A5369

HIV-1-Gag Conserved-Element DNA Vaccine (p24CE) as Therapeutic Vaccination in HIV-Infected Persons with Viral Suppression on Antiretroviral Therapy

A Multicenter Trial of the AIDS Clinical Trials Group (ACTG)

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National Institute of Allergy and Infectious Diseases

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Ichor Medical Systems, Inc.

IND #

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ACTG
AIDS CLINICAL TRIALS GROUP

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SIGNATURE PAGE

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 (US) Health and Human Services regulations (45 CFR 46); applicable U.S. Food and Drug Administration regulations; standards of the International Conference on Harmonisation 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 (e.g., US National Institutes of Health, Division of AIDS) and institutional policies.

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Print/Type

Signed: _____ Date: _____
Name/Title

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STUDY MANAGEMENT

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A response should generally be received within 24 hours (Monday through Friday).

Protocol E-mail Group
Sites should contact the User Support Group at the Data Management Center (DMC) as soon as possible to have the relevant personnel at the site added to the actg.protA5369 e-mail group. Include the protocol number in the e-mail subject line.

- Send an e-mail message to actg.user.support@fstrf.org.

Clinical Management:
For questions concerning entry criteria, toxicity management, concomitant medications, and co-enrollment, contact the core protocol team.

- Send an e-mail message to actg.coreA5369@fstrf.org. Include the protocol number, patient identification number (PID), and a brief relevant history.

Laboratory
For questions specifically related to immunologic or virologic laboratory tests, contact the protocol immunologist or virologist.

- Send an e-mail message to actg.teamA5369@fstrf.org (ATTENTION: Nicole Frahm or Alan Landay/Daniel Kuritzkes).

Data Management

- For nonclinical questions about transfers, inclusion/exclusion criteria, electronic case report forms (eCRFs), randomization/registration, and other data management issues, contact the data manager. Completion guidelines for eCRFs and participant-completed eCRFs can be downloaded from the FSTRF website at www.frontierscience.org.
- For transfers, reference the Study Participant Transfer SOP 119, and contact Dave Rusin (rusin@fstrf.org) and Loren Wright (wright@fstrf.org) directly.
- For other questions, send an e-mail message to actg.teamA5369@fstrf.org (ATTENTION: Dave Rusin and Loren Wright).
- Include the protocol number, PID, and a detailed question.

Randomization/Participant Registration
For randomization/participant registration questions or problems and study identification number SID lists:

- Send an e-mail message to rando.support@fstrf.org or call the DMC Randomization Desk at 716-834-0900, extension 7301.

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- Send an e-mail message to actg.user.support@fstrf.org or call 716-834-0900 x7302.

Protocol Document Questions
For questions concerning the protocol document, contact the Clinical Trials Specialist.
- Send an e-mail message to actg.teamA5369@fstrf.org (ATTENTION: Linda Boone).

Copies of the Protocol
- To request a hard copy of the protocol, send an e-mail message to ACTGNCC@s-3.com (ATTENTION: Diane Delgado).
- Electronic copies can be downloaded from the ACTG website at https://www.actgnetwork.org.

Product Package Inserts and/or Investigator Brochures
To request copies of product package inserts or investigator brochures, contact the DAIDS Regulatory Support Center (RSC) at RIC@tech-res.com or call 301-897-1708.

Protocol Registration
For protocol registration questions, send an e-mail message to Protocol@tech-res.com or call 301-897-1707.

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Call the Clinical Research Products Management Center (CRPMC) at 301-294-0741.

IND (Investigational New Drug) Number or Questions
The IND number will be available on the protocol-specific web page (PSWP) within 30 days of the submission to the Food and Drug Administration (FDA). For any questions related to the IND submission, contact the DAIDS RSC at Regulatory@tech-res.com or call 301-897-1706.

Expedited Adverse Event (EAE) Reporting/Questions
Contact DAIDS through the RSC Safety Office at DAIDSRSCSafetyOffice@tech-res.com or call 1-800-537-9979 or 301-897-1709; or fax 1-800-275-7619 or 301-897-1710.

Telephone Calls
Sites are responsible for documenting telephone calls made to A5369 team members.
- Send an e-mail message to actg.teamA5369@fstrf.org.

Protocol-Specific Web Page
Additional information about management of the protocol can be found on the protocol-specific web page (PSWP).

GLOSSARY OF PROTOCOL-SPECIFIC TERMS

AA	amino acids
ART	antiretroviral therapy
cART	combination antiretroviral therapy
CE	conserved elements
CTL	cytotoxic T lymphocytes
Gag	group-specific antigen
HLA	human leukocyte antigen
HVTN	HIV Vaccine Trials Network
IFN-γ	interferon gamma
IM/EP	intramuscular injection (or intramuscularly) followed by in vivo electroporation
INR	international normalized ratio
NIH	National Institutes of Health
pDNA	plasmid deoxyribonucleic acid
PT	prothrombin time
PTT	partial thromboplastin time
SIV	simian immunodeficiency virus
TDS-IM	Intramuscular TriGrid Delivery System (also known as “EP device”)
TNF-α	tumor necrosis factor alpha
VRC	vaccination report card

SCHEMA

A5369

HIV-1-Gag Conserved-Element DNA Vaccine (p24CE) as Therapeutic Vaccination in HIV-Infected Persons with Viral Suppression on Antiretroviral Therapy

DESIGN	A5369 is a phase I/IIa, randomized, double-blind, placebo-controlled study that will evaluate the safety, immunogenicity, and preliminary assessment of efficacy of a novel vaccine encoding "conserved elements" (CE) of the HIV-1 Gag core protein, p24Gag, as a therapeutic vaccine in HIV-1 infected persons on antiretroviral therapy (ART) with the aim to induce potent virus-specific cytotoxic T lymphocytes (CTL) responses.
DURATION	Participants will be on study for 48 weeks.
SAMPLE SIZE	40 participants randomized 2:1:1 to the p24CE/full-length Gag DNA vaccine arm versus full-length Gag DNA vaccine arm versus placebo arm.
POPULATION	HIV-infected individuals receiving ART with plasma HIV-1 RNA <50 copies/mL for at least 2 years (one "blip" allowed), current CD4 T cell counts >500 cells/mm^3, and nadir CD4 T cell counts >350 cells/mm^3 (by documentation or candidate recall).
STRATIFICATION	Randomization will be stratified by indication of willingness to have the leukapheresis procedure at study screening.
REGIMEN	Arm 1: p24CE1/2 pDNA vaccine at weeks 0 and 4, followed by p24CE1/2 pDNA admixed with full-length $p55^{gag}$ pDNA vaccine at weeks 12 and 24. Arm 2: full-length $p55^{gag}$ pDNA vaccine at weeks 0, 4, 12, and 24. Arm 3: placebo (Sodium Chloride for Injection, USP 0.9%) at weeks 0, 4, 12, and 24. The active vaccines/placebo will be administered by intramuscular injection/electroporation.

1.0 HYPOTHESIS AND STUDY OBJECTIVES

1.1 Hypothesis

The administration of p24CE Gag DNA vaccine followed by p24CE DNA + full-length Gag DNA vaccine boost to HIV-infected persons on antiretroviral therapy (ART) will focus newly induced immune responses to immunogenic conserved epitopes of Gag and away from decoy variable epitopes, compared to the administration of full-length Gag DNA vaccine alone, and compared to placebo. The vaccine regimes will be safe and well tolerated.

1.2 Primary Objectives

1.2.1 To compare the additional number of p24CEs recognized by a CD4 and/or CD8 T cell response in participants in each study arm at week 26 compared to baseline.

1.2.2 To evaluate the safety of the p24CE/full-length Gag DNA vaccine and the full-length Gag DNA vaccine in HIV-infected persons receiving ART.

1.3 Secondary Objectives

1.3.1 To compare the percent of individual study participants in each study arm with a CD4 and/or CD8 T cell response to an increased number of conserved elements (CEs) at week 26 compared to baseline.

1.3.2 To compare the percent of individual study participants in each study arm with a CD4 T cell response to an increased number of CEs at week 26 compared to baseline.

1.3.3 To compare the percent of individual study participants in each study arm with a CD8 T cell response to an increased number of CEs at week 26 compared to baseline.

1.3.4 To compare the change in total magnitude of CD4 T cell responses against each CE added together across study arms.

1.3.5 To compare the change in total magnitude of CD8 T cell responses against each CE added together across study arms.

1.4 Other Objectives

1.4.1 To further characterize the HIV-specific cellular immune responses induced by the p24CE/full Gag DNA vaccine compared to full-length Gag DNA vaccine alone and to placebo control using a viral inhibition assay.

1.4.2 To assess the HIV-specific humoral immune response induced by the p24CE/full Gag DNA vaccine compared to full-length Gag DNA vaccine alone and to placebo control.

1.4.3 To assess cellular and soluble markers of immune activation, exhaustion, and checkpoint inhibition in HIV-infected participants receiving the p24CE/full-length Gag DNA vaccine compared to participanats receiving the full-length Gag DNA vaccine alone or placebo.

1.4.4 To assess the effects of the p24CE DNA/full-length Gag DNA vaccine on measures of the latent cell reservoir of HIV-1 compared to full-length Gag vaccine alone or placebo.

1.4.5 To evaluate the tolerability of the intramuscular injection/electroporation (IM/EP) delivery of the DNA vaccines in HIV-infected individuals.

1.4.6 To evaluate the effect of the gut microbiome on immune responses to the vaccine.

2.0 INTRODUCTION

2.1 Background

Oral ART has been highly successful in the management of human immunodeficiency virus type 1 (HIV-1) infection, achieving dramatic viral suppression in most infected persons maintaining access to care. However, ART does not eradicate HIV-1 and morbidities still occur. When ART is discontinued, viral rebound occurs, usually within 2-4 weeks.

Therapeutic vaccination has a potential role either as a component of a strategy to eliminate cells latently infected with HIV-1, where cells need to express HIV antigen to be targeted by anti-HIV immunotherapy, or as a functional cure in itself to achieve indefinite host control of HIV-1 infection to undetectable levels off ART. HIV-1 vaccine development for both prevention and therapy has been hindered by the high rate of mutation of the virus and the tendency of immune responses to focus on epitopes that can readily mutate without negatively impacting virus viability and thus divert the immune system from responding against protective epitopes [1].

Thus, the approach taken here was to develop protective immunogens [1-3] that direct immune targeting to conserved regions of viral proteins while excluding variable immunodominant epitopes, thereby avoiding the potential negative effects of directing T cell responses to ‘‘decoy’’ epitopes that do not benefit virologic control [1,4]. The immunogen designs were based on selection of epitopes that are conserved among all HIV-1 sequences and are associated with virologic control in HIV-1 infected individuals [1-3, 5-13]. Cytotoxic T lymphocyte (CTL) activity against conserved viral epitopes have been noted in elite controllers and long-term non-progressors [3, 14]. Furthermore, mutations affecting the conserved regions of HIV-1 proteins often impair viral fitness [15-

20].
There is substantial evidence that Gag-specific CD4 and CD8 T cell responses correlate with control of viremia [3,21-24], including reduced viremia after infection in the Step preventive vaccine trial [25]. The pDNA vaccine to be studied here encodes seven CEs of HIV-1 p24Gag that were selected by database exploration and viral load/CTL response data. These protein sequences are typically found in >98% of the HIV-1 Group M viruses worldwide and represent 54% of the p24Gag protein [9].

2.2 Rationale

p24CE pDNA vaccine regimen
This trial will evaluate the safety, immunogenicity, and provide a preliminary assessment of the efficacy of a novel vaccine encoding CEs of the HIV-1 Gag core protein, p24Gag, as a therapeutic vaccine in HIV-1 infected persons on ART with the aim to induce potent virus-specific CTL responses and reduce virus rebound set point after discontinuation of ART. Induction of such CTL responses targeting conserved, subdominant epitopes has application in a therapeutic setting against HIV infection, and this vaccine regimen induces potent CTL responses targeting the presumed Achilles' heel of the virus.

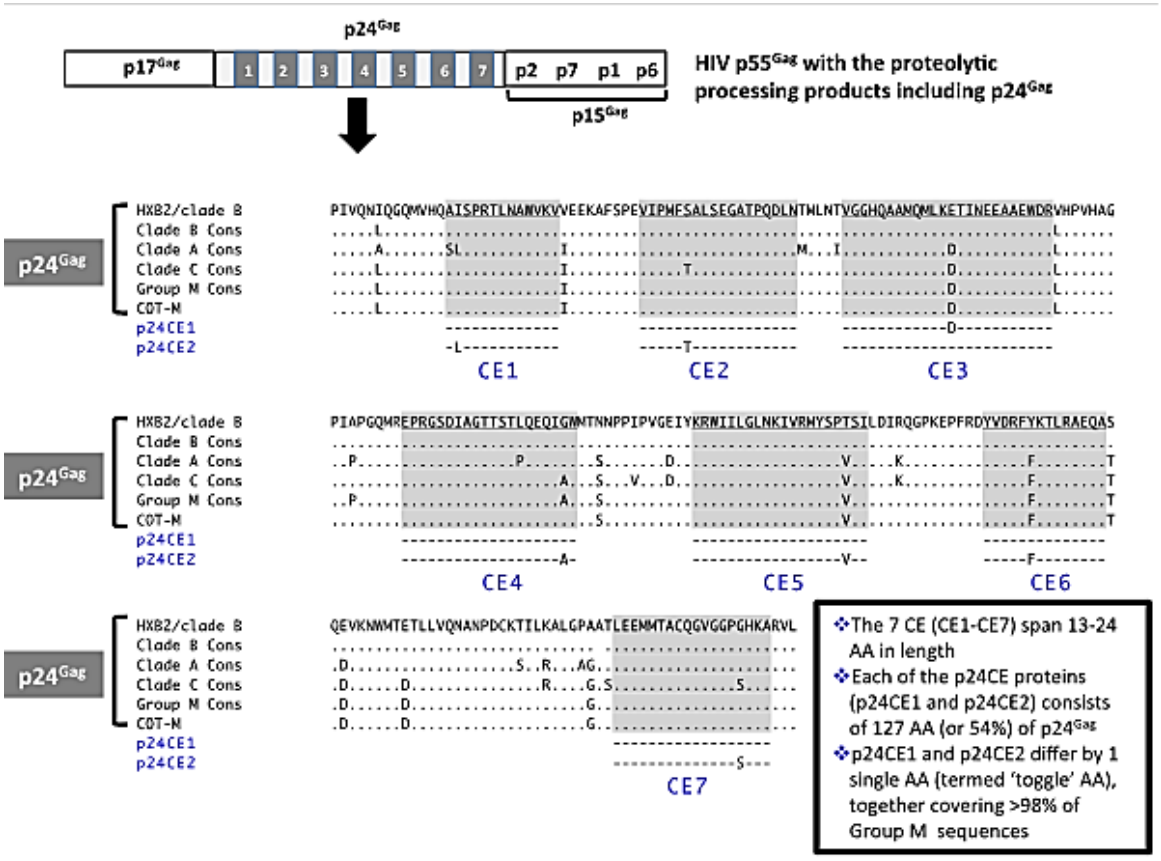
The study will use p24CE and $p55^{gag}$ plasmids in a pDNA prime-pDNA boost vaccine regimen comprising p24CE prime followed by p24CE+$p55^{gag}$ booster vaccination. This vaccine regimen has been selected for the clinical trial HVTN 119 to test safety and immunogenicity in HIV-naïve persons.

As noted above, HIV sequence diversity and potential "decoy" epitopes are hurdles in the development of an effective AIDS vaccine. To target immune responses towards critical viral epitopes, we engineered immunogens encoding CEs of HIV-1 selected on the basis of stringent conservation, functional importance, broad HLA-coverage, and association with viral control [1, 26, 27]. We engineered a pDNA vaccine targeting seven CEs within the HIV-1 p24Gag protein and demonstrated that this vaccine is able to induce potent cellular and humoral immune responses in mice and macaques [9, 10, 28]. CE-specific T cells are polyfunctional and highly cytotoxic. We further developed a novel, improved vaccine regimen that includes priming with HIV p24CE pDNA, critical to induce immune responses to subdominant epitopes, and p24CE pDNA together with a plasmid expressing the full-length immunogen p55Gag as a boost. We found this to be the most effective protocol to induce CE-targeted responses with the greatest breadth, magnitude, and cytotoxic capability in macaques [29]. Thus, this vaccine regimen alters the immunodominance hierarchy and induces robust immune responses to subdominant epitopes. In contrast, this result could not be achieved by vaccination with complete $p55^{gag}$ pDNA, which induced few and mostly monofunctional responses recognizing CE, these responses being found in only 50% of the vaccinated animals and having limited breadth [9, 10, 28]. The combination of pDNA vaccines expressing CE and full-length immunogen provides a novel strategy to maximize the breadth and magnitude of cellular and humoral immunity targeting the subdominant, highly conserved regions of HIV. Therapeutic vaccination by this method could induce novel responses unlikely to be overcome by viral mutations and has the potential to provide an additional critical step towards reduction of the long-term viral reservoir.

p24CE pDNA vaccine

The p24CE vaccine is composed of two gene segments (p24CE1 and p24CE2) on one plasmid, referred to as p24CE1/2 pDNA, expressing carefully selected portions of the HIV-1 capsid protein, p24Gag, the most abundant protein in HIV virions (Figure 2.2-1). p24Gag was chosen because of its availability as an immune target during infection, and because CTL responses to Gag, and in particular the p24Gag component of Gag, have been associated with greater control of HIV viremia in numerous studies [20-23, 30-37]. The sequences expressed by p24CE1/2 pDNA correspond to the portions of p24Gag found in nearly every HIV-1 (M group) strain observed to date throughout the world; while they elicit immune responses in HIV-infected individuals, these responses are subdominant to the more variable segments of Gag. Unlike most of the HIV-1 genes and their encoded proteins, CEs have rarely, if at all, been observed to mutate (for the most part they are composed of amino acids [AA] conserved in 98-100% of all HIV-1 M- group infections) and represent components that are enriched with those essential to virus infectivity. We hypothesize that by targeting these potentially critical regions of the HIV-1 p24Gag protein, this vaccine approach may elicit specific immune responses linked to protection and viral control, rather than immune responses that may include decoy responses with detrimental effects or limited antiviral potential. In a therapeutic vaccine application, this vaccine may induce new cellular immune responses not overcome by virus mutations and contribute to virus control. Allowing pre-existing anti-HIV immune responses to ebb, as occurs during ART, may also contribute to a more robust response to the CE.

Figure 2.2-1. Alignment of p24CE1, p24CE2, and p24Gag proteins. The schematic representation at the top of the shows the p55Gag and its proteolytic processing products including p24Gag protein. Below is the alignment of the amino acid (AA) sequences of the majority consensus $p24^{Gag}$ sequences of different HIV-1 clades and of the seven CE encoded by the p24CE1/2 pDNA vaccine. The AA sequences of the p24Gag of different HIV-1 clades (A, B, C), group M consensus, COT-M, and of the p24CE1 and p24CE2 proteins are shown (Note that consensus agreement, indicated by ".", does not necessarily indicate strong sequence conservation at that site). The CE1 to CE7 sequences with the single AA change per CE are indicated.

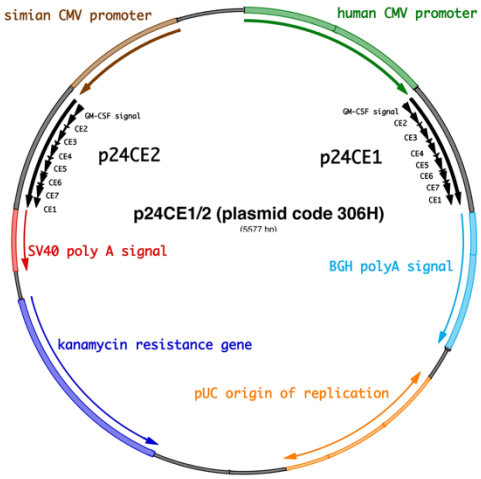


The p24CE1/2 pDNA is a dual promoter plasmid (Figure 2.2-2) generated to express the p24CE1 gene from the human cytomegalovirus (CMV) promoter and the p24CE2 gene from the simian CMV promoter in the opposite transcriptional orientation. There are 7 CE segments of 13, 18, 24, 20, 20, 14, and 18 AA in each of the p24CE constructs [1]. One "toggled" amino acid is included in each of the 7 CE segments, resulting in two protein coding elements (p24CE1 and p24CE2) differing by a total of 7 codons, each sharing 127 amino acids with p24Gag. Coding sequences were optimized to enhance expression in human cells [38-41]. The p24CE sequences represent 54% of p24Gag, including most extended coiled regions of the protein. CE regions are enriched for AA at p24Gag hexamer interfaces [42], which provides a structural rationale for their

conservation. Also, CE are enriched with AA sites that, if mutated to the second-most common AA at that site in the HIV database, would result in noninfectious virus [27, 42]. The plasmid also contains two polyadenylation signals (bovine growth hormone polyA signal for p24CE1 and the simian virus 40 polyA signal for p24CE2).

The CE segments are separated by linkers of 0-3 AA in length composed of Alanine, or Alanine and Lysine, or Alanine and Glycine. The total length of the proteins spans 157 AA, including 17 AA of the GM-CSF signal peptide and 13 AA of linker sequences. The linker sequences are designed for efficient proteolytic cleavage [9]. The length and sequence of the linker sequences were therefore set based on the existing knowledge of cleavage specificities [43], as well as to avoid fortuitous junctional homologies with HIV and the human proteome, the latter determined by searching against the HIV and human proteome databases [44].

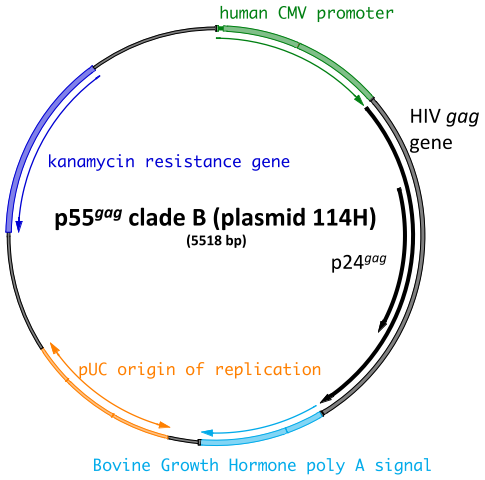
Figure 2.2-2: Schematic of the p24CE1/2 pDNA (plasmid code 306H): p24CE1/2 pDNA uses a plasmid backbone derived from pVR1012 [45], which has been used in previous DNA vaccine studies. p24CE1/2 pDNA was manufactured and produced at a concentration of 4 mg/mL, in 30 mM citrate buffer pH 6.5 containing 150 mM NaCl, 0.01% EDTA and 0.25% bupivacaine-HCl by Ajinomoto Althea, San Diego, CA. p24CE pDNA has been tested in macaques monitored for >2 years without any adverse effects.



p55^{gag} pDNA

The p55^{gag} pDNA (plasmid 114H) contains an expression-optimized full-length HIV-1 p55^{gag} gene from the HIV-1 molecular clone HXB2 (Genbank NP_057850) cloned into the pCMVkan plasmid between the human CMV promoter and the bovine growth hormone polyA signal (Figure 2.2-3). Related plasmids expressing HIV p55Gag have been tested in several clinical trials, including HVTN 060, 063, 070, 080, and 087, and no significant adverse effects related to vaccine were reported.

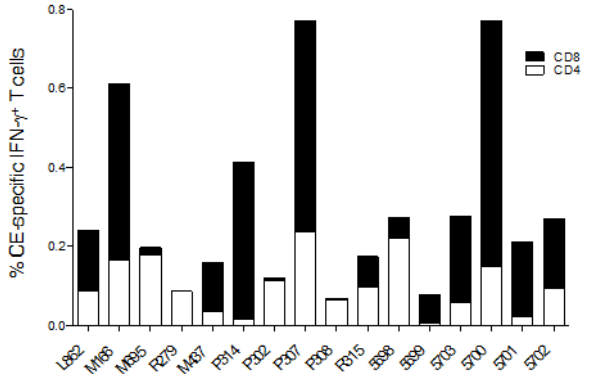
Figure 2.2-3. Schematic of p55^{gag} pDNA. p55^{gag} pDNA was manufactured and produced at a concentration of 4 mg/mL, in 30 mM citrate buffer pH 6.5 containing 150 mM NaCl, 0.01% EDTA and 0.25% bupivacaine-HCl by Ajinomoto Althea, San Diego, CA. p55^{gag} pDNA will be admixed with pCE1/2 pDNA to produce a single injectable solution for the booster vaccination.



Preclinical immunogenicity studies of p24CE pDNA in macaques

A variety of model systems were used to ensure that delivery of p24CE pDNA would induce strong immune responses [9, 10, 26, 29]. The p24CE pDNA studies conducted in macaques are shown in Figure 2.2-4. Cellular immune responses were measured in blood 2 weeks after the last vaccination by stimulation of peripheral blood mononuclear cells (PBMC) with peptides spanning the 7 CE [10, 29]. All animals developed CE-specific cellular responses, as measured by IFN-γ production, with a frequency ranging from 0.1% to 0.8% of total T cells in blood. These responses were elicited by both CD4 and CD8 T cells, of both central (CD28^{+}, CD95^{+}) and effector memory phenotype (CD28^{-}, CD95^{+}), and included cytotoxic and polyfunctional CE-specific T cells, as defined by their granzyme B content, ability to secrete two cytokines (IFN-γ and TNF-α), and ability to degranulate (CD107a). In addition to cellular immune responses, vaccination with p24CE plasmids induced robust antibody responses to CE, not achieved by vaccination with p55^{gag} DNA. These studies demonstrated that vaccination with CE pDNA induces potent immune responses to subdominant epitopes, notachieved by vaccination with pDNA expressing the full-length Gag (see Figure 2.2-5).

Figure 2.2-4. p24CE pDNA vaccine is immunogenic in macaques. Macaques (N=16) were vaccinated twice with a mixture of p24CE1&p24CE2 pDNA (N=6) or the dual-expression vector p24CE1/2 (N=10). The frequency of CE-specific IFN-γ$^+$ T cells was measured 2 weeks after the last priming vaccination using CE-specific peptide pools composed of a mixture of 15-mer overlapping by 11 AA and 10-mer peptides overlapping by 9 AA covering both p24CE1 and p24CE2 proteins. The CE-specific CD4 (open bars, labeled CD4) and CD8 (filled bars, labeled CD8) T cells are shown.

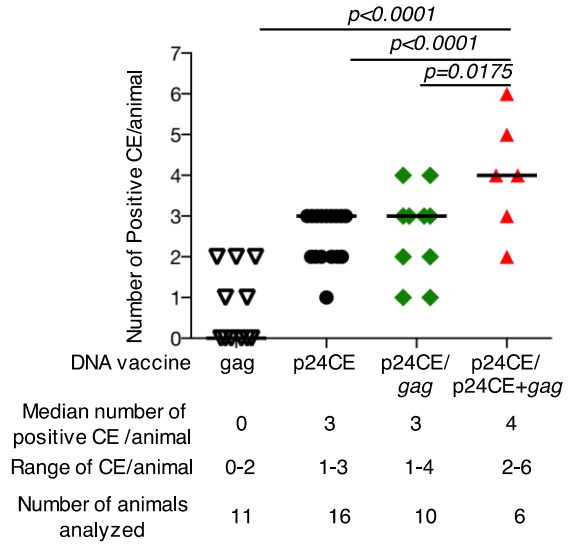


Preclinical macaque studies of p24CE1/2 DNA prime/co-delivery of p24CE1/2 and p55^{gag} pDNA boost vaccine regimen

A novel prime-boost regimen was developed to maximize magnitude, breadth, and the generation of cytotoxic T cell responses recognizing the CE [10, 26, 29]. This regimen includes p24CE1/2 and p55^{gag} plasmids in a pDNA prime-pDNA boost vaccine regimen comprising p24CE pDNA prime followed by p24CE+p55^{gag} pDNA booster vaccination. Booster vaccination including p55^{gag} pDNA significantly increased T cell responses and also induced highly cytotoxic CE-specific IFN-γ$^+$ T cells with Granzyme B$^+$ co-expression of >89%.

The breadth of the response, measured by the number of CE recognized per animal, was compared among groups that received different CE-based vaccines (CE pDNA only, CE/*gag* pDNA, and CE/CE+*gag* pDNA) (Figure 2.2-5). The CE+*gag* pDNA booster vaccination induced responses with significantly increased breadth (2-6 CE per animal), compared to CE or CE/*gag* pDNA vaccines with 1-3 or 1-4 CE per animal, with no difference between the latter groups. All CE-based DNA vaccines induced broader responses than the HIV p55^{gag} pDNA only vaccine, which induced responses to 0-2 CE per animal.

Figure 2.2-5. Increased breadth of the HIV CE-specific responses induced by a CE/CE+gag pDNA vaccine regimen (Note: gag = p55gag. The plot shows the number of CE recognized by each macaque immunized with different vaccine regimens, counting both CD4 and CD8 T cells recognizing CE peptides. Recognition was defined by being >2x the no peptide control and greater than 0.01% of the total T cells. The median number CE, range of CE responses, and the number of animals analyzed are indicated. A comparison of the CE breadth using different vaccine regimens are shown including HIV CE pDNA only, HIV CE prime/gag pDNA boost, HIV CE prime/CE+gag pDNA boost, or HIV gag pDNA. P values are from ANOVA (Dunnett's test).



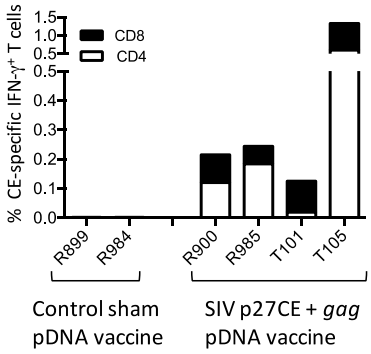
Immunogenicity of the CE pDNA vaccine regimen in SIV-infected ART-treated macaques.

We previously showed that therapeutic SIV pDNA vaccination induces potent immune responses able to significantly reduce viremia after ART interruption [46, 47]. Importantly, in these studies we reported that despite initial rebounds after ART interruption, viral loads then decreased dramatically in many DNA-vaccinated animals, resulting in significant long-term decreases in viremia.

We hypothesize that CE pDNA vaccine could contribute to cure by inducing broad, effective immunity with less likelihood of immune escape. To test the CE vaccine concept as a potential therapeutic approach in the macaque model, we engineered SIV p27CE homologs to the HIV p24CE immunogens [29]. Analogous immunogenicity studies were conducted with the SIV p27CE in macaques and the results mirrored those obtained with the HIV constructs. The immunogenicity of the p27CE pDNA vaccine regimen was further tested in SIV_{mac251}-infected macaques treated with cART (Gilead, ViiV) for 10 months. Four of the animals received the SIV p27CE DNA vaccine regimen (priming with p27CE, two-booster vaccination with p27CE+$p57^{gag}$ pDNA), two macaques received sham pDNA (Figure 2.2-6), and all were analyzed for CE-specific T cell responses 2 weeks later. The two sham DNA vaccinated macaques showed no T cell

responses to CE (Figure 2.2-6) or total Gag (not shown), as expected, due to successful cART treatment. Robust T cell responses to CE reaching more than 1% of T cells in blood (Figure 2.2-6) as well as to complete Gag (not shown) were induced upon vaccination.

Figure 2.2-6: Immunogenicity of CE of pDNA vaccine in cART-treated macaques. Six macaques were infected with SIV_{mac251} for 3 months and treated with cART (Gilead, ViiV) for 10 months, and were vaccinated 2x with p27CE pDNA vaccine regimen (N=4) or sham DNA (N=2) via IM electroporation. Animals were primed with 1 mg of p27CE pDNA and boosted with 1 mg p27CE and 1 mg *gag* pDNA. T cell responses were measured 2 weeks after the third vaccination using a CE-specific peptide pool.



Rationale for Placebo

Unlike in HIV-negatives, the continued but variable expression of HIV-1 antigens from cells otherwise latently infected with HIV-1, and perhaps continued low-level viral replication in tissues, probably continue to stimulate immune responses in persons on ART, possibly including those we would like to induce with this vaccine regimen. This natural fluctuation in HIV-antigen-specific immune responses over time in HIV-infected individuals necessitates having a control group receiving placebo to which the immune responses induced in the vaccine groups will be compared.

Rationale for Full-length gag DNA vaccine alone arm of the study

We hypothesize that the initial administration of p24CE *gag* DNA vaccine to HIV-infected persons on ART will focus newly induced immune responses to immunogenic conserved epitopes of Gag and away from decoy variable epitopes. The inclusion of the comparison arm in which only full-length *gag*DNA vaccine is given is critical to test this hypothesis.

Rationale for the microbiome section of the study

The gut microbiome appears to play a role in directing T cell responses. Mice in a melanoma model with high spontaneous antitumor immunity were noted to have a gut microbiota enriched with Bifidobacterium [48]. Oral administration of Bifidobacterium improved tumor control similar to administration of a PD-1 blocker, and this effect was amplified when mice were treated with both PD-1 blocker and oral Bifidobacterium. Tumor control appears to be driven by CD8 T cell infiltration into the tumor. Two species

of Bacteroides also appear to be associated with response to CTLA-4 blockade [49]. Germ-free mice and those treated with antibiotics did not have tumor response to CTLA-4 blockade. Response to CTLA-4 blockade was restored by immunization and gavage with *Bacteroides fragilis* polysaccharides. It remains unclear whether T cells are primed by interaction with microbiota and their products or whether there is cross-reactivity between microbial and melanoma antigens.

The influence of gut microbiota on B cell responses is complex and understudied. Cross-reactivity between bacterial and HIV antigens reduced the efficacy of antibody responses to previous HIV vaccine. Analysis of antibody responses to an HIV-1 DNA prime vaccine demonstrated that vaccine-induced monoclonal antibodies against gp41 were non-neutralizing and frequently polyreactive with host and environmental antigens [50]. The vaccine induced anti-gp41 antibody reacted with whole cell lysates of several anaerobic and aerobic intestinal microbiota. There appears to be a pre-existing pool of B cells that are cross reactive between intestinal microbiota and gp41. Studies in mice suggest that interaction with commensal microbes may be necessary for the development of neutralizing antibody to influenza vaccine [51]. Toll-like receptor (TLR)-deficient mice had inadequate plasma cell responses to influenza vaccine; this trend was also seen in germ-free and antibiotic-treated mice. Flagellated *E. coli* administered orally restored vaccine response but aflagellated *E. coli* did not, consistent with TLR-based sensing of flagellar proteins.

Stool microbiota analysis in elite controllers has suggested that gut microbes may contribute to CD8 T cell control of HIV. Nowak et al. found in principal coordinate analysis based on beta-diversity results that elite controllers clustered tightly together separate from either healthy controls or viremic participants [52]. These elite controllers had significantly lower weighted unifrac diversity. Elite controllers also had a significantly higher prevalence of the phylum Bacteroidetes and lower prevalence of Proteobacteria and Actinobacteria compared to viremic participants.

We hypothesize that participants with a more robust T cell response to p24CE1/2 pDNA vaccine will have a higher prevalence of the genus Bifidobacterium and/or the genus Bacteroides and lower weighted unifrac diversity of the stool microbiome.

3.0 STUDY DESIGN

A5369 is a phase I/IIa, randomized, double-blinded, placebo-controlled study in which 40 study participants will be randomized 2:1:1 to the p24CE/full-length *gag*DNA vaccine arm versus full-length *gag*DNA vaccine arm versus placebo arm. HIV-specific immunologic assays will be done at baseline and after the last dose of vaccine. Cells and plasma will also be obtained after the second prime vaccine dose, processed, and stored. Immunologic assays may be performed at this timepoint depending on the results of the assays done at baseline and after the last dose of vaccine. HIV-1 reservoir assays will be done at baseline and after the last dose of vaccine. Enrollment slots for participants agreeing to have leukaphereses will be reserved for a minimum of 20 participants. These participants are required to have leukapheresis at both the pre-entry and week 26 visit.

4.0 SELECTION AND ENROLLMENT OF PARTICIPANTS

4.1 Inclusion Criteria

4.1.1 HIV-1 infection, documented by any licensed rapid HIV test or HIV enzyme or chemiluminescence immunoassay (E/CIA) test kit at any time prior to study entry and confirmed by a licensed Western blot or a second antibody test by a method other than the initial rapid HIV and/or E/CIA, or by HIV-1 antigen, plasma HIV-1 RNA assay.

NOTE: The term “licensed” refers to a U.S. FDA-approved kit, which is required for all IND studies.

WHO (World Health Organization) and CDC (Centers for Disease Control and Prevention) guidelines mandate that confirmation of the initial test result must use a test that is different from the one used for the initial assessment. A reactive initial rapid test should be confirmed by either another type of rapid assay or an E/CIA that is based on a different antigen preparation and/or different test principle (e.g., indirect versus competitive), or a Western blot or a plasma HIV-1 RNA viral load.

4.1.2 Receiving a stable ART regimen for a minimum of 2 years prior to study entry and with no changes in the components of their antiretroviral therapy for at least 90 days prior to study entry. One of the agents must include an integrase inhibitor, non-nucleoside reverse transcriptase inhibitors (NNRTI), or a boosted-protease inhibitor (PI).

NOTE: Changes in the ART regimen for reasons other than virologic breakthrough during the 2-year period are acceptable.

4.1.3 CD4 cell count >500 cells/mm^3 obtained within 60 days prior to study entry at any U.S. laboratory that has a CLIA certification or its equivalent.

4.1.4 Nadir CD4 cell count >350 cells/mm^3.

NOTE: Candidate recall or documentation is acceptable.

4.1.5 One documented plasma HIV-1 RNA that is below the limit of detection of an FDA-approved assay between 24 and 36 months prior to the screening HIV-1 RNA, one documented plasma HIV-1 RNA that is below the limit of detection of an FDA-approved assay between 12 and 24 months prior to the screening HIV-1 RNA, and one documented HIV-1 RNA that is below the limit of detection of an FDA-approved assay collected fewer than 12 months prior to the screening HIV-1 RNA (see section 4.1.6).

NOTE: A single, unconfirmed plasma HIV-1 RNA above the limit of detection but <400 copies/mL is allowed if followed by an HIV-1 RNA below detectable limits, but not in the 6 months prior to screening.

4.1.6 Plasma HIV-1 RNA level that is below the limit of detection of an FDA-approved assay within 60 days prior to study entry.

4.1.7 The following laboratory values obtained within 60 days prior to entry by any U.S. laboratory that has a CLIA certification or its equivalent:

- Absolute neutrophil count (ANC) ≥750 cells/mm^3
- Hemoglobin ≥10.0 g/dL for men and ≥9.0 g/dL for women
- Platelet count ≥100,000/mm^3
- Prothrombin time (PT), partial thromboplastin time (PTT), and INR <1.5 x upper limit of normal (ULN)
- Creatinine clearance ≥50 mL/min estimated by the Cockcroft-Gault equation

NOTE: A program for calculating creatinine clearance by the Cockcroft-Gault method is available on www.fstrf.org.

- Alanine aminotransferase (ALT) (SGPT) ≤2.5 x ULN
- Total bilirubin <1.6 x ULN (if on atazanavir ≤5 x ULN)

4.1.8 HCV antibody negative result within 60 days prior to study entry or, if the HCV antibody result is positive, a negative HCV RNA result prior to study entry.

4.1.9 Negative HBsAg result obtained within 60 days prior to study entry.

4.1.10 Men and women age ≥18 to ≤65 years

4.1.11 Documentation of the availability of the stored pre-entry peripheral blood mononuclear cell (PBMC) specimens for T cell assays. Sites must receive confirmation from the processing lab via phone, e-mail, or fax that specimens have been entered into the ACTG Laboratory Data Management System (LDMS).

4.1.12 Ability and willingness of participant or legal guardian/representative to provide informed consent

4.1.13 Ability and willingness of participant to continue cART throughout the study.

4.1.14 For females of reproductive potential, negative serum or urine pregnancy test within 15 days prior to entry by any clinic or laboratory that has a CLIA certification or its equivalent, or is using a point of care (POC)/CLIA-waived test.

NOTE: Reproductive potential is defined as girls who have reached menarche and women who have not been post-menopausal for at least 12 consecutive months with follicle-stimulating hormone (FSH) ≥40 IU/mL or 24 consecutive

months if an FSH is not available, or have not undergone surgical sterilization (e.g., hysterectomy, bilateral oophorectomy, tubal ligation, or salpingectomy).

4.1.15 If participating in sexual activity that could lead to pregnancy, willingness of female participants to use two forms of effective contraception while receiving study medication and for 3 months after stopping study medication is required.

NOTE A: Effective forms of contraception include:

- Barrier methods (condoms [male or female] with or without a spermicidal agent, diaphragm, or cervical cap [with spermicide])
- Hormone-based contraception (oral, patch, parenteral, implants, or vaginal ring)
- Intrauterine device (IUD)

NOTE B: If the female participant is not of reproductive potential (women who are post-menopausal as defined above, or women who have undergone surgical sterilization [e.g., hysterectomy, bilateral oophorectomy, tubal ligation or salpingectomy]), she is eligible without requiring the use of a contraceptive method. Acceptable documentation of surgical sterilization and menopause is participant-reported history.

4.1.16 Indication of willingness to have the leukapheresis procedure.

NOTE: Leukapheresis will be required if the study has reached 50% of the accrual target and less than 20 participants have agreed to have the leukapheresis procedure.

4.2 Exclusion Criteria

4.2.1 History of malignancy within the last 5 years prior to study entry or current malignancy requiring cytotoxic therapy.

NOTE: A history of non-melanoma skin cancer (e.g., basal cell carcinoma or squamous cell skin cancer) is not exclusionary.

4.2.2 History of HIV-related opportunistic infections within the last 5 years prior to study entry.

NOTE: The CDC classifications are available on the A5369 protocol-specific webpage (PSWP).

4.2.3 History of or active autoimmune disorders including but not limited to inflammatory bowel diseases, scleroderma, severe psoriasis, myocarditis, uveitis, pneumonitis, systemic lupus erythematosus, rheumatoid arthritis, optic neuritis, myasthenia gravis, adrenal insufficiency, autoimmune thyroiditis, or sarcoidosis.

NOTE: For questions related to the definition of autoimmune disorders, sites should contact the A5369 core team per the Study Management section.

4.2.4 Bleeding diathesis or condition associated with prolonged bleeding time that would contraindicate IM injection.

4.2.5 A skin-fold measurement of the cutaneous and subcutaneous tissue for eligible injection sites (on the medial deltoid muscles) that exceeds 40 mm.

NOTE: The skin-fold measurement must be conducted in accordance with the procedure described in the TDS-IM Instructions for Use (see A5369 MOPS).

4.2.6 Use of any prior HIV vaccine (prophylactic and/or therapeutic) within 1 year prior to study entry.

NOTE: A documented study placebo recipient may participate.

4.2.7 Use of any investigational treatment within 6 months prior to study entry.

4.2.8 Any licensed or experimental non-HIV vaccination (e.g., hepatitis B, influenza, pneumococcal polysaccharide) within 4 weeks prior to study entry.

NOTE: Participants with anticipated need to receive non-HIV vaccinations within 2 weeks prior to the scheduled study vaccination #2 (week 4), or #3 (week 12), or #4 (week 24) injection should be excluded.

4.2.9 Use of any infusion blood product or immune globulin within 3 months prior to study entry.

4.2.10 Acute or serious illness, in the opinion of the site investigator, requiring systemic treatment and/or hospitalization within 30 days prior to entry.

4.2.11 Use of immunomodulators (e.g., interleukins, interferons, cyclosporine), systemic cytotoxic chemotherapy, or investigational therapy within 60 days prior to study entry.

NOTE: Participants receiving stable physiologic glucocorticoid doses, defined as prednisone ≤10 mg/day or the equivalent, will not be excluded. Stable physiologic glucocorticoid doses should not be discontinued for the duration of the study. In addition, participants receiving inhaled or topical corticosteroids will not be excluded.

4.2.12 Intent to use immunomodulators (e.g., IL-2, IL-12, interferons, or TNF modifiers) during the course of the study.

4.2.13 Known or suspected hypersensitivity to any vaccine component, including hypersensitivity to amide-type local anesthetics, such as lidocaine (Xylocaine),

mepivacaine (Polocaine/Carbocaine), etidocaine (Duranest), bupivacaine (Marcaine), or prilocaine.

4.2.14 Current use of any electronic stimulation device, such as cardiac demand pacemakers, automatic implantable cardiac defibrillator, nerve stimulators, or deep brain stimulators.

4.2.15 History of cardiac arrhythmia or palpitations (e.g., supraventricular tachycardia, atrial fibrillation, frequent ectopy, or sinus bradycardia [i.e., <50 beats per minute on exam]) prior to study entry.

NOTE: Sinus arrhythmia is not excluded.

4.2.16 History of syncope or fainting episode within 1 year of study entry.

4.2.17 Seizure disorder or any history of prior seizure.

4.2.18 Extensive tattoos covering the site of administration (upper left and right medial deltoid muscles).

4.2.19 Presence of any surgical or traumatic metal implants at the site of administration (medial deltoid muscles).

4.2.20 Immune deficiency other than HIV.

4.2.21 Breastfeeding or pregnancy.

4.2.22 Active drug or alcohol use or dependence that, in the opinion of the site investigator, would interfere with adherence to study requirements.

4.2.23 Current HCV antiviral therapy.

4.2.24 Type I or type II diabetes mellitus.

4.2.25 Weight <50 kg or >200 kg.

4.2.26 Known to have been started on antiretroviral therapy within 3 months of the presumed or known date of first acquiring HIV-1 infection; i.e., treated during acute HIV-1 infection.

4.3 Study Enrollment Procedures

4.3.1 Prior to implementation of this protocol, and any subsequent full version amendments, each site must have the protocol and the protocol consent form(s) approved, as appropriate, by their local institutional review board (IRB)/ethics committee (EC) and any other applicable regulatory entity (RE). Upon receiving final approval, sites will submit all required protocol registration documents to the DAIDS Protocol Registration Office (DAIDS PRO) at the Regulatory Support

Center (RSC). The DAIDS PRO will review the submitted protocol registration packet to ensure that all of the required documents have been received.

Site-specific informed consent forms (ICFs) WILL be reviewed and approved by the DAIDS PRO, and sites will receive an Initial Registration Notification from the DAIDS PRO that indicates successful completion of the protocol registration process. A copy of the Initial Registration Notification should be retained in the site's regulatory files.

Upon receiving final IRB/EC and any other applicable RE approvals for an amendment, sites should implement the amendment immediately. Sites are required to submit an amendment registration packet to the DAIDS PRO at the RSC. The DAIDS PRO will review the submitted protocol registration packet to ensure that all required documents have been received. Site-specific ICF(s) WILL NOT be reviewed and approved by the DAIDS PRO, and sites will receive an Amendment Registration Notification when the DAIDS PRO receives a complete registration packet. A copy of the Amendment Registration Notification should be retained in the site's regulatory files.

For additional information on the protocol registration process and specific documents required for initial and amendment registrations, refer to the current version of the DAIDS Protocol Registration Manual.

Once a candidate for study entry has been identified, details will be carefully discussed with the candidate. The candidate (or, when necessary, the legal representative if the candidate is under guardianship) will be asked to read and sign the approved protocol consent form.

For participants from whom a signed informed consent has been obtained, an ACTG Screening Checklist must be entered through the DMC Participant Enrollment System.

4.3.2 Protocol Activation

Prior to enrollment, sites must complete the Protocol Activation Checklist found on the ACTG Member website. This checklist must be approved prior to any screening of participants for enrollment.

4.3.3 Randomization/Participant Registration

For participants from whom informed consent has been obtained, but who are deemed ineligible or who do not enroll into the initial protocol step, an ACTG Screening Failure Results form must be completed and keyed into the database. Participants who meet the enrollment criteria will be registered to the study according to standard ACTG DMC procedures.

4.4 Co-enrollment Guidelines

- US sites are encouraged to co-enroll participants in A5128, “Plan for Obtaining Informed Consent to Use Stored Human Biological Materials (HBM) for Currently Unspecified Analyses.” Co-enrollment in A5128 does not require permission from the A5369 protocol chairs.
- For specific questions and approval for co-enrollment in other studies, sites should first check the PSWP or contact the protocol team via e-mail as described in the Study Management section.

5.0 STUDY TREATMENT

CRS pharmacists should consult the Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks for standard pharmacy operations.

5.1 Regimens, Administration, and Duration

ARM 1: p24CE1/2 pDNA 4 mg administered by one injection/electroporation at week 0 and week 4

Then

p24CE1/2 pDNA 2 mg admixed with full-length p55^{gag} pDNA 2 mg administered by one injection/electroporation at week 12, and week 24

ARM 2: full-length p55^{gag} pDNA 4 mg administered by one injection/electroporation at week 0, week 4, week 12, and week 24

ARM 3: Placebo (Sodium Chloride for Injection, USP 0.9%) 1 mL administered by one injection/electroporation at week 0, week 4, week 12, and week 24

5.2 Study Product Formulation and Preparation

Study Product

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

Store at -20˚C

The product is contraindicated in participants with known hypersensitivity to bupivacaine. Provided by DAIDS, NIAID, NIH, DHHS (Bethesda, MD, USA).

Please refer to the Investigator’s Brochure for additional information.

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

Store at -20˚C

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

Please refer to the Investigator's Brochure for additional information.

Provided by DAIDS, NIAID, NIH, DHHS (Bethesda, MD, USA)

Placebo
Sodium Chloride for Injection, USP 0.9% will not be provided by the study and must be obtained by the site. Pharmacists must record the lot numbers of the Sodium Chloride for Injection, USP 0.9% used to prepare the placebo injections.

Empty Sterile Mixing Vials will be required for this protocol and will not be provided by the study.

Intramuscular TriGrid Delivery System (TDS-IM v2.0) (ICHOR Medical Systems, San Diego, California), a device for electroporation mediated intramuscular administration of DNA based-biologic candidates. TDS-IM v2.0 Stimulator and Applicator which are reusable components, will be provided to the clinic by arrangement with the ACTG Leadership and Operations Center.

TDS-IM v2.0 Cartridges are sterile, single-use components specifically designed for use with the TDS-IM v2.0 system. Each Cartridge consists of a plastic, injection-molded body, which encloses the 4-electrode TriGrid™ array and the 22-gauge injection needle. Each Cartridge interfaces with a compatible glass 1 mL syringe containing the dose for delivery. TDS-IM v2.0 single-use Cartridge and single-pack sterile glass syringes will be provided by ICHOR through arrangement by the ACTG Leadership and Operations Center.

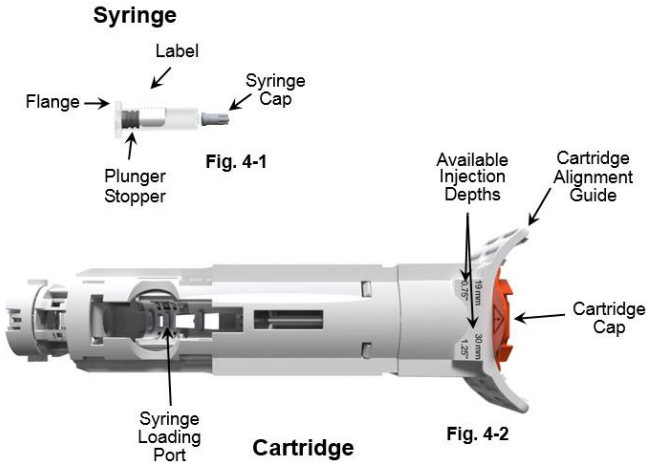
Refer to the Ichor Intramuscular TriGrid Delivery System Instructions for more detailed information.

Preparation of Study Products
Prior to mixing the study products, the pharmacist will inspect the sterile pouch containing the TriGrid™ Application Cartridge for integrity and verify the Cartridge has not passed expiration. If any tears or gaps in the sterile pouch are noted or if the expiration date has been reached, the Application Cartridge should be placed in quarantine and the protocol pharmacist should be informed. Note: In the event that the Cartridge Cap has become detached from the Cartridge in the sterile pouch, the Cartridge may still be used if the Cartridge Cap can be replaced without contaminating

the patient contact portion of the Cartridge.

Open the cartridge in the aseptic preparation area with the Tyvek shield down and ridged plastic cover up so that the syringe loading port is up.
See Figure 5.2-1 below.

Figure 5.2-1: Directions for Preparing the Glass Syringe for each Treatment Arm.



With the syringe tip up, carefully unscrew the plunger barrel clockwise to detach the plunger barrel from the plunger stopper. Note: At first, the barrel feels like it will not release but keep turning and it will unscrew.

Load the glass syringe into the cartridge. The flange on the glass syringe has two flat sides. Hold the flat sides with your finger tips and line the flange up with the slots in the port (away from the orange cartridge cap) and gently drop into place. Gently push down on the barrel of the syringe until you feel the syringe click into place. The syringe tip does NOT have to be inserted into the slip-tip of the needle but instead it will be lined up with the needle. The applicator will push the syringe forward to attach with the needle.

Label the cartridge as ACTG 5369 Study Product 4 mg or Placebo 1 mL and include a 4-hour expiration date from the time the vial is removed from the refrigerator/freezer. The label must also contain the words “Administer as soon as possible.” Attach the label so that it can be easily removed for loading into the applicator. It is suggested to use the outside of the curved cartridge alignment guide. Attach a duplicate label to a zip-lock plastic bag, and place the cartridge in the plastic bag to dispense to the clinician. Any unused portion of reconstituted vials or expired prefilled syringes must be disposed of in accordance with institutional or pharmacy policy.

ARM 1: Week 0 and Week 4 p24CE1/2. Two vials of p24CE1/2 pDNA (4 mg/mL) will be needed to prepare the syringe.

Remove two vials of p24CE1/2 pDNA from the freezer and allow to thaw at room temperature for 15-30 minutes until no crystals are observed.

Gently swirl and invert the vials for at least 10 inversions (do not shake vigorously). Inspect the vial prior to use. If the vial contains material different from its description above, do not use the vial and contact the protocol pharmacist.

Remove syringe cap from the glass syringe and add a needle. Using aseptic technique, withdraw sufficient quantity fluid from each of the two vials of p24CE1/2 pDNA (4mg/mL) to load the glass syringe with 1 mL. Holding glass syringe upright, remove the needle. Pull the plunger out partway to allow air space to develop at the top. Push the fluid to the top of the syringe.

ARM 1 Week 12 and Week 24 p24CE1/2 admixed with $p55^{gag}$. One vial of p24CE1/2 pDNA (4 mg/mL), one vial of $p55^{gag}$ pDNA (4 mg/mL), and one empty 5 mL sterile vial will be needed to prepare the dose.

Remove one vial of p24CE1/2 pDNA and one vial of $p55^{gag}$ pDNA from the freezer and allow to thaw at room temperature for 15 to 30 minutes until no crystals are observed.

Gently swirl and invert the vials for at least 10 inversions (do not shake vigorously). Inspect the vials prior to use. If the vial contains material different from its description above, do not use the vial and contact the protocol pharmacist.

Using aseptic technique, withdraw 0.6 mL from the vial of p24CE1/2 pDNA (4 mg/mL) with a 1 mL low void 25 Ga syringe and inject into an empty mixing sterile vial. Withdraw 0.6 mL from the vial of $p55^{gag}$ pDNA (4 mg/mL) with a 1 mL low void 25 Ga syringe and inject this into the mixing. Gently swirl and invert the vial for 10 inversions to mix.

Remove syringe cap from the glass syringe and add a needle. Withdraw 1 mL of the admixture. Holding glass syringe upright, remove the needle. Pull the plunger out part way to allow air space to develop at the top. Push the fluid to the top of the glass syringe. Load the glass syringe into the cartridge. See directions above.

Label the cartridge as ACTG 5369 Study Product 4 mg or Placebo 1 mL and include a 4-hour expiration date from the time the vial is removed from the refrigerator/freezer. The label must also contain the words “Administer as soon as possible.” Any unused portion of reconstituted vials or expired prefilled syringes must be disposed of in accordance with institutional or pharmacy policy.

ARM 2: Week 0, Week 4, Week 12, and Week 24 $p55^{gag}$ pDNA. Two vials of $p55^{gag}$ pDNA (4 mg/mL) will be needed to prepare the dose.

Remove two vials of $p55^{gag}$ pDNA from the freezer and allow to thaw at room temperature for 15 to 30 minutes until no crystals are observed.

Gently swirl and invert the vials for at least 10 inversions (do not shake vigorously). Inspect the vials prior to use. If the vial contains material different from its description above, do not use the vial and contact the protocol pharmacist.

Remove syringe cap from the glass syringe and add a slip-tip needle. Using aseptic technique, withdraw sufficient quantity fluid from each of the two vials of p24CE1/2 pDNA (4 mg/mL) to load the glass syringe with 1 mL. Holding glass syringe upright, remove the needle. Pull the plunger out partway to allow air space to develop at the top. Push the fluid to the top of the syringe.

Load the glass syringe into the cartridge. See directions above.

Label the cartridge as ACTG 5369 Study Product 4 mg or Placebo 1 mL and include a 4-hour expiration date from the time the vial is removed from the refrigerator/freezer. The label must also contain the words “Administer as soon as possible.” Any unused portion of reconstituted vials or expired prefilled syringes must be disposed of in accordance with institutional or pharmacy policy.

ARM 3: Placebo Week 0, Week 4, Week 12, and Week 24. One vial Sodium Chloride for Injection, USP 0.9% will be needed to prepare the syringes.

The vial should be visually inspected prior to use.

Remove syringe cap from the glass syringe and add a needle. Using aseptic technique, withdraw 1 mL from the vial with the glass syringe to load the glass syringe with 1 mL. Holding glass syringe upright, remove the needle. Pull the plunger out partway to allow air space to develop at the top. Push the fluid to the top of the syringe

Load the glass syringe into the cartridge. See directions above.

Label the cartridge as ACTG 5369 Study Product 4 mg or Placebo 1 mL and include a 4-hour expiration date from the time the vial is removed from the refrigerator/freezer. The label must also contain the words “Administer as soon as possible.” Any unused portion of reconstituted vials or expired prefilled syringes must be disposed of in accordance with institutional or pharmacy policy.

5.3 Pharmacy: Product Supply, Distribution, and Accountability

5.3.1 Study Product Acquisition/Distribution

p24CE1/2 pDNA (4 mg/mL) and p55^{gag} pDNA (4 mg/mL) will be available through the NIAID Clinical Research Products Management Center (CRPMC). The site pharmacist should obtain the study products for this protocol by following the instructions in the manual *Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks.*

5.3.2 Study Product Accountability

The site pharmacist is required to maintain complete records of all study products received from the NIAID CRPMC and subsequently dispensed. All unused study products in U.S. CRSs must be returned to the NIAID CRPMC (or as otherwise directed by the sponsor) after the study is completed or terminated. The procedures to be followed are in the manual *Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks.*

5.4 Concomitant Medications

Whenever a concomitant medication or study agent is initiated or a dose changed, investigators must review the concomitant medication's and study agent's most recent package insert, Investigator's Brochure, or updated information from DAIDS to obtain the most current information on drug interactions, contraindications, and precautions.

Additional drug information may be found on the ACTG Precautionary and Prohibited Medications Database located at http://tprc.pharm.buffalo.edu/home/di_search/.

5.4.1 Required Medications

All participants are required to take their current antiretroviral (ARV) regimen until study completion. ARV medications will not be provided by the study.

If the participant develops toxicity related to his/her previously-stable ART, then the protocol team should be consulted (preferably before appropriate therapy modification).

5.4.2 Prohibited Medications

For a list of prohibited medications, refer to the A5369 PSWP.

5.4.3 Precautionary Medications

A list of precautionary medications can be generated using the ACTG Precautionary and Prohibited Medications Database located at http://tprc.pharm.buffalo.edu/home/di_search/.

6.0 CLINICAL AND LABORATORY EVALUATIONS

6.1 Schedule of Evaluations

Evaluation	Screening	Pre-Entry	Entry (Week 0)	Post-Entry Evaluations (Weeks)													Premature Treatment/Study Discontinuation Evaluations
				Post Vaccination #1	4	Deferred Week 4 (See section 6.3.10)	Post Vaccination #2	6	12	Deferred Week 12 (See section 6.3.10)	Post Vaccination #3	24	Deferred Week 24 (See section 6.3.10)	Post Vaccination #4	26	48	
Documentation of HIV	X																
Medical History	X		X														
Medication History	X		X														
Clinical Assessment	X		X		X	X			X	X		X	X		X	X	X
Skin Pinch	X																
EKG	X	If Clinically Indicated															
Hematology	X		X		X	X			X	X		X	X		X	X	X
Liver Function Tests	X		X		X	X			X	X		X	X			X	X
Blood Chemistries	X		X		X	X			X	X		X	X			X	X
Calculated Creatinine Clearance	X		X		X	X			X	X		X	X			X	X
Urinalysis	X		X		X	X			X	X		X	X				X

Evaluation	Screening	Pre-Entry	Entry (Week 0)	Post-Entry Evaluations (Weeks)													Premature Treatment/Study Discontinuation Evaluations
				Post Vaccination #1	4	Deferred Week 4 (See section 6.3.10)	Post Vaccination #2	6	12	Deferred Week 12 (See section 6.3.10)	Post Vaccination #3	24	Deferred Week 24 (See section 6.3.10)	Post Vaccination #4	26	48	
PT, PTT, and INR	X																
Pregnancy Testing	X	X	X		X	X			X	X		X	X				
Hepatitis B and C Assessment	X																
CD4+/CD8+	X	X	X						X	X		X	X				X
Advanced Flow Cellular Immune Responses		X	X					X							X	X	X
Phenotyping of Immune Activation Markers		X	X					X							X	X	X
Humoral Immune Responses		X	X					X							X	X	X
Soluble Activation Markers		X	X					X							X	X	X
Viral Inhibition Assay		X	X					X							X	X	X
HLA-Typing		X															
Stored Plasma and PBMC		X	X					X							X	X	X
HIV-1 RNA	X		X						X	X		X	X			X	X

Evaluation	Screening	Pre-Entry	Entry (Week 0)	Post-Entry Evaluations (Weeks)													Premature Treatment/Study Discontinuation Evaluations
				Post Vaccination #1	4	Deferred Week 4 (See section 6.3.10)	Post Vaccination #2	6	12	Deferred Week 12 (See section 6.3.10)	Post Vaccination #3	24	Deferred Week 24 (See section 6.3.10)	Post Vaccination #4	26	48	
Plasma HIV-1 RNA Single Copy Assay (SCA)		X													X		
CD4 T Cell Associated HIV-1 RNA/DNA		X													X		
Whole Viral Genome Sequencing		X													X		
CD4 T Cell Purification from PBMC		X													X		
Study Vaccine/ Placebo Administration and Evaluation			X		X	X			X	X		X	X				
Telephone Contact				X			X				X			X			
Vaccination Report Card Distribution and Collection			X		X	X			X	X		X	X				X
Tolerability Assessment			X		X	X			X	X		X	X				X
Leukapheresis (Optional)		X													X		

Evaluation	Screening	Pre-Entry	Entry (Week 0)	Post-Entry Evaluations (Weeks)													Premature Treatment/Study Discontinuation Evaluations
				Post Vaccination #1	4	Deferred Week 4 (See section 6.3.10)	Post Vaccination #2	6	12	Deferred Week 12 (See section 6.3.10)	Post Vaccination #3	24	Deferred Week 24 (See section 6.3.10)	Post Vaccination #4	26	48	
Stool (Optional)		X													X		X

6.2 Timing of Evaluations

6.2.1 Screening and Pre-Entry Evaluations

Screening and pre-entry evaluations must occur at least 24 hours apart and prior to the participant starting any study treatments.

Screening
Screening evaluations to determine eligibility must be completed within 60 days prior to study entry unless otherwise specified.

In addition to data being collected on participants who enroll into the study, demographic, clinical, and laboratory data on screening failures will be captured in a Screening Failure Results form and entered into the ACTG database.

Pre-Entry
Pre-entry evaluations must be completed at least 24 hours after screening evaluations have been completed and at least 24 hours prior to entry evaluations unless otherwise specified.

6.2.2 Entry Evaluations

Entry evaluations must occur at least 24 hours after pre-entry evaluations unless otherwise specified. Participant must begin treatment within 72 hours after study enrollment.

6.2.3 Post-Entry Evaluations

Post-entry evaluations occur after the study entry visit and vaccination #1 administration at week 0. Study visits are scheduled as indicated in the SOE.

The visit window for the post-entry evaluations is ±14 days.

NOTE A: The deferred study vaccinations #2, #3, and #4 should be performed within 14 days from the time of the previously scheduled week 4, week 12, and week 24 visit, respectively (see section 6.3.10).

NOTE B: After study entry, administration of non-HIV vaccines must occur more than 2 weeks prior to the scheduled study vaccination #2 (week 4), #3 (week 12), and #4 (week 24) injections.

Study Completion Evaluations
The week 48 evaluations will be completed as the participant's final on study visit.

6.2.4 Discontinuation Evaluations

Evaluations for Randomized Participants Who Do Not Start Vaccination at Entry
Participants who are enrolled, but who do not receive vaccination #1 will be taken off study. No further evaluations are required. These participants will be replaced.

All eCRFs must be keyed for the period up to and including the entry visit.

Premature Treatment Discontinuation Evaluations
Participants who prematurely discontinue study treatment prior to receiving vaccinations #2 or #3 should remain on study (off study treatment) until the final visit. No further vaccinations should be administered. The participant should complete the premature treatment/study discontinuation evaluations as specified in the SOE. If the premature treatment discontinuation visit coincides with a regularly scheduled visit, the evaluations should be combined. Participants should complete the safety evaluations (clinical assessments, hematology, chemistries, and VRC review) per protocol until study completion.

NOTE: Participants who discontinue study treatment prior to receiving vaccination #2 may be replaced. Sites should contact the core team.

Premature Study Discontinuation Evaluations
Participants who discontinue the study prior to the final visit should complete the premature treatment/study discontinuation evaluations as specified in the SOE.

6.3 Instructions for Evaluations

All clinical and laboratory information required by this protocol is to be present in the source documents. Sites must refer to the Source Document Guidelines on the DAIDS website for information about what must be included in the source document: https://www.niaid.nih.gov/sites/default/files/sourcedocappndx.pdf .

All stated evaluations are to be recorded on the eCRF unless otherwise specified. Refer to section 7.0 for information on the DAIDS AE Grading Table and AE reporting of adverse events requirements.

6.3.1 Documentation of HIV-1

Section 4.1.1 specifies assay requirements for HIV-1 documentation. HIV-1 documentation is not recorded on the eCRF.

6.3.2 Medical History

The medical history must include all signs and symptoms regardless of grade and all diagnoses identified by the ACTG criteria for clinical events and other

diagnoses regardless of grade within the past 30 days. In addition, the following diagnoses should be recorded regardless of when the diagnosis was made:

- AIDS-defining conditions
- Bone fractures (verbal history accepted)
- Coronary heart disease
- Cancer (exclusive of basal/squamous cell skin cancer)
- Diabetes
- Tuberculosis
- Chronic hepatitis C
- Chronic hepatitis B

Any allergies to any medications and their formulations must also be documented.

Document the nadir CD4 count if available. If nadir documentation is not available, then collect and record participant recall.

6.3.3 Medication History

A medication history must be present, including start and stop dates. The table below lists the medications that must be included in the history.

Table 6.3.3-1: Medication History

Medication Category	Complete History or Timeframe
Antiretroviral therapy	2 years prior to entry
Immune-based therapy	1 year prior to entry
Blinded study treatment	1 year prior to entry
HIV-1-related vaccines	Complete history
Prescription drugs for treatment of opportunistic infections	1 year prior to entry
Prescription drugs for prophylaxis of opportunistic infections	1 year prior to entry
Prescription drugs (other)	1 year prior to entry
Nonprescription drugs	30 days prior to entry
Alternative therapies	30 days prior to entry
Dietary supplements	30 days prior to entry
Sex-hormone medications or sex-hormone analogues or antagonists*	Last 12 months except as noted below

*Includes: hormone-releasing IUDs (e.g., Mirena inserted in the last 5 years); oral, injectable, implanted, or patch contraceptives; vaginal ring, creams, or inserts; estrogen, progesterone, or testosterone therapy; leuprolide or other

synthetic gonadotropin-releasing hormone; tamoxifen, raloxifene, aromatase inhibitors or any other androgen, estrogen, or progesterone analogue or antagonist therapy.

6.3.4 Clinical Assessments

Complete Physical Examination
A complete physical examination is done at screening and is to include at a minimum an examination of the skin, head, mouth, and neck; auscultation of the chest; cardiac examination; abdominal examination; and examination of the lower extremities for edema. The complete physical examination will also include signs and symptoms, diagnoses, and vital signs (height, weight, temperature, pulse, respiration rate, and blood pressure).

Targeted Physical Examination
A targeted physical examination is done at all visits after screening and is to include vital signs (weight, temperature, pulse, respiration rate, and blood pressure), and is to be driven by any previously identified or new adverse event/targeted condition , that the participant has experienced since the last visit or at this visit.

At screening, pre-entry, and entry, refer to section 6.3.2, Medical History, for reporting requirements.

Post entry, record the following targeted event:
- Uterine Pregnancy

Post-entry, refer to section 7.2 for AE collection requirements.

Concomitant Medications
Post-entry, the following new and discontinued concomitant medications must be recorded on the eCRFs:
- Sex-hormone medications or sex-hormone analogues or antagonists (see section 6.3.3 for examples)
- New and discontinued prescription medications

ARV Medications
Post-entry record all modifications, including initial doses, participant-initiated modifications, inadvertent and deliberate delay, and discontinuation.

6.3.5 Skin Pinch

The skin pinch test measures the thickness of the cutaneous and subcutaneous tissue at the injection site of both upper arms (medial deltoid muscles).

This measurement is also used to determine the depth of injection for each eligible participant to ensure IM administration of the vaccine/placebo prior to the

first vaccination. The skin thickness ranges and corresponding depth settings on the TDS-IM are described in the TDS-IM Instructions for Use (see A5369 MOPS).

6.3.6 Electrocardiogram (EKG)

EKG will be performed within 60 days prior to study entry. If clinically indicated, a follow-up EKG may be performed.

6.3.7 Laboratory Evaluations

At screening, pre-entry, and entry all laboratory values must be recorded on the eCRF. For post-entry assessments, record on the eCRF all laboratory values that lead to a change in treatment regardless of grade; record abnormal findings as per section 7.2.

Hematology
Hemoglobin, hematocrit, white blood cell count (WBC), differential WBC, absolute neutrophil count (ANC), and platelet count, will be performed in real time at the local laboratory.

Liver Function Tests
Total bilirubin, AST (SGOT), ALT (SGPT), alkaline phosphatase, and indirect bilirubin will be performed in real time at the local laboratory.

Blood Chemistries
Electrolytes (sodium, potassium, chloride, and bicarbonate), glucose, phosphate, creatinine, total protein, and albumin will be performed in real time at the local laboratory.

Calculated Creatinine Clearance
Calculated CrCl is required as estimated by the Cockcroft-Gault equation. This requires the recording of all serum creatinine values regardless of grade.

NOTE: A program for calculating creatinine clearance by the Cockcroft-Gault method is available on www.fstrf.org.

Urinalysis
Dipstick only.

PT, PTT, and INR
PT, PTT, and INR must be <1.5 x ULN at screening.

Pregnancy Testing
For women with reproductive potential: Serum or urine β-HCG (urine test must have a sensitivity of <25 mIU/mL) must be performed as indicated in the SOE and whenever pregnancy is suspected. Record pregnancy and pregnancy outcomes per section 8.2.

Hepatitis B and C Assessments
HBsAg and HCV antibody performed by a CLIA-certified laboratory or its equivalent.

6.3.8 Immunologic Studies

CD4/CD8
Obtain absolute CD4/CD8 T cell count and percentages within 60 days prior to entry from a laboratory that possesses a CLIA certification or equivalent.

For entry and post-entry evaluations, all laboratories must possess a CLIA certification or equivalent and must be certified for protocol testing by the DAIDS Immunology Quality Assurance (IQA) Program.

Advanced Flow Cellular Immune Responses
Advanced flow analysis requires a CD4/CD8 and WBC with differential from a sample obtained at the same time.

PBMCs will be stored according to the laboratory processing chart (LPC) and utilized for the following advanced flow assays:

- Vaccine-specific CD4 and CD8 T cell responses following stimulation of PBMCs with synthetic HIV peptides that span the proteins encoded by the vaccine construct, including pools covering individual CE regions.
- ICS parameters will include cytokines such as IFN-γ, IL-2, and TNF-α, and may include other cytokines and phenotypic markers to identify T cells of specific functionality (such as Th2 and Tfh).
- Markers of cytotoxic potential (e.g., granzyme B) may also be included. Data will be reported as percentages of CD4 or CD8 T cells responding to a specific peptide pool.
- Additional cell surface markers, cytokines, or functional markers may also be analyzed.

Phenotyping of Immune Activation Markers
PBMCs will be stored according to the LPC and utilized for the following advanced flow assays:

- Activated (HLA-DR+/CD38+) CD4 and CD8 T Lymphocytes
- Memory and Naïve (CCR7, CD45RA) T Lymphocyte subsets
- Exhaustion Markers/Checkpoint Inhibitors (including PD-1, TIM-3, TIGIT, LAG-3)

Humoral Immune Responses
Plasma will be stored according to the LPC and utilized for:

- Detection of Gag-specific binding IgG using ELISA or a Binding Antibody Multiplex Assay (BAMA)

Soluble Activation Markers
Plasma will be stored according to the LPC to test for markers including:

- sCD14, IL-6, hs-CRP, 2D-dimer, sCD163, IP10

Viral Inhibition Assay
A viral inhibition assay using autologous $CD4^+$ T cell targets that are superinfected with a reporter virus or a panel of cross-clade viruses to measure the ability of HIV-specific $CD8^+$ T cells to suppress viral replication in vitro both pre- and post-vaccination.

Human Leukocyte Antigen (HLA) Typing
HLA typing will be performed on stored PBMCs or plasma that was collected at pre-entry.

Stored Plasma and PBMCs
PBMCs and plasma will be stored for future analysis. For storage and shipping, please see the A5369 LPC.

6.3.9 Virologic Studies

Plasma HIV-1 RNA
Screening HIV-1 RNA must be performed within 60 days prior to study entry by a laboratory that possesses a CLIA certification or equivalent. Eligibility will be determined based on the screening value.

On study evaluations will be performed at Quest Diagnostics per the SOE.

Plasma HIV-1 RNA Single Copy Assay (SCA)
Plasma will be stored for determination of HIV-1 RNA by SCA per the SOE. See the LPC for details on processing plasma samples for the SCA test.

CD4 T Cell Associated HIV-1 RNA/DNA
PBMCs will be stored for determination CD4 T cell-associated HIV-1 RNA/DNA per the SOE. See the LPC for details on processing PBMC samples.

Whole Viral Genome Sequencing
Plasma/PBMC will be stored for whole viral genome sequencing per the SOE. See the LPC for details on processing plasma/PBMC samples.

CD4T Cell Purification from PBMC
PBMCs will be stored for CD4 T Cell purification per the SOE. See the LPC for details on processing PBMC samples.

6.3.10 Study Vaccine/Placebo Administration and Evaluation

Vaccine Administration
All vaccinations including modifications and permanent discontinuations must be documented. The vaccine is to be administered via IM injection in the outer aspect of the upper arm (deltoid). Details of all vaccinations, including the time the vaccine vial is removed from the freezer, reconstituted, administered, and route of administration are to be documented in the source documents.

NOTE A: Analgesic medication can be provided to the participant after vaccination, if needed.

NOTE B: Participants must remain at the clinic for observation for 30 minutes after each vaccination.

Criteria for Deferment
The following criteria for deferment must be assessed prior to all vaccinations:

- Recent (<72 hours) history of febrile illness (≥101°F [≥38.3°C] oral or equivalent) defer the second or third vaccination until at least 24 hours after resolution of febrile illness.

In addition the following criteria for deferment must be assessed prior to the second, third, and fourth vaccinations:

- Any medical condition that, in the opinion of the investigator, may interfere with the evaluation of the study objectives.
- Medical necessity for administration of vaccines other than study pDNA vaccine or placebo.
- Plasma HIV-1 RNA increase to >1000 copies/mL.

Deferment of the second vaccination must be for no longer than 14 days beyond the scheduled vaccination #2 (week 4). Deferment of the third vaccination must be no longer than 14 days beyond the scheduled vaccination #3 (week 12). Deferment of the fourth vaccination must be no longer than 14 days beyond the scheduled vaccination #4 (week 24). The reason for deferral must be recorded in the eCRF.

Laboratory evaluations and specimen collections should be deferred until the deferred vaccination visit. The post-vaccination laboratory evaluations and specimen collections scheduled for weeks 6 and 26 should also be deferred until two weeks after the deferred vaccination visit. If the week 4, 12 or 24 evaluations and collection of specimens were not performed at the time of the previously scheduled week 4, 12, or 24 visit, they must be performed immediately prior to administration of the deferred vaccination. The pregnancy test will need to be repeated, and negative results received, at the time of the vaccination.

If the vaccination #2, #3, or #4 is deferred, the originally scheduled post-vaccine evaluations will be performed at the equivalent time interval following the vaccination.

If the vaccination #2, #3, or #4 must be deferred for longer than 14 days, then the vaccination will not be administered. The participant will be followed in an off study treatment/on study status. The participant should complete the treatment discontinuation evaluations as specified in the SOE.

NOTE A: If vaccination #2 was missed (i.e., extends beyond the 14-day visit window), then vaccinations #3 and #4 will not be administered. The participant will be followed in an off study treatment/on study status.

NOTE B: If the deferred vaccination #3 was missed (i.e., extends beyond the 14-day visit window), then vaccination #4 will not be administered. The participant will be followed in an off study treatment/on study status.

The vaccination report card (VRC, described below) data will continue to be collected and reviewed during a deferral period.

The core team (actg.corea5369@fstrf.org) must be notified of deferments within 72 hours.

6.3.11 Telephone Contact

Participants will be contacted by telephone (2 to 3 days post-vaccination) to assess for changes in health status and to answer questions about vaccination-related signs and/or symptoms. See pre-specified telephone script in the A5369 MOPS.

NOTE: If participants prefer not to be contacted by telephone, then they may return to the clinic and the same procedures should be followed as noted above.

6.3.12 Vaccination Report Card (VRC) Distribution and Collection

All participants will be given a new VRC at the time of each vaccination to record daily temperatures (oral or equivalent) for 4 days following each vaccination, injection-site reactions for 5 days after each vaccination, and systemic reactions during the 26-week follow-up. A VRC will be given at week 0 and a new VRC will also be given to the participant at weeks 4, 12, and 24 study visits. The VRC will be a source document containing data that will be transferred to the appropriate eCRFs.

All non-study vaccines or medications taken during the study must be documented on the VRC.

All VRCs should be reviewed at all study visits. The following data should be transcribed from the VRC to the eCRF:

- Any oral/equivalent temperature ≥101.0°F (≥38.3°C)
- All injection site reactions such as pain, redness, and swelling
- All systemic reactions
- All rash reactions
- All medications taken
- Any vaccines received

NOTE: Participants should take their daily temperature (oral or equivalent) measurements at about the same time each day for consistency.

Clinic personnel will review the VRC for completeness, accuracy, and clarity at all visits. (In case of premature study discontinuation, the VRC will be reviewed for completeness, accuracy, and clarity at the time of discontinuation.) If either vaccination #2, #3, or #4 is deferred, then the VRC will be completed until the vaccination is administered. All comments are to be reviewed by the study personnel and, if necessary, discussed with the participant for clarification.

Any information gained by contact with the participant should be clearly documented, initialed, and dated at the bottom of the VRC. The VRC is considered a source document and no original information recorded by the participant should be crossed-out or altered in any manner by study personnel.

Participants will be asked to notify the study personnel immediately if any unexpected or severe reaction occurs. The participant will be asked to notify immediately the study investigator if he/she experiences a rash. The rash should be examined by the site investigator or clinic personnel within 72 hours of rash onset.

6.3.13 Tolerability Assessment

The tolerability assessment measures the acceptability of the vaccine administration procedure. The tolerability questionnaire consists of questions that capture the participant's level of discomfort experienced at different time points during the vaccination process. Participants will mark their responses for each designated time point immediately after completion of the vaccine administration procedure.

6.3.14 Leukapheresis (Optional)

Leukapheresis will be performed at pre-entry and week 26. Enrollment slots for participants agreeing to have leukapheresis will be reserved for a minimum of 20 participants. Participants agreeing to have leukapheresis performed at pre-entry are also required to have leukapheresis performed at week 26.

6.3.15 Stool Sample (Optional)

Stool samples will be collected and stored per the SOE and MOPS.

7.0 ADVERSE EVENTS AND STUDY MONITORING

7.1 Definition of Adverse Events

An adverse event (AE) is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or diagnosis that occurs in a study participant during the conduct of the study REGARDLESS of the attribution (i.e., relationship of event to medical treatment/study product/device or procedure/intervention). This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition.

7.2 Adverse Event Collection Requirements for this Protocol

All AEs defined below must be recorded on the eCRFs if any of the following criteria have been met:

- All grade ≥3 AEs
- All AEs that led to a change in study treatment/intervention regardless of grade
- All AEs meeting SAE definition or EAE reporting requirement

NOTE: All grade ≥ 3 AEs should be recorded on the eCRF within 48 hours of site awareness of the event.

NOTE: SAEs or events meeting EAE reporting requirements should also be entered into the DAIDS Adverse Experience Reporting System (DAERS), an Internet-based reporting system.

All AEs that are reported must have their severity graded. To grade AEs, sites must refer to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), corrected Version 2.1, July 2017, which can be found on the DAIDS RSC website at http://rsc.tech-res.com/clinical-research-sites/safety-reporting/daids-grading-tables.

Serious Adverse Events (SAEs)

An SAE is defined as any untoward medical occurrence that:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect

- Is an important medical event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above.

7.3 Expedited Adverse Event (EAE) Reporting to DAIDS

7.3.1 Expedited Adverse Event Reporting to DAIDS

Requirements, definitions and methods for expedited reporting of Adverse Events (AEs) are outlined in Version 2.0 of the DAIDS EAE Manual, which is available on the RSC website at http://rsc.tech-res.com/clinical-research-sites/safety-reporting/manual.

The DAIDS Adverse Experience Reporting System (DAERS), an internet-based reporting system, must be used for EAE reporting to DAIDS. In the event of system outages or technical difficulties, EAEs may be submitted using the DAIDS EAE Form. This form is available on the DAIDS RSC website at http://rsc.tech-res.com/clinical-research-sites/safety-reporting/daids/paper-eae-reporting.

For questions about DAERS, please contact NIAID CRMS Support at CRMSSupport@niaid.nih.gov. Please note that site queries may also be sent from within the DAERS application itself.

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

7.3.2 Reporting Requirements for this Study

- The SAE Reporting Category, as defined in Version 2.0 of the DAIDS EAE Manual, will be used for this study.
- The study agents for which expedited reporting are required are: p24CE1/2 pDNA vaccine, full-length p55^{gag} pDNA vaccine, and placebo for p24CE1/2 pDNA vaccine and full-length p55^{gag} pDNA vaccines.
- In addition to the SAE Reporting Category identified above, other adverse events that must be reported in an expedited manner are: Grade 3 or higher AEs or SAEs related to the electroporation or leukapheresis procedures.

7.3.3 Reporting of Error Codes for the TDS-IM

Error codes for the EP device must be reported to the A5369 protocol team in real time. These error codes should be recorded on the CRF and keyed in the database in a timely manner.

7.3.4 Grading Severity of Events

The Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), corrected Version 2.1, July 2017, must be used and is available on the DAIDS RSC website at http://rsc.tech-res.com/clinical-research-sites/safety-reporting/daids-grading-tables.

7.3.5 Expedited AE Reporting Period

- The expedited AE reporting period for this study is as per the EAE manual.
- After the protocol-defined AE reporting period, unless otherwise noted, only suspected, *unexpected* serious adverse reactions (SUSARs), as defined in Version 2.0 of the EAE Manual, will be reported to DAIDS if the study staff become aware of the events on a passive basis (from publicly available information).

7.4 Study Monitoring

SDAC prepared reports, pooled over treatment arms, accrual, baseline characteristics, study conduct (including premature treatment and study discontinuations), and AEs will be regularly monitored during the trial by the protocol core team. The protocol core team, blinded to active treatment versus placebo assignment, will review the individual safety data frequently to assess the relation of all reported AEs to study treatment.

The DAIDS clinical representative will review and assess EAE reports for potential impact on the study participant safety and protocol conduct as per DAIDS policies, guidance documents, and SOPs, as applicable.

The study will undergo interim review at least annually by an independent ACTG-appointed Study Monitoring Committee (SMC). The SMC will review accrual, baseline characteristics, conduct of the study (including premature treatment and study discontinuations), data completeness, and AEs by treatment arm. The first interim review will occur approximately 6 months after the enrollment of the first study participant, or after 20 participants enroll, whichever occurs first.. An interim review may also be convened if a concern is identified by the DAIDS clinical representative, the study chairs, or study statisticians in consultation with the team. See section 10.0 for statistical considerations related to interim monitoring.

Detailed plans for study monitoring will be outlined in a Study Monitoring Plan developed by the Statistical and Data Management Center (SDMC) prior to enrollment of the first participant.

8.0 CLINICAL MANAGEMENT ISSUES

8.1 Toxicity

Only toxicities considered to be related to the study-provided vaccines or EP procedure

using the TDS-IM will be managed directly by this protocol.

NOTE: In the event that a participant develops toxicity related to his/her previously-stable ART, consultation with the core team via email (actg.corea5369@fstrf.org), is required (preferably before appropriate therapy modification).

8.1.1 Vaccine or EP Toxicity Management

Participants must remain at the clinic for observation for 30 minutes after each vaccination. The protocol team should be contacted within 48 hours for any Grade 3 or 4 reactions thought definitely, possibly, or probably related to study vaccine or TDS-IM.

8.1.1.1 Grade 1 or 2 Local or Systemic Reactions

Local reactions of mild (Grade 1) or moderate (Grade 2) severity will usually resolve spontaneously. If needed, they may be managed with local application of cold packs, oral acetaminophen, oral nonsteroidal anti-inflammatory agents, or a combination of these measures as appropriate.

8.1.1.2 Grade 3 or 4 Local Reactions

For severe (Grade 3) or potentially life-threatening (Grade 4) local reactions, the protocol team must be notified within 48 hours. For Grade 4 local reactions, definitive medical and/or surgical intervention should be undertaken as appropriate. Further vaccinations should not be administered prior to consultation with the core team via email (actg.corea5369@fstrf.org, ATTN: Protocol chair and vice chair).

8.1.1.3 Systemic Reactions

The protocol team should be contacted within 48 hours for any nonlocal Grade 3 or 4 reaction thought definitely, possibly, or probably related to vaccinations. Further vaccines should not be administered prior to consultation with the core team via email (actg.corea5369@fstrf.org, ATTN: Protocol chair and vice chair).

8.2 Pregnancy

Pregnancy and pregnancy outcome will be recorded on the eCRFs. Pregnancies that occur on study should be reported prospectively to The Antiretroviral Pregnancy Registry. More information is available at www.apregistry.com. Telephone: 800-258-4263; Fax: 800-800-1052.

Pregnant women will discontinue study medication and will be encouraged to continue on study/ off study treatment. At the end of the pregnancy, outcome and AEs for

participant and infant will be recorded on the outcome eCRF.

If a woman has completed the study or chooses to discontinue from the study before the end of the pregnancy, then site staff should request permission to contact her regarding pregnancy outcomes at the end of pregnancy. If the information is obtained, pregnancy outcomes will be submitted on an eCRF at the end of the pregnancy.

9.0 CRITERIA FOR DISCONTINUATION

9.1 Permanent and Premature Treatment Discontinuation

- Drug-related toxicity (see section 8.1 Toxicity).
- Requirement for prohibited concomitant medications (see section 5.4).
- Pregnancy (participants who choose to remain on study will be followed off treatment/on study).
- Breastfeeding.
- Occurrence of a primary safety endpoint (see section 10.2.1.2).
- Request by participant to terminate study treatment.
- Failure by the participant to attend two consecutive clinic visits. The core team should be consulted before the participant is discontinued.
- Request of the primary care provider if she or he thinks the study treatment is no longer in the best interest of the participant.
- Clinical reasons believed life-threatening by the physician, even if not addressed in the toxicity section of the protocol.

9.2 Premature Study Discontinuation

- Failure to receive the first vaccination.
- Poor adherence to anti-HIV medications as judged by the site investigator.
- Participant fails to comply with the protocol.
- Request by the participant to withdraw.
- At the discretion of the IRB/EC, FDA, NIAID, Office for Human Research Protections (OHRP), other government agencies as part of their duties, investigator, or industry supporter.

10.0 STATISTICAL CONSIDERATIONS

10.1 General Design Issues

A5369 is a phase I/IIa, randomized, double-blind, placebo-controlled study to evaluate the safety, immunogenicity, and efficacy of p24CE/full-length Gag DNA, as a therapeutic vaccine in HIV-1 infected persons. The study targets to enroll 40 participants. All participants are well-suppressed on ART, current CD4 T cell counts >500 cells/mm^3, and nadir CD4 T cell counts >350 cells/mm^3. Each participant will receive active vaccine/placebo at weeks 0, 4, 12, and 24 delivered by electroporation after intramuscular injection. The primary efficacy outcome will be based on HIV-specific

immunologic assays at baseline and week 26.

10.2 Outcome Measures

Primary and secondary outcome measures listed below will be addressed in the study's primary Statistical Analysis Plan, which will define the content of the Primary Analysis Report. This report will form the basis for the primary study manuscript and results reporting to ClinicalTrials.gov. Outcomes of interest for secondary and exploratory objectives intended for subsequent publications are to be listed under "Other Outcome Measures."

10.2.1 Primary Outcome Measures

10.2.1.1 Immunogenicity

The number of CEs with a CD4 and/or CD8 T cell response at week 26 compared to baseline in an individual study participant. A CE response (for a participant at a given timepoint) is defined by a criterion that uses Fisher's exact test to compare the proportion of peptide-stimulated CD4 and/or CD8 T cells that are cytokine positive to the proportion that are cytokine positive in the unstimulated sample (negative control), and applying a stringent p-value of 10e-5 as a cutoff.

NOTE: For this primary endpoint, we will sum the CD4 and CD8 responses.

10.2.1.2 Safety

Occurrence of at least one ≥ Grade 3 AE except injection site pain or tenderness of less than 48 hours duration, that is possibly, probably, or definitely related to study treatment (as judged by the core team, blinded to treatment arm) any time from the first day of study treatment until 28 days after the last study vaccine administration.

Grade 4 AEs and deaths at any time on study will be considered a primary safety outcome.

10.2.2 Secondary Outcome Measures

10.2.2.1 The number of CEs with a CD4 T cell response at week 26 compared to baseline in an individual study participant.

10.2.2.2 The number of CEs with a CD8 T cell response at week 26 compared to baseline in an individual study participant.

10.2.2.3 The total magnitude of CD4 T cell responses against each CE added together in each study arm at week 26 compared to baseline.

10.2.2.4 The total magnitude of CD8 T cell responses against each CE added together in each study arm at week 26 compared to baseline.

10.2.3 Other Outcome Measures

10.2.3.1 HIV-specific cellular responses.

10.2.3.2 HIV-specific humoral immune responses.

10.2.3.3 Cellular and soluble markers of immune activation, exhaustion, and checkpoint inhibition.

10.2.3.4 Measures of latent cell reservoir of HIV-1.

10.2.3.5 Tolerability

- At any visit answering “no” to the question, “In your opinion, would this study’s vaccination procedure be acceptable as part of a treatment for HIV, if it proved to be effective?”
- At any visit answering “no” to the question, “In your opinion, would this study’s vaccination procedure be acceptable if it could contribute to increased scientific knowledge about how best to administer vaccines to prevent or treat infections?”.
- For participants administered vaccine/placebo by the EP device: a) rated pain when the device was placed on the skin and the vaccine/placebo was injected, b) rated pain at the time of the electrical stimulation and muscle contraction, and c) rated pain: i) 10 minutes and ii) 30 minutes after the procedure was completed
- Premature treatment discontinuation for reasons related to study treatment or related to any real or perceived effect of study vaccination or its administration.

10.2.3.6 Gut microbiota

- Measures of microbial diversity (Shannon index, alpha, beta diversity).
- Prevalence of individual bacterial taxa.
- Measures of bacterial metabolic pathways.

10.3 Randomization and Stratification

Participants will be randomized 2:1:1 to the p24CE/full-length Gag DNA vaccine arm versus full-length Gag DNA vaccine arm versus placebo arm, using the permuted block method. Randomization will be stratified by indication of willingness to have the leukapheresis procedure.

10.4 Sample Size and Accrual

The primary comparison will be between the p24CE/full-length Gag DNA and placebo arms. Statistical power is estimated based on a two-sided, 5% significance level, Fisher's exact test of whether a participant has a larger number of CEs with a CD4 and/or CD8 T cell response at week 26 compared to baseline (i.e., a yes/no binary outcome), considering n=18 versus n=9 evaluable participants in the two arms, respectively. This is a conservative calculation because the primary analysis uses the more powerful Wilcoxon rank sum test (see section 10.6.1, Primary Immunogenicity).

The table below provides several scenarios to detect a difference (ranging from 0.50 to 0.75) between the two arms in the probability that a participant has increased CE responses.

Table 10.4-1: Power and Effect Size

Power	P_p24CE	P_placebo	Effect Size
72%	0.60	0.10	0.50
80%	0.64	0.10	0.54
82%	0.65	0.10	0.55
89%	0.70	0.10	0.60
90%	0.71	0.10	0.61
94%	0.75	0.10	0.65
97%	0.80	0.10	0.70
98%	0.85	0.10	0.75

Power is 80% to detect a difference of 0.64 versus 0.10 in the probability that a participant has more recognized CEs at week 26 compared to baseline. The protocol team believes that a difference of 0.54 in the probability of increased CE responses between the p24CE vaccine arm and the placebo arm for the primary comparison is a clinically relevant immunogenicity effect size that would lend support for further development of the p24CE vaccine. The targeted anti-HIV immune responses being induced in less than 50% of study participants receiving the p24CE vaccine compared to placebo would not be seen as being clinically meaningful and would discourage further studies of this vaccine regimen. Power to detect a difference of 0.85 versus 0.10 between the p24CE vaccine arm and the placebo arm is >98%.

Power is also calculated for a secondary analysis to compare the p24CE vaccine arm and the full-length Gag vaccine arm. Considering n=9 evaluable participants in the full-length Gag vaccine arm, the study has approximately 80% power to detect a difference of 0.85 versus 0.30 when comparing the p24CE and full-length Gag vaccine arms.

The sample size calculation is based on the assumption that in the absence of intervention, while an HIV+ individual remains on stable and suppressive ART (i.e., placebo arm), the HIV-specific immune responses to the CE elements would be relatively stable over time. Hence the team expects only a small probability that longitudinal assays for a placebo participant would identify an increase in the number of CEs recognized by CD4 or CD8 T cells.

NOTE: The sample size calculation may change pending the testing of A5321 samples.

Regarding the assessment of safety (see section 10.2.1.2), the sample size of 20 participants receiving the p24CE/Gag DNA vaccine will provide >90% probability of observing a p24CE/Gag DNA-related AE that would occur in 11% or more of treated participants. The sample size of 10 participants receiving the full-length *gag* DNA vaccine will provide >90% probability of observing a full-length *gag* DNA-related AE that would occur in 21% or more of treated participants.

It is anticipated that the study will take 6-9 months to reach accrual targets.

10.5 Study Suspension Rule

If at any time during the study:

- Two or more participants experience a primary safety outcome that is possibly or probably related to study treatment (as judged by the core team, blinded to treatment arm), or

- One or more participants experience a primary safety outcome that is definitely related to study treatment or that is Grade 4 or death and possibly or probably related to study treatment (as judged by the Core Team, blinded to treatment arm),

then enrollment into the study will be temporarily suspended and the Study Monitoring Committee (SMC, unblinded to treatment assignment) will be asked to review all safety data; review the relation to study treatment of the event(s) thought by the blinded Core Team to be a primary safety outcome; and recommend how the study should proceed with respect to resuming enrollment and continuing study treatment.

Pending full SMC review, the protocol team will decide, in consultation with the SMC chair or designee, whether to continue study treatment for subjects already enrolled.

10.6 Data and Safety Monitoring

At SMC reviews, data will be considered as detailed in section 7.4.

10.7 Analyses

10.7.1 Primary Immunogenicity

The planned analysis will compare Y=sum of CEs recognized by CD4 and CD8 T cells at week 26 minus the number at baseline between the p24CE/full-length Gag DNA and placebo arms, using a two-sided 5% level Wilcoxon rank-sum test, which is anticipated to have greater statistical power than analyzing the binary version of whether Y>0, for which statistical power was based. An exact 95% confidence interval (CI) for the the median of all paired differences between observations in the two arms (i.e., Hodges-Lehmann estimate) will be calculated to provide additional insight on the effect of p24CE/full-length Gag DNA vaccine.

The primary immunogenicity analysis for this pilot study will be per protocol, limited to participants who received all four study vaccination/placebo administrations and who did not take any medication prohibited by the study protocol on or prior to their week 26 visit.

10.7.2 Primary Safety

All participants who have been exposed to the study treatment will be included in this analysis. AEs attributed to study treatment based on the protocol core team review will be summarized separately by treatment arm, including the number and percentage of participants experiencing an AE with an exact binomial 95% CI.

10.7.3 Secondary Analyses

Secondary analyses will compare Arm 2, the gag-DNA alone vaccine arm, to each of the other arms. These analyses will be performed in the same manner as the primary analysis.

The percentage of participants in each study arm with a CD4 and/or CD8 cell response to an increased number of CEs at week 26 compared to baseline will be presented along with an exact binomial 95% CI. Pairwise comparisons among study arms will be conducted using Fisher’s exact test. Similar analyses will be performed for CD4 and CD8 cell responses separately.

The change in total magnitude of CD4 T cell responses against each CE added together among study arms will be compared using the two-sided Wilcoxon rank-sum test. The analysis for CD8 T cell responses will be conducted in a similar manner. All statistical tests will be two-sided at the 5% nominal level of significance without adjustment for multiple testing.

For each tolerability endpoint, all occurrences of the endpoint will be summarized descriptively (e.g., for each participant with the endpoint, the answers to all of the questions in that participant’s tolerability questionnaire will be reported), with an

exact binomial 95% CI on the probability of the tolerability endpoint for each treatment arm. Also, the participant ratings of pain will be described a) when the device was placed on the participant's skin and the vaccine/placebo was injected, b) at the time of the electrical stimulation and muscle contraction, c) 10 minutes and 30 minutes after the procedure was completed, by treatment arm, and the left and right deltoid separately.

11.0 PHARMACOLOGY PLAN

Not applicable.

12.0 DATA COLLECTION AND MONITORING

12.1 Records to Be Kept

Electronic case report form (eCRF) screens will be made available to sites for data entry. Participants must not be identified by name on any data submitted to the DMC. Participants will be identified by the patient identification number (PID) and study identification number (SID) provided by the ACTG DMC upon registration.

12.2 Role of Data Management

12.2.1 Instructions concerning entering study data on eCRFs will be provided by the ACTG DMC. Each CRS is responsible for keying the data in a timely fashion.

12.2.2 It is the responsibility of the ACTG DMC to ensure the quality of computerized data for each ACTG study. This role extends from protocol development to generation of the final study databases.

12.3 Clinical Site Monitoring and Record Availability

12.3.1 Site monitors under contract to the NIAID will visit participating clinical research sites to review the individual participant records, including consent forms, eCRFs, supporting data, laboratory specimen records, and medical records (physicians' progress notes, nurses' notes, individuals' hospital charts), to ensure protection of study participants, compliance with the protocol, and accuracy and completeness of records. The monitors also will inspect sites' regulatory files to ensure that regulatory requirements are being followed and sites' pharmacies to review product storage and management.

12.3.2 The site investigator will make study documents (e.g., consent forms, drug distribution forms, eCRFs) and pertinent hospital or clinic records readily available for inspection by the local IRB, the site monitors, the FDA, the NIAID, the OHRP, the industry supporter or designee, and other local, U.S., and international regulatory entities for confirmation of the study data.

13.0 PARTICIPANTS

13.1 Institutional Review Board (IRB) Review and Informed Consent

This protocol and the informed consent document (Appendix I) and any subsequent modifications will be reviewed and approved by the IRB or EC responsible for oversight of the study. A signed consent form will be obtained from the participant (or legal guardian or person with power of attorney for participants who cannot consent for themselves). The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the participant, or legal guardian and this fact will be documented in the participant's record.

13.2 Participant Confidentiality

All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified by coded number only to maintain participant confidentiality. All records will be kept locked. All computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by the ACTG, IRB/EC, FDA, NIAID, OHRP, other local, US, and international regulatory entities as part of their duties, or the industry supporters or designees.

13.3 Study Discontinuation

The study may be discontinued at any time by the ACTG, IRB/EC, FDA, NIAID, OHRP, other government agencies as part of their duties to ensure that research participants are protected, or the industry supporter.

13.4 Compliance with NIH Guidelines for Research Involving Products Containing Recombinant DNA

This protocol was submitted to the Office of Biotechnology Activities (OBA) in accordance with Appendix M of the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines 2009) http://oba.od.nih.gov/rdna/nih_guidelines_oba.html. The protocol (#1004-1038) was reviewed by the NIH Recombinant DNA Advisory Committee (RAC) as notified by the OBA on May 4, 2010.

The Principal Investigators and the institution are responsible for ensuring that no research subjects are enrolled in the protocol until Institutional Biosafety Committee (IBC) approval, IRB approval and all applicable regulatory authorizations have been obtained.

The current reporting requirements set forth in Appendix M-I-C-1 of the NIH Guidelines require the Principal Investigator to submit additional documentation as specified to the

OBA.

Within 20 working days of enrolling the first subject in the trial, the investigator at the first site must provide OBA with the documents and information listed below (per Appendix M-I-C-1 of the NIH Guidelines).

- A copy of the protocol approved by the IBC and IRB
- A copy of the informed consent approved by the IRB
- A copy of the IBC approval of the clinical site
- A document outlining the responses to the RAC's recommendations (if any) and any modification to the protocol required by the FDA
- The IND number
- The NIH grant number
- The date of the initiation of the trial

The guidelines allow for formal delegation of all or part of the investigator reporting requirements (Appendix M-I-C-4 of the NIH Guidelines).

When adding new sites to the clinical trial, no research subject should be enrolled at the site until the following documentation has been submitted to the OBA:

- IBC approval from the clinical site
- IRB approval
- IRB-approved informed consent document
- Curriculum vitae of the Principal Investigator(s) – no more than two pages in biographical sketch format
- NIH grant number(s) if applicable

During the conduct of the study, the principal investigator is responsible for providing OBA with safety reports and annual report. In such cases, OBA requires that these documents follow the timelines for submission and format of the homologous FDA mandatory reports.

All information communicated to the OBA is publically available unless it is clearly labeled as confidential. Since annual reports to the FDA may contain proprietary information, the product manufacturer will edit the annual report to remove any proprietary information prior to submission to OBA. The DAIDS medical officer will sign off on the redacted annual report before submission to OBA.

The safety reports can be sent simultaneously to the FDA and OBA by the RSC SAE Office.

14.0 PUBLICATION OF RESEARCH FINDINGS

Publication of the results of this trial will be governed by ACTG policies. Any presentation, abstract, or manuscript will be made available for review by the industry supporter prior to submission.

15.0 BIOHAZARD CONTAINMENT

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion 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 National Institutes of Health.

All dangerous goods and materials, including diagnostic specimens and infectious substances, must be transported using packaging mandated by CFR 42 Part 72. Please refer to instructions detailed in the International Air Transport Association (IATA) Dangerous Goods Regulations.

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APPENDIX I SAMPLE INFORMED CONSENT

Division of AIDS
AIDS CLINICAL TRIAL GROUP (ACTG)
For protocol A5369

HIV-1-Gag Conserved-Element DNA Vaccine (p24CE) as Therapeutic Vaccination in HIV-Infected Persons with Viral Suppression on Antiretroviral Therapy
FINAL Version 1.0, 03/09/18

SHORT TITLE FOR THE STUDY: HIV-1-Gag Conserved-Element DNA Vaccine (p24CE) as Therapeutic Vaccination

INTRODUCTION

You are being asked to take part in this research study because you are infected with the human immunodeficiency virus (HIV) and are currently taking anti-HIV medications. This study is sponsored by the National Institutes of Health (NIH). The doctor in charge of this study at this site is: (insert name of Principal Investigator). Before you decide if you want to be a part of this study, we want you to know about the study.

This is a consent form. It gives you information about this study. The study staff will talk with you about this information. You are free to ask questions about this study at any time. If you agree to take part in this study, you will be asked to sign this consent form. You will get a copy to keep.

WHY IS THIS STUDY BEING DONE?

The study will look to see if two HIV vaccines are safe in people infected with HIV. Additionally, the study will look at whether or not the HIV vaccines improve the immune system's ability to fight HIV. This study will also look at whether the intramuscular injection of the study vaccines given along with electroporation will be well tolerated.

The HIV vaccine used in this study is experimental. This means that this vaccine has not been approved by the Food and Drug Administration (FDA).

WHAT DO I HAVE TO DO IF I AM IN THIS STUDY?

To be in this study, you must continue taking your current anti-HIV drugs. Your anti-HIV drugs will not be provided to you by the study.

Screening
If you decide to take part in this research study, you will be asked to sign this consent form. You will come to the clinic to have a screening visit. Tests will be done at the screening visit to see if it is safe for you to join the study. The screening visit will take about 1 hour, but it may be shorter or longer.

At the screening visit:

- Your HIV-1 infection will be confirmed. If there is no record available, you will have another HIV-1 test. You may have to sign a separate consent form before having this test.
- You will have a complete physical exam and will be asked questions about how you are feeling.
- You will also be asked to answer questions about your medical history and medications you are taking now and have taken in the past.
- You will have a skin pinch test to measure the thickness of the skin on your upper arm muscles. This test will also measure the right depth when giving the injections in the upper arm muscles.
- You will have a pregnancy test done, if you are a woman able to become pregnant. You cannot take part in this study if you are pregnant or breastfeeding.
- You will have a urinalysis performed to evaluate the health of your kidneys.
- You will have an electrocardiogram (EKG) test done to measure the electrical activity of your heart. You will be asked to lie down very still and breathe normally during the test.

You will have about 2 tablespoons of blood drawn from a vein in your arm:
- To measure HIV viral load (a viral load test measures how much HIV is in your blood).
- To measure CD4/CD8 T cell counts (the number of white blood cells that fight infection).
- To test for hepatitis B and hepatitis C (viruses that can infect your liver).
- To conduct routine safety tests.

You will be told the results of the tests done at the screening visit.

If you do not enroll into the study
If you decide not to take part in this study or if you do not meet the eligibility requirements, we will still use some of your information. As part of this screening visit, some demographic (for example, age, gender, race), clinical (for example, disease condition, diagnosis), and laboratory (for example, CD4 cell count, viral load) information is being collected from you so that ACTG researchers may help determine whether there are patterns or common reasons why people do not join a study.

Pre-Entry
If you qualify for the study, you will return to the clinic about 2 weeks after your screening visit. The visit is expected to last between 3 to 4 hours.

At the pre-entry visit:

- You will have a total of about 3 tablespoons of blood drawn from a vein in your arm.
- You will have blood collected and stored for future immunologic testing (to measure the body's ability to fight infection).
- You will have a pregnancy test done, if you are a woman able to become pregnant.
- You will have blood collected to measure CD4/CD8 T cell counts (the number of white blood cells that fight infection).
- You will have blood collected for human leukocyte antigen (HLA) typing and testing.
- You will have blood stored for future tests.
- If you agree, you will have leukapheresis performed. You will also be required to have a leukapheresis performed at the week 26 visit. By collecting blood using this procedure, researchers are able to get many more white blood cells than is usually possible.
- If you agree, you will have stool samples collected to study the different kind of bacteria in your stool. These samples will be stored and will be tested after the study is over. You will not be given the results. (This test is optional)

Leukapheresis Procedure

The leukapheresis procedure may be performed at *[insert site-specific details]*. The procedure will take about *[insert site-specific details]* and the full visit will last about *[insert site-specific details]*. You will have to remain in a semi-reclining or reclining position for most of this time.

Leukapheresis is a medical procedure that involves removing whole blood from an individual/donor and separating the blood into individual components so that leukocytes (white blood cells) can be removed. The remaining blood components are then put back into the bloodstream of the individual/donor. This will be done by inserting a needle attached to sterile tubing in one arm, and first sending your blood through a machine. This machine spins your blood to separate the red blood cells (cells that carry oxygen), the white blood cells (cells that fight infection) and the platelets (cells that help form clots). The white blood cells will be kept for testing. The rest of your blood will be returned to your body through another needle and tube in your other arm. Not all of your white blood cells are removed, and your body will make more white cells within a few days. Losing the number of white blood cells that are collected does not pose a danger to your health.

Entry

You will return to the clinic at least 24 hours after your pre-entry visit. The entry visit (week 0) will last about 1-2 hours, but may be shorter or longer.

At the entry visit:

- You will have a brief physical exam.
- You will be asked about how you are feeling and any medications you have taken since the last visit.
- You will have a pregnancy test done, if you are a woman able to become pregnant.
- You will have a total of about 4 tablespoons of blood drawn from a vein in your arm.
- You will have blood drawn for routine safety tests.
- You will have a urinalysis performed to evaluate the health of your kidneys.

- You will have blood drawn for CD4/CD8 T cell counts, HIV viral load, and future immunologic testing.

You will be told the results of the safety tests, HIV viral load, and CD4/CD8 T cell counts done during the study.

Study Injections

When you enter the study, you will be placed into one of the three groups. You will be randomized 2:1:1 (by chance) to receive either the p24CE1/2 pDNA vaccine (an HIV vaccine) followed by p24CE1/2 + full-length Gag pDNA vaccine (a different HIV vaccine), OR the full-length Gag pDNA vaccine alone, OR placebo (the placebo is a salt solution that does not contain any vaccine, medicine, or drugs).

Neither you nor the study staff will know whether you will receive the vaccine or placebo. You will receive the injections at the clinic at weeks 0 (entry), 4, 12, and 24.

Electroporation (EP) Procedure

To improve the effectiveness of the vaccine, instead of a regular needle and syringe, a small, hand-held device will be used to inject the vaccine or placebo into your upper arm muscle. To give the vaccine or placebo, the study staff will press the device against your skin and press a button. Although you will not be able to see them, the device will put an injection needle and four thin wires into your muscle. The vaccine or placebo will be given through the injection needle into your muscle. After the injection, the device will give a very short electrical signal to your muscle at the spot of the injection. The electrical signal will last for about one half second. You will feel twitching in your muscle, which is often painful. Previous participants have described the feeling as a short “cramp” or “punch” in their muscle. Right after the electrical signals are finished, the device will be removed from your muscle. Your muscle may be sore to the touch after the vaccination. If this occurs, it usually does not last for more than 30-60 minutes.

Before Injection

Due to the risk of dizziness/lightheadedness or in rare cases, fainting, the injection will be given to you while you are in a secure, seated position.

After Injection

You will be observed by the study staff when coming to a standing position following the injection. You may ask for pain-relieving medication after each injection if you need it.

After each injection, you will be asked to stay at the clinic for 30 minutes to be observed and to complete a brief survey (Procedure Tolerability Questionnaire). This survey will ask you questions on the level of discomfort you may have experienced during the vaccination, including the overall acceptability of the procedure.

You will be called 2 to 3 days after each injection to see how you are doing. The telephone call will take about 5-10 minutes. If you prefer not to be contacted by telephone, then you will be asked to return to the clinic within 2 to 3 days after each vaccination. The study staff may ask you to come to the clinic if you are having any side effects from the vaccination.

Study Diary
You will be given a diary to record:
- Your temperature for 4 days after each injection.
- Any side effects for 5 days after each injection.
- Any rash or skin irritation around and/or in the middle of the injection site.
- Any medications taken.
- Any non-study vaccines received.

The diary will be given to you at the entry visit. It will take you about 10-15 minutes to complete the diary each day. You will bring the diary back to the clinic at your next visit and at every visit afterwards until the 26-week follow-up visit. The diary will be collected by the study staff at the final visit (week 48); if you stop the study early, the diary will be collected your last study visit.

During the Study
After your entry visit, the next study visits will take place over a 12-month period (weeks 4, 6, 12, 24, 26, and 48). Each study visit will last about 1-2 hours, but may be shorter or longer.

At most visits, you will be asked about how you are feeling, what medications you are taking, and have a brief physical exam. At the injection visits (weeks 4, 12, and 24), you will have a pregnancy test done, if you are a woman able to become pregnant, before you have the injection. At weeks 4, 12, and 24, you will also have a urinalysis performed.

At most visits, you will have blood drawn from a vein for routine safety tests. At some visits, you will have blood drawn for CD4/CD8 T cell counts, HIV viral load, and future immunologic testing. You will have between 2 and 4 tablespoons of blood drawn at each visit.

If you agree, you will have stool sample collectesd at week 26 to study the different kind of bacteria in your stool.These samples will be stored and will be tested after the study is over. You will not be given the results. (This test is optional.)

If you agreed to have leukapheresis performed at the pre-entry visit you will be required to also have leukapheresis performed at the week 26 visit.

NOTE: Your blood that is drawn for future immunologic testing, which is required for this study, will be stored with usual protectors of your identity. Your blood samples will be processed in the laboratory to produce a certain type of white blood cells, called PBMC (peripheral blood mononuclear cells), and plasma.

Other
If you agree, some of your blood that is left over after all required study testing is done may be stored (with usual protectors of your identity) and used for future ACTG-approved HIV-related research.

You may refuse or withdraw your permission for storage of leftover samples without any impact on you taking part in the study or any penalty or loss of benefits to which you are entitled. You

can withdraw your permission at any time. If you do not agree to have the leftover samples stored or withdraw your permission, the leftover samples will be destroyed. These leftover samples may be stored for an indefinite period of time. You might not receive the results of testing performed on these leftover samples.

Please initial one of the following lines to indicate whether or not you wish to have your specimens stored for research in the future.

_____ YES _____ NO

HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?

About 40 people will take part in this study

HOW LONG WILL I BE IN THIS STUDY?

You will be in this study for about 12 months (48 weeks).

WHY WOULD THE DOCTOR TAKE ME OFF THIS STUDY EARLY?

The study doctor may need to take you off the study early without your permission if:
- The study is stopped or cancelled.
- You do not take your anti-HIV medications as prescribed.
- You do not comply with the protocol.
- You choose to stop your participation in the study.
- Your primary care doctor feels the treatment is no longer in your best interest.
- Failure to receive the first vaccination.

The study doctor may also need to take you off the study drug(s) without your permission if:
- You miss two consecutive clinic visits.
- Continuing to receive the study vaccine may be harmful to you.
- You need a drug or treatment that you may not take while on the study.
- You are not able to receive the study vaccine as required by the study.
- You have a bad reaction to the study vaccine and need treatment.
- You become pregnant or start breast-feeding.

If you must stop receiving the study-provided vaccine before the study is over, the study doctor may ask you to continue to be part of the study and return for some study visits and procedures.

If I have to permanently stop receiving the study-provided vaccine, or once I leave the study, how would vaccine be provided?

During the study:

If you must permanently stop receiving the study-provided vaccine before your study participation is over, the study staff will discuss other options that may be of benefit to you.

After the study:

After you have completed your study participation, the study will not be able to continue to provide you with the vaccine you received on the study.

WHAT ARE THE RISKS OF THE STUDY?

The vaccine or placebo used in this study may have side effects, some of which are listed below. Please note that these lists do not include all the side effects seen with the study vaccine. These lists include the more serious or common side effects with a known or possible relationship. There may be more serious or common side effects that we do not know about yet. Therefore, it is important that you report any side effects to the study staff. If you have questions concerning additional study vaccine side effects, please ask the medical staff at your site.

There is a risk of serious and/or life-threatening side effects when non-study medications are taken with the study vaccine. For your safety, you must tell the study doctor or nurse about all medications you are taking before you start the study and also before starting any new medications while on the study. Also, you must tell the study doctor or nurse before enrolling in any other clinical trials while on this study.

The following side effects can occur from either the vaccine or the placebo in this study. If you do have a reaction, it does not mean that you received the study vaccine.

General Vaccination Risks
The possible risks for vaccines in general include fever, chills, rash, aches and pains, nausea, headache, dizziness, and fatigue. We know these side effects can occur with other vaccines. They can occur whether you receive the vaccine or the placebo in this study. The side effects do not usually last long.

As with all vaccines or drugs, you could have an immediate allergic reaction, including itchy rash, hives, low blood pressure, sudden body swelling, or even difficulty breathing. Allergic reactions can be life threatening; therefore, the study staff will watch you for 30 minutes after each injection. There may be other side effects, even serious ones that we do not know about yet. Therefore, it is important that you report any side effects to the study staff as soon as they occur.

For people infected with HIV, vaccinations can cause a temporary increase in the HIV viral load, but this has not been seen when people are also taking medications that lower the amount of HIV in the blood. The long-term effects of temporary increases in HIV viral load levels are unknown.

Risks of Injections
- Arm discomfort
- Bleeding or bruising at the spot where the needle enters your body
- Small risk of fainting or infection
- Stinging, pain, soreness, redness, itching, swelling, burning, warmth at injection site
- Induration (hardness under the skin) at the site where the vaccine/placebo is given

Risks of Drawing Blood
Blood tests for screening and study visits will be done by inserting a needle into one of your veins and this can cause temporary mild pain or discomfort at the needle site (common), local bruising at needle site (rare), infection, and fainting (very rare).

Risks of HIV DNA Vaccines, including pDNA Study Vaccine
HIV-1 DNA vaccines have been studied in humans and appear to be well tolerated, but we do not have long-term follow-up on people taking part in these studies.

Possible risks related to DNA vaccines include muscle damage, or the production of antibodies (proteins made by the body's immune system in response to a foreign substance) to DNA. Other potential risks include insertion of the vaccine DNA into the body's DNA (leading to cancer) or into the DNA of a bacteria or virus in your body. None of these possible risks of DNA vaccines have been seen in laboratory tests or in animals or humans so far, but you need to be aware of these possible risks. During the study, regular blood tests and check-ups will be done to monitor these possible side effects.

The pDNA vaccine consists of artificial DNA. Since the vaccine in this study has not been given to humans for treating HIV infection, all the possible risks or side effects are not known.

The pDNA vaccine was tested in animals at doses similar to or larger than those planned to be given in this study without serious side effects. However, people may respond differently to the vaccine than animals.

Information about local injection site reactions in humans after injection of the pDNA vaccine is not available at this time. However, the local reactions related to intramuscular (IM) injections such as pain, hardness under the skin, redness, and swelling may occur and are expected to be mostly mild to moderate in severity based on results from earlier HIV clinical trials with related products. Information about systemic events (which affect your whole body, such as fever or not feeling well) with the pDNA vaccine are not available at this time. Based on results from clinical trials with related products in healthy adults and in HIV-infected persons, systemic events are expected to be mild to moderate in severity.

It is unknown whether receiving this HIV vaccine will change your response to any future HIV therapies or vaccines that you might receive. This might prevent you from taking part in other experimental vaccine or immune studies.

Risks of EP Procedure

This section describes the risks and restrictions we know about. There may also be unknown risks, even serious ones. If we learn about new risks during this study, we will tell you.

- Brief twitching/contraction of the upper arm muscle where the vaccination is given will occur during the the EP procedure. This may result in a painful sensation, which should last only a few seconds.
- It is possible that the EP procedure could cause you to become dizzy or lightheaded. If severe enough, this could lead you to faint. Should this happen, you may require additional tests and/or hospitalization.
- After the EP device is removed, slight bleeding may occur in the skin at the vaccination site. This bleeding, if any, should only last for a few minutes.
- Delivery of the vaccine with the EP device is likely to result in muscle soreness or pain of mild to moderate severity at the vaccination site. Redness, swelling, and/or bruising in the area of the vaccination could also occur. It is possible that these reactions could persist for several days or more after administration.

Other Expected Risks

Common:

- Muscle pain with severe muscle contractions during vaccination administration
- Mild to moderate injection site pain, tenderness, redness, bruising, or edema (swelling under the skin)
- Muscle aches or headache in the first few days following vaccination

Less common:

- Severe vaccination site pain or tenderness
- Joint pain
- Vaccination site bruising (a mass of blood in the tissue caused by injury), laceration (cut or slash), or bleeding related to the vaccination procedure
- Dizziness/lightheadedness

Uncommon or rare:

- Fainting
- Severe reaction of the vaccination site including sterile abscess (lumps caused by nonliving irritants such as drugs) or secondary bacterial infection
- Allergic reaction, including rash, urticaria (hives), angioedema (swelling of the skin), bronchospasm (narrowing of the muscles that help you breathe), or anaphylaxis (life-threatening allergic reaction)

Unknown frequency or theoretical risks:
- Muscle damage at the vaccination site
- Insertional mutagenesis (a mutation caused by new genetic material put into a normal gene)
- Autoimmune reaction (a process in which your immune system attacks your body)

Risks of Bupivacaine
The HIV pDNA vaccine contains bupivacaine. Bupivacaine helps the DNA get into the muscle cells. It is an anesthetic, similar to the numbing medicine used by dentists. Bupivacaine, like all medicines, can have side effects. Bupivacaine can cause problems with the nervous system and heart. The nervous system side effects include confusion, dizziness, blurred vision, shaking, or seizures. The heart side effects can include decreased heart pumping, fast heart rate, low blood pressure, abnormal heartbeats, or even death. Other possible side effects include nausea, vomiting, or chills. All of these side effects are very rare, but may happen as a result of accidental injection into the bloodstream.

Risks of Leukapheresis
Leukapheresis has been shown to be safe in HIV-infected donors and does not affect CD4+ T-cell count or immune status of short-term donors. The needle used is larger than normal blood draw and may be uncomfortable. Rarely, a participant may feel faint during or after leukapheresis. This sort of reaction can be handled by changing the participant's position or administering intravenous fluids. You may experience chills, nausea, and heartburn caused by the citrate anticoagulant that is used during the procedure to keep the collected cells from clumping together in the bag. This chemical may use up some of the calcium in your blood stream, and tingling in the face, lips, or hands may be noted. If this happens, study staff may slow the rate of infusion of this chemical and may offer you one or two calcium carbonate tablets to correct the calcium loss. Participants will be observed closely by an experienced blood bank technician during the procedure.

ARE THERE RISKS RELATED TO PREGNANCY?

The vaccine in this study may be unsafe for unborn babies. If you are having sex that could lead to pregnancy, you must agree not to become pregnant or make a woman pregnant for at least three months after the final study vaccination. Because of the risk involved, you and your partner must agree to use two methods of birth control that you discuss with the study staff. You may choose two of the birth control methods listed below:
- Condoms (male or female) with or without a spermicidal agent
- Diaphragm or cervical cap with spermicide
- Intra-uterine device (IUD)
- Hormone-based contraceptive

If you can become pregnant, you must have a pregnancy test before you enter this study. The test must show that you are not pregnant. If you think you may be pregnant at any time during the study, tell your study staff right away. The study staff will talk to you about your choices. If you become pregnant while on the study, you will be asked if you wish to continue in the study

and come for study visits for routine safety evaluations. You will not receive any further injections that are scheduled.

Safety information regarding the performance of this pDNA vaccine in pregnant women is not available at this time. Therefore, this vaccine is not to be given to pregnant women or nursing mothers.

If you become pregnant while on study, the study staff would like to obtain information from you about the outcome of the pregnancy (even if it is after your participation in the study ends). If you become pregnant during the study and do not give birth by the time of the final study visit, and if you agree, you will be contacted at the end of the pregnancy regarding pregnancy outcomes.

If you are taking anti-HIV drugs when you become pregnant, your pregnancy will be reported to an international database that collects information about pregnancies in women taking anti-HIV drugs. This report will not use your name or other information that could be used to identify you.

ARE THERE BENEFITS TO TAKING PART IN THIS STUDY?

If you take part in this study, you should expect no direct benefit. Information learned from this study may help others who have HIV.

WHAT OTHER CHOICES DO I HAVE BESIDES THIS STUDY?

Instead of being in this study you have the choice of:
- treatment with prescription drugs available to you
- treatment with experimental drugs, if you qualify
- no treatment

Please talk to your doctor about these and other choices available to you. Your doctor will explain the risks and benefits of these choices.

WHAT ABOUT CONFIDENTIALITY?

We will do everything we can to protect your privacy. In addition to the efforts of the study staff to help keep your personal information private, we have gotten a Certificate of Confidentiality from the U.S. Federal Government. This certificate means that researchers cannot be forced to tell people who are not connected with this study, such as the court system, about your participation. Also, any publication of this study will not use your name or identify you personally.

Your records may be reviewed by the U.S. Food and Drug Administration (FDA), the ACTG, the U.S. Office for Human Research Protections (OHRP), or other local, U.S., and international regulatory entities as part of their duties, (insert name of site) institutional review board (IRB) (a committee that protects the rights and safety of participants in research), National Institutes of Health (NIH), study staff, study monitors, the drug company supporting this study, and their designees. Having a Certificate of Confidentiality does not prevent you from releasing information about yourself and your participation in the study.

Even with the Certificate of Confidentiality, if the study staff learns of possible child abuse and/or neglect or a risk of harm to yourself or others, we are required to tell the proper authorities.

A description of this clinical trial will be available on www.ClinicalTrials.gov, as required by U.S. law. 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.

WHAT ARE THE COSTS TO ME?

Taking part in this study may lead to added costs to you and your insurance company. In some cases it is possible that your insurance company will not pay for these costs because you are taking part in a research study.

The HIV pDNA vaccine or placebo that you may receive will be supplied by the study. Your anti-HIV drugs will not be provided by the study, so you must get these drugs through your primary care provider; you must also pay for them through some other manner, such as your insurance or local AIDS Drug Assistance Program (ADAP).

WILL I RECEIVE ANY PAYMENT?

[*Sites: Please indicate whether you will provide payment to participants. If so, please describe the amount to be paid or reimbursed, the payment schedule, and any prorated schedule should the participant decide to withdraw or is withdrawn early by the investigator*.]

WHAT HAPPENS IF I AM INJURED?

If you are injured as a result of being in this study, you will be given immediate treatment for your injuries. The cost for this treatment will be charged to you or your insurance company.

There is no program for compensation either through this institution or the National Institutes of Health. You will not be giving up any of your legal rights by signing this consent form.

WHAT ARE MY RIGHTS AS A RESEARCH PARTICIPANT?

Taking part in this study is completely voluntary. You may choose not to take part in this study or leave this study at any time. Your decision will not have any impact on your participation in other studies conducted by NIH and will not result in any penalty or loss of benefits to which you are otherwise entitled.

We will tell you about new information from this or other studies that may affect your health, welfare, or willingness to stay in this study. If you want the results of the study, let the study staff know.

WHAT DO I DO IF I HAVE QUESTIONS OR PROBLEMS?

For questions about this study or a research-related injury, contact:
- name of the investigator or other study staff
- telephone number of above

For questions about your rights as a research participant, contact:
- name or title of person on the Institutional Review Board (IRB) or other organization appropriate for the site
- telephone number of above

SIGNATURE PAGE

If you have read this consent form (or had it explained to you), all your questions have been answered and you agree to take part in this study, please sign your name below.

Participant's Name (print)	Participant's Signature and Date
Participant's Legally Authorized Representative (print) (As appropriate)	Legally Authorized Representative Signature and Date
Study Staff Conducting Consent Discussion (print)	Study Staff's Signature and Date
Witness's Name (print) (As appropriate)	Witness's Signature and Date