12.5	M13	M17	M24.5	
Procedure	Scr.														
Study procedures															
Assessment of understanding	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Complete Physical Exam	X														
Signed protocol consent	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Medical history	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Risk reduction counseling[2]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	—
Confirm eligibility	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Obtain demographics (for mother)	X	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Social impact assessment	—	X	X	X	X	X	X	X	X	X	X	X	X	X	—
Outside testing of baby questionnaire	—	—	—	—	—	—	—	—	—	—	—	—	—	X	—
Belief questionnaire	—	—	—	—	X	—	—	—	X	—	—	—	—	X	
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	—
Specimen Collection [3]	X	X	—	—	—	X	—	X	—	X	—	X	—	—	—
Poststudy															
Provide unblinding information to mother	—	—	—	—	—	—	—	—	—	—	—	—	—	—	X

[1] Screening may occur over the course of several contacts/visits up to and including day 0.
[2] Risk reduction counseling, see Section 9.6 and HVTN 135 SSP.
[3] For specimen collection requirements, see Appendix E, Laboratory procedures- Mother.

Appendix H 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 135 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, myelo radiculomyelitis) • 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 I Infant visit windows

Visit Number	Visit Type	Lower Allowable Window	Lower Target Day	Target Day[1]	Upper Target Day	Upper Allowable Window
01.0	Screening	-5	-	-	-	+0
02.0	**Enrollment/Vaccination 1**	-	-	0	-	-
03.0	Follow-up	-	-	1	+2	-
04.0	2 Weeks Post-Vaccination	-	-4	14	+4	+7
05.0	**Vaccination 2**	-	-9	56	+9	+14
06.0	2 Weeks Post-Vaccination	-	-4	70	+4	+7
07.0	**Vaccination 3**	-21	-14	112	+14	+21
08.0	2 Weeks Post-Vaccination *Primary Immunogenicity*	-	-4	126	+4	+7
09.0	**Vaccination 4**	-28	-14	224	+14	+28
10.0	2 Weeks Post-Vaccination	-	-4	238	+4	+7
11.0	**Vaccination 5**	-28	-14	379	+14	+28
12.0	2 Weeks Post-Vaccination *Primary Immunogenicity*	-	-4	393	+4	+7
13.0	Follow-up	-	-14	516	+14	+21
14.0	Final Visit	-28	-14	743	+14	+28

[1]Target dates are relative to Day 0 (Enrollment), with the exception of postvaccination visits 4.0, 6.0, 8.0, 10.0, and 12.0 which are relative to the prior vaccination visit.

Appendix J Mother visit windows

Visit Number	Visit Type	Lower Allowable Window	Lower Target Day	Target Day[1]	Upper Target Day	Upper Allowable Window
01.0	Screening	-273	-	-	-	+0
02.0	**Enrollment/Infant Vaccination**	-	-	0	-	-
03.0	Follow-up	-	-	1	+2	-
04.0	Follow-up	-	-4	14	+4	+7
05.0	Follow-up	-	-9	56	+9	+14
06.0	Specimen Collection	-	-4	70	+4	+7
07.0	Follow-up	-21	-14	112	+14	+21
08.0	Specimen Collection	-	-4	126	+4	+7
09.0	Follow-up	-28	-14	224	+14	+28
10.0	Specimen Collection	-	-4	238	+4	+7
11.0	Follow-up	-28	-14	379	+14	+28
12.0	Specimen Collection	-	-4	393	+4	+7
13.0	Follow-up	-	-14	516	+14	+21
14.0	Final Visit	-28	-14	743	+14	+28

[1]Target dates are relative to Day 0 (Enrollment), with the exception of visits 4.0, 6.0, 8.0, 10.0, and 12.0 which are relative to the prior visit.

Appendix K Protocol Signature Page

A phase 1 clinical trial to evaluate the safety and immunogenicity of the HIV-1 CH505 transmitted/founder gp120 adjuvanted with GLA-SE in healthy, HIV-exposed uninfected infants

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); 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

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

DAIDS Protocol Number: HVTN 135

DAIDS Protocol Version: Version 3.0

Protocol Date: December 18, 2020