A Phase I Randomized, Double-Blind, Placebo-Controlled Study of a Multi-Antigen DNA Vaccine Prime Delivered by In Vivo Electroporation, rVSV Booster Vaccine in HIV-Infected Patients Who Began Antiretroviral Therapy During Acute/Early Infection

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List of Abbreviations

ALT	Alanine Aminotransferase
AE	Adverse Event/Adverse Experience
AR	Adverse Reaction
ART	Antiretroviral Therapy
AST	Aspartate Aminotransferase
ATI	Analytical Treatment Interruption
BGH	Bovine Growth Hormone
bp	Base Pair
cART	Combination Antiretroviral Therapy
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CPK	Creatine Phosphokinase
CRIMSON	Clinical Research Information Management System of the NIAID
CSO	Clinical Safety Office
CT	Cytoplasmic Tail
DAIDS	Division of AIDS
DCR	Division of Clinical Research
DSMB	Data and Safety Monitoring Board
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic Acid
EIA	Enzyme Immunoassay
ELISpot	Enzyme-Linked Immunosorbent Spot Assay
EP	Electroporation
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HBsAg	Hepatitis B Surface Antigen
HCMV	Human Cytomegalovirus
HCV	Hepatitis C Virus
HIV-1	Human Immunodeficiency Virus Type 1
HIV-MAG pDNA	HIV-1 Multiantigen Plasmid DNA
ICH	International Conference on Harmonization
ICS	Intracellular Cytokine Staining
IFN	Interferon
IL	Interleukin
IL-12 pDNA	Interleukin 12 Plasmid DNA
IM	Intramuscular
IND	Investigational New Drug
IRB	Institutional Review Board
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NNRTIs	Non-Nucleoside Reverse Transcriptase Inhibitors
OCRPRO	Office of Clinical Research Policy and Regulatory Operations

OHRP	Office for Human Research Protections
PBMCs	Peripheral Blood Mononuclear Cells
PCR	Polymerase Chain Reaction
pDNA	Plasmid DNA
Pfu	Plaque-Forming Units
PolyA	Polyadenylation
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
rVSV	Recombinant Vesicular Stomatitis Virus
rVSV HIV *gag*	Recombinant Vesicular Stomatitis Virus Vector Containing the HIV-1 *Gag* Gene
$rVSV_{IN}$	Recombinant Indiana Serotype Vesicular Stomatitis Virus
SAE	Serious Adverse Event/Serious Adverse Experience
SAR	Suspected Adverse Reaction
SCMV	Simian Cytomegalovirus
SIV	Simian Immunodeficiency Virus
SLE	Systemic Lupus Erythematosis
SRCP	Safety Review and Communication Plan
SUSAR	Serious And Unexpected Suspected Adverse Reaction
SV	Simian Virus
TDS	TriGrid® Delivery System
ULN	Upper Limit of Normal
UP	Unanticipated Problem
VPA	Valproic Acid
VSV	Vesicular Stomatitis Virus
VSV_{IN}	Indiana Serotype Vesicular Stomatitis Virus

Protocol Summary

Full Title:	A Phase I randomized, double-blind, placebo-controlled study of a multi-antigen DNA vaccine prime delivered by in vivo electroporation, rVSV booster vaccine in HIV-infected patients who began antiretroviral therapy during acute/early infection.
Short Title:	HIV-MAG pDNA prime, rVSV boost.
Clinical Phase:	Phase I.
IND Sponsor:	OCRPRO
Conducted by:	LIR/NIAID.
Principal Investigator:	Michael C. Sneller, MD.
Sample Size:	n=30 subjects with human immunodeficiency virus type 1 (HIV-1).
Accrual Ceiling:	n=50.
Study Population:	Adults (18-65 years of age) on combination antiretroviral therapy (cART) who started therapy during early or acute HIV-1 infection.
Accrual Period:	18 months.
Study Design:	This is a randomized, 2-arm (1:1), double-blind, placebo-controlled study evaluating the safety and efficacy of an HIV-1 multiantigen plasmid DNA (HIV-MAG pDNA) vaccine prime in combination with an interleukin 12 plasmid DNA (IL-12 pDNA) adjuvant, delivered by intramuscular (IM) electroporation (EP), and a recombinant vesicular stomatitis virus vector containing the HIV-1 *gag* gene (rVSV HIV *gag*) booster vaccine delivered by conventional IM injection.
Study Duration:	Start Date: August 2013. End Date: November 2016.
Study Agents/ Intervention Description:	**HIV-MAG pDNA vaccine** prime (Profectus Biosciences, Inc.; Tarrytown, NY) will be administered at a dose of 3000 µg (1500 µg of the HIV-1 *gag/pol* plasmid and 1500 µg of

	the HIV-1 *net/tat/vif*, *env* plasmid) at week 0, 4, 12, and 36. Each construct of HIV-MAG pDNA vaccine (1500 µg each) will be mixed and combined with 1000 µg of the IL-12 pDNA adjuvant. The resulting mixture will be divided and administered as 2 IM injections, 1 into each deltoid, with EP using the Ichor Medical Systems (San Diego, CA) TriGrid® Delivery System (TDS) device. **IL-12 pDNA adjuvant** (GENEVAX®; Profectus Biosciences, Inc.; Tarrytown, NY) will be mixed with the HIV MAG pDNA vaccine, as noted above, and administered at a dose of 1000 µg (500 µg in each IM injection) at week 0, 4, 12, and 36. **rVSV HIV *gag* booster vaccine** (Profectus Biosciences, Inc.; Tarrytown, NY) will be administered as 2 conventional IM injections (1 mL each), 1 into each deltoid, for a total dose of $1x10^7$ plaque-forming units (pfu) at week 24 and 48.
Primary Objective:	To evaluate safety and tolerability of the study vaccines in subjects who began cART during acute or early HIV-1 infection.
Secondary Objectives:	To evaluate the efficacy of the study vaccines as determined by its effect on rebound viremia following analytical treatment interruption (ATI).
Exploratory Objectives:	1. To assess the effect of the study vaccines on the rate of decay of the HIV-infected, CD4+ T-cell reservoir. 2. To determine the immunogenicity of the study vaccines.
Primary Endpoint:	The rate of occurrence of grade 3 or higher AEs, including serious adverse events (SAEs) that per standard criteria (see safety section) are: • At least possibly related to the test article, and • Definitely NOT related to a factor other than the test article.
Secondary Endpoint:	The difference in HIV-1 viral load at the end of the ATI between the vaccine and placebo groups.
Exploratory Endpoints:	1. The frequency and rate of decay of the infectious HIV reservoir between the vaccine and placebo groups.

2. Change from baseline (mean of entry and pre-entry) to post-vaccination in the number of interferon (IFN)-γ/ interleukin (IL)-2-generating CD8+ and CD4+ T-cells/million peripheral blood mononuclear cells (PBMCs); in response to *gag, pol, env, nef, tat* and *vif*, as measured by intracellular cytokine staining (ICS).

Précis

The advent of combination antiretroviral therapy (cART) has dramatically improved the clinical outcome in human immunodeficiency virus (HIV)-infected individuals through sustained reduction in viral replication. However, it has become clear that cART alone cannot eradicate HIV in infected individuals, likely in part due to the persistence of viral reservoirs in peripheral blood and various tissue compartments. Consequently, a major thrust of HIV research over the past several years has been to develop therapeutic strategies that can eliminate persistent viral reservoirs and boost host immunity to control viral replication upon discontinuation of cART. Therapeutic HIV vaccination is one approach that could potentially achieve these goals through vaccine-induced improvement in HIV-specific immune responses and/or by direct reactivation of HIV-specific $CD4^+$ memory T cells that harbor latent HIV. An effective therapeutic vaccine could augment immunologic control of HIV infection and potentially obviate the need for chronic cART.

The current study is an exploratory randomized, 2-arm (1:1), double-blind, placebo-controlled trial evaluating the safety and efficacy of an HIV-1 multiantigen plasmid DNA (HIV-MAG pDNA) vaccine prime in combination with an interleukin-12 plasmid DNA (IL-12 pDNA) adjuvant delivered by in vivo electroporation followed by a recombinant vesicular stomatitis virus vector containing the HIV-1 *gag* gene (rVSV HIV *gag*) booster vaccine in subjects on cART who started therapy during acute or early HIV infection.

Subjects will be randomized to receive placebo or the HIV-MAG pDNA (3000 µg) vaccine prime and IL-12 pDNA adjuvant (1000 µg) at week 0, 4, 12, and 36, and the rVSV HIV *gag* booster vaccine ($1x10^7$ plaque-forming units) at week 24 and 48. The HIV-MAG pDNA vaccine prime and IL-12 pDNA adjuvant will be administered as 2 IM injections, 1 into each deltoid, with electroporation using the Ichor TDS device, while the rVSV HIV *gag* booster vaccine will be administered as 2 conventional IM injections, 1 into each deltoid. After the week 56 visit, all subjects will undergo an analytical treatment interruption to determine if the vaccination strategy results in an improved immune control of viral replication, as evidenced by a blunted or absent rebound in HIV plasma viremia. All subjects will be followed through week 96 for safety and efficacy parameters.

The study population includes HIV-infected adults who began cART during acute or early infection. Subjects must be receiving an effective cART regimen, with a CD4 cell count of >450 cells/mm^3 at screening, and they must have documented viral suppression below the limit of detection for >1 year. The rationale for testing the study vaccine regimen in this subject population is because these individuals may have a relatively preserved immune function, which could be augmented by therapeutic vaccination.

1 Background Information and Scientific Rationale

1.1 Background Information

Prolonged suppression of plasma viremia is now achievable in the majority of HIV-infected individuals receiving cART.[1] However, complete eradication of HIV has not been possible using cART alone, likely due to the persistence of various viral reservoirs.[2-4] Previous studies have demonstrated that HIV persists in latently infected, CD4+ T cells in the peripheral blood of virtually all infected individuals receiving clinically effective doses of cART.[5-7] In addition, evidence from a number of recent studies has suggested that low levels of HIV replication may persist in such individuals in the absence of detectable viremia.[8-10] Given that plasma viremia rapidly rebounds in virtually all HIV-infected individuals upon cessation of cART, regardless of the duration and timing of therapy,[11,12] therapeutic strategies aimed at destroying persistently infected cells are needed to achieve complete eradication of HIV.[13]

Nearly a decade ago, several clinical studies tested the feasibility of purging latent HIV reservoirs in infected individuals receiving cART, with the use of non-specific immune-activating agents, such as IL-2[14] and an anti-CD3 antibody.[15] In a non-randomized study, subjects who received cART plus repeated cycles of intermittent IL-2 exhibited a marked diminution of the pool of latently infected, resting CD4+ T cells, compared with those receiving cART alone;[14] nonetheless, rapid rebound of plasma viremia was observed following interruption of cART.[12] More recently, valproic acid (VPA), a histone deacetylase inhibitor, was tested in humans as a potential virus-“purging” agent and was shown in one study to diminish the size of the latent viral reservoir in infected individuals receiving ART.[16] However, subsequent studies demonstrated no appreciable reduction of the latent viral reservoir following treatment with VPA.[17,18]

Other therapeutic strategies aimed at achieving eradication are under investigation. These include genetic manipulation of CD4+ T cells (deletion of one of the HIV co-receptors chemokine receptor type 5)[19,20] and stem-cell transplantation.[21] Although it appears that eradication of HIV was achieved in one individual who underwent multiple rounds of chemotherapy and stem-cell transplantation,[21] it is unlikely that this approach can be safely applied to a substantial proportion of the HIV-infected population. Considering the difficulties encountered with these modalities, a more realistic approach is needed to explore the therapeutic strategies that harness host immunity to control HIV replication in infected individuals in the absence of cART (a “functional” cure).[4] One such strategy is therapeutic vaccination.

The goal of therapeutic vaccination is to induce or augment virus-specific immune responses using a planned exposure to HIV antigens. A successful therapeutic vaccination regimen would sufficiently augment the immune response to HIV such

that control of viral replication could be achieved in the absence of cART, thus, mimicking the unique immune response of a small portion of HIV-infected individuals who are able to control viral replication in the absence of treatment (variably termed elite controllers or long-term non-progressors). Early attempts at therapeutic vaccination in HIV-1-infected individuals during the pre-cART era were largely unsuccessful; however, these results were not unexpected. The introduction of a small amount of exogenous antigen in the form of a therapeutic vaccine is unlikely to have a significant immunostimulatory effect in chronically infected individuals with uncontrolled viral replication and ongoing CD4 cell death.

More recent pre-clinical and clinical studies, performed in the cART era, have shown improvement of virus-specific immune responses after therapeutic vaccination. The induction of broad T-cell responses was observed in a macaque simian immunodeficiency virus (SIV) model using DNA vaccine during suppressive cART.[22] Vaccination reduced virus burden in the gut and blood, compared with unvaccinated controls and provided durable protection from viral rebound and disease progression following ART interruption. In the ACTG study 5197, vaccination of subjects receiving suppressive cART with an Ad5 HIV *gag* vector induced moderate increases in HIV-specific T-cell responses.[23] Vaccinated subjects showed a modest decrease in their viral load set point at the end of the ATI, compared with unvaccinated controls; however, the decrease did not reach the pre-specified level of significance (P <0.025). In a recently published randomized controlled study by Garcia et al,[24] subjects receiving the active dendritic cell vaccine (autologous dendritic cells pulsed with autologous heat inactivated virus) had a significant decrease in viral set point following ATI compared to controls.

The studies presented above suggest that therapeutic vaccination could become a promising immune-based modality for the treatment of HIV-1 infection, if more immunogenic vaccination strategies can be developed. Our trial will utilize a pDNA prime/live virus vector boost vaccination strategy that has been shown to elicit a strong HIV-specific cellular immune response in pre-clinical animal models. This vaccination regimen has additional features designed to maximize immunogenicity. For example, the HIV-MAG pDNA vaccine prime-IL-12 pDNA adjuvant will be administered to study participants via an in vivo EP delivery system. EP is a technology in which a transient electric field is applied to enhance the cellular uptake of large molecules, such as DNA, and it has been shown to provide an efficient method of delivering DNA into the cell.[25] Preclinical studies in nonhuman primates provide compelling evidence that in vivo EP enhances the potency of the HIV-MAG pDNA/IL-12 pDNA vaccine (see Section 1.1.2). In addition, clinical studies indicate that EP delivery of pDNA vaccines is safe and well tolerated.[26] Secondly, the vaccine regimen will utilize an IL-12 pDNA adjuvant that, when administered by in vivo EP, is designed to enhance the immunogenicity of the pDNA vaccine. Thirdly, the booster component of the vaccine utilizes a

recombinant vesicular stomatitis virus (rVSV)-vector encoding HIV *gag*. Wild-type vesicular stomatitis virus (VSV) rarely causes infections in humans; thus, the use of an rVSV vector will minimize any interference of pre-existing immune responses in study subjects.

1.1.1 Description of the Study Agents

HIV-MAG pDNA vaccine prime

The HIV-MAG pDNA vaccine consists of 2 DNA expression vectors (HIV-1 *gag/pol* [WLV-151M] and HIV-1 *nef/tat/vif*, *env* [WLV-255M]) encoding multiple HIV-1 clade B antigens (Figure 1). WLV-151M is a 7,487-base pair (bp) plasmid expressing an HIV-1 clade B ($HIV\text{-}1_{HXb2}$) Gag/Pol fusion protein controlled by the human cytomegalovirus (HCMV) immediate early promoter and the bovine growth hormone (BGH) polyadenylation (polyA) signal. WLV-255M is a dual-promoter plasmid (8,750 bp) expressing an HIV-1 clade B primary isolate ($HIV\text{-}1_{6101}$) Env gp160 controlled by the simian cytomegalovirus (SCMV) promoter and the BGH polyA, and an HIV-1 clade B ($HIV\text{-}1_{NL43}$) Nef/Tat/Vif fusion protein utilizing the HCMV promoter and the simian virus (SV)40 polyA tail.

IL-12 pDNA adjuvant

IL-12 pDNA adjuvant is a dual-promoter expression plasmid (Figure 2) that is 6,259 bp long and consists of 2 genes encoding the human IL-12 proteins p35 and p40. Each cistron contains one of the two IL-12 subunits, p35 or p40, and each subunit is controlled by independent regulatory elements. The p35 subunit is controlled by the HCMV promoter/ enhancer and the SV40 polyA signal. The p40 subunit is regulated by the SCMV promoter and the BGH polyA signal.

Figure 1: Map of HIV-1*gag/pol* pDNA (WLV-151M) and HIV-1 *nef/tat/vif, env* pDNA (WLV-255M)

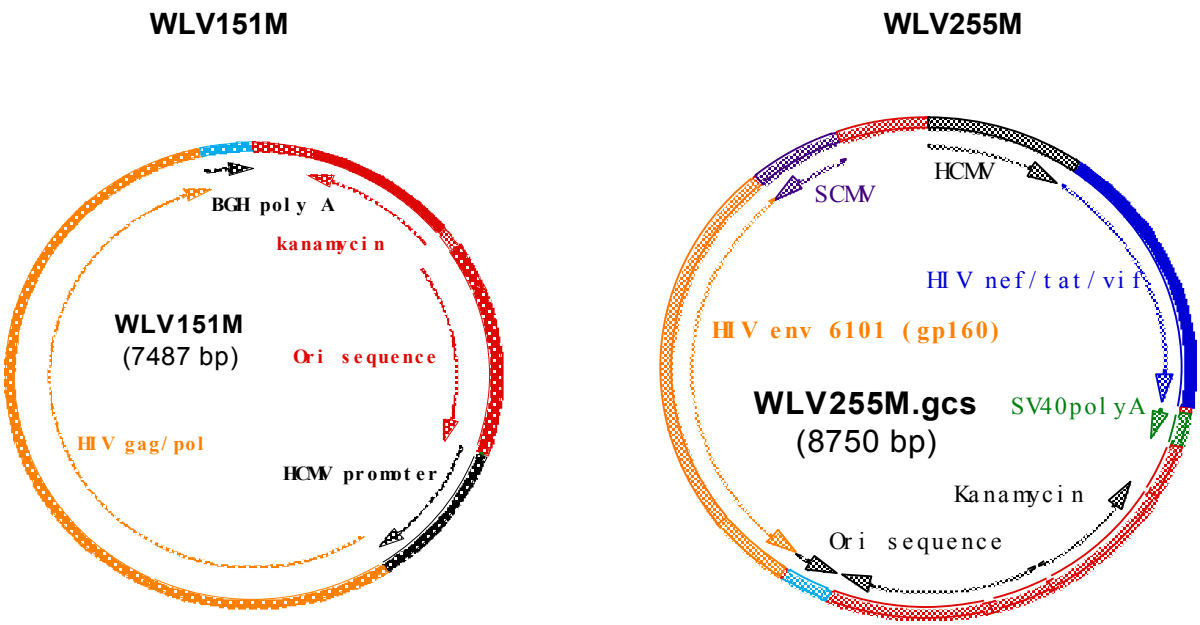
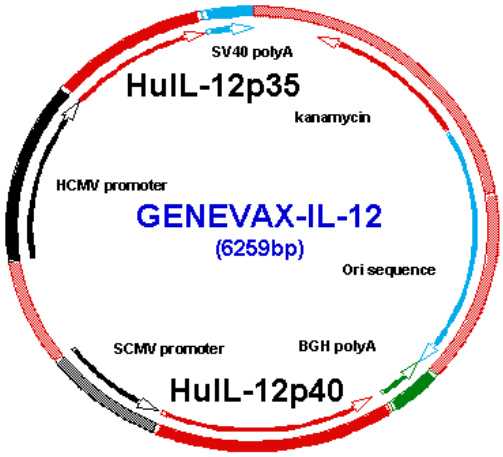


Figure 2: Map of IL-12 pDNA

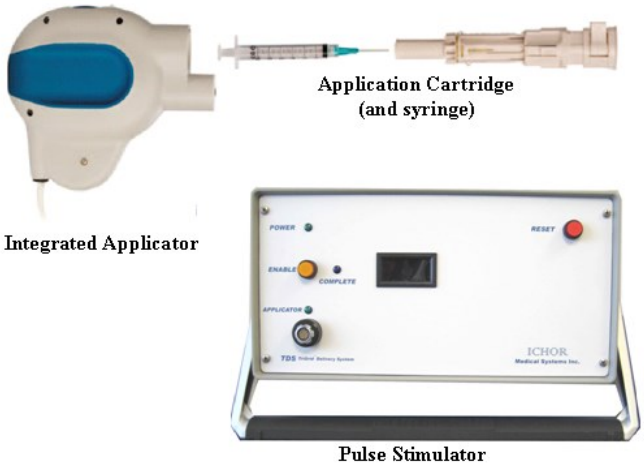


Ichor TriGrid delivery system device

The Ichor TDS device is designed for IM administration of DNA. Specifically, the device is designed to propagate EP inducing transient electrical fields at the site of administration in the presence of DNA, thus, enhancing its cellular uptake. The TDS device has been shown to be an efficient method for delivering DNA into cell,[25] and preclinical studies have suggested that in vivo EP enhances the potency of a DNA vaccine.[27,28]

The TDS device consists of 3 components depicted in Figure 3. They include a single-use application cartridge, an integrated applicator, and a pulse stimulator. The current configuration of the device is designed for the administration of investigational agents in the context of early phase human clinical studies. To provide the degree of flexibility necessary to support novel product candidates entering the clinical-phase testing, the TDS device has been designed to interface with a standard, "off-the-shelf" syringe and can accommodate injection volumes of 0.5 mL to 1.2 mL.

Figure 3: The Ichor TDS device

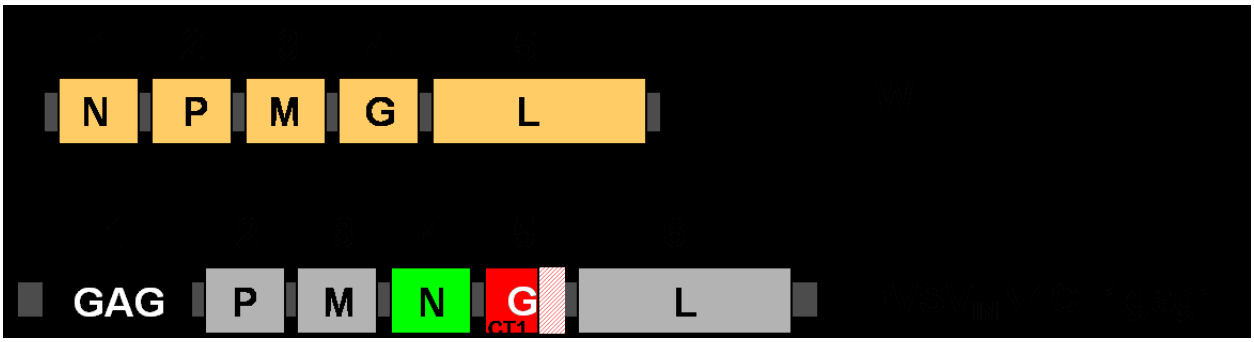


rVSV HIV *gag* booster vaccine

The rVSV HIV *gag* booster vaccine preparation contains an attenuated, replication-competent form of the lab-adapted recombinant Indiana serotype vesicular stomatitis virus ($rVSV_{IN}$) that expresses the HIV-1 clade B (strain HXB2) Gag protein. The vaccine vector was attenuated by translocation of the virus N gene from the first position (N1) in the genome to the fourth position (N4) and truncation of the virus G protein cytoplasmic tail (CT) from 29 amino acids to 1 amino acid (CT1). The HIV-1 *gag* gene was inserted at

the first position (*gag1*) in the rVSV genome adjacent to the virus messenger RNA transcription promoter to maximize Gag protein expression. The vaccine vector has been named to reflect the major attenuating mutations, the position of the *gag* gene, and the vector serotype (Figure 4).

Figure 4: Genomic organization of wild type VSV and the vaccine vector, $rVSV_{IN}$N4CT1gag1



WT=wild type; $rVSV_{IN}$=recombinant Indiana serotype vesicular stomatitis virus.
Numbering (e.g. N4, gag1) denotes the relative positions of genes within the viral genomes. The subscript IN (Indiana) describes the vector serotype. CT1 indicates that the length of the G protein cytoplasmic tail is a single amino acid.

1.1.2 Summary of Previous Pre-Clinical Studies

An overview of pharmacokinetic, toxicological, and immunogenicity studies relevant to the clinical testing of the HIV-MAG pDNA vaccine alone, or in combination with human IL-12 pDNA adjuvant (with or without TDS EP injector) and the rVSV HIV *gag* booster vaccine, is presented below. More detailed information can be found in the investigator's brochure documents.

Biodistribution and persistence/integration study of the HIV-MAG pDNA in combination with the human IL-12 pDNA adjuvant delivered by IM or IM EP injection in rats

A good laboratory practice study was carried out to characterize the systemic biodistribution and persistence/integration of the HIV-MAG pDNA vaccine in combination with the human IL-12 pDNA adjuvant in rats. The formulated pDNA vaccine was administered bilaterally (tibialis anterior muscles) in 2 groups of 22 rats each (11 rats/sex/group) at a dose of 80 µg (32 µg HIV-1 *gag/pol*, 32 µg HIV-1 *nef/tat/vif, env*, 16 µg IL-12 pDNA) by conventional IM injection or IM EP delivery using the Ichor TDS device (for a total dose of 160 µg per animal). Additionally, a group of 12 rats (6 rats/sex) was injected with saline using the TDS device. Real-time quantitative polymerase chain reaction (PCR) was used to estimate the levels of the vaccine pDNA in tissue specimens. The presence of the vaccine pDNA in the total DNA (genomic +

plasmid) isolated from the blood and various tissues was examined in this study at day 7 and day 60 post-vaccination. PCR analyses of tissue specimens obtained at day 7 showed that the distribution of the vaccine pDNA was largely confined to the tissues at the site of administration (muscle and skin) following IM or IM EP delivery. On day 60, the vaccine pDNA was detected in tissues at the site of administration in both the IM and IM EP delivery groups at levels of <7,000 copies/µg. Plasmid levels decreased by approximately 1 to 2 orders of magnitude compared to the levels detected on day 7. These analyses indicate that IM and IM EP delivery of the vaccine pDNA resulted in an initial uptake of the plasmid, which was localized to the injection site and then decreased substantially over time. No significant differences were observed in the biodistribution or persistence of the vaccine pDNA between the IM and IM EP delivery groups.

Biodistribution and persistence study of the rVSV HIV *gag* vaccine delivered by IM injection in mice

A virus biodistribution study has been performed in mice to investigate the degree of virus replication and dissemination in a permissive host following IM vaccination.[29] Mice were inoculated with the $rVSV_{IN}$N4CT9*gag1* vaccine at a dose of 10^8 pfu (10 x the anticipated clinical dose); the $rVSV_{IN}$N4CT9*gag1* is a closely related but more competent vector than $rVSV_{IN}$N4CT1*gag*1 vector. Vector replication and spread was monitored by assaying the infectious virus and by virus- specific quantitative PCR performed on tissue, blood, and organ samples collected at different time intervals post-injection (day 0-2, 4, 8, and 10). Results from these studies showed that all measurable virus replication was localized at the IM injection site and in the major, draining poplilteal lymph node. Viral replication peaked at 1 to 2 days post-injection, and there was no evidence of viral gene expression at 10 days post-injection.

Repeat-dose toxicity study of the HIV-MAG pDNA vaccine with or without the human IL-12 pDNA adjuvant and the rVSV HIV *gag* booster vaccine delivered by IM or IM EP injection in rabbits

In a repeat-dose toxicology study, NZW rabbits were administered the HIV-MAG pDNA vaccine as an IM injection at a dose of 4,000 µg with or without the human IL-12 pDNA adjuvant (1,000 µg) on day 1, 22, 43, 64, 85, and 106. A subgroup of animals treated with the HIV-MAG pDNA vaccine and IL-12 pDNA adjuvant also received the rVSV HIV *gag* booster vaccine on day 64 and 85 by IM delivery. Animals in all groups exhibited minor changes in body weight, food consumption, and clinical and anatomical pathology, generally with a complete or partial resolution of these changes by the end of the recovery period. Based on these results, it was concluded that the toxicity effects were localized or reflective of an inflammatory response targeted at the injection/EP sites rather than being systemic in nature.

Toxicology study of the rVSV HIV *gag* vaccine delivered by IM injection in rabbits

A good manufacturing practice toxicology study was performed in 20 rabbits using the rVSV HIV *gag* vaccine. No AEs were observed either at the injection site or systemically following the IM injection of the rVSV HIV *gag* vaccine at a total dose of 10^8 pfu. Blood chemistry panels remained normal throughout the study and tissue/organ histopathology profiles were unremarkable in animals treated with the vaccine, compared with those in the placebo group.

Immunogenicity study of the HIV-MAG pDNA vaccine plus a rhesus IL-12 pDNA adjuvant with and without in vivo EP in rhesus macaques

To determine the effect of EP on the immunogenicity of HIV-MAG pDNA vaccination, a preclinical study was conducted in rhesus macaques immunized with an HIV-MAG pDNA vaccine (8500 µg) plus a rhesus IL-12 pDNA adjuvant (1500 µg) by conventional IM injection or IM EP delivery (1200 µg HIV MAG pDNA + 300 µg IL-12 pDNA).[30] Animals immunized by IM EP delivery exhibited stronger cellular responses, compared with those immunized by conventional IM injection. At 8 and 22 weeks after the final pDNA immunization, there was a 10- and 45-fold increase, respectively, in HIV-specific IFN-γ responses, as measured by an enzyme-linked immunosorbent spot (ELISpot) assay, in the IM EP delivery group, compared with the IM delivery group. The 10- and 45-fold increase translated into an apparent 50- or 225-fold increase in pDNA vaccine potency, respectively. Importantly, the IM EP delivery enhanced the immune response against the less immunogenic antigens (*nef-tat-vif*), resulting in a more balanced immune response. In addition, antibody responses against the *env* antigen showed that the IM EP delivery led to an ~2.5-log increase in antibody titer, compared with the IM delivery group.

Immunogenicity studies of an rVSV SIV or HIV Gag p55 vaccine in rhesus macaques

The first non-human primate experiments to evaluate rVSV as a vector system for HIV infection were conducted by Rose and colleagues at Yale. In a pioneering study, rhesus macaques (n=7) were immunized with a combination of 2 prototypic rVSV vectors expressing an HIV-1–89.6 *env* gp160/VSV-G fusion polypeptide and an SIV Gag p55 protein or control (n=8).[31] In this study, all animals in the combination vaccine group remained disease-free after the challenge for >4 years. In contrast, all the macaques in the control group progressed to AIDS within an average time of 8 months. The protection from AIDS in this study correlated with large differences in peak viral loads, low or undetectable viral loads at set point, and the preservation of CD4+ T cells in the vaccine recipients relative to controls. This encouraging level of post-challenge vaccine efficacy suggested that

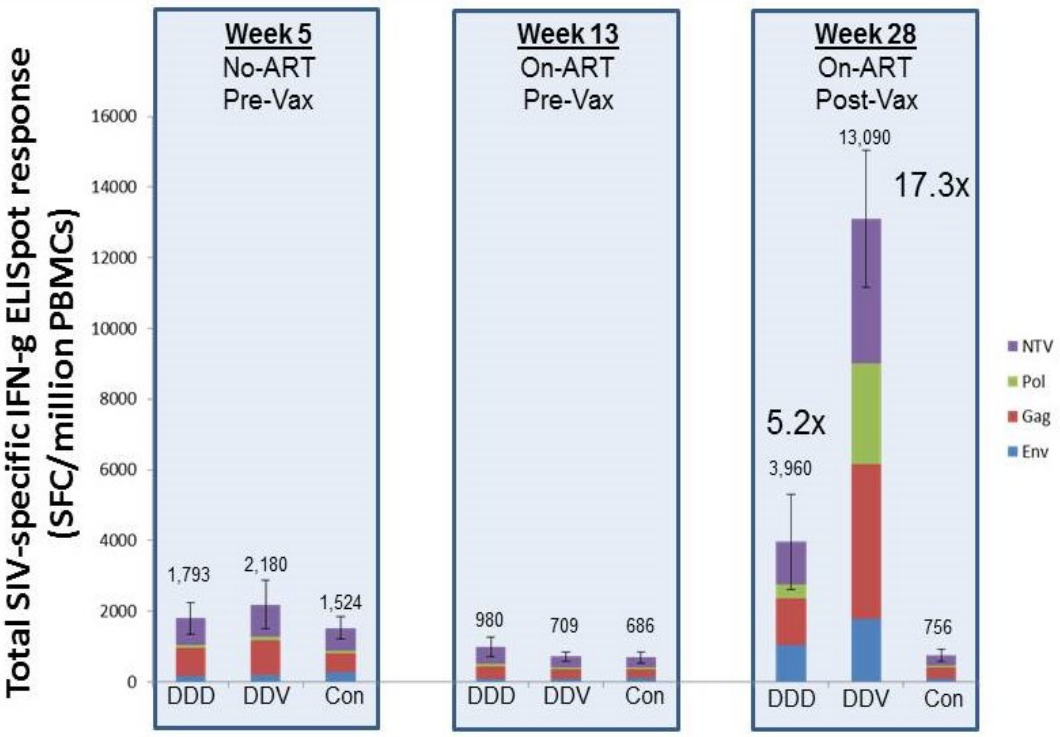
rVSV vectors expressing HIV genes might be an effective AIDS vaccine in humans.

In a separate unpublished study conducted by Profectus, groups of rhesus macaques were immunized at weeks 0 and 8 with a highly attenuated replication competent rVSV vaccine vector encoding the HIV-1 Gag p55 protein (rVSV HIV *gag*) at a dose of $1x10^7$ pfu or a much more virulent prototypic rVSV vector (rVSV-HIV *gag*5) vaccine at a dose of $1x10^7$ pfu by conventional IM injection; the rVSV-HIV *gag*5 vaccine was previously shown by Rose and colleagues[31] to protect macaques against simian-AIDS in a pathogenic SHIV (simian/human immunodeficiency virus) challenge model. The data demonstrate that the highly attenuated rVSV vector was as good as the more virulent vector in eliciting HIV *gag*-specific IFN-γ ELISpot responses following vaccination ($rVSV_{IN}$N4CT1gag1 vaccine Profectus Bioscience investigator's brochure).

Immunogenicity studies of an IL-12-enhanced SIV-MAG pDNA vaccine plus an rVSV *gag* booster vaccine in rhesus macaques

In a pre-clinical, immunogenicity study conducted by Profectus, rhesus macaques were infected with SIV and subsequently placed on cART until they reached suppressive viremia. Macaques were then immunized with an IL-12-enhanced SIV-MAG pDNA vaccine alone or in combination with a heterologous rVSV booster vaccine. Unimmunized control animals exhibited SIV-specific immune responses barely detectable by ELISpot and polyfunctional ICS assays. Animals vaccinated with the IL-12-enhanced SIV-MAG pDNA vaccine demonstrated a 5-fold increase in total SIV-specific ELISpot responses, compared with control animals.. In contrast, animals vaccinated with the IL-12-enhanced SIV-MAG pDNA vaccine plus the rVSV booster vaccine demonstrated a 17-fold increase in total SIV-specific ELISpot responses, compared with control animals (Figure 5).

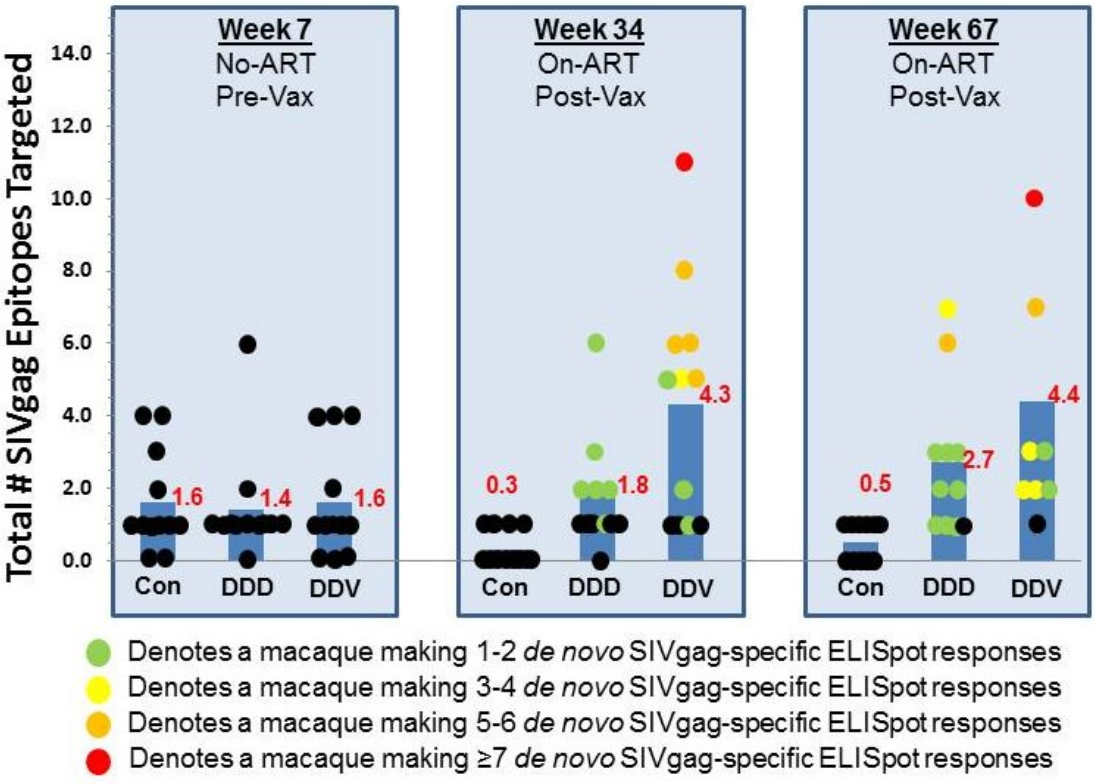
Figure 5: SIV-specific IFN-γ ELISpot responses in immunized SIV-infected rhesus macaques



DDD=animals vaccinated with an IL-12-enhanced SIV-MAG pDNA vaccine; DDV= animals vaccinated with IL-12-enhanced SIV-MAG pDNA vaccine plus an rVSV booster vaccine; Con=control animals; SIV=simian immunodeficiency virus; IFN-g=interferon γ; ELISpot=enzyme-linked immunospot spot assay; SFC=spot- forming cells; PBMCs= peripheral blood mononuclear cells; ART=antiretroviral therapy; Vax=vaccination.

In additional studies utilizing a polyfunctional ICS assay, the breadth and magnitude of SIV-specific cellular responses returned to pre-cART levels in vaccinated animals (data not shown). Furthermore, animals vaccinated with the IL-12-enhanced SIV-MAG pDNA vaccine plus the rVSV booster vaccine demonstrated de novo SIV-specific immune responses resulting in an increase in the epitope breadth of SIV *gag*-specific immune responses, compared with animals vaccinated with the IL-12-enhanced SIV-MAG pDNA vaccine alone (Figure 6). These data demonstrate that therapeutic immunization of SIV-infected rhesus macaques with a cytokine-enhanced pDNA prime, rVSV boost vaccination regimen augments virus-specific immunity and elicits de novo immune responses.

Figure 6: SIV *gag*-specific epitope breadth in immunized SIV-infected rhesus macaques



DDD=animals vaccinated with an IL-12-enhanced SIV-MAG pDNA vaccine alone; DDV= animals vaccinated with IL-12-enhanced SIV-MAG pDNA vaccine plus an rVSV booster vaccine; Con=control animals; SIV=simian immunodeficiency virus; ELISpot=enzyme-linked immunospot spot assay; ART=antiretroviral therapy; Vax=vaccination.

1.1.3 Summary of Relevant Clinical Studies

Table 1 below provides a summary of clinical studies evaluating the HIV-MAG pDNA vaccine, IL-12 pDNA vaccine, TDS device, and the rVSV HIV *gag* vaccine. Detailed information about these studies can be found in the investigator's brochure documents.

Table 1: Previous clinical use of the HIV-MAG pDNA vaccine, IL-12 pDNA, TDS device, and the rVSV HIV gag vaccine

Vaccine component	Previous clinical experience	Notes
HIV-MAG pDNA	Healthy adults: IAVI B004; ClinicalTrials.gov identifier: NCT01496989 HVTN-087; ClinicalTrials.gov identifier: NCT01578889	To date, approximately 105 healthy adult volunteers have received HIV-MAG pDNA vaccine delivered by IM EP injection.
	HIV-positive adults: ACTG-5281; ClinicalTrials.gov identifier: NCT01266616	To date, approximately 50 HIV-positive subjects have received the HIV-MAG pDNA vaccine delivered by IM EP injection.
IL-12 pDNA	Healthy adults: HVTN-060; ClinicalTrials.gov identifier: NCT00111605 HVTN-063; ClinicalTrials.gov identifier: NCT00115960 HVTN-070; ClinicalTrials.gov identifier: NCT00528489 HVTN-080; ClinicalTrials.gov identifier:NCT00991354 IAVI B004; ClinicalTrials.gov identifier: NCT01496989 HVTN-087; ClinicalTrials.gov identifier: NCT01578889	To date, approximately 120 healthy adult volunteers have received IL-12 pDNA delivered by IM injection and approximately 88 have received IL-12 pDNA delivered by IM EP injection.
	HIV-positive adults: ClinicalTrials.gov identifier: NCT00195312 ACTG-5281;ClinicalTrials.gov identifier: NCT01266616	To date, 10 HIV-positive subjects have received IL-12 pDNA delivered by IM injection and approximately 50 subjects have received IL-12 pDNA delivered by IM EP injection.

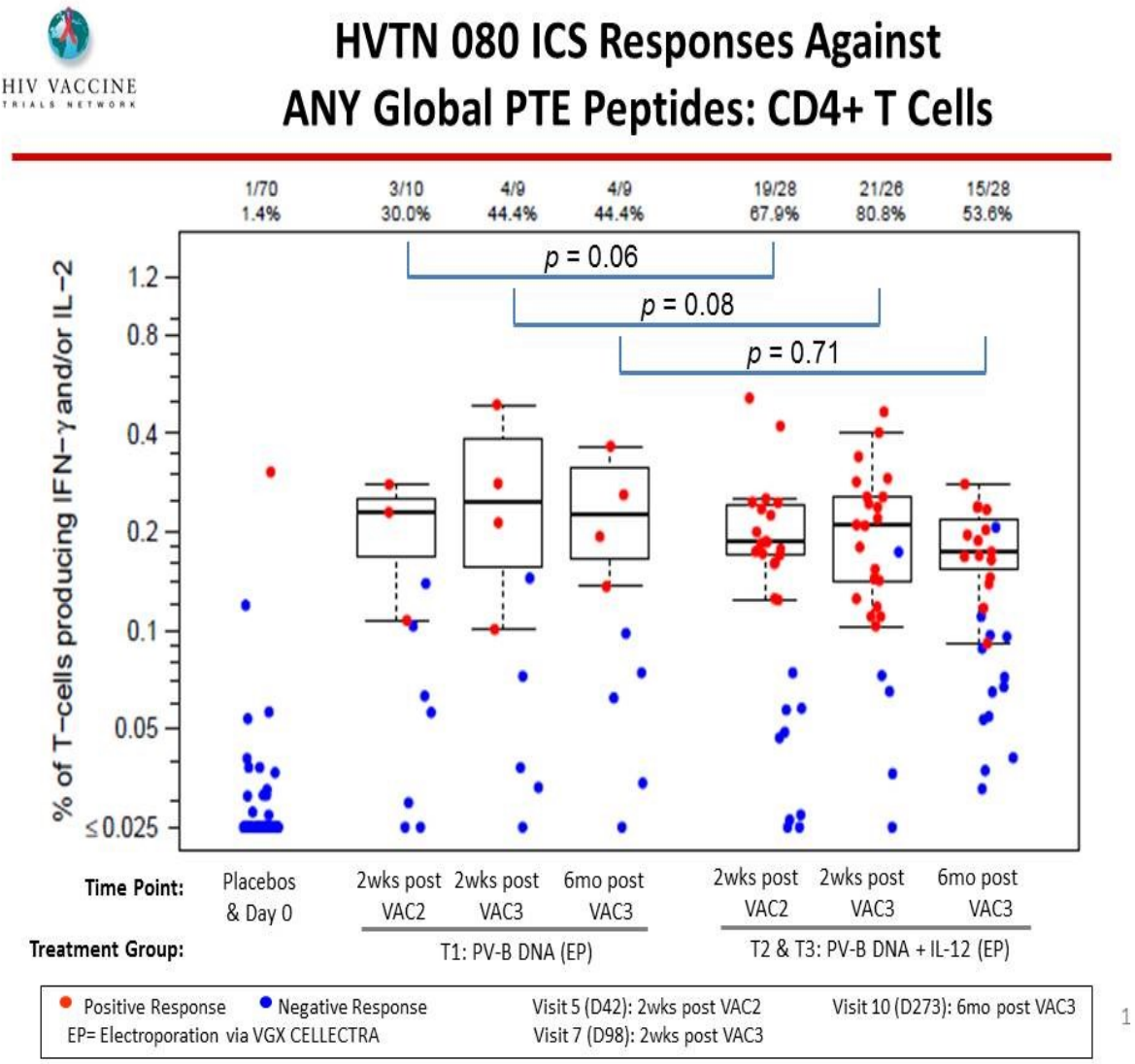
Vaccine component	Previous clinical experience	Notes
TDS device	Healthy adults: Rockefeller University Hospital; ClinicalTrials.gov identifier: NCT00545987	24 subjects have been administered a DNA vaccine encoding HIV-1 subtype B'/C antigens *env, gag, pol, nef,* and *tat* via the TDS device. Subjects have received up to 3 administrations at total DNA doses of up to 4,000 µg. An additional 8 subjects received placebo via the TDS device.
	IAVI B004; ClinicalTrials.gov identifier: NCT01496989	75 subjects have been administered the HIV-MAG pDNA vaccine with or without the IL-12 pDNA adjuvant via the TDS device.
	Emory Vaccine Trials Unit; ClinicalTrials.gov identifier: NCT01169077	30 subjects have been administered a DNA vaccine encoding epitopes from *P* falciparum antigens via the TDS device. Subjects have received up to 3 administrations at total DNA doses of up to 4,000 µg. An additional 9 subjects received placebo via the TDS device.
	Walter Reed Army Institute of Research; ClinicalTrials.gov identifier: NCT01502345	30 subjects have been administered a DNA vaccine encoding antigens from the Hantaan and Puumala viruses via the TDS device. Subjects have received up to 3administrations at total DNA doses of up to 2,000 µg.
	HIV-positive adults: ACTG-5281; ClinicalTrials.gov identifier: NCT01266616	To date, approximately 50 HIV-positive subjects have received the HIV-MAG pDNA vaccine with or without the IL-12 pDNA adjuvant delivered by IM injection via the TDS device.
	Stage IIB-IV melanoma subjects: Memorial Sloan Kettering Cancer Center; ClinicalTrials.gov identifier: NCT00471133	16 subjects have been administered a DNA vaccine encoding epitopes derived from the tyrosine-related protein 2 delivered by IM injection via the TDS device. Subjects received up to 6 administrations at total DNA doses of up to 4,000 µg.
	Hepatitis B virus-positive adults: ClinicalTrials.gov identifier: NCT01641536	To date, 9 subjects with chronic hepatitis B virus infection have received an antigen-encoding DNA vaccine by IM EP injection.
rVSV HIV *gag*	Healthy adults: HVTN-090; ClinicalTrials.gov identifier: NCT01438606	To date, approximately 50 healthy adult volunteers have received rVSV HIV *gag* vaccine delivered by IM injection.

IM=intramuscular; EP= electroporation; HIV-MAG pDNA=HIV-1 multiantigen plasmid DNA; IL-12

pDNA= interleukin 12 plasmid DNA; rVSV HIV *gag*=recombinant vesicular stomatitis virus vector containing the HIV-1 *gag* gene; TDS=TriGrid delivery system.

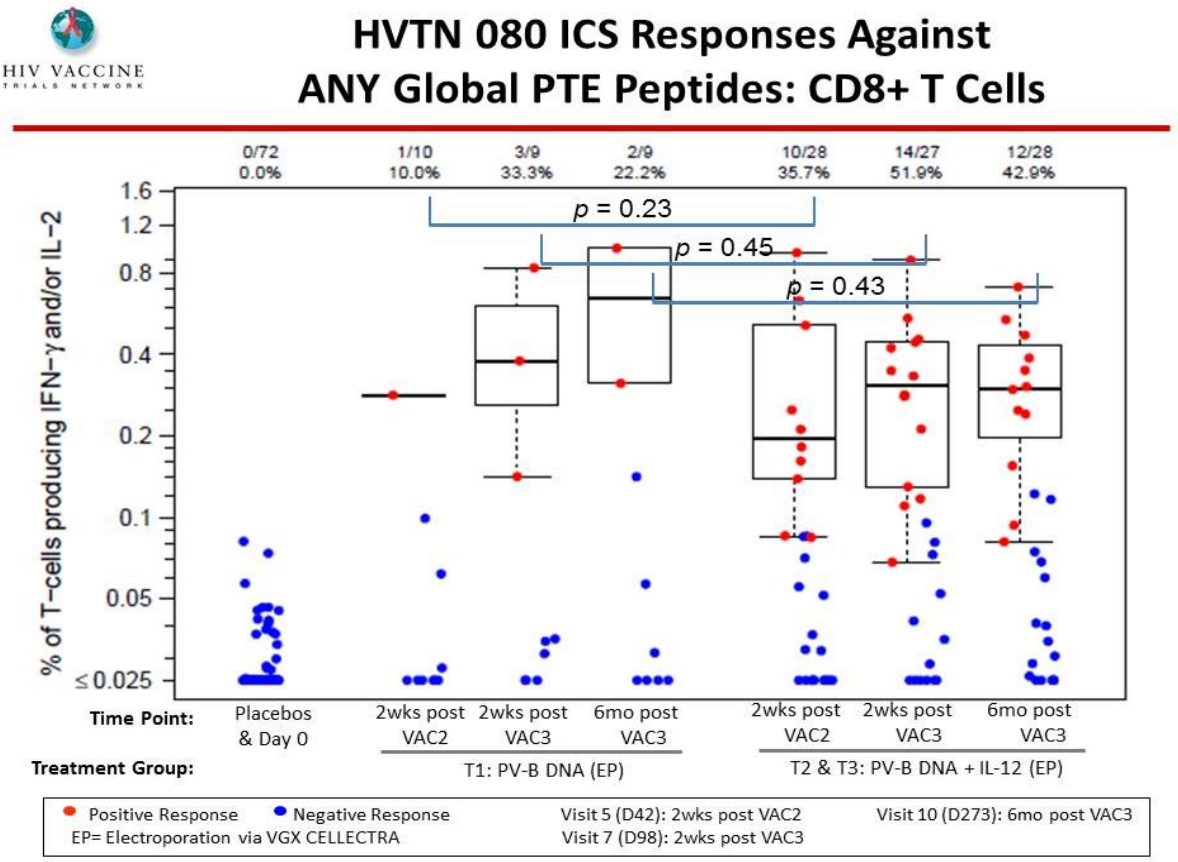
The safety and adjuvant activity of the IL-12 pDNA alone, or in combination with the HIV-MAG pDNA vaccine has been evaluated in several phase I clinical trials. The **HVTN-080** phase I study evaluated the safety and immunogenicity of the PENNVAX™-B (*gag, pol, env*) vaccine, with or without an IL-12 pDNA adjuvant, delivered by IM EP injection in 48 healthy, HIV-negative adults. Participants who received the *gag, pol, env* vaccine alone (group 1), or in combination with the IL-12 pDNA adjuvant (group 2 & 3), developed both HIV-specific CD4+ (Figure 7) and CD8+ (Figure 8) T-cell responses. After 3 vaccinations, 44.4% (4/9) of subjects in group 1 and 80.8% (21/26) of subjects in group 2 and 3 responded (Figure 7). The latter response rate was higher than after 4 vaccinations with the *gag, pol, env* vaccine with the IL-12 adjuvant delivered by IM injection without EP (P=.005; HVTN-070). The response rate for the *gag, pol, env* vaccine with the IL-12 adjuvant IM+EP was still relatively high (53.6% 15/28) 6 months after the third vaccination. These findings validate the activity of the IL-12 pDNA adjuvant in combination with a DNA plasmid vaccine. No serious vaccine-related safety concerns or AEs were reported.

Figure 7: HIV-specific CD4+ intracellular cytokine staining responses from the HVTN 080 study



IFN=interferon; IL=interleukin; VAC=vaccination.

Figure 8: HIV-specific CD8+ intracellular cytokine staining responses from the HVTN-080 study



IFN=interferon; IL=interleukin; VAC=vaccination.

Several other ongoing clinical studies evaluating the HIV-MAG pDNA vaccine or the rVSV HIV *gag* vaccine provide interim safety data. The **IAVI-B004** study is a double-blind, randomized, placebo-controlled trial assessing the safety and immunogenicity of an HIV-MAG pDNA vaccine with a recombinant IL-12 pDNA adjuvant followed or preceded by a recombinant Ad35-GRIN/ENV HIV vaccine in healthy, HIV-negative participants. Enrollment is complete and vaccination of the first participant occurred in December 2011. To date, no safety concerns have been reported. Interim data following the HIV-MAG pDNA vaccine revealed a 36% combined response rate, and the full data set is anticipated in January 2013. The ongoing **ACTG-5281** trial is a placebo-controlled, dose-escalation study evaluating the safety and immunogenicity study of a cytokine-enhanced HIV-MAG pDNA vaccine delivered IM or IM EP injection in individuals with chronic HIV infection. Interim data show a 30% response rate. Enrollment is complete and vaccination of the first subject occurred in April 2011; no grade 3 or 4 AEs have been observed.

The safety and immunogenicity of the rVSV HIV *gag* vaccine was evaluated in a phase I, randomized, double-blind, placebo-controlled, dose-escalation study **(HVTN-090)** of healthy, HIV-negative adults; the study began in October 2011. Groups of 12 participants received the rVSV HIV *gag* vaccine (n=10/group) at 5 nominal dose levels (10^4, 10^5, 10^6, 10^7, and 10^8 pfu) or placebo (n=2/group) delivered by IM injection at 0 and 2 months. Reactogenicity over 7 days, AEs, and viral cultures from whole blood, urine, saliva, and swabs of oral lesions were collected. HIV-1-specific CD4+ and CD8+ T-cell responses to the Gag peptides were measured 1 and 2 weeks post-vaccination using an ICS assay.

As of July 31, 2012, all the vaccinations for this study were completed. As of September 11, 2012, the study is still ongoing, and the data are blinded. The median age for the participants is 24 years; 47% of participants are female and 37% are nonwhite. Local (Figure 9) and systemic (Figure 10) reactogenicity was self-limited, mild-to-moderate in intensity, and increased with the dose; headache was the common AE (52%) followed by malaise/fatigue (43%).

At the highest dose, 92% of the subjects reported having systemic symptoms, including flu-like syndrome (41%), fever (41%), and moderate chills (33%). Of the AEs considered related to the study product and reported by 30% of participants, the following were experienced by >1 participant: lymphadenopathy (3), decreased neutrophil count (3), mouth ulceration (3), other oral lesions (3), presyncope (3), and upper respiratory tract infection (2). Other AEs considered related to the study product and reported by 1 person each were asthenia, axillary pain, injection-site hematoma, increased levels of AST, myalgia, dizziness, hypoesthesia (at the injection site), oropharyngeal pain, and hot flushes. No severe reactogenicity, encephalitis, or product-related SAEs were reported. Viral-infectivity assays were performed on blood, urine, and saliva samples at baseline, 3 days post-vaccinations, and any time symptoms of a viral syndrome were reported within 1 week of the vaccination. Saliva samples and swabs were collected for all oral ulcerations or oral lesions detected during the exam. Cultures of VSV tested negative at all the doses and sites analyzed. Low-frequency, HIV-specific, CD4+ (9%), and CD8+ (3%) T-cell responses were detected post-vaccination in the first 3-dose levels. At the 10^7 pfu dose level, HIV *gag*-specific cell-mediated immune responses were detected in 6 of the 10 (60%) vaccine recipients, as measured by IFN-γ ELISpot or IFN-γ/IL-2 ICS assay. These data indicate that immunization with an attenuated, replicating rVSV HIV *gag* vaccine has an acceptable reactogenicity and safety profile to date.

The safety and immunogenicity of the HIV-MAG pDNA vaccine in combination with the IL-12 adjuvant delivered by IM EP injection and followed by the rVSV HIV *gag* boost vaccine is being evaluated in **HVTN 087**, an ongoing phase I study of HIV-negative participants. Administration of the rVSV HIV *gag* vaccine

boost began in February 2012. Thirty of the expected 100 subjects have been enrolled to date, and interim data are anticipated in May 2013. No safety-related concerns have been reported thus far. In a substudy of innate immunity, 3 subjects who had blood collected 24 hours after vaccination with rVSV were noted to have transient lymphopenia. The lymphopenia was not associated with any clinical symptoms and had resolved by the second blood collection 72 hours post-vaccination. A similar transient lymphopenia (presumably a result of transient changes in lymphocyte migration from blood to lymphoid tissue) has been reported following immunization with inactivated influenza virus.[32]

Figure 9: HVTN 090: Maximum local reactogenicity by treatment group

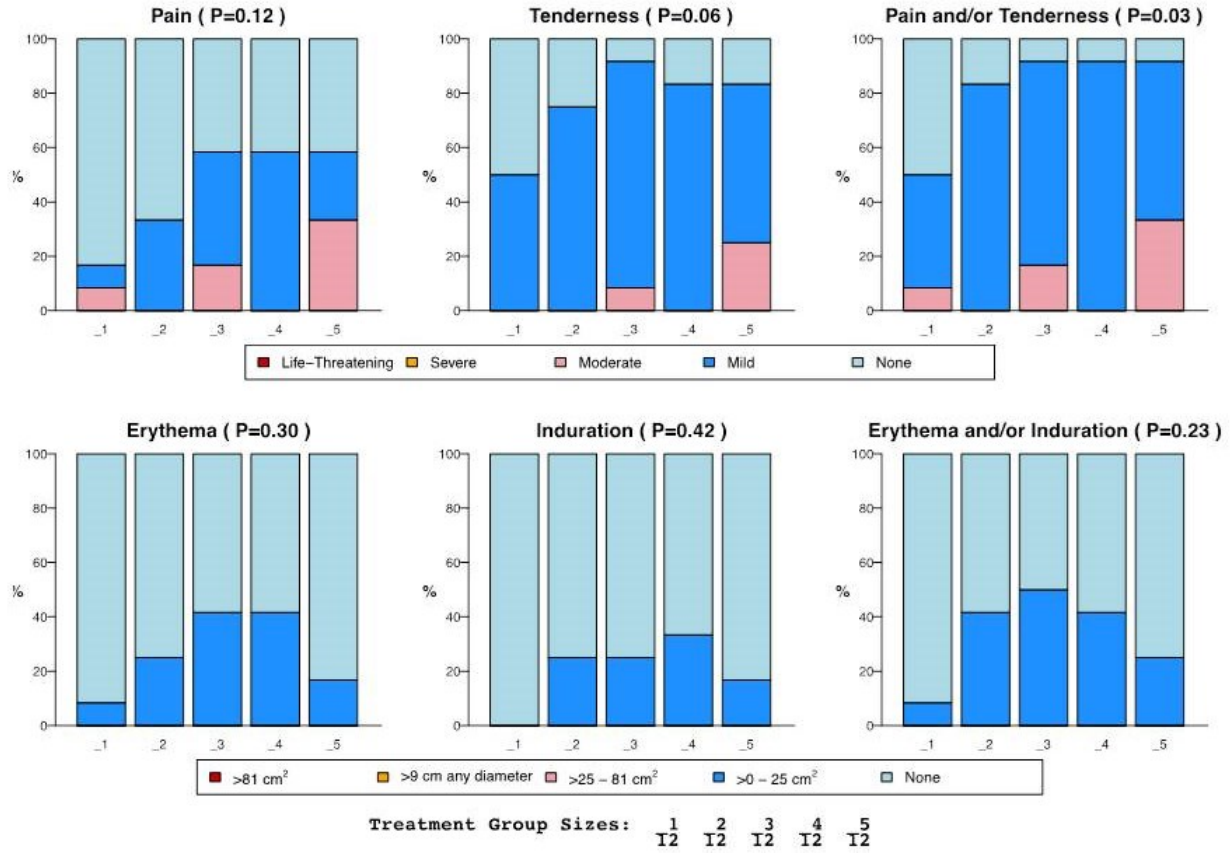
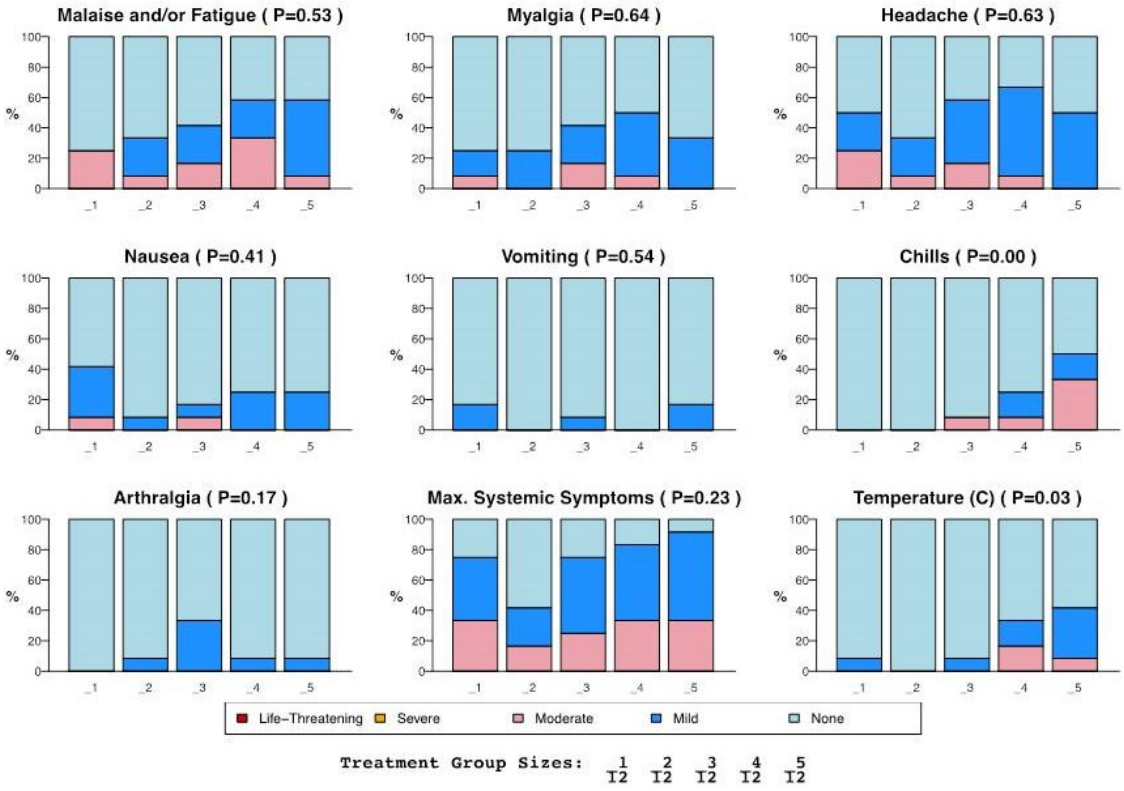


Figure 10: HVTN 090: Maximum systemic reactogenicity by treatment group



1.2 Rationale

The objectives of this study are to evaluate the safety and efficacy of the HIV-MAG pDNA vaccine prime plus the IL-12 pDNA adjuvant delivered by IM injection with EP in a prime-boost combination with the rVSV HIV *gag* booster vaccine. Up to 30 participants will be enrolled in the study, half of whom will receive the study vaccines and the other half will receive placebo. Only study participants who began cART during the acute/early phase of HIV infection are eligible to participate. These individuals have been shown to have a relatively preserved immune function, with smaller numbers of persistently infected $CD4^+$ T cells, compared with those who initiated cART during the chronic phase of infection.[33-36]

The rationale for using the proposed doses of the HIV-MAG pDNA vaccine prime and IL-12 pDNA adjuvant is based on the safety and immunogenicity data generated from the HVTN-080 and ACTG-5281 trials described in the previous section. Likewise, the proposed dose for the rVSV HIV *gag* vaccine is based on the safety and immunogenicity data from HVTN 090 trial. Additionally, the TDS device will be used in

the current study for EP of the DNA prime vaccine/placebo. In vivo EP is believed to enhance the potency of DNA vaccines by facilitating cellular uptake of DNA.[25,27,28]

Various assays measuring HIV-specific immune responses have been used to assess vaccine immunogenicity. Yet, to date, none of the assays are clinically validated to predict vaccine efficacy. Thus, ATI has been used in the past to evaluate the impact of therapeutic vaccination on the viral rebound that occurs following cART treatment interruption. The use of ATI in the design of this study will allow us to determine if vaccination results in a clinically significant improvement of the immunologic control of viral replication, as evidenced by a blunted or absent plasma viral rebound following ATI in the vaccine recipients, compared with individuals in the control group. Based on results from prior therapeutic vaccine studies using ATI,[23,24,37,38] as well as a recent subgroup analysis of the SMART study,[39] we feel that a 16-week ATI, with close clinical and laboratory monitoring, is a safe and acceptable strategy to evaluate the efficacy of the study vaccination regimen in this population of HIV-infected adults.

2 Study Objective

2.1 Primary Objective

To evaluate the safety and tolerability of the study vaccines in subjects who began cART during acute or early HIV-1 infection.

2.2 Secondary Objectives

To evaluate the efficacy of the study vaccines as determined by its effect on rebound viremia following ATI.

2.3 Exploratory Objectives

1. To assess the effect of the study vaccines on the rate of decay of the HIV-infected, CD4+ T-cell reservoir.
2. To determine the immunogenicity of the study vaccines.

3 Study Design

3.1 Description of the Study Design

This is a randomized, 2-arm (1:1), double-blind, placebo-controlled study evaluating the safety and efficacy of an HIV-MAG pDNA vaccine prime with EP in combination with an IL-12 pDNA adjuvant and an rVSV HIV *gag* booster vaccine delivered by conventional IM injection, in subjects who began cART during acute or early HIV-1 infection. Efficacy will be measured in terms of the effect of the study vaccinations on the plasma viral rebound at 16 weeks following ATI. Because the study is blinded, references to active vaccines included below refer EITHER to the active vaccine OR matching placebo, unless otherwise specified.

Vaccination schedule

Subjects will be randomized to receive placebo or the HIV-MAG pDNA vaccine prime (3000 µg) and IL-12 pDNA adjuvant (1000 µg) at week 0, 4, 12, and 36, and the rVSV-HIV *gag* booster vaccine ($1x10^7$ pfu) at week 24 and 48 (see Appendix B).

Each construct (HIV-1 *gag/pol* plasmid or the HIV-1 *net/tat/vif*, *env* plasmid) of the HIV-MAG pDNA vaccine (1500 µg each) will be mixed with 1000 µg of the IL-12 pDNA adjuvant and administered as 2 IM injections, 1 into each deltoid, with EP. The rVSV HIV *gag* booster vaccine will be administered as 2 IM injections (1 mL each), 1 into each deltoid.

Analytical treatment interruption

After the week 56 visit, all subjects meeting ATI criteria (described below) will undergo an approximately 16-week ATI to determine if the vaccination strategy results in an improved immune control of the viral replication, as evidenced by a blunted or absent viral rebound in the vaccine recipients, compared with the placebo recipients.

1. ATI will be initiated only in subjects whose CD4 cell counts are >400 cells/ mm^3 and plasma viral levels are below the limit of detection at week 56. HIV plasma viremia and CD4 cell counts will be assessed every 2 weeks during the ATI.
2. Subjects who do not receive at least one dose of the rVSV HIV *gag* boost or matching placebo will not undergo ATI, but they will continue to be followed for safety outcomes.
3. Any subject who develops oral lesions that are positive for VSV by culture or PCR will not undergo ATI until the lesions resolves (for at least 4 weeks).
4. Individuals taking non-nucleoside reverse transcriptase inhibitors (NNRTIs) will be advised to switch to a protease inhibitor or an integrase inhibitor-based regimen 2 weeks prior to the discontinuation of cART to ensure that the washout period of antiretroviral agents is roughly equal.
5. Subjects whose plasma viral levels are below the limit of detection at week 72 (the end of the ATI period) may elect not to restart cART until their viral levels rise above the limits of detection.
6. Subjects will be restarted on cART if they meet any of the following criteria during the ATI:
   - A confirmed >30% decline in baseline CD4 cell count or an absolute CD4 cell count <350 cells/mm^3.
   - A sustained (≥4 weeks) HIV RNA level of >50,000 copies/mL.
   - Any HIV-related symptoms.

3.2 Study Endpoints

3.2.1 Primary Endpoint

The rate of occurrence of grade 3 or higher AEs, including SAEs, that, per standard criteria (see safety section) are:

- At least possibly related to the test article, and
- Definitely NOT related to a factor other than the test article.

3.2.2 Secondary Endpoint

The difference in HIV-1 viral load at the end of the ATI between the vaccine and placebo groups.

3.2.3 Exploratory Endpoints

1. The frequency and rate of decay of the infectious HIV reservoir between the vaccine and placebo groups.
2. Change from baseline (mean of entry and pre-entry) to post-vaccination in the number of IFN-γ/IL-2-generating CD8+ and CD4+ T-cells/million PBMCs in response to *gag, pol, env, nef, tat* and *vif*, as measured by ICS.

4 Study Population

4.1 Rationale for Subject Selection

Individuals who begin cART during early/acute HIV infection have been shown to have a relatively preserved immune function, with smaller numbers of persistently infected $CD4^+$ T cells, compared with those who initiated cART during the chronic phase of infection. Limiting enrollment to this pool of subjects maximizes safety, and it helps to determine whether therapeutic vaccination can significantly augment HIV-specific immune responses in a clinically meaningful way.

4.2 Recruitment Plan

Subjects will be recruited from known cohorts of individuals who started treatment during acute/early HIV infection. Additional local and national recruitment will be done using direct mailing to infectious disease physicians, internet ad campaigns, social media outlets, and print ads.

4.3 Subject Inclusion Criteria

1. Age, 18-65 years.
2. Institution of cART within 12 weeks of being diagnosed with acute or early HIV-1 infection.

   Acute HIV-1 infection is defined as:
   a. Detectable plasma HIV-1 RNA levels of >2000 copies/mL with a negative result from an HIV-1 EIA, or
   b. Positive result from an HIV-1 EIA with a negative or indeterminate result from an HIV-1 western blot that subsequently evolves to a confirmed positive result, or
   c. Negative result from an HIV-1 EIA within the past 4 months and HIV-1 RNA levels of >400,000 copies/mL, in the setting of a potential exposure to HIV-1.

Early HIV-1 infection is defined as:

a. Negative result from an HIV-1 EIA within 6 months prior to a positive result from an HIV-1 EIA and an HIV-1 western blot.
b. Negative result from a rapid HIV-1 test within 1 month prior to a positive result from an HIV-1 EIA and an HIV-1 western blot.
c. Presence of low level of HIV antibodies as determined by having a positive EIA or a positive Western blot with a non-reactive detuned EIA according to a serologic testing algorithm for recent infection.

3. CD4+ cell count >450 cells/mm^3 at screening.
4. Documentation of continuous cART treatment with suppression of plasma viral level below the limit of detection for >1 year. Subjects with a single "blip" (i.e., detectable viral levels on cART) prior to randomization may be included provided they satisfy the following criteria:
   a. The blips are <400 copies/mL, and
   b. Succeeding viral levels return to levels below the limit of detection on subsequent testing.
5. Willingness to undergo ATI.
6. Laboratory values within pre-defined limits at screening:
   - Absolute neutrophil count >1,000/mm^3.
   - Hemoglobin levels >10.0 g/dL for men and >9.0 g/dL for women.
   - Platelet count >100,000/mm^3.
   - Prothrombin time (PT) and partial thromboplastin time (PTT) <1.5 upper limit of normal (ULN).
   - Estimated or a measured creatinine clearance rate of >60 mL/min as determined by the NIH Clinical Center laboratory.
   - AST and ALT levels of <2.5 x ULN.
7. Willingness to have samples stored for future research.
8. Women of childbearing potential must have a negative pregnancy test result.
   - They must agree to use an adequate form of contraception:
     - Hormonal contraception.
     - Male or female condoms with or without a spermicidal.
     - Diaphragm or cervical cap with a spermicidal.
     - Intrauterine device.

4.4 Subject Exclusion Criteria

1. Allergy to amide-type local anesthetics (bupivacaine [Marcaine], lidocaine [Xylocaine], Mepivacaine [Polocaine/Carbocaine], etidocaine [Duranest], prilocaine [Citanest, EMLA cream]).
2. Chronic hepatitis B, as evidenced by a positive test for hepatitis B surface antigen (HBsAg), or chronic hepatitis C virus (HCV) infection, as evidenced by a positive test for HCV RNA. Subjects with a positive test for HCV antibody and a negative test for HCV RNA are eligible.
3. Changes in cART regimen due to virologic breakthrough.

4. HIV immunotherapy or vaccine(s) received within 1 year prior to screening.
5. Any licensed or experimental non-HIV vaccination (e.g., hepatitis B, influenza, pneumococcal polysaccharide) received within 4 weeks prior to study entry.
6. Interruption of cART for >3 months since its initiation.
7. Pregnancy or planned pregnancy during the study period or breastfeeding.
8. Any active malignancy that may require systemic chemotherapy or radiation therapy.
9. Immunosuppressive medications received within 6 months before the first study vaccination (Not excluded: [1] corticosteroid nasal spray for allergic rhinitis; [2] topical corticosteroids for mild, uncomplicated dermatitis; or [3] oral/parenteral corticosteroids administered for non-chronic conditions not expected to recur [length of therapy ≤10 days, with completion in ≥30 days prior to enrollment]).
10. Evidence of hepatic decompensation in subjects with cirrhosis: history of ascites, hepatic encephalopathy, or bleeding esophageal varices, or screening laboratory results with any of the following:
    a. International normalized ratio of ≥1.5 x ULN.
    b. Serum albumin <3.2 g/dL.
    c. Serum total bilirubin >1.8 x ULN, unless history of Gilbert's disease or deemed related to treatment with atazanavir.
11. History or other clinical evidence of:
    a. Significant or unstable cardiac disease (e.g., angina, congestive heart failure, recent myocardial infarction, significant arrhythmia).
    b. Severe illness, malignancy, immunodeficiency other than HIV, or any other conditions that, in the opinion of the investigator, would make the subject unsuitable for the study.
    c. AIDS-defining condition.
12. Known allergy or sensitivity to the components of the investigational therapy.
13. History of significant cardiac arrhythmia (e.g., supraventricular tachycardia, ventricular tachycardia, and atrial fibrillation/flutter).
14. Active drug or alcohol use or any other pattern of behaivor that, in the opinion of the investigator, would interfere with adherence to study requirements.
15. Any active systemic inflammatory or autoimmune disease or condition.
16. Presence of implanted electronic medical device (e.g., pacemaker, implantable cardiac defibrillator) or surgical/traumatic metal implant in the upper limb and/or upper torso.
17. Neurological or neuropsychiatric disorder that may interfere with the assessment of safety (e.g., frequent recurring headaches, for example, a pattern of >1 headache/month affecting activities of daily living/work, frequent or severe/complicated migraines, cluster headaches); or history of encephalitis, narcolepsy, stroke with sequelae, moderate/severe major depressive disorder, moderate/severe bipolar disorder, seizure disorder.
18. Deltoid skinfold measurements (by caliper) of >40 mm.
19. Body mass index >40.

Co-enrollment guidelines: Co-enrollment in other trials is restricted, other than enrollment on observational studies or those evaluating the use of a licensed medication. Study staff should be notified of co-enrollment, as it may require the approval of the principal investigator.

5 Justification for Exclusion of Women and Children (Special Populations)

Exclusion of women:

- **Pregnancy:** Pregnant women are excluded from this study because the effects of the HIV-MAG pDNA vaccine prime/IL-12 pDNA adjuvant/rVSV HIV *gag* booster vaccine on the developing human fetus are unknown with the potential for teratogenic or abortifacient effects.
- **Breastfeeding:** Because there is an unknown but potential risk for AEs in nursing infants secondary to the treatment of the mother with the HIV-MAG pDNA vaccine prime/IL-12 pDNA adjuvant/rVSV HIV *gag* booster vaccine, breastfeeding should be discontinued if the mother is treated with these study agents.

Exclusion of children: Because there are insufficient data regarding the dosing or AEs available in adults for the study agents to judge the potential risk in children, they are excluded from this study.

6 Study Agent/Interventions

6.1 Disposition and Dispensation

The study agents will be distributed by Profectus, Inc. to the NIH Central Pharmacy according to standard pharmacy procedures.

6.1.1 Formulation, Packaging, and Labeling

Each study product (i.e., HIV-MAG pDNA vaccine prime, IL-12 pDNA adjuvant, and rVSV-HIV *gag* booster vaccine) will be individually vialed and labeled with the study product name, lot number, concentration, and recommended storage conditions.

6.2 Study Agent Storage and Stability

The HIV-MAG pDNA vaccine prime and the IL-12 pDNA adjuvant should be stored and refrigerated at 2˚C to 8˚C, if not immediately administered. When constructs are mixed and administered in combination, it is recommended that the combined formulation be used within 4 hours after mixing. The proposed expiration period for the HIV-MAG pDNA vaccine prime and the IL-12 pDNA adjuvant is 12 months from the date of formulation. Batches may be evaluated for stability at the expiration time point to justify their use passed the expiration date.

Vials containing the rVSV-HIV *gag* booster vaccine vector should be stored at -80˚C. No more than 4 hours prior to use, vaccine vials should be placed at room

temperature to thaw the vaccine formulation rapidly. Thawed vaccine can either be used immediately for inoculation or be stored at 2˚C-8˚C for up to 4 hours prior to inoculation. The proposed expiration period for the booster vaccine is 12 months from the date of formulation. Batches may be evaluated for stability at the expiration time point to justify their use passed the expiration date.
Sodium chloride for injection (USP 0.9%) will be used as a control substance for the placebo group. The vials must be stored as directed by the manufacturer of the product.

6.3 Preparation, Administration, and Dosage of Study Agent/Intervention(s)

Description

HIV-MAG pDNA vaccine prime composed of the HIV-1 *gag/pol* plasmid and HIV-1 *nef/tat/vif, env* plasmid

HIV-MAG pDNA HIV-1 gag/pol plasmid
The HIV-MAG pDNA HIV-1 *gag/pol* plasmid is a clear, colorless solution enclosed in 2-mL single-use vials. Each 2-mL vial contains 0.8±0.04 mL of the HIV-1 *gag/pol* pDNA at a concentration of 3 mg/mL in 30 mM citrate buffer pH 6.5, containing 150 mM NaCl, 0.015 ethylenediaminetetraacetic acid (EDTA), and 0.25% bupivacaine-HCl.

The product is contraindicated in participants with known hypersensitivity to bupivacaine or any other amide-type local anesthetic.

HIV-MAG pDNA HIV-1 nef/tat/vif, env plasmid
The HIV-MAG pDNA HIV-*1 nef/tat/vif, env* is a clear, colorless solution packaged in 2-mL single-use vials. Each 2-mL vial contains 0.8±0.04 mL of the HIV-1 *nef/tat/vif, env* pDNA at a concentration of 3 mg/mL in 30 mM citrate buffer pH 6.5, containing 150 mM NaCl, 0.015 EDTA, and 0.25% bupivacaine-HCl.

The product is contraindicated in participants with known hypersensitivity to bupivacaine or any other amide-type local anesthetic.

IL-12 pDNA adjuvant
The IL-12 pDNA adjuvant is a clear, colorless solution packaged in 2-mL single-use vials. Each 2-mL vial contains 0.9±0.04 mL of IL-12 pDNA at a concentration of 2 mg/mL in 30 mM citrate buffer pH 6.5, containing 150 mM NaCl, 0.01% EDTA, and 0.25% bupivacaine-HCl.

The product is contraindicated in participants with known hypersensitivity to bupivacaine or any other amide-type local anesthetic.

rVSV HIV *gag* booster vaccine
The rVSV-HIV *gag* booster vaccine is a clear, colorless solution packaged in 3-mL single-use vials. Each 3-mL single-use vial contains 1.3 mL ($5x10^6$ pfu/mL) of the

vaccine formulated in an aqueous liquid phosphate buffer containing gelatin as a virus stabilizer.

Control product
Sodium chloride for injection (USP 0.9%) will be used as a control substance for the

placebo group. The volume of sodium chloride administered will be the same as that for the study vaccine components.

Dosing and administration

HIV-MAG pDNA vaccine prime will be administered at a dose of 3000 µg (1500 µg of the HIV-1 *gag/pol* plasmid and 1500 µg of the HIV-1 *net/tat/vif*, *env* plasmid) at week 0, 4, 12, and 36. Each construct of HIV-MAG pDNA vaccine (1500 µg each) will be mixed and combined with 1000 µg of the IL-12 pDNA adjuvant. The resulting mixture will be divided into 2 IM injections and administered as 0.75 mL IM injection in the left deltoid **and** 0.75 mL IM injection in the right deltoid with EP using the TDS device.

IL-12 pDNA adjuvant will be mixed with the HIV-MAG pDNA vaccine prime, as noted above, and administered at a dose of 1000 µg (500 µg in each IM injection) at week 0, 4, 12, and 36.

rVSV HIV *gag* booster vaccine--The total dose, $1x10^7$ pfu, will be administered as 1 mL ($5x10^6$ pfu) IM injection in the left deltoid **and** 1 mL ($5x10^6$ pfu) IM injection in the right deltoid at week 24 and 48.

Placebo for the IL-12 pDNA adjuvant and HIV-MAG pDNA vaccine (sodium chloride for injection, USP 0.9%) will be administered as 0.75 mL IM injection in the left deltoid **and** 0.75 mL IM injection in the right deltoid at weeks 0, 4, 12, and 36 with EP using the TDS device.

Placebo for the rVSV HIV *gag* (sodium chloride for injection, USP 0.9%) will be administered as 1 mL IM injection in the left deltoid **and** 1 mL IM injection in the right deltoid at week 24 and 48.

Dose Adjustments/Modifications/Delays

Delaying vaccinations for a participant
Under certain circumstances, a participant's scheduled vaccination will be delayed. The factors to consider when such a decision needs to be made include, but are not limited to, the following:

- Live attenuated vaccines, other than influenza vaccine, received within 30 days prior to the study vaccination(s).

- Influenza vaccine or any vaccines that are not live attenuated (e.g., pneumococcal vaccines) received within 14 days prior to the study vaccination(s).
- Allergy treatment with antigen injections received within 30 days prior to the study vaccination(s).
- Abnormal vital signs or clinical symptoms prior to the study vaccinations that may interfere with assessment of the vaccine reaction.

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

Participant departure from the vaccination schedule

Every effort should be made to follow the vaccination schedule according to the protocol. With the exception of the first pDNA vaccination, if a participant misses a vaccination and the 14-day window period for the vaccination has passed, that vaccination cannot be administered. The participant should be asked to continue with the remaining study visits. The participant should resume the vaccination schedule for the next vaccination unless there are circumstances that require further delay or permanent discontinuation of the vaccination.

If a participant receives only 1 of the 2 scheduled injections (i.e. half of the vaccine/ placebo dose), the principal investigator will determine whether the vaccination schedule can be resumed with the next scheduled injection.

Tracking of dose

The vials for the HIV-MAG pDNA vaccine prime, IL-12 pDNA adjuvant, and rVSV-HIV *gag* booster vaccine will be labeled as described in Section 6.1.1 with the assigned study subject numbers and no other forms of subject identifiers, and they will be kept as directed by the sponsor. Tracking will be based on the number of vials used for each subject, accounting for any amount left in the last vial for each subject.

6.4 Concomitant Medications and Procedures

All concomitant prescription medications taken during study participation will be documented in Clinical Research Information Management System of the NIAID (CRIMSON). For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in CRIMSON documents are concomitant prescription medications,

over-the-counter medications, and non-prescription medications taken at the time of AEs (all grades).

6.5 Prohibited Prescription Medications and Procedures

Treatment with immunosuppressive medications received within 6 months before the first study vaccination (not including [1] corticosteroid nasal spray for allergic rhinitis; [2] topical corticosteroids for mild, uncomplicated dermatitis; or [3] oral/parenteral corticosteroids given for non-chronic conditions not expected to recur-length of therapy ≤10 days) will not be permitted unless discussed with and approved by the principal investigator.

7 Study Schedule

For all the study visits, unless otherwise specified, subjects will come to the NIH Clinical Center to undergo the procedures. Unless otherwise specified, the visit window for the post-entry study visits is ± 5 days.

7.1 Screening

Screening may occur over the course of several contacts/visits, up to and including, before the first vaccination on day 0 (week 0). All inclusion and exclusion criteria must be assessed within 8 weeks before enrollment, unless otherwise specified in the eligibility criteria.

After signing informed consent, subjects will undergo the following procedures:

- Medical history and a targeted physical examination, including weight, vital signs, and a symptom-directed evaluation based on symptoms or complaints reported by each participant.
- Assessment of concomitant medications.
- Deltoid skinfold measurement.
- Blood collection for:
    - HBsAg.
    - Rapid plasma reagin test.
    - Anti-HIV and anti-HCV antibody tests.
    - Complete blood count (CBC) with differential, PT, activated PTT.
    - Chemistry panel to include: ALT, AST, alkaline phosphatase, creatinine, CPK, total and direct bilirubin, and serum albumin levels.
    - Flow cytometry panel (includes CD4+ cell count).
    - Plasma HIV and HCV viral RNA levels.
    - Storage of PBMCs.
- Urinalysis.
- Serum or urine pregnancy test (for women of child-bearing potential).
- Electrocardiogram (ECG).

7.2 Enrollment/Baseline

During enrollment at week 0, eligible subjects will be randomized in a 1:1 ratio to receive placebo or the HIV-MAG pDNA vaccine prime plus the IL-12 pDNA adjuvant and the rVSV-HIV *gag* booster vaccine. The first dose of placebo or the vaccine regimen will be administered at this visit, and enrollment is defined as the day of receipt of the first study vaccine.

During week 0, subjects will undergo the following procedures (prior to the first study vaccination):

- Medical history and a targeted physical examination, including weight, vital signs, and a symptom-directed evaluation based on symptoms or complaints reported by each participant.
- Assessment of concomitant medications.
- HIV transmission risk behavior assessment and counseling
- Leukapheresis for research studies (see Section 8.2)
- Blood collection for:
    - Flow cytometry panel (includes CD4+ cell count).
    - Plasma HIV viral RNA levels.
    - Serum baseline VSV serology.
    - HLA typing.
    - Storage of serum and PBMCs.
    - CBC with differential.
    - Chemistry panels, to include: ALT, AST, alkaline phosphatase, creatinine, CPK, total and direct bilirubin, and serum albumin levels.
- Serum or urine pregnancy test (for women of child-bearing potential).
- Assessment of reactogenicity parameters (following the first study vaccination) as described in Section 8.1.
- Instructions on the use of a symptom log/diary card.

7.3 Randomization

The Pharmaceutical Development Section of the NIH Clinical Center pharmacy will generate a 1:1 randomization scheme for the protocol. A table of random numbers will be used. A set of numbers, equivalent to the proposed “N” will be selected. An appropriate block will be chosen, based on the “N”. Within that block, the highest numbers will be assigned to one treatment assignment (vaccine), while the lowest numbers will be assigned to the alternate treatment (placebo).

7.4 Vaccination Phase

Subjects must continue on their current cART regimen during the vaccination phase.

Subjects will be administered either placebo or the HIV-MAG vaccine plus the IL-12 pDNA adjuvant at week 0, 4, 12, and 36, and the rVSV HIV *gag* booster vaccine ($1x10^7$ pfu) at week 24 and 48.

During the visits at week 4, 12, 24, 36, 48, and 56, subjects will undergo the following procedures:

- Medical history and a targeted physical examination, including weight, vital signs, and a symptom-directed evaluation based on symptoms or complaints reported by each participant.
- Assessment of concomitant medications.
- Assessment of reactogenicity parameters (at week 4, 12, 24, 36, and 48 only; see Section 8.1).
- Assessment of any new or unresolved AEs/intercurrent illnesses.
- Blood collection for:
    - CBC with differential.
    - Chemistry panels, to include: ALT, AST, alkaline phosphatase, creatinine, CPK, total and direct bilirubin, and serum albumin levels.
    - Flow cytometry panel (includes CD4+ cell count).
    - Plasma HIV viral RNA levels.
    - VSV serology (at week 26 and 50 only).
- Leukapheresis for research studies including, but not limited to, quantitative co-cultures of CD4+ cells and related immunologic and virologic studies (at week 36 and 56 only).
- Serum or urine pregnancy test (for women of child-bearing potential).
- Evaluation of symptom log/diary card.
- HIV transmission risk behavior assessment and counseling (week 56 only)

During the visits at week 14, 26, 38, and 50, blood will be collected for flow cytometry, CBC, and storage of plasma and PBMCs for assessment of immunogenicity. For select subjects residing outside the local (Bethesda, MD) area, blood samples for CD4 counts, and storage, may be collected at their local clinics and sent to the NIH Clinical Center for testing and storage.

Any participant who develops oral ulcers within 7 days post-vaccination with the rVSV vaccine/placebo will have swabs from the oral lesion(s) collected and sent for VSV culture and PCR (see section 8.1).

7.5 Post-Vaccination ATI Phase

The ATI phase will begin after the visit at week 56 and will continue until week 72. During this phase, CD4 counts and HIV viral RNA levels will be measured every 2 weeks.

For select subjects residing outside the local (Bethesda, MD) area, blood samples for CD4 counts and HIV viral RNA levels may be collected at their local clinics and sent to the NIH Clinical Center via overnight shipment for testing.

All subjects will return to the NIH Clinical Center at week 64 and 72 for the following procedures:

- Medical history and a targeted physical examination, including weight, vital signs, and a symptom-directed evaluation based on symptoms or complaints reported by each participant.
- Assessment of any new or unresolved AEs/intercurrent illnesses.
- Blood collection for:
    - CBC with differential.
    - Chemistry panels to include: ALT, AST, alkaline phosphatase, creatinine, total and direct bilirubin, and serum albumin levels.
    - Flow cytometry panel (includes CD4+ cell count).
    - Plasma HIV viral RNA levels.
    - Serum or urine pregnancy test (for women of child-bearing potential).
    - HIV genotype (week 72 only, or prior to restarting cART).
    - Storage of serum and PBMCs.

If a subject develops one or more criteria for ending ATI (see Section 3.1), he/she will be asked to return for an unscheduled visit for assessment and confirmation of the laboratory/clinical abnormality. If the criteria for ending ATI are met, the subject will restart cART and will continue to be followed for 6 months after restarting cART, and he/she will undergo the schedule of procedures listed in Section 7.6 below under week 84 and 96.

7.6 Post-Vaccination, Post-ATI Phase

After the visit at week 72, subjects will restart their cART regimen and will continue treatment through week 96 (end of study).

At week 76 and 80, blood will be collected for CD4+ cell counts and HIV viral RNA levels. For select subjects residing outside the local area, blood samples may be collected at local clinics and sent to the NIH Clinical Center via overnight shipment for testing.

All subjects will return to the NIH Clinical Center at week 84 and 96 to undergo the following procedures:

- Medical history and a targeted physical examination, including weight, vital signs, and a symptom-directed evaluation based on symptoms or complaints reported by each participant.
- Assessment of any new or unresolved AEs/intercurrent illnesses.
- Blood collection for:
    - CBC with differential.
    - Chemistry panels, to include: ALT, AST, alkaline phosphatase, creatinine, total and direct bilirubin, and serum albumin levels.
    - Flow cytometry panel (includes CD4+ cell count).

- Plasma HIV viral RNA levels.
- Storage of serum and PBMCs.

If HIV viral RNA levels are detectable at week 84, the testing will be repeated at week 88 and week 92 (if detectable at week 88).

For subjects who meet criteria to re-start cART prior to week 72 (described in Section 3.1), HIV RNA levels will be done every 4 weeks until levels fall below the limit of detection.

As described in Section 3.1, subjects whose HIV RNA levels are below the limit of detection (<40 copies/ml) at week 72 may elect to continue on the ATI phase of the study and have their CD4 count and HIV RNA levels monitored every 2 weeks until HIV RNA levels become detectable or until the final study visit at week 92.

If a subject with HIV RNA levels above the limit of detection (>40 copies/ml) at week 72 refuses to restart cART (despite the protocol team's recommendation to do so), the subject may continue to have CD4 count and HIV viral levels monitored every 2 weeks until cART is restarted. If the subject continues to have measurable virema at the week 84 visit and continues to refuses to restart cART, the subject will be removed from the study and returned to the care of their private physician.

For purposes of data analysis in the subjects described above, the HIV RNA level obtained at week 72 will be used for the secondary endpoint analysis.

8 Study Procedures/Evaluations

8.1 Clinical Evaluations

- **Medical history and physical examination**
- **Deltoid skinfold measurement:** 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 will be used to determine the depth of the injection for each eligible subject to ensure IM administration of the vaccine/ placebo prior to the first vaccination. The skin-thickness range and corresponding depth settings on the TDS-IM are described in the *TDS-IM: Instructions for Use Manual*.
- **Phlebotomy:** Blood will be collected for routine serologic, hematologic, and clinical chemistry evaluations as described in Section 7.
- **Oral swabs:** Subjects who develop oral lesions up to 7 days post-vaccination with rVSV/placebo will have the lesions tested for the presence of VSV shedding via RT/PCR analysis and in vitro virus culture via the rVSV viral infectivity assay. Vero cell cultures will be incubated with participant samples and examined by

microscopy for the presence of VSV-induced cytopathic effects. If cytopathic effects are observed, VSV titrations will be performed by a plaque assay to quantify the amount of virus being shed in test samples. Nucleotide sequence analysis will be performed on amplified virus from test sample(s) to verify the presence of VSV. All viral infectivity assays will be performed by Profectus Biosciences.

- **Assessment of reactogenicity:** For all participants, reactogenicity assessments will be performed at baseline prior to any study vaccinations and subsequently after each vaccination. All reactogenicity symptoms will be followed until resolution and graded according to the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 1.0, December 2004 (Clarification August 2009).

  The reactogenicity assessment period is 3 full days following each HIV-MAG pDNA vaccine/placebo injection and 7 full days following the rVSV- HIV *gag* vaccine/ placebo injection according to the schedule shown in Table 2 below. Participants will be instructed to record symptoms using a post-vaccination symptom log/diary card (see Appendix D) and to contact the site daily during the assessment period. Participants will also be instructed to contact the clinic for events that occur during the period between each vaccination and the next scheduled visit. In general, any participant who self-reports a post-vaccination reaction greater than moderate will be evaluated by a clinician within 48 hours after onset, unless the reaction is improving and/or has completely resolved.

Table 2: Schedule of reactogenicity assessments

Day	Time	Performed by
0[a]	Pain assessment immediate: post-vaccination	OP8 staff
	Pain assessment 25-60 minutes: post-vaccination	OP8 staff
1	Reactogenicity between 12:00 AM and 11:59 PM day 1	OP8 staff or participant
2	Reactogenicity between 12:00 AM and 11:59 PM day 2	OP8 staff or participant
3	Reactogenicity between 12:00 AM and 11:59 PM day 3	OP8 staff or participant
4-7	Reactogenicity between 12:00 am and 11:59 pm days 4-7, **for VSV HIV *gag* vaccine/placebo only**	OP8 staff or participant

VSV=vesicular stomatitis virus.
[a]Day of vaccination.

8.2 Research Evaluations

Leukapheresis: Will be performed for research studies including, but not limited to, measurements of the frequency of CD4+ T cells carrying replication-competent HIV by quantitative coculture assays. These studies will determine the rate of decay of the $CD4^+$ T-cell reservoir. If leukapheresis cannot be performed for technical reasons (e.g. poor venous access) 80 ml of heparinized blood will be drawn instead.

Other research evaluations measuring the effect of the vaccinations on the HIV pathogenesis may include:

- Frequency of $CD4^+$ T cells carrying HIV proviral DNA and cell-associated HIV RNA.
- HIV burst size in $CD4^+$ T cells in the presence of autologous $CD8^+$ T cells.
- Magnitude and breadth of HIV-specific $CD4^+$ and $CD8^+$ T-cell responses.
- T-cell activation and soluble markers of inflammation.
- Residual (1-40 copies/mL) plasma viremia.

- **VSV serology:** The VSV neutralizing antibody titer will be evaluated to determine the magnitude of the immune response elicited to the vector.
- **HIV viral RNA levels**.
- **Flow cytometry with CD4+ cell count**.

9 Potential Risks and Benefits

9.1 Potential Risks

General risks associated with vaccination/DNA vaccination
Possible risks associated with vaccines include fever, chills, rash, arthalgias/myalgias, nausea, headache, dizziness, fatigue, and anaphylaxis. The theoretical risks of DNA vaccination could include the possible integration of plasmid DNA into the host genome, resulting in mutations, problems with DNA replication, triggering of autoimmune responses, and activation of cancer-causing genes. To date, there is no clear evidence from numerous clinical trials for integration of plasmid DNA into the host genome triggering autoimmune disease/neoplastic disease.

HIV-MAG pDNA vaccine
The HIV-MAG pDNA vaccine has been administered to approximately 150 individuals to date, and it appears to be well tolerated. The vaccine plasmids HIV-1 *gag*/*pol* and HIV-1 *nef*/*tat*/*vif*, *env* are formulated in 0.25% bupivacaine, thus, there is a risk of allergic reaction from bupivacaine, including nausea, vomiting, chills, rash, urticaria, angioedema, bronchospasm, or anaphylaxis. Significant AEs, including cardiac arrest and death, have occurred following intravenous delivery of bupivacaine. In most cases, the events occurred following use of bupivacaine at a dose of 1.6 mg/kg. In the current study, bupivacaine will be administered IM at a dose of 2.5 mg/mL (maximum 2 mL or 5 mg). A 70-kg person would receive a dose of 0.07 mg/kg per dose. Participants with a history of allergic reaction to amide-type local anesthetics (bupivacaine, lidocaine, mepivacaine, etidocaine, prilocaine) will be excluded from the study.

IL-12 pDNA adjuvant

The IL-12 plasmid adjuvant is also formulated in 0.25% bupivacaine, so the risks associated with bupivacaine are also associated with this product (see HIV-MAG pDNA above).

In addition, it is possible that administration of the IL-12 pDNA adjuvant might elicit antibodies that could affect the native IL-12 cytokine. In the HVTN-080 study, an assay was performed to test for the presence of IL-12 neutralizing antibody following administration of the IL-12 pDNA (1,000 µg or placebo). Results showed that 1 subject tested positive for IL-12 neutralizing antibodies following vaccination (49 NU/mL), but the subject also had a high levels (39 NU/mL) at baseline. Another subject was positive for IL-12 neutralizing antibodies at baseline (31 NU/mL) followed by lower post-vaccination levels (9 NU/mL).

Electroporation

EP has been associated with transient muscle contractions and injection-site discomfort, such as pain, soreness, bruising, redness, swelling, itching, and stiffness of the upper arm. The procedure also carries the theoretical risks of electrical injury or infection at the injection site, disruption of the function of the implanted electronic medical devices (e.g., pacemaker, implantable cardiac defibrillator), or the exacerbation of cardiac arrhythmias. Participants with a history of clinically significant cardiac arrhythmia (excluding sinus arrhythmia), pacemakers, implantable cardiac defibrillator, or certain metal implants will be excluded. Electroporation with the TDS device has been used in about 280 people to date who participated in clinical trials. Adverse responses reported in association with use of the device have been largely limited to acute discomfort during the procedure application, and transient injection-site soreness of mild–to-moderate severity for up to 1 week following administration.

rVSV HIV *gag*

Results from a randomized, double-blind, placebo-controlled, dose-escalation study of 60 healthy volunteers (HVTN 090) showed that local (Figure 9) and systemic (Figure 10) reactogenicity was self-limited, mild-to-moderate in intensity, and increased with dose; headache was the common AE (52%) followed by malaise/fatigue (43%). At the highest dose (10^8 pfu), 92% of the subjects reported having systemic symptoms, including flu-like syndrome (41%), fever (41%), and moderate chills (33%). Of the AEs considered related to the study product and reported by 30% of participants, the following were experienced by >1 participant: lymphadenopathy (3), decreased neutrophil count (3), mouth ulceration (3), other oral lesions (3), presyncope (3), and upper respiratory tract infection (2). Other AEs considered related to the study product and reported by 1 person each were asthenia, axillary pain, injection-site hematoma, increased levels of AST, myalgia, dizziness, hypoesthesia (at the injection site), oropharyngeal pain, and hot flushes. No severe reactogenicity, encephalitis, or product-related SAEs were reported.

VSV infection in humans is rare, but it can occur when animal handlers and veterinarians come in close contact with infected livestock and through inadvertent

exposure of laboratory personnel. The most common portals of infection in humans are skin and mucous membranes of the nose, mouth, and eyes, although there is also serological evidence that suggests that some vesiculoviruses may be directly transmitted to humans through insect bites. Human infection with VSV can either be asymptomatic, or it may lead to disease symptoms, which include myalgia, headache, fatigue, oral ulcers, and fever that resolve in 3–5 days without complications. Because the vaccine candidate is a live, replication competent vector, there is a theoretical risk of human disease, including neurologic manifestations. The vaccine vector was specifically attenuated to eliminate neurovirulence, which has been tested in sensitive animal models (refer to the investigator's brochure for more information on neurovirulence testing). Furthermore, no evidence of encephalitis or other neurologic events was observed following administration of the vaccine in subjects participating in the HVTN 090 trial. However, as a precaution, subjects may not participate in the current study if they have a neurological/neuropsychiatric disorder that could be confused with reactions to the VSV HIV *gag* vaccine, or could interfere with the assessment of safety.

There is also a hypothetical risk of eliciting autoimmune responses in vaccine recipients. This risk is based on the identification of a peptide octamer and other shorter peptides in the Indiana serotype VSV (VSV_{IN}) N protein that are very similar in sequence to peptides found in Ro60kD, which bind the anti-Ro/SSA antibodies found in sera of subjects with systemic lupus erythematosis (SLE).[40] Antibodies that bind the Ro protein can also be elicited in rabbits that are hyperimmunized with purified VSV_{IN} N protein.[41] In addition, antibodies have been detected in SLE subjects that cross react with the VSV_{IN} M and, to a lesser extent, N core proteins, but at a much lower frequency to the VSV_{IN} G protein.[42] However, it is not clear what these observations mean since antibodies that react to the peptide octamer in Ro60kD account only for a small minority of the anti-Ro60kD auto-antibodies in SLE subjects.[43] Also, the detection of antibodies in the sera of SLE subjects that react with the VSV_{IN} M and N proteins, but not with the G protein, is puzzling, because the VSV G protein has strong antigenic properties and typically elicits a very robust and durable humoral immune response. Furthermore, the epidemiology of SLE does not support a specific role for VSV_{IN} as a causative agent, since VSV_{IN} is found only in the Americas, and the frequency of SLE in the United Kingdom and the USA is very similar.[44] Based on the literature currently available, there is no convincing evidence that infection with VSV_{IN} leads to the development of SLE.

Analytical treatment interruption

The risks from a 16-week ATI performed under close virological and immunological monitoring are minimal in this subject population. There is a theoretical risk that ATI could lead to the development of HIV drug resistance. This may be a particular concern for individuals taking NNRTIs. However, this potential risk with NNRTIs is essentially eliminated by undertaking the procedures described in Section 3.1. Given the study population, the short duration of the ATI, the frequency of immunological

and virological monitoring, and strict criteria for restarting cART, it is extremely unlikely that the ATI will lead to the development of any opportunistic infections or AIDS-defining conditions.

Leukapheresis
The potential risks associated with leukapheresis include lightheadedness, dizziness, possible fainting, tingling around the mouth and in the fingers and toes, nausea, chills, vomiting, mild muscle cramps, loss of <1 pint of blood, or pain, bruising, or discomfort at the needle insertion sites. More serious, but rare, complications include nerve damage at the needle insertion site, seizures and air embolism. Most procedures are performed without an incident. Blood components removed during leukapheresis are generally replaced by the body within a few hours or a few days. No infections associated with this procedure have been reported in thousands of cases performed over the last 10 years at the NIH.

Phlebotomy
This may be associated with discomfort, bruising, local hematoma formation and, on rare occasions, infections, lightheadedness, and fainting. The amount of blood drawn for research purposes will be within the limits allowed for adult subjects by the NIH CC (Medical Administrative Policy 95-9: Guidelines for Limits of Blood Drawn for Research Purposes in the Clinical Center: http://internal.cc.nih.gov/policies/list_policies.asp?index=med_chrono).

HLA typing
Some HLA types have been associated with an increased risk of certain diseases like arthritis and other rheumatologic disorders, or a faster progression to AIDS. HLA typing will be performed on samples collected from all the enrolled subjects. Results from the HLA typing will become part of each subject's medical record at the NIH. Medical records containing this information are maintained in a secure place.

9.2 Potential Benefits

Improvement of HIV-specific immune control from the vaccine regimen is a potential benefit for subjects randomized to the vaccine arm.

10 Research Use of Stored Human Samples, Specimens or Data

- **Intended use:** Stored blood samples and data collected under this protocol may be used to study the effect of the HIV-MAG pDNA vaccine prime in combination with the IL-12 pDNA adjuvant and the rVSV HIV *gag* booster vaccine on the virologic and immunologic parameters listed in Section 8.2. Samples may also be used to study other aspects of the immunopathogenesis of HIV infection or measure serum levels of antiretroviral agents during ATI.
- **Storage:** Access to stored samples will be limited using a locked room or a locked freezer. Samples and data will be stored using codes assigned by the investigators.

Data will be kept in password-protected computers. Only investigators will have access to the samples and data.

- **Tracking:** Samples will be tracked utilizing the repository operated by SAIC Frederick, Inc. Data will be stored and maintained in the NIAID CRIMSON database.
- **Disposition at the completion of the protocol:** At the completion of the protocol (termination), samples and data will either be destroyed, or after IRB approval, transferred to another existing protocol.
- **Reporting the loss or destruction of samples/specimens/data to the IRB:**
  - Any loss or unanticipated destruction of the samples or data (for example, due to freezer malfunction) that meets the NIH Intramural protocol deviation definition or results in a deviation that compromises the scientific integrity of the data collected for the study will be reported to the NIAID IRB.
  - Additionally, subjects may decide at any point not to have their samples stored. In this case, the principal investigator will destroy all known remaining samples and report what was done to the subject and the IRB. This decision will affect the subject's participation in this protocol, but it may not affect participation in other protocols at the NIH.

11 Remuneration Plan for Subjects

Subjects will receive financial compensation for leukapheresis according to the NIH Clinical Center volunteer guidelines: $100 for a 2-pass leukapheresis procedure or $200 for a 4-pass leukapheresis procedure.

12 Assessment of Safety

12.1 Documenting, Recording, and Reporting Adverse Events

At designated visits with the subject, information regarding AEs will be elicited by appropriate questioning and examinations, and it will be:

- Immediately documented in the electronic database and medical record.
- Reported as outlined below (e.g., IND sponsor, IRB, Food and Drug Administration [FDA]).

12.2 Definitions

Adverse event

An AE is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease temporally associated with the subject's participation in the research, whether or not considered related to the research.

Adverse reaction

An adverse reaction (AR) is an AE that is caused by an investigational agent (drug or biologic).

Suspected adverse reaction
A suspected AR (SAR) is an AE for which there is a reasonable possibility that the investigational agent caused the AE. 'Reasonable possibility' means that there is evidence to suggest a causal relationship between the drug and the AE. A SAR implies a lesser degree of certainty about the causality than an AR, which implies a high degree of certainty.

Serious adverse event
An SAE is an AE that results in one or more of the following outcomes:
- Death.
- A life-threatening (i.e., an immediate threat to life) event.
- An inpatient hospitalization or prolongation of an existing hospitalization.
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly/birth defect.
- A medically important event.*

*Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life threatening or result in death or hospitalization, but they may jeopardize the subject or may require intervention to prevent one of the other outcomes listed above.

Unexpected adverse event
An AE is unexpected if it is not listed in the investigator's brochure or package insert (for marketed products), or it is not listed at the specificity or severity that has been observed. It is the responsibility of the IND sponsor to make this determination.

Serious and unexpected suspected adverse reaction
A serious and unexpected suspected AR (SUSAR) is a SAR that is both serious and unexpected.

Unanticipated problem
An unanticipated problem (UP) is an event, incident, experience, or outcome that is—
1. Unexpected in terms of nature, severity, or frequency in relation to—
   a. The research risks that are described in the IRB-approved research protocol and informed consent document; investigator's brochure, or other study documents; and
   b. The characteristics of the subject population being studied; and
2. Possibly, probably, or definitely related to participation in the research; and
3. Places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or

recognized (per the IND sponsor, an AE with a serious outcome will be considered increased risk).

Unanticipated problem that is not an adverse event
An unanticipated problem that is not an AE is incident, experience, or outcome that is not associated with an AE, which meets the 3 criteria of a UP. Examples include breaches of confidentiality, accidental destruction of study records, and unaccounted-for study drug.

Protocol Deviation
Any change, divergence, or departure from the IRB approved study procedures in a research protocol. Protocol deviations are designated as serious or non-serious and further characterized as:

1. Those that occur because a member of the research team deviates from the protocol.
2. Those that are identified before they occur, but cannot be prevented.
3. Those that are discovered after they occur

Serious Protocol Deviation: A deviation that meets the definition of a Serious Adverse Event or compromises the safety, welfare or rights of subjects or others.

Non-compliance: The failure to comply with applicable NIH HRPP policies, IRB requirements, or regulatory requirements for the protection of human subjects. Non-compliance is further characterized as:

1. Serious: Non-compliance that
   a. Increases risks, or causes harm, to participants
   b. Decreases potential benefits to participants
   c. Compromises the integrity of the NIH-HRPP
   d. Invalidates the study data
2. Continuing: Non-compliance that is recurring
3. Minor: Non-compliance that, is neither serious nor continuing.

Protocol specified events
Protocol specified events are AEs specified in the protocol that the principal investigator, IND sponsor, or medical monitor would like to review in real time rather than weeks or months later, when they would otherwise appear in various line listings. These events may or may not also be SAEs.

12.3 Investigator Assessment of Adverse Events

If a diagnosis is clinically evident (or subsequently determined), the diagnosis rather than the individual signs and symptoms or lab abnormalities will be recorded as the AE.

All AEs occurring from the time when the first vaccine dose is administered through the specified study follow-up period, recorded, and reported. The principal investigator will evaluate all AEs with respect to **Seriousness** (criteria listed above), **Severity** (intensity or grade), and **Causality** (relationship to study agent and relationship to research) according to the following guidelines.

12.3.1 Severity

The principal investigator will grade the severity of each AE according to the Division of Aids Table for Grading the Severity of Adult and Pediatric Adverse Events Version 1.0, December, 2004, (Clarification August 20) which can be found at:
http://rsc.techres.com/Document/safetyandpharmacovigilance/Table_for_Grading_Severity_of_Adult_Pediatric_Adverse_Events.pdf

Subjective reactogenicity symptoms (such as malaise, fatigue, injection site pain, headache, chills) will not be given a grade 3 score unless they persist for >3 days.

Some grade 1 lab parameters on the DAIDS toxicity table (fibrinogen, low calcium levels, high fasting glucose, low phosphate levels, low potassium levels, low sodium levels, elevated uric acid levels [males only]) fall within the NIH lab reference range for normal values. These normal values will not be reported as grade 1 AEs. The grade 1 values for these tests will be reported as follows:

- Fibrinogen: 100–176 mg/dL.
- Phosphate (low): There is no grade 1 reportable value.
- Sodium (low): 130–134 mmol/L.
- Potassium (low): 3.0–3.2 mmol/L.
- Calcium (low): 1.95–2.04 mmol/L.
- Glucose (high, fasting): 116–125 mg/dL.
- Uric Acid (males): 8.7–10.0 mg/dL.

12.3.2 Causality

Causality (likelihood that the event is related to the study agent) will be assessed considering the factors listed under the following categories:

Definitely related

- Reasonable temporal relationship.
- Follows a known response pattern.
- Clear evidence to suggest a causal relationship.
- There is no alternative etiology.

Probably related

- Reasonable temporal relationship.

- Follows a suspected response pattern (based on similar agents).
- No evidence of a more likely alternative etiology.

Possibly related

- Reasonable temporal relationship.
- Little evidence for a more likely alternative etiology.

Unlikely related

- Does not have a reasonable temporal relationship.
  OR
- Good evidence for a more likely alternative etiology.

Not related

- Does not have a temporal relationship.
  OR
- Definitely due to an alternative etiology.

Note:
Other factors (e.g., dechallenge, rechallenge) should also be considered for each causality category when appropriate. Causality assessment is based on available information at the time of the assessment of the AE. The investigator may revise the causality assessment as additional information becomes available.

12.4 Investigator Reporting Responsibilities to the Sponsor

12.4.1 Adverse Events

Line listings, frequency tables, and other summary AE data will be submitted to the IND sponsor per the Safety Review and Communications Plan (SRCP – see below), or as needed for periodic safety assessments, review of IND annual reports, review of IND safety reports, and preparation of final study reports.

12.4.2 Serious Adverse Events

SAEs (whether or not they are also UPs) must be reported on the SAE/UP report form and sent to the sponsor Clinical Safety Office (CSO) by fax or e-mail attachment. Deaths and immediately life-threatening SAEs must be reported within 1 business day after the site becomes aware of the event. All other SAEs must be reported within 3 business days of site awareness.

Sponsor clinical safety office contact information:
OCRPRO Clinical Safety Office
5705 Industry Lane
Frederick, MD 21704

Phone 301-846-5301
Fax 301-846-6224
E-mail: rchspsafety@mail.nih.gov

12.4.3 Unanticipated Problems

Non-serious AEs that are UPs must also be reported on the SAE/UP report form and sent to the CSO by fax or e-mail attachment no later than 7 calendar days of site awareness of the event. UPs that are not AEs are not reported to the sponsor CSO.

12.4.4 Protocol Specified Events

Autoimmune disorders are AEs of special interest. A sample list of autoimmune disorders is provided in Appendix C. These protocol specified events must be reported to the CSO on a SAE/UP report form within 3 business days of site the awareness.

12.4.5 Pregnancy

Pregnancy itself is not an AE. However, complications of pregnancies are AEs and may be SAEs. Pertinent obstetrical information for all pregnancies, including pregnancies disclosed by the subject as occurring in a partner of a male subject, will be reported to the CSO via fax or e-mail within 3 business days from the site awareness of the pregnancy.

Pregnancy outcome data (e.g., delivery outcome, spontaneous or elective termination of the pregnancy) will be reported to the CSO within 3 business days of the site awareness on a protocol-specified form. In the event of pregnancy, the following steps will be taken:

- Discontinuation of the study agents.
- Unblind per the unblinding section of the protocol.
- Withdraw from the study but continue following for safety.
- Report to safety oversight committee (Data and Safety Monitoring Board [DSMB]) and the IRB.
- Advise research subject to notify the obstetrician of study agent exposure.

12.5 Investigator Reporting Responsibilities to the NIAID IRB

12.5.1 Expedited Reporting to the NIAID IRB

Serious and non-serious Unanticipated Problems, deaths, serious deviations, and serious or continuing non-compliance will be reported within 7 calendar days of investigator awareness. SAEs that are possibly, probably, or definitely related to the research will be reported to the NIAID IRB within 7 calendar days of investigator awareness, regardless of expectedness.

12.5.1.1. Waiver of Reporting Anticipated Protocol Deviations, Expected UP nonAEs and Deaths to the NIAID IRB

Anticipated deviations in the conduct of the protocol will not be reported to the IRB unless they occur at a rate greater than anticipated by the study team. Expected adverse events will not be reported to the IRB unless they occur at a rate greater than that known to occur in HIV(+) population. If the rate of these events exceeds the rate expected by the study team, the events will be classified and reported as though they are unanticipated problems. Deaths related to the natural history of HIV (+) population will be reported at the time of continuing review.

12.5.2 Annual Reporting to the NIAID IRB

The following items will be reported to the NIAID IRB in summary at the time of continuing review:

- Serious and non-serious unanticipated problems
- Expected serious adverse events that are possibly, probably, or definitely related to the research
- Serious adverse events that are not related to the research
- All adverse events, except expected AEs and deaths granted a waiver of reporting.
- Serious and Non-Serious Protocol deviations
- Serious, continuing, and minor non-compliance
- Any trends or events which in the opinion of the investigator should be reported

12.6 Follow-up of Adverse Events and Serious Adverse Events

AEs that occur following enrollment of subjects (i.e., after the first vaccination) are followed until the final outcome is known or until the end of the study follow-up period.

SAEs that have not resolved by the end of the follow-up period are followed until the final outcome is known. If it is not possible to obtain a final outcome for an SAE (e.g., the subject is lost to follow up), the reason a final outcome could not be obtained will be recorded by the investigator on the SAE/UP report form.

SAEs that occur after study completion (month 24) that are reported to and are assessed by the investigator to be possibly, probably, or definitely related must be reported to the CSO, as described above.

12.7 Sponsor's Reporting Responsibilities

SUSARs as defined in 21 Code of Federal Regulations (CFR) 312.32 and determined by the IND sponsor will be reported to the FDA and all participating investigators as IND safety reports.

The IND sponsor will also submit an IND annual report of the progress of the investigation to the FDA as defined in 21 CFR 312.33.

12.8 Halting Criteria for the Protocol

Halting the study requires immediate discontinuation of the study agents administered for all subjects and suspension of enrollment until a decision is made about whether or not to continue study agent administration.

The halting criteria (as determined by the study principal investigator and IND sponsor secondary to aggregate data review) for this study include:

- Any SAE or grade 4 AE that is possibly, probably, definitely related to the study agent; OR
- Any death; OR
- Any safety issue that the study principal investigator or IND sponsor determines should halt the study.

Any related AE that is ≥ grade 3 (not including transient, subjective reactogenicity symptoms such as malaise, fatigue, injection site pain, headache, chills) will be reviewed within 48 hours of site awareness, by the principal investigator and IND sponsor medical monitor, to consider the need for halting the protocol.

The IRB, the IND sponsor, DSMB, or the FDA may halt the study at any time due to safety concerns.

Halting is carried out by or through the principal investigator (or designee if unavailable) within 24 hours of the decision to halt the trial, regardless of the party initiating the halt, whether the halt is due to the criteria defined above or another issue.

12.8.1 Reporting of Study Halting

If the study is halted, a description of the event(s) or safety issue must be reported by the principal investigator within 1 business day to the sponsor CSO and the NIAID IRB by fax or e-mail.

12.8.2 Resumption of a Halted Study

The IND sponsor, in collaboration with the principal investigator and the DSMB, will determine if it is safe to resume the study. The IND sponsor will notify the principal investigator of this decision. The conditions for resumption of the study will be defined in this notification. The principal investigator will notify the IRB of the decision to resume the study.

12.9 Pausing Criteria for a Subject or Group

The decision to suspend administration of the study agent(s) for a single subject, or for all the subjects in the study, requires discontinuation of the study agent administrated for the study subject(s) until a decision is made whether or not to continue study agent administration.

The pausing criteria for a single subject or for all the subjects in this study include:

- A subject or 3 subjects experience an SAE or grade 3 or greater AE (not including transient, subjective reactogenicity symptoms such as malaise, fatigue, injection site pain, headache, chills, or total bilirubin in subjects taking atazanavir) that is unexpected (as determined by the IND sponsor) and is possibly, probably, or definitely related to the study agent;

OR

- 2 subjects experience grade 4 local or systemic reactogenicity symptom(s) or AE that is considered to be related to the study vaccination.

OR

- Any safety issue that the site investigator determines should pause administration of the study agent to a single subject or to all the subjects in the study.
- The IND sponsor, in collaboration with the principal investigator , may also pause for an individual subject or the entire group if a safety concern is identified during routine aggregate data analysis.

12.9.1 Reporting of Pausing for a Subject or Group

If a pausing requirement is met, a description of the AE(s) or safety issue must be reported by the principal investigator by fax or e-mail within 1 business day to the sponsor CSO, principal investigator, IRB, and the DSMB.

12.9.2 Resumption of Pausing for a Subject or Group

The IND sponsor in collaboration with the principal investigator and/or the relevant DSMB will determine if it is safe to resume administration of the study agents to the subject/group. The IND sponsor will notify the principal investigator of this decision. The principal investigator will notify the IRB of the decision to resume administration of the study agent prior to resumption.

12.10 Withdrawal Criteria for an Individual Subject

An individual subject will be withdrawn for any of the following:

- An individual subject's decision.
  (The investigator will attempt to determine the reason for the subject's decision, and will strongly suggest a follow-up plan to help ensure the subject safely returns to baseline or better, if possible).
- Co-enrollment in a study with an investigational research agent (rare exception allowing the continuation of the study vaccinations granted by the principal investigator).

- 2 consecutive viral loads >400 copies/mL while on cART during the vaccination phase of the study.
- Development of an AIDS defining opportunistic infection (OI). Any subject who develops an AIDS OI will have repeat assessments of of CD4 counts weekly until counts rise to >200 cells/mm^3 on at least 2 successive assessments".
- 2 consecutive CD4 counts showing a >30% decline from pre-vaccination baseline or an absolute CD4 cell count <350 cells/mm^3 while on cART during the vaccination phase of the study.
- Any SAE or grade 4 local or systemic reactogenicity symptom, lab abnormality, or AE that is subsequently considered to be related to the study vaccination(s).
- Clinically significant type 1 hypersensitivity reaction associated with the study vaccination(s). In the event of a type 1 hypersensitivity reaction that is NOT considered to be clinically significant, (e.g., brief, mild, and self limited skin reaction without other symptoms), the principal investigator, in consultation with the sponsor medical monitor, may consider possible additional vaccinations with appropriate precautions.
- Any clinical AE, laboratory abnormality, or other medical condition or situation such that continued participation in the study would not be in the best interest of the subject. Subjects will be followed for the duration of the study for indicated safety assessments.
- Non-compliance with study procedures to the extent that it is potentially harmful to the subject or to the integrity of the study data.
- Pregnancy.
- Participant misses more than 1 study vaccinations.

If possible, all subjects who discontinue the study treatment prematurely will be followed through week 56 for all the study evaluations.

12.11 Replacement for Withdrawn Subjects

Any subject who withdraws from the study, or who discontinues the study vaccinations, prematurely before the 28^{th} day of the last study vaccination, and whose reasons for withdrawing from the study or discontinuing study vaccination administration are unrelated to any real or perceived effect of the study vaccinations or their administration, will be replaced.

12.12 Safety Oversight

12.12.1 Safety Review and Communications Plan

An SRCP has been developed for the protocol. The SRCP is an internal communications document between the principal investigator and the IND sponsor CSO, which delineates the safety oversight responsibilities of the principal investigator, the CSO, and other stakeholders. The SRCP also

includes the overall plan for conducting periodic safety surveillance assessments.

12.12.2 Sponsor Medical Monitor

A medical monitor, representing the IND sponsor (OCRPRO), has been appointed for the safety oversight in this clinical study. The sponsor medical monitor will be responsible for performing safety assessments as outlined in the SRCP.

12.12.3 Data and Safety Monitoring Board

The NIAID intramural DSMB will review the study prior to initiation and twice a year thereafter. The Board may convene additional reviews as necessary. The Board will review the study data to evaluate the safety, efficacy, study progress, and conduct of the study. All SAEs, all UPs, and all IND safety reports will be reported by the principal investigator to the DSMB at the same time they are submitted to the IRB or IND sponsor. The principal investigator will notify the DSMB of any cases of intentional or unintentional unblinding as soon as possible. The principal investigator will notify the Board at the time when pausing or halting criteria are met and obtain a recommendation concerning continuation, modification, or termination of the study. The principal investigator will submit the written DSMB summary reports with recommendations to the IRB.

13 Clinical Monitoring Structure

13.1 Site Monitoring Plan

As per International Conference on Harmonization (ICH) Good Clinical Practice (GCP) 5.18 FDA 21 CFR 312.50, if under IND, clinical protocols are required to be adequately monitored by the study sponsor. This study monitoring will be conducted according to the NIAID Intramural Clinical Monitoring Guidelines. Monitors under contract to the NIAID/OCRPRO will visit the clinical research site to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent documents and documentation of the informed consent form process for each monitored subject; 2) to verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs; 3) to compare abstracted information CRIMSON data abstracts with individual subjects' records and source documents (subjects' charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and 4) to help ensure that investigators are in compliance with the protocol. The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (Office for Human Research Protections [OHRP]), FDA, and applicable guidelines (ICH-GCP) are being

followed. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

The investigator (and/or designee) will make the study documents (e.g., consent forms, CRIMSON data abstracts, and pertinent hospital or clinical records readily available for inspection by the local IRB, the FDA, the site monitors, and the NIAID staff for confirmation of the study data.

A specific protocol monitoring plan will be discussed with the principal investigator and study staff prior to enrollment. The plan will outline the frequency of the monitoring visits based on factors such as the study enrollment, data collection status, and regulatory obligations.

13.1.1 Study Blinding and Unblinding

Study participants and site staff (except for the NIH pharmacists) will be blinded to the participant treatment arm assignments (e.g., active vaccine or control). Any discussion about the study product assignment between the pharmacy staff and any other protocol staff is prohibited. The DSMB members also are unblinded to the treatment assignment for the review of trial safety.

When a participant leaves the trial prior to study completion, the participant will be told he or she must wait until the all the participants are unblinded to learn about his or her treatment assignment.

Emergency unblinding decisions will be made by the principal investigator. if time permits, the sponsor medical monitor will be consulted before emergency unblinding occurs.

14 Compliance With NIH Guidelines for Research Involving Products Containing Recombinant DNA

Because this study is evaluating products containing recombinant DNA, it must comply with regulations set forth in the NIH's *Guidelines for Research Involving Recombinant DNA Molecules*. Information about the study must be submitted to site Institutional Biosafety Committees and must be approved before participants are enrolled in the study. IBC review and approval must be documented by the investigator and submitted as part of protocol registration for this trial.

The NIH guidelines also require that human gene transfer trials conducted at or sponsored by institutions that receive NIH funds must be submitted to the NIH Office of Biotechnology Activities for review by the Recombinant DNA Advisory Committee.

The study team and DAIDS will ensure that reporting requirements to the Recombinant DNA Advisory Committee, as outlined in *Appendix M-I-C-1.Initiation of the Clinical Investigation*, *Appendix M-I-C-3.Annual Reports*, and *Appendix M-I-C-4.Safety Reporting* are satisfied per the NIH guidelines.

15 Statistical Considerations

15.1 Study Hypotheses

The study will test the hypothesis that therapeutic vaccination with the HIV-MAG pDNA vaccine prime in combination with the IL-12 pDNA adjuvant and the rVSV HIV *gag* booster will have acceptable safety and tolerability in a population of virally suppressed HIV-infected subjects who began cART during acute or early infection. The study will also test the hypothesis that HIV-specific immune responses induced by this vaccination strategy will have clinically significant antiviral activity in the absence of cART.

15.2 Sample Size Justification

The primary outcome for this study is the occurrence of SAEs or grade 3 or higher AEs. The probability of at least 1 such event among 15 vaccine recipients is 1-(1-*P)*,[15] where *P* is the probability that a given person has the event. Table 3 shows, for different values of *P*, the probability of observing at least 1 vaccine participant with an AE of probability *P*. For instance, *P*=.05 corresponds to an AE that occurs in about 5% of participants; the probability of this AE being observed for at least 1 of the 15 vaccine recipients is approximately 54%. This means that we cannot be confident of observing relatively rare events with a probability of 5% or less. On the other hand, the probability of observing at least 1 vaccine participant with an AE of probability 15% is approximately 91%. This means that if we do not observe anyone with a given type of AE, we can be relatively confident that it occurs in fewer than 15% of the population.

Table 3: Probability of observing at least 1 vaccine recipient out of 15 with an AE that has probability P of occurring in a given participant

P =.05	*P* =.10	*P* =.15	*P* =.20	*P*=.25
54%	79%	91%	96%	99%

The power for a comparison of the 2 arms with respect to a given AE is low unless the event probability is high in the vaccine arm and very low in the placebo arm. For example, if the AE probabilities in the 2 arms are .55 and .05, then power using Fisher's exact test is approximately 82%.

To compute the power for the secondary outcome of difference in plasma viremia at the end of the ATI phase of the study, we needed to estimate the standard deviation of rebound plasma viremia in $\log_{10}$ units. We used data from Rosenberg et al[38] and Grijsen et al.[45] The Rosenberg study had a similar design to the one being contemplated here, namely randomization to vaccine or placebo among acutely infected participants followed by ART interruption and determination of viral levels 17-23 weeks following interruption. Rosenberg et al do not give the standard deviation, but back calculation of this quantity from the confidence interval given

suggests a standard deviation between 0.64 and 0.90. The Grijsen study had an estimated standard deviation of close to 1 in the ART treatment arms, though their measure of rebound plasma viremia was determined following a longer period of time (36 weeks) from the treatment interruption. To be conservative, we decided to adopt the larger standard deviation of 1. Although the Wilcoxon rank sum test will be used to compare the 2 groups with respect to viremia, power for the Wilcoxon test is very close to that of the t-test (the asymptotic relative efficiency is approximately 95%). Therefore, we approximated power for the Wilcoxon test by that of the t-test. The power based on a 2-sample t-test with a 2-tailed alpha of .05 and a total sample size of 30 is approximately 91% to detect a 1.25 $\log_{10}$ reduction in the rebound plasma viremia.

16 Ethics/Protection of Human Subjects

16.1 Informed Consent Process

Informed consent is a process where information is presented to enable persons to decide voluntarily whether or not to participate as a research subject. It is an on-going conversation between the human research subject and the researchers, which begins before consent is given and continues until the end of the subject's involvement in the research. Discussions about the research will provide essential information about the study and include: purpose, duration, experimental procedures, alternatives, risks, and benefits. Subjects will be given the opportunity to ask questions and have the questions answered.

The subjects will sign the informed consent document prior to undergoing any research procedures. The subjects may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the subjects for their records. The researcher will document the process of signing the consent form in the subject’s medical record. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

16.2 Subject Confidentiality

All records will be kept confidential to the extent provided by federal, state, and local law. The study monitors and other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the investigator, including, but not limited to, medical records. Records will be kept locked and all computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the IRB, FDA, NIAID, OHRP, pharmaceutical supporter(s), or the sponsor’s designee.

17 Data Handling and Record Keeping

17.1 Data Management Responsibilities

The investigator is responsible for assuring that the data collected are complete, accurate, and recorded in a timely manner. Source documentation (the point of initial recording of information) should support the data collected in the electronic data system and must be signed and dated by the person recording and/or reviewing the data.

17.2 Data Capture Methods

Study data will be collected at the study site and maintained on an electronic data system (CRIMSON). These forms or systems are to be completed on an ongoing basis during the study. Data entered into electronic data systems shall be performed by authorized individuals. Corrections to electronic data systems shall be tracked electronically (password protected or through an audit trail) with the time, date, individual making the correction, and what was changed.

17.3 Types of Data

Source documents include, but are not limited to, subjects' medical records, laboratory reports, ECG tracings, x-rays, radiologist's reports, biopsy reports, ultrasound photographs, progress notes, pharmacy records, and any other similar reports or records of procedures performed during the subjects' participation in the study.

17.4 Source Documents and Access to Source Data/Documents

Source documents include all recordings of observations or notations of clinical activities, and all reports and records necessary for the evaluation and reconstruction of the clinical trial. Data from the CRIMSON Data System will be collected directly from subjects during study visits and telephone calls, or will be abstracted from subjects' diaries/memory cards and medical records. Each subject's medical record must record his/her participation in the clinical trial and, after unblinding, study treatment/vaccination (with doses and frequency) or other medical interventions or treatments administered, as well as any AEs experienced during the trial.

17.5 Record Retention

The investigator is responsible for retaining all the essential documents listed in the ICH GCP guidelines. All essential documentation for all the study subjects are to be maintained by the investigators in a secure storage facility for a minimum of 3 years per NIAID policies. The FDA requires study records to be retained for up to 2 years after marketing approval or disapproval (21 CFR 312.62), or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational agents for a specific indication. These records are also to be maintained in compliance with IRB, state, and federal medical records-retention

requirements, whichever is longest. All stored records are to be kept confidential to the extent required by federal, state, and local law.

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Appendix B: Schedule of Evaluations

See attached document.

Appendix C: AEs of Special Interest

AEs of special interest for this protocol include, but are not limited to, autoimmune disorders; representative examples of AES of special interest are listed below.

Neuroinflammatory disorders

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

Musculoskeletal disorders

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

Gastrointestinal disorders

Crohn's disease
Ulcerative colitis
Celiac disease

Metabolic diseases

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

Skin disorders

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

Others

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

Appendix D: Vaccine Diary Card

See attached document.