A Pilot Study to Evaluate the Safety and Tolerability of Escalating Doses of Autologous CD4 T-Cells Modified with Lentiviral Vector Expressing an HR2, C34-peptide conjugated to the CXCR4 N-terminus in HIV-infected Subjects

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ACRONYMS

AE	adverse event/experience
AIDS	acquired immunodeficiency syndrome
ALP	alkaline phosphatase
ALT	alanine aminotransferase (SGPT)
ANC	absolute neutrophil count
ARS	acute retroviral syndrome
ART	antiretroviral therapy
AST	aspartate aminotransferase (SGOT)
ATI	analytical treatment interruption (removal from HAART)
BLQ	below level of quantification
CAR	chimeric antigen receptor
CBC	complete blood count
CCI	Center for Cellular Immunotherapies
CDC	Centers for Disease Control and Prevention
CHPS	Center for Human Phenomic Science
CRF	case report form
CVPF	Clinical Cell and Vaccine and Production Facility
DLT	dose limiting toxicity
DSMB	Data and Safety Monitoring Board
ELISA	enzyme-linked immunosorbent assay
ENV	HIV envelope
EAE	expedited adverse event
FDA	Food and Drug Administration
HAART	Highly Active Antiretroviral Therapy
HBsAg	hepatitis B surface antigen
HCV	hepatitis C virus
HIC	Human Immunology Core
HIV	human immunodeficiency virus
HR2	Heptad Repeat 2
ICS	intracellular cytokine staining
IRB	institutional review board
IUD	intrauterine device
MRN	medical record number
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NNRTI	non-nucleoside reverse transcriptase inhibitor
OHRP	Office for Human Research Protections (formerly OPRR)
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PID	patient identification number
RCL	replication-competent lentivirus
RNA	ribonucleic acid
RNAi	RNA interference
SAE	serious adverse event
SADR	serious adverse drug reaction
SCID	severe combined immunodeficiency
SOE	schedule of events
STI	Structured treatment interruption (removal from HAART)

TCSL	Translational and Correlative Studies Laboratory
ULN	upper limit of normal
UPenn	University of Pennsylvania
VL	viral load
Wt	wild-type

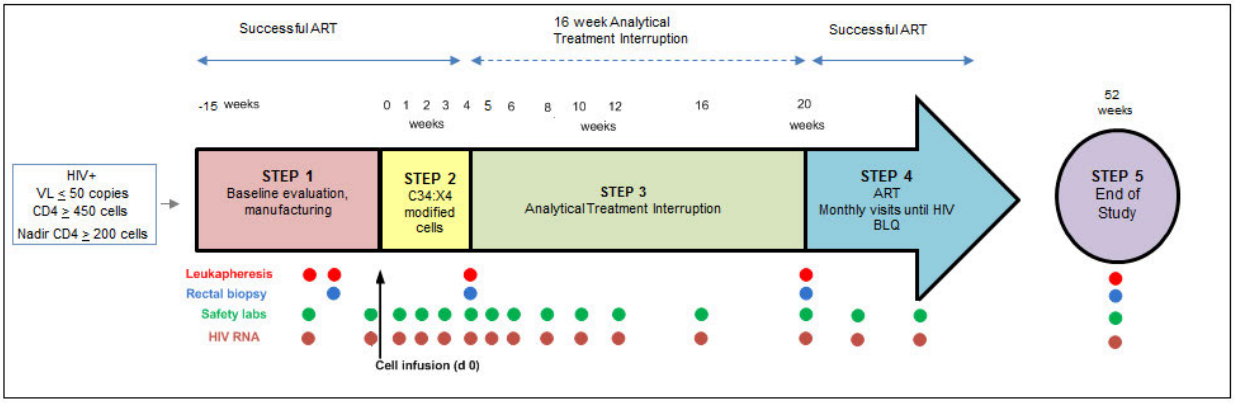
STUDY SUMMARY

Title	A Pilot Study to Evaluate the Safety and Tolerability of Escalating Doses of Autologous CD4 T-Cells Modified with Lentiviral Vector Expressing an HR2, C34-peptide conjugated to the CXCR4 N-terminus in HIV-infected Subjects
Short Title	C34-CXCR4-modified CD4+ T-cells in HIV Therapy
Protocol Number	Sponsor #C34-CXCR4; IRB #826035
Phase	Pilot
Methodology	This is a single cohort, open-label pilot study of the safety and tolerability of a single infusion of autologous CD4+ T-cells genetically modified with an HR2, C34-peptide conjugated to the CXCR4 N-terminus using a lentiviral vector in HIV-infected subjects. This is a first in human study of C34-CXCR4-T cells. There will be a single cohort in this study, which consists of subjects with well-controlled HIV replication on HAART. Within this cohort will be 3 escalating doses of T-cell infusions. A modified 3+3+3 dose-escalation design will be followed, in which the standard dose-escalation algorithm is stopped when a maximum of 9 evaluable subjects or a DLT stopping point has been reached, whichever comes first. At each dose level, three patients are treated. For dose levels 1 and 2, if 0/3 subjects have a dose limiting toxicity (DLT), then the dose is escalated. If 1/3 has a DLT (grade 3 or higher unexpected, related adverse event [AE]) at a dose level then 3 additional patients are treated at that dosage before escalating, and if <2/6 have DLT (i.e. no additional DLT is observed) then the dose is escalated to the next planned dose level and patients treated until a maximum of 9 evaluable subjects has been reached. The study will comprise of 5 steps: In Step 1, all participants will undergo leukapheresis to obtain CD4 positive T-cells that will be genetically modified. A second leukapheresis and an optional rectal biopsy will provide baseline specimens to evaluate the size of the HIV reservoir In Step 2, all participants will receive a single infusion of C34-CXCR4-modified CD4+ T-cells at one of 3 dose levels. The first 3 subjects will receive Dose Level 1 of 0.8-1x10^9 transduced CD4+T-cells. Provided no dose limiting toxicity (DLT) is seen at the first dose level, the next 3 subjects will receive infusion at Dose Level 2 of 2.4-3x10^9 transduced CD4+ T-cells. If no DLT occurs at that dose, the final 3 subjects will receive an infusion at Dose Level 3 of 0.8-1x10^{10} transduced CD4+ T-cells. In the event of a DLT (grade 3 or higher unexpected, related AE) recruitment will be paused pending DSMB decision. At the end of step 2 all participants will undergo mini-leukapheresis and an optional rectal biopsy. In Step 3 all participants will participate in a 16-week analytical

	treatment interruption beginning 4 weeks after T-cell infusion. At the end of step 3 all participants will undergo mini-leukapheresis and an optional rectal biopsy In Step 4 all participants will be advised to resume antiretroviral therapy and will be followed until plasma HIV RNA falls below the limit of detection. In Step 5 all participants will undergo leukapheresis and an optional rectal biopsy at 52 weeks post infusion. At the completion of the study, participants will be asked to participate in a long-term follow-up study as required by regulatory authorities.
Study Duration	Approximately 1 year and 3 months from screening visit. At the completion of the study participants will roll over into a long-term gene therapy safety follow-up study.
Study Center(s)	University of Pennsylvania Medical Center
Objectives	The primary objective of this clinical study is to evaluate the safety and tolerability of a single dose of this novel biological drug in HIV positive subjects. Secondary objectives focus mainly on persistence and enrichment of C34-CXCR4-modified autologous CD4+T-cells, correlating the number of transduced T-cells infused with effects on CD4+ T-cell count and HIV viral load, trafficking of vector modified cells in mucosal tissue, immunogenicity of C34-CXCR4, and effects on immune function.
Number of Subjects	Maximum of 9 evaluable subjects. Evaluable subjects are defined as those who have received T-cell infusion and undergo analytical treatment interruption.
Diagnosis and Main Inclusion Criteria	HIV-1-positive women and men, ≥18 years old with HIV-1 RNA levels undetectable by ultrasensitive HIV PCR assay, CD4+ T-cells counts ≥450 cells/ mm^3, with a documented CD4 nadir of not lower than 200 cells/ mm^3, and who have a recorded viral load set point prior to starting therapy.
Study Product, Dose, Route, Regimen	Autologous CD4+ T-cells modified with lentiviral vector expressing an HR2, C34-peptide conjugated to the CXCR4 N-terminus (C34-CXCR4-T), given at a single dose of either: **1)** 0.8-1x10^9, **2)** 2.4-3x10^9, or **3)** 0.8-1x10^{10} transduced T-cells via intravenous infusion.
Duration of administration	Based on the total volume to be infused and the recommended infusion rate of approximately 10mL-20mL/minute. Study subjects will be treated with only a single infusion of modified CD4+T-cells.
Statistical Methodology	The primary focus of this trial is establishing safety. As such, with three evaluable subjects in each dose level, we can be reasonably assured of observing an adverse event that occurs in the population at a rate of at least 80%. As this is a pilot study, there will be limited statistical power to evaluate efficacy and related biological endpoints. Therefore, analyses will be primarily descriptive. In general, an evaluable sample size of three

	subjects will provide 80% power, at a two-sided significance level of 5%, to identify an effect size of 3.3 (corresponding to 3.3 standard deviations) for the within person change for normally distributed outcomes. In this exploratory study we will evaluate the impact of C34-CXCR4-modified CD4+ T-cells in HIV-positive subjects. In order to have an evaluable sample size, subjects who prematurely discontinue the study prior to the infusion or analytical treatment interruption will be replaced with another subject.

Figure 1: Study Schema



1.0 INTRODUCTION

1.1 Background

1.1.1 Current Status of "the cure" of HIV Infection

There are an estimated 36.9 million people living with HIV and a corresponding 1.2 million deaths annually worldwide (UNAIDS, 2015). In North America and Western and Central Europe, it is estimated that 2.4 million people are infected with HIV, almost 26,000 people die each year, and more than 85,000 become infected annually. The use of highly active antiretroviral therapy (HAART) in HIV infected individuals has been associated with prolonged suppression of HIV viremia. However the persistence of HIV in latently infected resting CD4+ T-cells creates an almost insurmountable challenge in the long-term control of HIV infection in the absence of antiretroviral therapy and in the prospects for eradication and the "cure" of HIV infection.

The recent case of a German patient who underwent an allogeneic stem cell transplantation using CD34+ selected peripheral blood progenitor cells from a donor identified to be homozygous for CCR5-Δ32, after conditioning with the FLAMSA regimen has challenged the widespread perception that HIV eradication, or at least a "functional" cure was not possible (Hutter, Nowak et al. 2009). That particular individual had been treated with TDF/FTC/EFV for 4 years before the acute myeloid leukemia appeared. After relapse of his leukemia he underwent a bone marrow transplant from an HLA-identical donor who had been screened for homozygosity for the *CCR5* Δ 32 allele. Antiretroviral therapy was discontinued on the day of the procedure. GVHD prophylaxis followed standard regimens and engraftment was achieved on day +13. Complete chimerism as detected by competitive PCR was observed on day +60. The virus load was measured both by RNA-PCR and proviral DNA-PCR. DNA-PCR was negative from day +68. Although HAART has now been discontinued for over 5 years, HIV-1 has not been detected, as determined by RNA and proviral DNA PCR assays of peripheral blood, bone marrow, and rectal mucosa. This patient has raised the interest in treatment strategies aimed at eradication (either biological or functional) of HIV infection (Allers, Hutter et al. 2010). In the field of HIV gene therapy, particular interest surrounds the idea of making cells refractory, or protected, from HIV infection.

Recently two Boston patients received an allogeneic bone marrow transplant. As with the Berlin patient, they also received aggressive conditioning chemotherapy and developed graft versus host disease, however they did not receive delta 32 bone marrow. Both patients discontinued antiretroviral therapy after no evidence of residual HIV infection could be found. In both cases the virus returned after several weeks. These two cases demonstrate that protecting cells in order to prevent the return of viremia is critical in any strategy of eradicating HIV infection (Henrich, Hanhauser et al. 2014).

One potential factor believed to have contributed to the "functional cure" of HIV infection in the case of the Berlin patient was the transplantation of CCR5 Δ32 cells, which due to a lack of a functional CCR5 coreceptor were unable to be HIV infected. The ultimate goal of our approach is similar in that it also intends to protect CD4 cells from HIV infection. Our hypothesis is that adoptive cell transfer of autologous CD4 T-cells genetically modified to express C34 peptide conjugated to the CXCR4 N-terminus in HIV-infected individuals will preserve CD4 cells that will be immunologically functional and can help control viremia, and that these cells will survive and be enriched in the absence of antiretroviral drugs, potentially contributing to a delay in the return of viral load.

Curing HIV will take a combinatorial approach involving activation of the HIV from its reservoir, targeting the activated reservoir by the immune system, and protecting the uninfected cells during the process. Although these strategies will most likely be used in the future in combination, it will require that each strategy is tested separately before being used in a combined approach. Our study aims to protect the HIV uninfected cells during the process of activating the reservoir by making the CD4+ T-cells resistant to HIV, thus preserving the immunological response against HIV.

Gene therapy for HIV-1 infection has been proposed as an alternative to antiretroviral drug regimens (Sarver and Rossi 1993, Dropulic and June 2006, Leibman and Riley 2015). A number of different genetic vectors with antiviral payloads have been utilized to combat HIV-1, including antisense RNA, transdominant proteins, ribozymes, RNA decoys, single chain antibodies, and RNAi (RNA-interference) (Dropulic and June 2006). Payloads targeting entry of HIV have also been investigated both in preclinical studies and in human trials (Li, Kim et al. 2005, van Lunzen, Glaunsinger et al. 2007). Blocking HIV at an early step may be important for conferring a selective advantage to genetically modified cells in the body, and hence allow outgrowth of HIV–resistant T-cells in the body (von Laer, Hasselmann et al. 2006). Our proposed study will investigate the inhibition of HIV entry using CD4 T-cells expressing the C34 peptide of gp41 conjugated to CXCR4 N terminus and the ability of those modified cells to confer such a selective advantage.

1.1.2 Lentiviral Vectors for Gene Therapy

The early vectors used for human gene transfer research have included murine oncoretrovirus-derived and adenovirus-derived vectors, and the more recent vectors are those derived from adeno-associated virus, herpes simplex virus, and now lentivirus (Kootstra, Matsumura et al. 2003, Nathwani, Davidoff et al. 2005, Verma and Weitzman 2005). Murine oncoretrovirus-derived vectors were initially found to be useful for long-term gene expression because of their ability to integrate into host DNA. However, with the evidence that this vector could produce lymphoma due to insertional mutagenesis (Hacein-Bey-Abina, Von Kalle et al. 2003, Hacein-Bey-Abina 2006), oncoretroviruses have been used less as a vehicle for cell transduction.

Lentiviral vectors are an alternative to oncoretroviral vectors and are known to have higher transduction efficiencies. However, an important question has been whether lentivirus vectors are safe, and the use of T-cell-based approaches has been a useful step in vector evaluation. Murine retrovirus vectors, although used in hundreds of persons and found to be generally safe, were known to induce lymphomas in animals, principally by means of insertional mutagenesis (Tsichlis 1987). The T-cell proliferation actually seen in two trials of human retrovirus gene therapy for severe combined immunodeficiency disease is likely due not only to insertional mutagenesis but also to the effects of a ‘growth’ promoting transgene used in this treatment (Hacein-Bey-Abina et al., 2003) (unpublished data on www.asgt.org). Lentivirus vectors integrate into sites of active gene transcription (Bushman 2002, Mitchell, Beitzel et al. 2004), but the relevance of this process to oncogenesis is unknown. The lentiviral vector poly-A signal more strongly inhibits transcriptional read through than oncoretroviral vectors (Zaiss, Son et al. 2002). Additionally, a study in tumor prone mice comparing the tumorigenicity of retroviral vectors to lentiviral vectors demonstrated that lentiviral vector gene transfer into hematopoietic stem cells of up to an average of 6 copies per cell was not tumorigenic in contrast to retroviral vectors which were tumorigenic at an average copy number of only 3 per cell (Montini, Cesana et al. 2006). Lentivirus vectors can result in multiple integrations per cell and can even integrate in known oncogenes (Woods, Muessig et al. 2003). Yet, there is little association between HIV-1 infection and T-cell leukemia, and the increased rate of B cell lymphoma in AIDS is commonly

known to result from immunodeficiency rather than from HIV-1 integration events (Kaplan, Shiramizu et al. 1995, Sandler and Kaplan 1996).

Recent evidence suggests that variations in cellular sites of integration account for varying levels of HIV expression from such sites (Lewinski, Bisgrove et al. 2005). Provirus integrations occur in HIV-1 infected persons at rates that vary with progression of infection, and for advanced disease, provirus integration is seen in 1 per 700-3500 peripheral blood cells (Simmonds, Balfe et al. 1990). Yet, considering the long, high, and continuous exposure to HIV-1 in infected patients, the relative lack of significant clinical problems due to insertional mutagenesis, per se, is supported by laboratory evidence suggesting that lentivirus vectors may be safe in this regard (Mitchell, Chiang et al. 2003, Mitchell, Beitzel et al. 2004).

1.1.3 Adoptive T-cell Therapy for HIV

The objectives of T-cell immunotherapy have been to augment HIV-specific T-cells and to reverse or decrease the progressive destruction of CD4 T-cells that leads to clinical AIDS. Levine and colleagues have developed a bead based system for stimulating T-cells using the anti-CD3 and CD28 specific monoclonal antibodies (Levine, Bernstein et al. 2002). In our 2002 study, polyclonal peripheral blood CD4 T-cells from HIV patients were co-stimulated with anti-CD3/CD28 ex vivo and were infused into patients at doses of up to 3×10^{10} activated T-cells. In the single infusion dose-escalation portion of this study, CD4 T-cell counts were increased for 2–3 months. With three repeat infusions, CD4 T-cells were increased for approximately 1 year.

Extensive safety data indicate that genetically engineered T-cells are safe in patients with congenital immunodeficiency and AIDS. Probably the most illuminating case of safety is a patient who has adenine deaminase (ADA) deficiency. Beginning in 1990, she was treated by Dr. R. Blaese and colleagues with 11 infusions comprising a total of $1.4x10^{11}$ retrovirally modified T-cells (Blaese, Culver et al. 1995). More than twenty years after her first infusion, she has sustained correction of her immunodeficiency, and 25% of her T-cells still express the transgene, illustrating that high level persistence can occur following infusions of retroviral-modified T-cells (Muul, Tuschong et al. 2003). In collaboration with Gary Nabel (Ranga, Woffendin et al. 1998) and scientists at Cell Genesys (Mitsuyasu, Anton et al. 2000, Walker, Bechtel et al. 2000, Deeks, Wagner et al. 2002) we first began infusions of retrovirally modified CD4 cells in the early 1990s. In collaboration with ViRxSys Corp, our group has treated 22 patients with lentiviral engineered T-cells at the University of Pennsylvania (**Table 1.1.3**). **The extensive experience with retroviral and lentiviral gene modified T-cells indicates that at least 497 T-cell infusions (at least 205 patients) have been given, and importantly, there has not been a single SAE due to the use of retroviral or lentiviral vector engineered T-cells.**

Table 1.1.3. Patients infused with retroviral or lentiviral modified T-cells for immunodeficiency

Indication	Transgene	Retro / Lenti	Sponsor	# Patients infused (# infusions)	Comments
Congenital immunodeficiency (SCID)	Adenosine deaminase	Murine retrovirus	NIH	2 (23 infusions)	Persistence at high levels in 1 of 2 patients for at least 10 years. No SAE.(Blaese, Culver et al. 1995, Muul, Tuschong et al. 2003)
HIV	neoR CTL	Murine retrovirus	Fred Hutchinson Cancer Research Center	3 (6 infusions)	Persistence for up to 1 month. No SAE.(Brodie, Lewinsohn et al. 1999)

Table 1.1.3. Patients infused with retroviral or lentiviral modified T-cells for immunodeficiency

Indication	Transgene	Retro / Lenti	Sponsor	# Patients infused (# infusions)	Comments
HIV	HyTK CTL	Murine retrovirus	Fred Hutchinson Cancer Research Center	6 (24 infusions)	Host immune response limited persistence to 6 weeks in 5 of 6 patients. No SAE (Riddell, Elliott et al. 1996)
HIV	RevM10	Murine retrovirus	University of Michigan	3 (3 infusions)	No SAE reported (Ranga, Woffendin et al. 1998)
HIV	CD4 CAR coupled with the CD3 ζ	Murine retrovirus	Cell Genesys	70 (306 infusions)	3 trials reported (Mitsuyasu, Anton et al. 2000, Walker, Bechtel et al. 2000, Deeks, Wagner et al. 2002). No SAE.
HIV	Anti-HIV-1 tat ribozyme (Rz2)	Murine retrovirus	Johnson and Johnson	4 (4 infusions)	Transgene persistence detected in all patients for 4 years (Macpherson, Boyd et al. 2005). No SAE
HIV	Trans-dominant rev and/or transdominant rev with TAR antisense	Murine retrovirus	NIH	10 (19 infusions)	6 of 6 patients studied had transgene persistence detected for at least 2 years (Morgan, Walker et al. 2005). No SAE
HIV	CD4 CAR coupled with the CD3 ζ	Murine retrovirus	University Pennsylvania	10 (10 infusions)	9 of 9 patients studied had transgene persistence detected for at least 4 years (Aronson, Benstein et al. 2008). No SAE
HIV	gp41 fusion peptide inhibitor	Murine retrovirus	Fresenius AG, Germany	10 (10 infusions)	4 of 10 patients had transgene persistence for at least 9 months (van Lunzen, Glaunsinger et al. 2007). No SAE
HIV	Anti HIV-1 antisense against the envelope gene	HIV-derived lentivirus, conditionally replicating	University Pennsylvania	22 (92 infusions)	14 of 22 had transgene persistence detected for at least 1 year (Levine, Humeau et al. 2006, Collman, Shaheen et al. 2009, Wang, Levine et al. 2009). No SAE
HIV	Anti HIV-1 antisense against the envelope gene	HIV-derived lentivirus, conditionally replicating	ViRxSys	65	Patients given 4 or 8 repeat infusions ClinicalTrials.gov NCT00131560. No SAE
HIV	SL9 gag TCR	HIV-derived lentivirus	Adaptimmune, NIH (at University of Pennsylvania)	2	2 of 2 subjects had transgene persistence detected for at least 6 months. No SAE
HIV	MazF endoribonuclease	Murine retrovirus	Takara Bio (at University of Pennsylvania)	10	8 subjects have completed main study to date with persistence out to 90 days.

Our investigation plans to utilize the CD3/CD28 stimulation of CD4 T-cells which has been shown to be safe in HIV patients for single or multiple doses (Mitsuyasu, Anton et al. 2000, Walker, Bechtel et al. 2000, Deeks, Wagner et al. 2002, Levine, Bernstein et al. 2002, Levine, Humeau et al. 2006). Long term persistence of genetically altered autologous T-cells has also been observed for cells manufactured using the CD3/28 protocol at the University of Pennsylvania (Deeks, Wagner et al. 2002, Levine, Humeau et al. 2006). In the Deeks protocol,

gene-modified cell persistence in peripheral blood was seen in all patients for at least 24 weeks (0.1%–10%). There were no treatment-related serious adverse events in over 120 infusions, and T-cell-related adverse events were mild and included fever (10%), chills (17%), asthenia (10%), headache (10%), and nausea (10%). In the Levine study, 5 patients were administered 6 doses of gene modified cells and 2 of the 5 patients had circulating levels of gene modified cells at one year post-infusion. No serious adverse events related to the treatment were observed.

In a follow up study testing the lentivirally transduced antisense VRX496-modified T-cells, we observed antiviral effects during analytic treatment interruption in a subset of 13 patients. VRX496-T was associated with significant decreases in the viral load set point. In addition, A-to-G transitions were enriched in HIV sequences post infusion, consistent with a model where transduced CD4 T-cells exert anti-sense mediated genetic pressure on HIV during infection. The engraftment half–life in the blood was approximately 5 weeks, with stable persistence in some patients for up to five years. No evidence of clonal selection of lentiviral vector transduced T-cells or integration enrichment near oncogenes could be detected. This was the first demonstration that gene modified cells can exert genetic pressure on HIV (Tebas, Stein et al. 2013). Thus gene modified T-cells have the potential to decrease the fitness of HIV-1.

Recently our group published results from a study using zinc finger nucleases to permanently disrupt CCR5 expression in autologous CD4 T-cells of HIV-1 infected subjects. Six subjects in this study entered into an analytical treatment interruption intended to last 12 weeks. In the 4 subjects who completed the 12 week ATI, viral load decreased by an average of 1.2 $\log_{10}$ during the interruption from the peak level without HAART. Notably, HIV RNA became undetectable in 1 of those 4 evaluable subjects. CCR5-modified CD4 cells had an estimated half-life of 48 weeks, and could be detected in all subjects at all subsequent long-term follow up time points. Additionally, CCR5-modified cells could be detected in the rectal biopsies of all 11 subjects that consented, indicating trafficking to gut mucosa. This first in human study of gene editing demonstrated that the protection of CD4 T-cells is an important component for the development of a “functional cure” (Tebas, Stein et al. 2014).

Our group has also reported long-term persistence of genetically modified T-cells from three clinical trials evaluating gammaretroviral vector-engineered T-cells for HIV. The vector encoded a chimeric antigen receptor (CAR) composed of CD4 linked to the CD3ζ signaling chain (CD4ζ). CAR T-cells were detected in 98% of samples tested for at least 11 years after infusion at frequencies that exceeded average T-cell levels after most vaccine approaches. The CD4ζ transgene retained expression and function. The CD4ζ T-cells had stable levels of engraftment, with decay half-lives that exceeded 16 years, in marked contrast to previous trials testing engineered T-cells. These findings indicate that host immunosuppression before T-cell transfer is not required to achieve long-term persistence of gene-modified T-cells (Scholler, Brady et al. 2012). These previous human studies in HIV patients support the manufacturing methodology for, and the safety and long-term persistence of, adoptively transferred anti-CD3/CD28-stimulated T-cells for immunotherapy in HIV.

1.1.4 Rationale for testing C34-CXCR4 in T-cells in HIV-infected subjects

In collaboration with the Hoxie laboratory at UPENN, Sangamo Therapeutics, Inc. has developed a novel approach to render primary CD4+ T-cells highly resistant to infection by diverse HIV-1 clades. CXCR4, when edited to contain a 34 amino acid peptide from the gp41 HR2 domain (C34) conjugated to the coreceptor amino terminus, potently inhibits HIV-1 entry (Leslie, et al. submitted). This effect occurs with high specificity, is effective against HIVs that are resistant to soluble T20, and remarkably, occurs irrespective of tropism when stably

transduced (i.e., C34-conjugated CXCR4 will inhibit both R5- and X4-tropic viruses). This inhibition is highly dependent on the positioning of the peptide on the coreceptor (CXCR4) and occurs at molar ratios of approximately one C34-CXCR4 to 10-20 unconjugated CXCR4. T-cell proliferation and cytokine expression were similar to non-transduced cells, and C34-CXCR4 expressing cells migrated in response to CXCL12 or beta chemokines in chemotaxis assays. The breadth and potency of HIV inhibition was demonstrated *in vitro* on primary PBMCs where cells expressing C34-CXCR4 showed a clear survival advantage during HIV-1 infection. By inhibiting both R5 and X4 viruses this novel strategy offers advantages to approaches that simply delete the CCR5 gene. In addition, by tethering the HR2 peptide directly to molecules required for HIV-1 entry, the strategic location of this inhibitory peptide can be expected to exhibit greater potency than when delivered non-specifically on the cell surface on constructs where high levels of expression are required for antiviral effects.

In order to assess the protective ability of these C34-CXCR4 modified CD4+ T-cells against HIV-1, subjects will undergo a 16-week analytical treatment interruption. Risks of ATI are discussed in **Section 1.5.3**, Risks of Analytical Treatment Interruption.

1.2 Investigational Agent

The investigational agent tested under this protocol is autologous CD4+ T-cells genetically modified with lentiviral vector to express the C34-peptide conjugated to the CXCR4 N-terminus. The investigational cell therapy will be administered to HIV positive patients.

1.2.1 C34-CXCR4 fusion

C34 is a peptide from the HIV-1 heptad repeat (HR2) domain of gp41 that inhibits formation of the gp41 6-helix bundle during viral entry. In this study, C34 is conjugated to the N-terminus of CXCR4, a coreceptor for HIV-1 entry. *In vitro*, the C34 peptide conjugated to CXCR4 (C34-CXCR4) is inhibitory for HIV-1 entry irrespective of HIV-1 tropism and clade.

1.2.2 HIV-derived Lentiviral Vector Expressing C34-CXCR4

The plasmids used in virus production include pTRPE C34-CXCR4 (the vector plasmid), pC-GP2 (the plasmid encoding *gag/pol*), pC-Rev2 (the plasmid encoding HIV *rev*), and pVSV-G (the plasmid encoding the pseudotyping-envelope VSV-G-protein of the vesicular-stomatitis virus).

The gene transfer vector pTRPE C34-CXCR4 is an HIV derived self inactivating (SIN) vector that comprises a 5' LTR and a 3' U3 deleted LTR. Transgene transcription is driven off of the mammalian ef-1α promoter (Kim, Uetsuki et al. 1990). The vector also contains the central polypurine tract and central termination sequence (cppt/CTS) (Sirven, Pflumio et al. 2000) for improved transduction efficiency, the rev response element (RRE) for RNA transport, the WPRE element for improved RNA translation (Zufferey, Nagy et al. 1997, Donello, Loeb et al. 1998), and the packaging sequence. A schematic representation is presented in **Figure 1.2.2**.

The clinical grade vector, pTRPE C34-CXCR4, will be provided by Sangamo Therapeutics, Inc. Frozen aliquots of vector will be shipped to the CVPF at the University of Pennsylvania for use in the production of the cell products for this study.

Figure 1.2.2. C34-CXCR4 lentivirus components

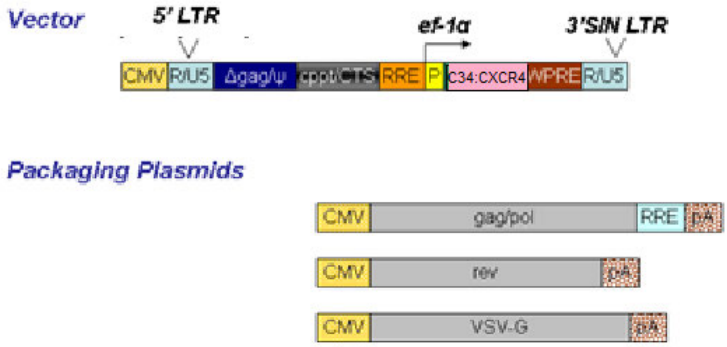


Figure 1.2.2 Schematic representation of the lentiviral vector and packaging plasmids. The C34-CXCR4 transgene is inserted into a lentiviral vector backbone derived from the Dull vector (Dull, Zufferey et al. 1998), and is expressed by the ef-1a promoter which has efficient expression with low levels of gene silencing. The packaging system consists of three plasmids (gag/pol, rev, and envelope), similar to that previously published (Zufferey et al, 1997).

This protocol will use a vesicular stomatitis virus G glycoprotein (VSV-G) pseudotyped replication defective HIV-based lentiviral vector to insert the C34-CXCR4 fusion construct into CD4 cells. The basis of using retroviruses as vehicles for efficient therapeutic gene delivery into mammalian cells was established in 1981 when it was shown that a replication competent murine oncoretrovirus could incorporate a herpes simplex virus thymidine kinase (tk) gene into the genome of a mouse cell, and to convert NIH-3T3 TK- cells into TK+ transformants. Today, HIV based lentiviral vectors are more advantageous, and may have less leukemogenic potential, since natural disease associated with HIV infection does not lead to leukemia. High-titer concentrated lentiviral vectors (>10e9 infectious units [IU]/mL) can now be generated. The ability to pseudotype retrovirus vectors with a variety of envelope proteins, including the VSV-G, significantly broadens the tropism of replication-defective lentiviral vectors.

VSV is a negative sense, single stranded RNA virus that replicates entirely in the cytoplasm (Lichty, Power et al. 2004). VSV consists of five genes that encode the five major viral proteins: the nucleoprotein (N), the phosphoprotein (P), the matrix protein (M), the glycoprotein (G), and the large polymerase protein (L). The glycoprotein of VSV serves both to bind the surface of the host T-cell and to fuse viral and cellular membranes, enabling the release of the viral genome and replicase into the cytoplasm. The VSV glycoprotein binds to phosphatidylserine, a near-universal component of cell-surface membranes, enabling VSV to infect virtually all animal cells.

The cell product infused in this protocol is tested for residual VSV-G DNA, and must be negative for infusion to proceed. However, it is possible that low levels of VSV-G DNA may be present at levels below the limit of detection of the assay. In this case, it is possible the patient may become seropositive for VSV-G, while such a conversion would not indicate VSV infection. Seropositivity for VSV-G has no known adverse health consequences. In prior gene therapy protocols, where lentiviral vectors pseudotyped with VSV-G were also used for gene transfer, monitoring for VSV-G antibody was used as a surrogate for RCL detection. In those trials, patients enrolled on the study were tested at baseline for VSV-G seropositivity and only those who were seronegative were enrolled. It is important to note that in our previous VRX496 study, 7/13 subjects did become seropositive for VSV-G. Although positive for VSV-G antibodies, there were no adverse infusion reactions associated with the seropositivity, and the presence of VSV-G antibody did not correlate with VRX496-T persistence (Tebas, Stein et al. 2013). In this trial, RCL is monitored by PCR for VSV-G DNA, which is an acceptable alternative method for RCL monitoring in accordance with current FDA regulations on RCR/RCL testing (November 2006;
http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGene

Therapy/ucm072961.htm). Therefore VSV-G seronegativity is not a requirement for inclusion in this this trial, and there are no plans to monitor for antibodies to VSV-G.

1.2.3 Autologous CD3/28 Bead Expanded T-cells

As in previous studies, autologous CD4 selected cells will be prepared at University of Pennsylvania (UPENN), and transduction with pTRPE C34-CXCR4 will be done at the Clinical Cell and Vaccine Production Facility (CVPF) at UPENN. These cells will be expanded in a closed system using CD3/CD28 polystyrene beads for ~8-14 days and then cryopreserved. Quality control assays will also be performed for the transduced T-cells prior to release for patient infusion.

1.3 Pre-Clinical Data

In collaboration with Sangamo Therapeutics, Inc., our group has developed a novel approach to render primary CD4+ T-cells highly resistant to infection by diverse clades of HIV-1. By conjugating a 34 amino acid peptide from the gp41 HR2 domain (C34) to the amino terminus of CXCR4, we have observed potent inhibition of HIV entry. This inhibition is highly specific, is effective on viruses resistant to soluble enfuvirtide (Fuzeon®, T20), and inhibits infection by both X4 and R5-tropic HIV-1 when CD4 cells are stably transduced by lentiviral vector.

This approach to protecting CD4 T-cells is based, in part, on our success in giving CD4 cells a selective advantage in HIV infection using CCR5 zinc finger nucleases. In that study (NCT00842634), which was recently published in the New England Journal of Medicine, 12 aviremic, chronic HIV-positive subjects on HAART were given a single infusion of 10 billion autologous CD4 T-cells where between 11 and 28% of the cells were genetically modified with CCR5 ZFN. Six of these subjects underwent an analytical treatment interruption beginning 4 weeks after infusion of modified cells. CD4 T-cell counts increased in all 12 subjects infused, from a median of 448 per cubic millimeter at baseline to 1517 per cubic millimeter at week 1. CCR5-modified cells were detected in the gut mucosa of the 11 subjects that assented to biopsy, demonstrating homing and persistence in the rectal mucosal tissue. In the 4 subjects who completed the 12 week treatment interruption, the viral load decreased by an average of 1.2 $\log_{10}$ from the peak level during the treatment interruption. In one subject who was determined to be a delta 32 heterozygote, increase in viral load did not occur until week 6 of the ATI, at which time the viral load peaked at 6247 copies/ml (pretreatment setpoint was 165,000 copies/ml). The modified cells were long-lived, with an estimated half-life of 48 weeks, and CCR5-modified T-cells could be detected in all subjects at all subsequent long-term follow up visits (Tebas, Stein et al. 2014). In brief, the collective data indicate that CCR5-modified CD4 cells can be safely infused in patients and that those cells persist long-term and have a survival advantage in the presence of HIV infection. This data serves as a partial proof-of-concept that approaches which render patients intrinsically resistant to HIV infection so that they do not require HAART are feasible and promising. Therefore, this type of approach which generates HIV resistant T-cell populations warrants further investigation as a potential therapeutic intervention in the treatment of HIV/AIDS and as a part of a combination strategy for a functional cure of HIV infection. Altogether, these data support the rationale for this protocol to test C34-CXCR4-modified CD4+ T-cells in HIV infected subjects for safety, as well as secondary objectives such as immune resonstitution and survival in the presence of HIV.

1.3.1 Antiviral activity of C34-CXCR4 against laboratory HIV strains

Preclinical *in vitro* studies have been performed to assess the ability of C34-CXCR4 cells to permit normal coreceptor function, while also being non-permissive to HIV entry in CD4+ T-

cells. The hypothesis was that viral fusion would be blocked using an HR2, C34-peptide conjugated to the CXCR4 N-terminus. To assess the antiviral activity of the C34-CXCR4 fusion, CXCR4-negative CD4+ SupT1 T-cell lines (termed A66) expressing either CXCR4, CCR5, or C34-CXCR4 were generated and infected with the HxB2 (X4-tropic) laboratory strain of HIV. Results indicated the modified cells were highly resistant to HIV-1 infection (Figure 1.3.1.1 B). Furthermore, when C34-CXCR4 was transiently expressed along with CD4 on Cf2-luc reporter cells to assess viral entry, again using HxB2, the percent of virus entry in C34-CXCR4-expressing cells mimicked that of cells expressing the non-permissive CCR5 receptor (Figure 1.3.1.1 C).

Figure 1.3.1.1

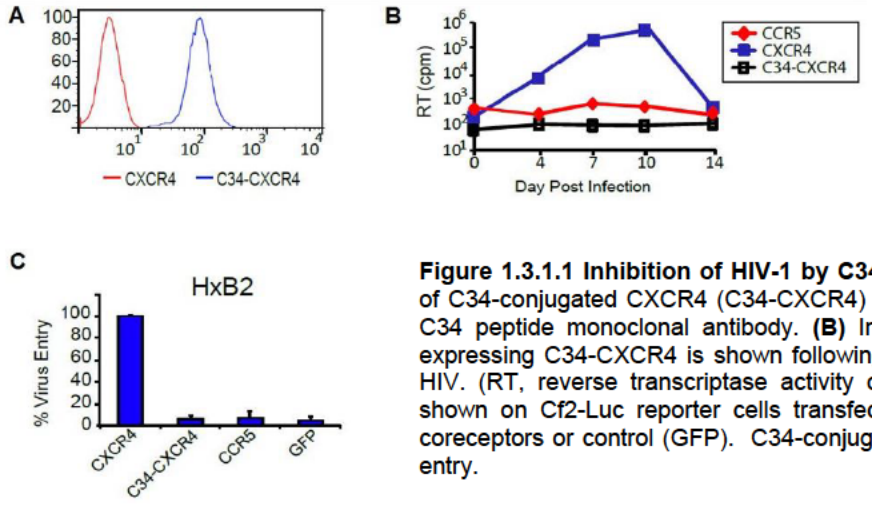


Figure 1.3.1.1 Inhibition of HIV-1 by C34-CXCR4. (A) Surface expression of C34-conjugated CXCR4 (C34-CXCR4) is shown by FACS using an anti-C34 peptide monoclonal antibody. **(B)** Infection of CD4+A66 cells stably expressing C34-CXCR4 is shown following inoculation with X4-tropic HxB2 HIV. (RT, reverse transcriptase activity over time). **(C)** Entry of HxB2 is shown on Cf2-Luc reporter cells transfected with CD4 and the indicated coreceptors or control (GFP). C34-conjugated CXCR4 does not permit HIV entry.

BaL (R5-tropic) or HxB2 (X4-tropic) HIV-1 strains. Relative light units were quantified as an indicator of HIV entry. As shown in Figure 1.3.1.2, inhibition of HIV infection by C34-CXCR4 was evident for both R5- and X4-tropic HIV-1 strains in the presence of the corresponding coreceptor in a ratio-dependent manner. Relative to HIV fusion on unconjugated CCR5 or CXCR4, for both BaL and HxB2, respectively, entry was comparable to cells transfected with GFP control only, and inhibition was observed up to a 1:5 molar ratio of C34-CXCR4 to unconjugated coreceptors. Subtracting background, at a ratio of 1:10 HxB2 was inhibited by C34-CXCR4 at a level >95%. Similarly, at ratios of up to 1:10 C34-CXCR4 to CCR5, Bal was inhibited approximately 85%. Both homologous (C34-CXCR4 inhibiting X4-tropic HIV-1 from using unconjugated CXCR4) and heterologous (C34-CXCR5 inhibiting R5-tropic HIV-1 from using unconjugated CCR5) inhibition were progressively lost at higher dilutions of C34-CXCR4.

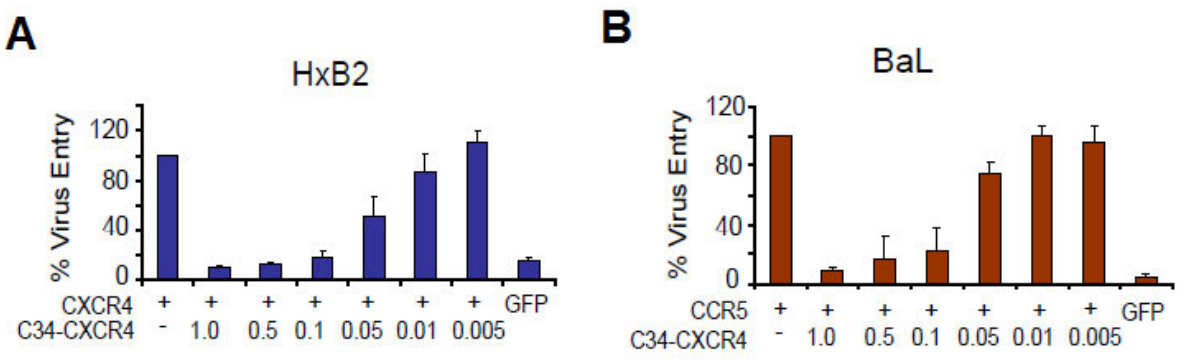


Fig. 1.3.1.2 C34-CXCR4 inhibits both X4- and R5- tropic HIV in the presence of each respective coreceptor. (A) C34-CXCR4 plasmids were transfected at the indicated ratios with unlabeled CXCR4 in transfected CF2-Luc cells and inoculated with HIV-1 HxB2. **(B)** C34-CXCR4 plasmids were transfected at the indicated ratios with unlabeled CCR5 in transfected CF2-Luc cells and inoculated with HIV-1 BaL. Both homologous (A) and heterologous (B) *trans*-inhibition was observed up to a ratio of 1:10 conjugated to unconjugated coreceptors.

To evaluate the inhibitory effect of C34-CXCR4 in primary cells, CD4+ T cells from healthy donors, stimulated with CD3/CD28 beads and maintained in media containing IL-2, were transduced with C34-CXCR4 or GFP control, inoculated or not inoculated with R5-tropic HIV-1 JRFL, and monitored using flow cytometry with an anti-C34 monoclonal antibody to assess expression over time. HIV infection was also monitored via intracellular p24-Gag expression (Figure 1.3.1.3). Uninfected CD4 T cells transduced with C34-CXCR4 maintained expression levels 95% of total T cells for up to 14 days (Figure 1.3.1.3, A). In cells inoculated with JRFL (Figure 1.3.1.3 B), 57.1% and 45.4% p24-Gag cells (ie, HIV+ cells), respectively, were seen for non-transduced and GFP-transduced cells. In contrast, in C34-CXCR4 transduced cells, p24-Gag expression was markedly reduced to 1.3%, and over time this inhibition persisted with<1% p24-Gag^{+} cells at Day 14 post-inoculation. Consistent with the results in cell lines, CD4 T-cells transduced with C34-CXCR4 failed to become HIV infected, as compared to untransduced primary CD4+ controls.

These primary CD4 cell assays were repeated with various strains of HIV, including -X4, -R5, and R5/X4 dual tropic viruses. As shown in Table 1.3, primary CD4 T-cells transduced with C34-CXCR4 were able to inhibit HIV infection, regardless of HIV tropism by 98-99%.

Figure 1.3.1.3

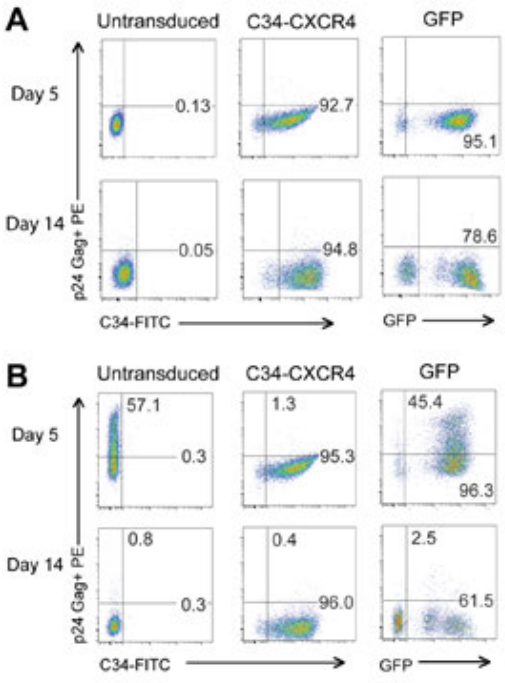


Table 1.3 HIV strains assessed for inhibition by C34:CXCR4	
Virus	**% Inhibition C34:X4**
JRFL (R5-topic)	**99%**
BaL (R5 Tropic)	**98%**
US1 (R5-topic)	**99%**
CMU01 (X4-topic)	**98%**
MN (X4-topic)	**99%**
R3A (R5/X4-topic)	**99%**
SF2 (R5/X4-topic)	**99%**

Figure 1.3.1.3 C34-CXCR4 expression inhibits HIV infection in transduced primary CD4 cells. Primary human CD4 T cells were transduced with C34-CXCR4 or GFP control, inoculated or not inoculated with HIV-1 JRFL, and monitored by flow cytometry with an anti-C34 peptide antibody. HIV infection was assessed by intracellular p24-Gag expression. **(A)** Expression of C34-CXCR4 in cells not inoculated with HIV-1 at Days 5 and 14. **(B)** Cells inoculated with HIV-1 JRFL show stable expression of C34-CXCR4 at Days 5 and 14 with marked inhibition of p24-Gag expression in that culture relative to GFP-transduced cells.

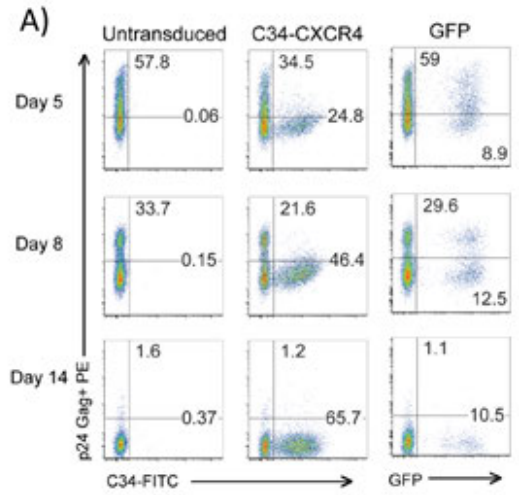
1.3.2 Selective advantage and enrichment of C34-CXCR4 cells

As previously observed with cells protected from HIV infection via CCR5 ZFN treatment, we aimed to assess the ability of C34-CXCR4 cells to be selected for in the presence of an actively replicating HIV infection. To assess the survival advantage of C34-CXCR4-modified primary CD4 cells, modified cells were diluted with untransduced CD4 T-cells at a ratio of 1:3 for a final percentage of 25% transduced cells and challenged with the JRFL (R5-tropic) strain of HIV-1. As a control, GFP-transduced CD4 cells were also diluted with untransduced CD4 cells. C34-CXCR4-modified CD4+ T-cells were assessed by C34 surface staining, while HIV infection was monitored concurrently over time by intracellular Gag co-staining. The course of infection was monitored for a period of 14 days. As shown in Figure 1.3.1.4, C34-CXCR4 cells enriched from approximately 25% of the culture to 66% of the culture at Day 14. Correspondingly, GFP+ cells declined to 10.6% of the culture at Day 14. Results of the entire timecourse are graphed in panel B.

The HIV infection peaked at Day 5 in the culture with 29.5% Gag-positive cells. In comparison, GFP transduced CD4 T-cells reached their maximum level of HIV infection also at Day 5 with 59% of cells staining Gag-positive. This experiment was repeated with multiple HIV strains of various tropisms. Those results are provided in tabular form in Figure 1.3.1.4, panel C.

Collectively, these data suggest that C34-CXCR4 can inhibit both R5 and X4 tropic HIV-1 isolates, and that C34-CXCR4-modified CD4+ T-cells may have a selective advantage in the presence of active HIV replication.

Figure 1.3.1.4



C)

Enrichment of C34:CXCR4 exposed to various HIV strains		
Virus	Enrichment C34:X4 Start	Final
JRFL (R5-topic)	25%	66%
BaL (R5 Tropic)	27%	53%
US1 (R5-topic)	27%	60%
CMU01 (X4-topic)	25%	59%
MN (X4-topic)	27%	78%
R3A (R5/X4-topic)	27%	76%
SF2 (R5/X4-topic)	25%	69%

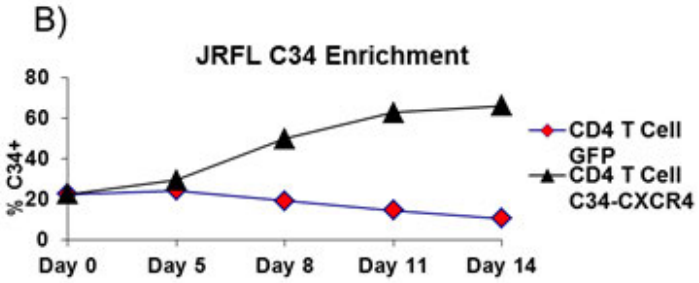


Figure 1.3.1.4 Selective Enrichment of C34:CXCR4 T-cells. A) Cells transduced with C34-CXCR4 or GFP were added to untransduced cells to reach a final percentage of 25% transduced cells and inoculated with JRFL. Cells were monitored for enrichment using flow cytometry for GFP or C34, and also monitored for HIV infection by intracellular gag staining. Expansion of C34-CXCR4 cells is shown over time. No expansion of GFP-transduced cells was seen. B) Enrichment over time is shown graphically. C) This experiment was replicated with multiple strains of HIV with various tropisms, each resulting in dramatic enrichment of the C34:CXCR4-modified cells.

1.3.3 C34-CXCR4 inhibition of enfirvutide (T-20)-resistant HIV-1

Acquired HIV-1 resistance to the fusion inhibitor enfirvutide (T-20) has been observed both *in vitro* and *in vivo*, and is generally associated with mutations within a 10 amino acid motif (aa 26-45) in the viral gp41 (Perez-Alvarez, Carmona et al. 2006, Melikyan 2008). Three mutations associated with T-20 resistance in HIV-infected patients receiving antiretroviral therapy were tested to determine whether infection by these mutated strains was susceptible to inhibition by C34-CXCR4. As shown in Figure 1.3.3, all T-20 resistant strains tested were highly susceptible to C34-CXCR4 inhibition.

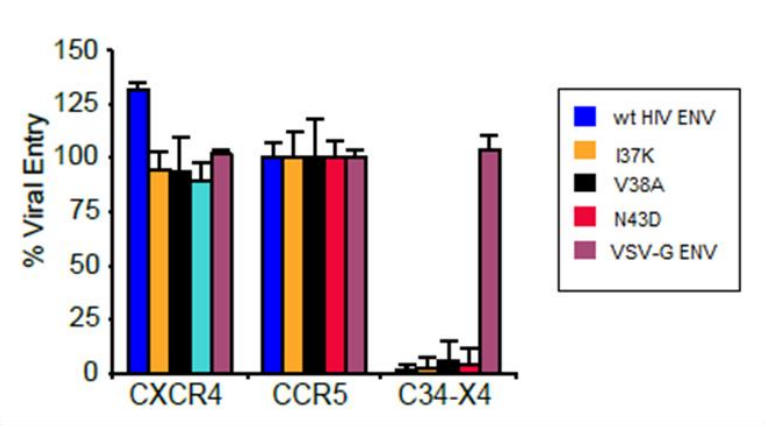


Fig. 1.3.3 Sensitivity of T20-resistant viruses to inhibition by C34-CXCR4. Gp41 mutations known to confer HIV-1 resistance to T20 (I37K, V38A, or N43D) were introduced into dual-tropic HIV-1 R3A (wt HIV ENV). Entry of pseudoviruses bearing these Envs was assessed on the indicated coreceptors. All viruses were inhibited by C34-CXCR4. VSV-G pseudotype served as a control.

1.3.4 *In vivo* data in humanized NSG mice

To evaluate the ability of C34-linked chemokine co-receptors to protect CD4 T cells *in vivo*, experiments were performed using a previously described NSG model that has been used to evaluate the ability of a wide range of antiviral agents to protect T cells from HIV-1 infection (Perez, Wang et al. 2008, Richardson, Carroll et al. 2008, Mukherjee, Plesa et al. 2010, Wilen, Wang et al. 2011, Didigu, Wilen et al. 2014, Richardson, Guo et al. 2014). We infused 10 million untransduced, GFP-transduced, or C34-CXCR4 transduced T cells into cohorts of mice. Once T cell engraftment was confirmed and enumerated (Fig 1.3.4 A), the mice were challenged with HIV. After eight days, viral load was measured at a time when CD4 T cell levels were comparable (Fig 1.3.4 B). We observed that mice engrafted with C34-CXCR4 expressing T cells had significantly lower viral loads than mice engrafted with either untransduced or GFP-transduced T cells (Fig 1.3.4 C). After an additional 20 days, the mice were sacrificed and the number of human CD4 T cells in the spleens was measured (Fig 1.3.4 D). The spleens were chosen because they contain the majority of human T cells in this model. Mice engrafted with C34-CXCR4 expressing T cells had remarkably more T cells than mice infused with untransduced or GFP-transduced T cells. The *in vitro* data indicated that C34-CXCR4 was superior in protecting T cells from HIV infection when compared to C34-CCR5. In a second experiment, we wished to directly compare whether C34-CCR5 or C34-CXCR4 transduced T cells had a survival advantage *in vivo* following HIV-1 infection over control T cells transduced with GFP. After infusion of T cells and HIV infection, mice were then bled at 10-day intervals to assess T cell survival and expansion (Figure 1.3.4 E). Remarkably, CD4 T cells expressing C34-CCR5 survived poorly, similar to T cells expressing GFP. However, in marked contrast, C34-CXCR4 transduced cells persisted throughout the period of HIV-1 infection in both the peripheral blood and in the spleens (Fig 1.3.4 F, G). Together, this data demonstrates that T cells expressing C34-CXCR4 are highly resistant to HIV-1 infection *in vivo*.

Figure 1.3.4

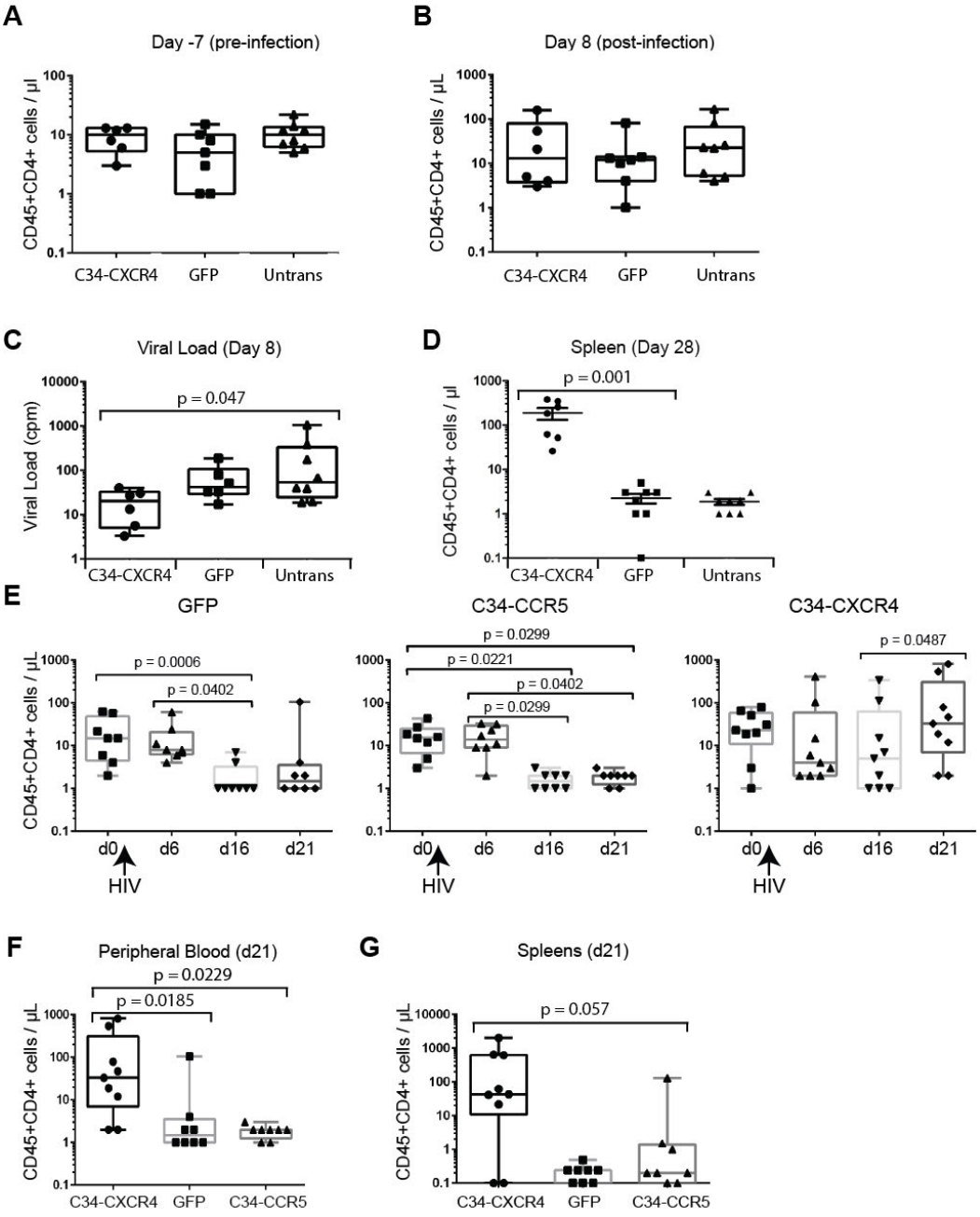


Figure 1.3.4. C34-CXCR4 expressing cells are protected from HIV mediated destruction in vivo. A) NSG mice were infused with 10 million CD4 T cells that were either untransduced, GFP expressing, or C34-CXCR4 expressing CD4 T cells. ~85% of each transduced population expressed the indicated marker. Engraftment was assessed (Day -7) and one week later all mice were infected with a viral mix of HIV-1 US1 (R5) and CMU-02 (X4). 8 days later mice were bled and both **B)** Number of modified T cells **C)** and viral load were determined. **D)** 28 days post HIV-1 infection the mice were sacrificed and the number of T cells in the spleen were enumerated. In separate experiment, NSG mice were infused with 10 million CD4 T cells that were either GFP, C34-CCR5 or C34-CXCR4 expressing CD4 T cells. Engraftment was checked (Day 0) and two days later mice were infected with a viral mix of HIV-1 US1 (R5) and CMU-02 (X4). After 3 weeks of HIV infection, the mice were sacrificed and the number of T cells in the peripheral blood **(F)** and spleen **(G)** were enumerated.

1.4 Clinical Data to Date

There are no available clinical research data to date on the investigational product. However there are extensive early phase clinical trial data regarding the use of autologous T-cell infusions for HIV as described earlier in the background section of this protocol.

1.5 Rationale and Risks/Benefits

1.5.1 Study Population Rationale

This protocol is designed to determine the safety of a single administration of C34-CXCR4 modified cells in individuals with well controlled HIV replication. This patient population was selected because it is ultimately the target population in which these types of interventions will be used if they prove to be effective. It is also the population that we have successfully enrolled in our prior trials. Additionally from our experience, individuals with well controlled HIV replication that are willing to participate in gene therapy studies are highly motivated, are willing to take the risk(s) associated with this type of study, and have the commitment to adhere to the close clinical monitoring required. We believe that selecting this particular population is what makes the study feasible.

Immune activation has long been correlated with HIV disease progression (Fahey, Taylor et al. 1998), and the etiology of immune activation and its role in HIV pathogenesis is an active area of investigation which suggests that reducing immune activation may be a critical adjunct approach to preventing HIV disease progression (Brenchley, Price et al. 2006, Chehimi, Azzoni et al. 2007, Meier, Alter et al. 2007, Milush, Reeves et al. 2007). Chronic immune activation is thought to be the cause of the increased susceptibility of PBMCs to apoptosis in HIV infected individuals. In HIV-1 and HIV-2 infected subjects, there is a statistically increased rate of apoptosis in PBMCs that is positively correlated with both disease stage (3/4, 2, or normal donor) and CD4 counts (>500, 200-500, and <200) (Gougeon 1996, Michel, Balde et al. 2000). Taking these data into consideration, it follows that later stage patients or patients with more advanced HIV disease are less likely to have healthy immune cells capable of supporting cell-based immunotherapy over the long-term. A primary endpoint of this study is to evaluate the long-term safety of C34-CXCR4-modified CD4+ T-cells, which will require long-term persistence of these cells. Therefore, subjects with a current or prior AIDS diagnosis, or those with a CD4 nadir lower than 200 cells/mm^3 will be excluded from the study.

We will enroll one cohort in this study. Within the cohort will be a maximum of 3 escalated doses of C34-CXCR4-modified CD4+ T-cells: Dose Level 1 = 0.8-1x10^9; Dose Level 2 = 2.4-3x10^9; and Dose Level 3 =0.8-1x10^{10}. If no DLT are observed, three subjects will be treated at each dose, for a total of 9 subjects in the study. For dose levels 1 and 2, if 0/3 has a dose limiting toxicity (DLT), then the dose will be escalated. If 1/3 has a DLT at a dose level then 3 additional patients will be treated at that dosage before escalating, and if <2/6 have DLT (i.e. no additional DLT is observed) then the dose will be escalated to the next planned dose level until the maximum of 9 evaluable subjects has been reached.

In Step 3, all participants will participate in a 16-week analytical treatment interruption beginning 4 weeks after T-cell infusion. After the analytical treatment interruption (ATI), resumption of antiretroviral therapy will be recommended and participants will be closely followed until their HIV RNA falls below the limit of quantification. One year after T-cell infusion subjects will undergo leukapheresis to evaluate the long-term persistence of C34-CXCR4-modified CD4+ T-cells. After completing this study the patients will be transferred to a destination protocol for the long term follow-up of individuals that have been exposed to gene therapies.

Risks associated with C34-CXCR4-T are in Section 12.1. Risks associated with treatment interruption are discussed in Section 1.5.3.

1.5.2 Dose Rationale

The starting dose was established based on: 1) Guidance for Industry: S6 Preclinical Safety Evaluation of Biotechnology-derived Pharmaceuticals; 2) Joint taskforce report from the BioIndustry Association (BIA) and the Association of the British Pharmaceutical Industry (ABPI) and 3) the available literature on phase I trials to date conducted with autologous T-cells in patients with HIV. The FDA guidance on safe starting doses (FDA Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers) does not apply well for cell and gene therapies, and as such was not used to guide the selection of the starting dose for this study.

Engraftment of 1-2% of genetically modified cells in the peripheral circulation has been observed following infusions of about 10 billion cells (Levine, Humeau et al. 2006), and higher cell doses result in higher levels of engraftment (Ranga, Woffendin et al. 1998, van Lunzen, Glaunsinger et al. 2007). In order to effectively detect any potential toxicity or tolerability issues, achieving measurable engraftment levels is necessary. However, exposure should be limited so as to reduce any potential C34-CXCR4 associated toxicities. Therefore, in the present study, we are proposing a dose escalation approach whereby participants receive a single dose of cells (either 0.8-1 $x10^9$, 2.4-3$x10^9$, or 0.8-1$x10^{10}$ cells; three subjects per dose), as this dose range is known to result in measurable but limited engraftment levels based upon our prior experience.

Safety. Safety is enhanced by using a 3+3+3 dose escalation. If there are no safety concerns regarding the 0.8-1$x10^9$ dose by completion of the Day 21 study visit of the 3rd subject, then this dose will be increased to 2.4-3$x10^9$ in the next 3 subjects. If no safety concerns are raised at that dose by the completion of the Day 21 study visit of the 3rd subject (6th subject overall) to receive the 2.4-3$x10^9$ dose, the dose will be increased once more to 0.8-1$x10^{10}$ in the final three subjects.

1.5.3 Risks of Analytical Treatment Interruption

Discontinuation of ART may result in viral rebound, immune de-compensation, increase in the size of the latent reservoir, and clinical progression. In HIV-infected subjects, an ART interruption is not recommended unless it is done in a clinical trial setting. Interruptions in a clinical trial setting have been termed “analytical treatment interruptions” (ATI) and are accepted tools in the evaluation of immunological interventions, gene therapy or therapeutic vaccines for the treatment of HIV infection (Kutzler and Jacobson 2008). In order to minimize the risk associated with treatment interruptions, the duration of these interruptions is kept to a maximum of 16 weeks. This duration allows the subject to reach a new viral load (VL) “set point,” which is defined by the AIDS Clinical Trial Group as the mean of week 12 and week 16 post treatment interruption values (Henry, Katzenstein et al. 2006). VL setpoint changes are the usual endpoint evaluated in these types of studies. Another frequently used endpoint is time to return of viremia, which uniformly occurs within 2 to 3 weeks of discontinuation of antiretroviral therapy, with kinetics that are well characterized. In most cases viral replication is re-supressed with resumption of ART (Davey, Bhat et al. 1999, Harrigan, Whaley et al. 1999, Papasavvas, Kostman et al. 2004, Henry, Katzenstein et al. 2006, Jacobson, Pat Bucy et al. 2006, Strategies for Management of Antiretroviral Therapy Study, El-Sadr et al. 2006).

Temporary treatment interruption to reduce inconvenience, potential long-term toxicity, and/or overall treatment cost was considered as a strategy for subjects on ART who had maintained high CD4+ counts. Several clinical trials were designed to determine the safety of such interruptions, in which reinitiation is triggered by predetermined CD4+ count thresholds (Maggiolo, Ripamonti et al. 2004, Ananworanich, Siangphoe et al. 2005, Cardiello, Hassink et al. 2005, Strategies for Management of Antiretroviral Therapy Study, El-Sadr et al. 2006, Holkmann Olsen, Mocroft et al. 2007, Pogany, van Valkengoed et al. 2007, Skiest, Su et al. 2007, Walmsley, Thorne et al. 2007). In these trials, various CD4+ count levels have been set to guide both treatment interruption and reinitiation. Several separate, randomized clinical trials of CD4+ count-guided treatment interruption have been reported. In the SMART study (Strategies for Management of Antiretroviral Therapy Study, El-Sadr et al. 2006), the largest of such trials with over 5,000 subjects, interrupting treatment with CD4+ counts >350 cells/μL and reinitiating when <250 cells/μL was associated with an increased risk of disease progression and death compared with the trial arm of continuous ART. However, most of these events tended to occur more than 16 weeks after the treatment interruption (**Figure 2.1**).

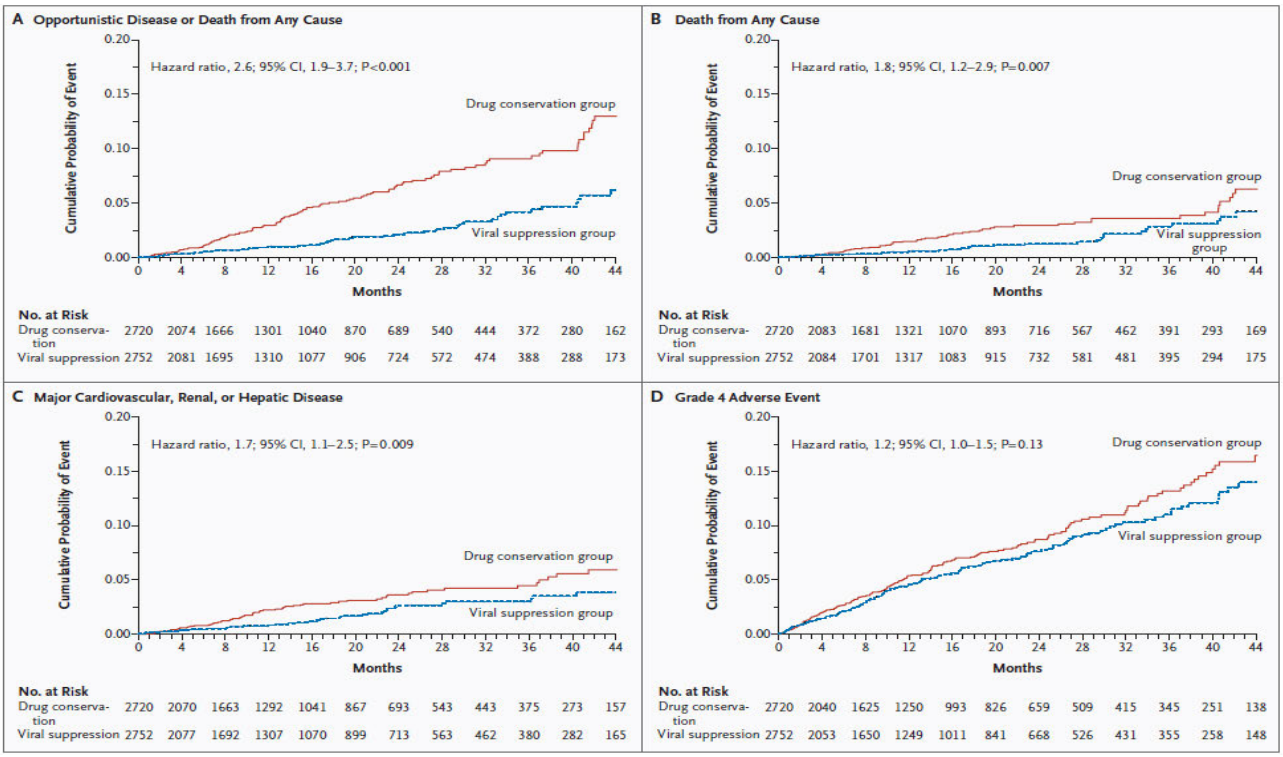


Figure 2.1 Cumulative probabilities of AEs following CD4-guided ARV interruption (Copied from El-Sadr et al, 2006). Data were collected from the SMART trial and show AEs in subjects on continual ARV (blue) or subjects with CD4+ guided ARV (red). AEs include opportunistic infection or death from any cause (A), death from any cause (B), major cardiovascular, renal, or hepatic disease (C), or grade 4 AEs (D). Of note, at 16 weeks (shown as 4 months) there is little difference between the two groups.

The participants in the SMART study had more advanced disease than the subjects we will include in our trial, and the treatment interruption was much more prolonged. We believe the subjects in this study will be at very low risk of developing clinical events during the brief treatment interruption proposed. For additional subject safety, both CD4+ T-cell counts and HIV-1 VLs will be monitored closely in the ATI period and subjects with a confirmed CD4 T-cell count ≤350 cells/ mm^3, will be advised to reinstitute ART. The threshold of 350 cells/μL, is higher than

250 cells/ mm^3 in the SMART study to provide a greater level of safety. Additionally, if viremia resumes at or above a threshold of 100,000 copies/ml, ART will be resumed. It is also possible that some subjects could experience symptoms compatible with retroviral rebound syndrome or rarely, other clinical events that occur before resumption of ART (eg, immune thrombocytopenia).

Although *in vitro* studies allow assessment of the antiviral activity of C34-CXCR4, the best way to evaluate the *in vivo* activity of these cells is to discontinue ART for a brief period of time in individuals with well-controlled viral replication. This then allows investigation of the ability of these gene modified cells to control or prevent the initiation of viral replication and to characterize the survival of C34-CXCR4-modified CD4+ T-cells (C34-CXCR4-T) in the presence of HIV infection.

A single ATI, as proposed in our study, remains appropriate to evaluate the antiviral activity of C34-CXCR4-modified CD4+ T-cells as a treatment intervention for three reasons:

1. Upon entering into the trial, all subjects will have CD4+ counts ≥450 cells/ mm^3 and will have a nadir CD4+ cell count of not lower than 200 cells/ mm^3. The subject will be advised to reinitiate HAART treatment as soon as the CD4+ T-cell count drops below 350 cells/ mm^3 *or* the HIV-1 VL increases to ≥100,000 copies/mL and remains as such for 3 successive weeks (see Section 7.1). Selecting subjects who were started on ART with CD4+ counts of not lower than 200 cells/ mm^3 will increase the likelihood that subjects will have reconstituted immune responses.

2. All subjects will receive C34-CXCR4-modified CD4+ T-cells; an intervention that we hope will improve the outcome of treatment interruption. In this proof of concept study we will not utilize a placebo group, as the kinetics and the frequency of reactivation during an ATI in chronically infected individuals are well known.

3. All subjects will be followed very closely after the treatment interruption, and therapy will be restarted if any problems appear. It is very unlikely that the participants will develop resistance to any of the antiretrovirals that they have been receiving.

Importantly, in our previous CCR5 ZFN Trial (NCT00842634) the ATI was for 12 weeks. At the end of the 12 weeks, although the viral load was declining, the protocol required that all subjects re-initiate ART. This may have prevented us from fully documenting the antiviral effects of the CCR5 ZFN treatment. By extending the ATI to 16 weeks, we believe that we can better define a new set point as well as results of the treatment, while not compromising subject safety.

In this protocol, CD4+ cell counts will be monitored closely in the treatment interruption period and patients in whom CD4+ cell counts drop to 350 cells/mm^3 or below, and who are confirmed to remain at or below 350 cells/ mm^3 with a second reading a week later, will be advised to reinstitute ART. Additionally, if a subject's viral load increases to ≥100,000 copies, and remains as such for 3 successive weeks, the subject will be advised to restart ART. Those subjects who fail to complete the 16 week ATI due to viral rebound will be tested for antiretroviral therapy resistance. The purpose of the treatment interruption is to serve as an analytic tool to evaluate both the effects of the study drug on the subject's virus and the virus on the genetically modified T-cells. The most stringent test of an immunologic intervention like the one proposed in this study would be its ability to lead to sustained suppression of plasma HIV-1 RNA after the

discontinuation of antiretroviral therapy, and/ or the protection of the genetically modified CD4 in the presence of ongoing viral replication.

During the treatment interruption, it is very likely subjects will have detectable viral loads. Given the positive correlation between viral load and HIV transmission (Quinn, Wawer et al. 2000), use of condoms during sexual activity will be stressed to subjects undergoing ATI to minimize the risk transmission.

It is also possible subjects may develop Acute Retroviral Syndrome (ARS), during which the HIV virus is duplicating at a rapid rate. Acute Retroviral Syndrome is a diagnosis that must be made by a site investigator or primary care provider. Signs and symptoms that may support the diagnosis of ARS include:

- Fever
- Sore throat
- Headache
- Rash
- Diarrhea
- Swollen lymph nodes
- Fatigue
- Joint or other body aches

Antiretroviral therapy will be restarted if the symptoms persist for more than one week, or affect the normal activities of daily living for more than one week.

1.5.4 Examination of Lymphoid Tissue by Rectal Mucosal Biopsy

It has been documented that HIV preferentially infects activated, memory CD4 cells. Memory cells are defined physiologically as those lymphocytes that have seen antigen, and phenotypically by CD45RO expression and high levels of CD62L expression. Generally these cells migrate to tertiary lymphatic sites such as the lamina propria and skin. The reason for the preferential infection by HIV-1 is probably related to the high density on these cells of the chemokine receptor CCR5, the co-receptor used by HIV throughout most of the course of infection. However, the cell culture process that is used in this study results in downregulation and undetectable expression of CCR5 for at least 6 weeks *in vivo* (Levine, Bernstein et al. 2002). In terms of survival of CD4 cells transduced with a retroviral vector, studies in a rhesus model showed persistence of marked cells in lymph nodes for 100 days (Bunnell et al., 1997; Donahue et al., 1998).

Lymphoid tissue, particularly gut-associated lymphoid tissue (GALT), rather than the blood is the dominant site of HIV-1 replication, and lymphoid tissue serves as the major reservoir of productively infected cells in the body. Only 2% of the body's total T cells circulate in the peripheral blood while the remainder are found in lymphoid tissue such as the lymph nodes, tonsils, and gut mucosa. The colon is known to have a large number of lymphoid nodules. In addition, the colonic mucosa has high concentrations of intra-epithelial lymphocytes (IEL), which are predominately CD8+T-cells and lamina propria lymphocytes (LPL) which are predominately CD4+ T-cells. Thus, the GALT may serve as an important and accessible reservoir of HIV-1 infected lymphocytes and C34-CXCR4 gene modified T-cells. The advantages of performing rectal mucosal biopsies as opposed to lymph node or tonsillar biopsies include greater patient acceptance as the procedure is less painful; relative ease of repetitions; and fewer associated complications (Rodriguez-Rosado, Jimenez-Nacher et al. 1998).

Following infection with HIV, depletion of CD4 T-cells occurs very rapidly and predominantly in the gastrointestinal tract (Lim, Condez et al. 1993, Brenchley, Schacker et al. 2004). Reconstitution of the GALT typically takes 8-12 months in chronically infected patients, although early treatment with HAART during primary infection avoids this problem (Guadalupe et al., 2003). The depletion is accompanied by an increased overall immune activation which is thought to contribute to the inability of CD4 T-cells to repopulate the GALT (Guadalupe, Sankaran et al. 2006). Chronic immune activation resulting from a breakdown of the gut immune system has been cited as a possible cause of chronic immune activation and thus disease progression in HIV-infected patients (Brenchley, Price et al. 2006). Notably, one study discovered that in a small group (3) of long term non progressors, such patients maintain their GALT CD4 population (Guadalupe, Reay et al. 2003). Reconstitution of the GALT appears to occur by homing of circulating cells to the GALT as opposed to local expansion of endogenous CD4 cells. Thus, enhancing or blocking the ability of circulating CD4 T-cells to home to the gut may have implications for overall disease progression. In kind, establishing a baseline effect on gut trafficking will be important in evaluating the overall potential of the proposed HIV cell based therapy.

Results from a phase II trial of CD4-zeta modified autologous T-cells demonstrate trafficking of CD4-zeta modified cells and quantifiable HIV-1 in the rectal mucosa of subjects with HIV-1 infection and undetectable viral replication in plasma (Deeks, Hoh et al. 2002). Others have successfully measured HIV proviral load in the GALT to evaluate HIV burden and its possible relationship with clinical outcome (Anton, Mitsuyasu et al. 2003). Similarly, in our ongoing HIV gene therapy trial utilizing lentiviral vector transduced CD4 T-cells, we have observed trafficking of gene modified cells to the GALT and are performing ongoing measurements on proviral load that reflect the data published in the Anton studies. In that study, we also worked out the procedures necessary to perform quantitative analysis in the compartment. In this protocol, we plan on performing sequential rectal mucosal biopsies on all patients in accordance with the Schedule of Events, and will analyze the mucosal T-cell viral load, C34-CXCR4 CD4 cell engraftment and, if enough cells are available, cellular responses as described previously (Shacklett, Yang et al. 2003).

The primary risks associated with rectal mucosal biopsies include mild rectal irritation and urgency and limited rectal bleeding for 2-3 days following the procedure. Infection or bowel perforations are extremely rare complications that may require antibiotics and/or surgical repair. Subjects will be followed in clinic for any complications. Although four rectal mucosal biopsies are planned for all subjects, the subject may decide at any point to have no further biopsy procedures. Prophylactic antibiotics should be given to subjects who would routinely be given such treatment prior to invasive procedures (e.g. cardiac valvular disease).

1.5.4.1 Viral Persistence in Mucosal Tissues and Its Clinical Relevance

Given the concentrated nature of susceptible $CD4^+$ T-cells in the gut mucosa, most HIV replication is thought to occur in this compartment. This is particularly true during acute HIV (and SIV) infection in which high-level viral replication results in depletion of 60% to 90% of $CD4^+$ T-cells during the first few weeks (Veazey, DeMaria et al. 1998, Guadalupe, Reay et al. 2003, Brenchley, Schacker et al. 2004, Mehandru, Poles et al. 2004, Li, Duan et al. 2005, Mattapallil, Douek et al. 2005). This process involves both activated and resting T-cells (at least initially) (Mehandru, Poles et al. 2007), and may even result in destruction of epithelial cells (Li, Duan et al. 2005) as well as the irreversible fibrotic changes to the lymphoid architecture (Brenchley, Schacker et al. 2004, Estes, Baker et al. 2008). The degree to which this process is reversed by HAART is controversial, with some studies suggesting irreversible damage to the

gut mucosa (Brenchley, Schacker et al. 2004, Mehandru, Poles et al. 2004, Mehandru, Poles et al. 2006, Brenchley and Douek 2008) while other studies suggesting that if therapy is started early enough (Estes, Baker et al. 2008),or if therapy is given for many years (Sheth, Chege et al. 2008), then normalization of the certain mucosal T-cell populations may occur.

The relationship between viral persistence and gut immune reconstitution during HAART is also poorly defined. HIV RNA and DNA can often be readily detected in gut tissue, even after years of HAART (Anton, Mitsuyasu et al. 2003). Viral DNA levels are typically higher in gut tissues than peripheral blood (as measured on a per $CD4^+$ T-cell basis) (Chun, Nickle et al. 2008, Macal, Sankaran et al. 2008). As has been observed in peripheral blood, viral burden in the gut mucosa appears to remain stable during long-term therapy (Poles, Boscardin et al. 2006). The degree to which viral persistence in gut mucosa reflects ongoing viral replication is not known, but the consistent association between measures of virus burden and either T-cell reconstitution or T-cell activation (Macal, Sankaran et al. 2008, Sheth, Chege et al. 2008) suggests that viral expression in these tissues may have clinical consequences (Sheth, Chege et al. 2008).

These observations indicate (1) that antiretroviral therapy does not fully restore normal immunity (Alexander, Ortiz et al. 2003), (2) that suboptimal CD4 gains during therapy may predict premature morbidity and mortality, (3) that suboptimal CD4 gains during therapy is common in clinical practice, especially as patients age and (4) that virus production persists during therapy, with much of this occurring in gut mucosa. Although the association between virus production in gut and immune reconstitution remains undefined, it is clear that many treated patients with poor immune reconstitution have elevated levels of markers of inflammation and of persistent microbial translocation. Indeed, in a series of recent papers, the inability to restore peripheral blood CD4 was predicted by persistent markers of microbial translocation of gut microbes across the gut mucosa (Macal, Sankaran et al. 2008, Jiang, Lederman et al. 2009, Nies-Kraske, Schacker et al. 2009). It is hypothesized that reconstitution of effective CD4 T-cell function in gut mucosa will be needed to restore long-term health in the antiretroviral treated patients.

2.0 STUDY OBJECTIVES

The study objectives were defined according to the hypothesis that forms the basis of this study. Our hypothesis is that autologous CD4+ T-cells genetically modified with lentiviral vector expressing an HR2, C34-peptide conjugated to the CXCR4 N-terminus will be safe and tolerable in HIV-1 positive subjects and will have a survival advantage in the presence of viremia.

2.1 Primary Objectives

The primary objective of this study is to evaluate the **safety and tolerability** of a single infusion ranging from $0.8x10^9$ to a maximum of $1x10^{10}$ of autologous CD4+ T-cells genetically modified with C34-CXCR4 in HIV-1-positive subjects.

2.2 Secondary Objectives

The secondary objectives of this study are:

1. To evaluate the **enrichment of C34-CXCR4-modified CD4+ T-cells** during analytical treatment interruption by comparing the percentage of modified cells in subjects prior to ATI (week 2 post infusion) to the percentage of modified cells just

prior to the reinitiation of antiretroviral therapy or at weeks 16 and 20 after the administration of modified CD4 positive T-cells (weeks 12 and 16 of the ATI), whichever comes first, using flow cytometry, and determine the half-life of modified cells compared to unmodified CD4 cells in the presence of HIV viremia during an analytical treatment interruption. Modified cells from both the peripheral blood and gut mucosa will be assessed.

2. To evaluate the effect of C34-CXCR4 modified CD4 cells on **CD4+ T-cell count** :

    a) Comparing the change between baseline CD4+ T-cell count (as above) and the average of two consecutive CD4+ T-cell count values after dosing and just prior to the analytical treatment interruption (Step 2) and prior to the reinitiation of antiretroviral therapy or at weeks 16 and 20 after the administration of the modified CD4 positive T-cells (weeks 12 and 16 of the analytical treatment interruption, Step 3), whichever comes first.

3. To evaluate the **antiviral effect** of C34-CXCR4-modified CD4+ T-cells by:

    a) Comparing the viral set point $\log_{10}$ HIV-1 RNA level (defined as the median of values available before the initiation of antiretroviral therapy) and the set point after the intervention, during the analytical treatment interruption. The new viral set point will be defined as the average of two consecutive viral load measurements of $\log_{10}$ HIV-1 RNA levels after dosing and just prior to the reinitiation of antiretroviral therapy or at weeks 16 and 20 after the administration of the modified CD4 positive T-cells (weeks 12 and 16 of the analytical treatment interruption), whichever comes first.

    b) Comparing the time and the frequency to recrudescence to >200 copies/ml viral load with historical controls.

4. To evaluate the **effects on immune function** of C34-CXCR4- modified CD4 cells by comparing the CD4+ and CD8+ T-cells responses following stimulation by HIV-specific and other viral antigens, at baseline (defined as the average of the Step 1 values), and after the infusion of C34-CXCR4-modified autologous T-cells. The responses will be evaluated by intracellular cytokine staining for cytokines and/or CD107a in response to stimulation with HIV peptide pools of 15 mers overlapping by 11aa, as well as peptide pools for Flu, EBV and CMV. In addition, proliferative capacity will be assessed.

    The immune response comparisons will be made using descriptive statistics and graphical methods for both phases of the study. Immune response measures will be correlated with dose level/treatment arm and participant outcome (i.e., adverse events) and with each other as well.

    For continuous immune response values, the actual and % change in the level of each of the biomarkers from baseline to post-baseline time points will be explored within each dose level/arm. For all translational endpoints, any notable statistical result will be viewed as an impetus for further study rather than as a definitive finding.

5. To evaluate the **immunogenicity** of C34-CXCR4-modified CD4 T-cells, analysis will

be done in parallel on cryopreserved patient sera from baseline and various post-infusion timepoints with CD4 T-cells transduced with lentiviral C34-CXCR4. The flow cytometry assay will utilize A66 SupT1 cells transduced with either the lentiviral C34-CXCR4 construct or a CXCR4 construct. Human antibodies binding to the former but not the latter will be considered C34-specific. The SupT1 cells will be incubated with dilutions of patient-derived sera to be empirically determined, washed, and cell aliquots then incubated separately with anti-human IgG, IgM, IgA and IgE reagents. The advantage of this cell-based assay over ELISA using plates coated with purified C34-CXCR4 is that antibodies to conformational epitopes are more likely to be detected. This flow assay will be based on an assay developed and qualified in the UPenn Translational and Correlative Studies Laboratory to evaluate human-anti chimeric antibodies (HACA) to mesothelin-specific chimeric antigen receptor mRNA-engineered T-cells (Beatty, Haas et al. 2014).

2.3 Exploratory Objectives

1. Studies will be initiated to evaluate the effects of C34-CXCR4-modified CD4+ T-cells on the **HIV reservoir**. The size of the reservoir will be measured using the following techniques:

    a) Number of latently-infected resting CD4 T-cells quantified using a limiting dilution co-culture assay (comparing Step 1 to Step 2).

    b) Frequency of integrated provirus as measured by Alu-PCR (comparing Step 1 to Step 2). (Liszewski, Yu et al. 2009)

3.0 STUDY DESIGN

This is a single cohort, open-label pilot study of the safety and antiviral activity of a single infusion of autologous CD4+ T-cells genetically modified with lentiviral vector expressing an HR2, C34-peptide conjugated to the CXCR4 N-terminus in HIV-infected subjects. There will be 1 cohort in this study consisting of subjects with well-controlled HIV replication on HAART. Infusions will be staggered by at least 21 days, to allow for the previous subject to be sufficiently evaluated for adverse events before infusion of the next subject. This 21 day inter-subject interval will allow for the capture of any delayed adverse events. If any serious adverse sevents arise that pose a safety concern, or fall under the criteria for pausing or stopping the study, a DSMB meeting will be convened to review study safety data.This study will have five steps:

In Step 1, all participants will undergo 2 leukaphereses and an optional rectal biopsy to obtain CD4 positive T-cells that will be genetically modified and to have baseline specimens to evaluate the size of the HIV reservoir.

In Step 2, all participants will receive a single infusion of C34-CXCR4-modified CD4+ T-cells at one of 3 dose levels. The first 3 subjects will receive dose level 1 of $0.8\text{-}1x10^9$ transduced CD4+T-cells. Provided no dose limiting toxicity (DLT) is seen at the first dose level at the completion of the Day 21 study visit of the 3rd subject, the next 3 subjects will receive infusion at the 2nd dose level of $2.4\text{-}3x10^9$ transduced CD4+ T-cells. If no DLT occurs at that dose by the completion of the Day 21 study visit of the 6th overall subject infused, the final 3 subjects will receive the 3rd dose level of $0.8\text{-}1x10^{10}$ transduced CD4+ T-cells. In the event of a DLT (grade

3 or higher unexpected, related AE) recruitment will be paused pending DSMB review and decision to proceed.

At the end of Step 2 all participants will undergo mini-leukapheresis and optional rectal biopsy.

In Step 3 all participants will participate in a 16 week analytical treatment interruption beginning 4 weeks after T-cell infusion. At the end of Step 3 all participants will undergo mini-leukapheresis and optional rectal biopsy.

In Step 4 all participants will be advised to resume antiretroviral therapy and be followed until plasma HIV RNA fall below the limit of detection.

> Note: For subjects with viral loads ≤1000 copies/ml at the end of 16 weeks, re-initiation of antiretroviral therapy will be recommended. However, resumption of ART medications will be left to the discretion of the subject and their physician. If a subject declines to restart antiretroviral medications, the subject will be followed in Step 3 with monthly visits until ART is resumed, in an effort to fully capture the impact of the intervention. These monthly visits should capture all procedures outlined in the Week 16 visit of the schedule of events. The study team will discuss re-initiation of ART with the subject at each visit. The subject may choose to restart ART at any time, and thus would move to Step 4 of the study when antiretroviral medication is resumed. All procedures outlined in the ATI Discontinuation visit should be performed just prior to restarting antiretroviral medications (Step 4), if possible.

In Step 5 all participants will undergo leukapheresis and an optional rectal biopsy at 52 weeks post infusion and then be followed long-term as required by regulatory authorities.

This study will consist of subjects who are well controlled on HAART. Each will receive a single dose of *C34-CXCR4-modified CD4+ T-cells,* followed by analytical treatment interruption of 16 weeks. Dosing of C34-CXCR4-modified cells will occur in a 3+ 3 +3 dose escalation format, capped at 9 evaluable subjects. Dose level 1 consists of 0.8-1x10^9 cells, dose level 2 consists of 2.4-3x10^9 cells, and dose level 3 contains 0.8-1x10^{10} cells. At each dose level, given no observed DLT, three patients will be treated. For dose levels 1 and 2, if 0/3 has a dose limiting toxicity (DLT), then the dose is escalated. If 1/3 has a DLT at a dose level then 3 additional patients will be treated at that dosage before escalating, and if <2/6 experience DLT (i.e. no additional DLT is observed) then the dose will be escalated to the next planned dose level, until the 9 evaluable subjects have been reached.

After enrollment, subjects will undergo leukapheresis (approximately a 10L volume) to obtain large numbers of PBMCs. To ensure sufficient T-cells in the event of a manufacturing failure, a second leukapheresis will be performed. If the second leukapheresis is not needed for manufacturing, a small scale apheresis (approximately a 5L volume) will be performed instead to serve as a cell bank for baseline controls for immunological evaluations. The second leukapheresis will be scheduled approximately 3 weeks after the first leukapheresis. CD4+ T-cells will be purified from the PBMCs, genetically modified using lentiviral transduction with the C34-peptide conjugated to the CXCR4 N-terminus, expanded *in vitro* and then frozen for future administration. Within approximately 15 weeks after enrollment confirmation, subjects will receive a single infusion of *C34-CXCR4-modified CD4+ T-cells*.

Subjects will have clinical blood tests to assess safety. Research tests such as HIV-1 RNA levels and CD4+ T-cell counts, HIV-1-specific CD4+ and CD8+ T-cell responses, and the proportion of circulating CD4+ T-cells that contain the genetically modified cells will be assessed at regular intervals as detailed in the schedule of events section. Subjects will be enrolled in the study in order to obtain a maximum of 9 evaluable subjects who complete the infusion of *C34-CXCR4-modified CD4+ T-cells* and undergo treatment interruption. Any subject who prematurely discontinues the study prior to the infusion will be replaced with another subject. Evaluable subjects are defined as those who receive infusion of C34-CXCR4 modified CD4 T-cells and undergo treatment interruption. Although it is standard across our protocols to allow for a 20% dropout rate, there were no such discontinuations in our previous CCR5 ZFN study (NCT00842634). Subjects who discontinue ATI prior to the 16 week duration will not be replaced.

3.1 Monitoring for delayed adverse events associated with lentiviral vector gene transfer

After the completion of the analytical treatment interruption, subjects will have their white blood cells monitored for vector sequences to evaluate whether there is an increase in marking, which may indicate a growth advantage, possibly from a transforming event associated with integration. In addition, patients will be monitored for evidence of a vector derived RCL. Monitoring for these events will occur for 12 months under this protocol, and then for up to 15 years under a separate long-term follow-up protocol, as described under the ***Clinical Management Issues*** (Section 7) section of this protocol.

4.0 SELECTION, ENROLLMENT, AND WITHDRAWAL OF SUBJECTS

4.1 Inclusion Criteria

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

NOTE: The term “licensed” refers to a US FDA-approved kit.

WHO (World Health Organization) and CDC (Centers for Disease Control and Prevention) guidelines mandate that confirmation of the initial test result must use a test that is different from the one used for the initial assessment. A reactive initial rapid test should be confirmed by either another type of rapid assay or by an E/CIA that is based on a different antigen preparation and/or different test principle (e.g., indirect versus competitive), or a Western blot or a plasma HIV-1 RNA assay. Alternatively, if a rapid HIV test or any FDA-approved HIV-1 Enzyme or Chemiluminescence Immunoassay (E/CIA) test kit is not available, two HIV-1 RNA values ≥ 2000 copies/mL at least 24 hours apart performed by any laboratory that has CLIA certification, or its equivalent, may be used to document infection.

4.1.2 Ability and willingness of subject to provide informed consent.

4.1.3 Men and women ages ≥18 years.

4.1.4 Clinically stable on their first or second HAART regimen. Changes while the patient HIV viral load is undetectable does not count toward the number of ART regimens used, only changes made for virologic failure (for example an individual switching from an NNRTI-based regimen to an integrase inhibitor based regimen while the HIV viral load is undetectable will still be in their first regimen). **Site investigator anticipates that a fully active alternative ART regimen could be constructed in the event of virologic failure on the current ART regimen.**

The current regimen should have no changes within 4 weeks of enrollment. Subjects must be willing to continue on current antiretroviral therapy for the duration of the study except for the duration of the 16 week analytical treatment interruption. (NOTE: changes to safely begin the treatment interruption –see section 5.6– are permitted).

4.1.5 Screening HIV-1 RNA that is ≤50 copies/mL using a FDA-approved assay performed by any laboratory that has a CLIA certification or its equivalent within 30 days prior to enrollment.

4.1.6 HIV-1 RNA ≤50 copies/mL using a FDA-approved assay for at least 24 weeks prior to enrollment performed by any laboratory that has a CLIA certification or its equivalent.

NOTE: HIV-RNA must be measured at least once in the last 24 weeks and at least 3 days before the screening measure. Single determinations that are between >50 and <400 copies/mL (ie, blips) are allowed as long as the preceding and subsequent determinations are ≤50 copies/mL. The screening value may serve as the subsequent determination ≤50 copies/mL following a blip.

NOTE: subjects who have participated in other trials using ATI's will be permitted since detectable virus during the interruption does not represent virologic failure. These subjects should have at least 24 weeks of VL <50 copies/mL.

4.1.7 Screening CD4+ T-cell count ≥450 cells/ mm^3 within 30 days of enrollment.

4.1.8 Started ART with nadir CD4+ ≥200 cells/ mm^3.

4.1.9 The following laboratory values obtained within 30 days prior to enrollment meeting the following criteria:

- Absolute neutrophil count (ANC) ≥1000 cells/mm^3
- Hemoglobin:≥10.0(males); ≥9.5 (females) g/dL
- Platelet count ≥ 100,000/mm^3
- Calculated creatinine clearance ≥50 mL/min estimated by the Cockcroft-Gault equation
  NOTE: A program for calculating creatinine clearance by the Cockcroft-Gault method is available at

http://reference.medscape.com/calculator/creatinine-clearance-cockcroft-gault

- Alanine aminotransferase (ALT) ≤ 2.0 x ULN

4.1.10 Negative HBsAg within 6 months prior to enrollment.

4.1.11 Negative HCV serology, or if positive, negative HCV RNA within 6 months prior to enrollment.

4.1.12 Adequate venous access and no other contraindications for leukapheresis.

4.1.13 Have a Karnofsky Performance Score of 70 or higher.

4.1.14 Have a recorded viral load set point prior to starting antiretroviral therapy

4.2 Exclusion Criteria

4.2.1. Acute or chronic hepatitis B or hepatitis C infection

4.2.2. Current or prior AIDS diagnosis.

4.2.3. History of cancer or malignancy, with the exception of successfully treated basal cell or squamous cell carcinoma of the skin

4.2.4. History or any features on physical examination indicative of active or unstable cardiac disease or hemodynamic instability.

NOTE: Subjects with a history of cardiac disease may participate with a physician's approval.

4.2.5 History or any features on physical examination indicative of a bleeding diathesis.

4.2.6 Have been previously treated with any HIV experimental vaccine within 6 months prior to enrollment, or any previous gene therapy using an integrating vector.

NOTE: Subjects treated with placebo in an HIV vaccine study will not be excluded if documentation that they received placebo is provided.

4.2.7 Use of chronic systemic corticosteroids, hydroxyurea, or immunomodulating agents (e.g., interleukin-2, interferon-alpha or gamma, granulocyte colony stimulating factors, etc.) within 30 days prior to enrollment.

NOTE: Recent or current use of inhaled steroids is not exclusionary. If subjects are prescribed a brief course of oral corticosteroids, the use should be limited to less than 7 days. Use of steroids before apheresis and immune assessment blood draws should be discouraged as it will affect white blood cell function.

4.2.8 Breast-feeding, pregnant, or unwilling to use acceptable methods of birth control.

4.2.9 Anticipated use of aspirin, dipyridamole, warfarin or any other medication that is likely to affect platelet function or other aspects of blood coagulation during the 2-week period prior to leukapheresis.

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

4.2.11 Serious illness requiring systemic treatment and/or hospitalization within 30 days prior to enrollment.

4.2.12 Asymptomatic baseline serum chemistry elevations in LFTs, bilirubin, and serum creatinine due to HAART medication are not exclusionary, when in the opinion of the investigator, the abnormalities are not attributable to intrinsic hepatorenal disease. Such baseline elevations must be due to HAART.

4.2.13 Receipt of vaccination within 30 days prior to enrollment.

NOTE: It is recommended that subjects enrolling into this study should have completed their routine vaccinations (hepatitis A, hepatitis B, pneumococcus, and tetanus diphtheria booster) at least 30 days prior to enrollment.

4.2.14 Have an allergy or hypersensitivity to study product excipients (human serum albumin, DMSO and Dextran 40).

4.3 Study Enrollment Procedures

4.3.1 Prior to implementation of this protocol, the site must have the protocol and consent form approved by their local institutional review board (IRB).

Once a candidate has been identified, details will be carefully discussed with the subject. The subject will be asked to read and sign the approved consent form before any study-specific procedures have been performed.

Note: For the purposes of this study, enrollment is defined as the date the Investigator confirms subject eligibility.

4.3.1.1 Prisoner Participation

This protocol does not meet Federal requirements governing prisoner participation in clinical trials and prisoners should not be considered for recruitment purposes.

4.3.1.2 Birth Control

Female subjects of reproductive potential (women who have reached menarche or women who have not been post-menopausal for at least 24 consecutive months, i.e., who have had menses within the preceding 24 months, or have not undergone a sterilization procedure [hysterectomy or bilateral oophorectomy]) must have a negative serum pregnancy test at the time of enrollment and the Safety Evaluation Visit. A urine pregnancy test must also be performed on Day 0 (T cell infusion day).

Due to the high risk level of this study, while enrolled, all subjects must agree not to participate in a conception process (e.g., active attempt to become pregnant or to impregnate, sperm donation, *in vitro* fertilization). Additionally, if participating in sexual activity that could lead to pregnancy, the study subject must agree to use at least two reliable methods of contraception during the follow-up period of the protocol, including at least one barrier method of contraception (diaphragm, cervical cap with spermicide, or condom).

Acceptable birth control includes a combination of two of the following methods, where at least one must be barrier contraception:

- Condoms* (male or female) with or without a spermicidal agent (barrier contraception).
- Diaphragm or cervical cap with spermicide (barrier contraception)
- Intrauterine device (IUD)
- Hormonal-based contraception

***** Condoms are recommended because their appropriate use is the only contraception method effective for preventing HIV transmission.

Subjects who are not of reproductive potential (women who have been post menopausal for at least 24 consecutive months or have undergone hysterectomy, salpingotomy, and/or bilateral oophorectomy or men who have documented azoospermia) are eligible without requiring the use of contraception. Acceptable documentation of sterilization, azoospermia, and menopause is specified below.

Written or oral communication by clinician or clinician's staff of one of the following:

- Physician report/letter
- Operative report or other source documentation in the subject record (a laboratory report of azoospermia is required to document successful vasectomy)
- Discharge summary
- Laboratory report of azoospermia
- Follicle stimulating hormone measurement elevated into the menopausal range

4.4 Co-enrollment Guidelines

For specific questions and approval for co-enrollment in other studies, site must contact the Sponsor Project Manager at [redacted].

4.5 Premature Subject Discontinuation

Subjects may be withdrawn for the following reasons:

- Request by the subject to withdraw.
- Request of the primary care provider if he or she thinks the study is no longer in the best interest of the subject.

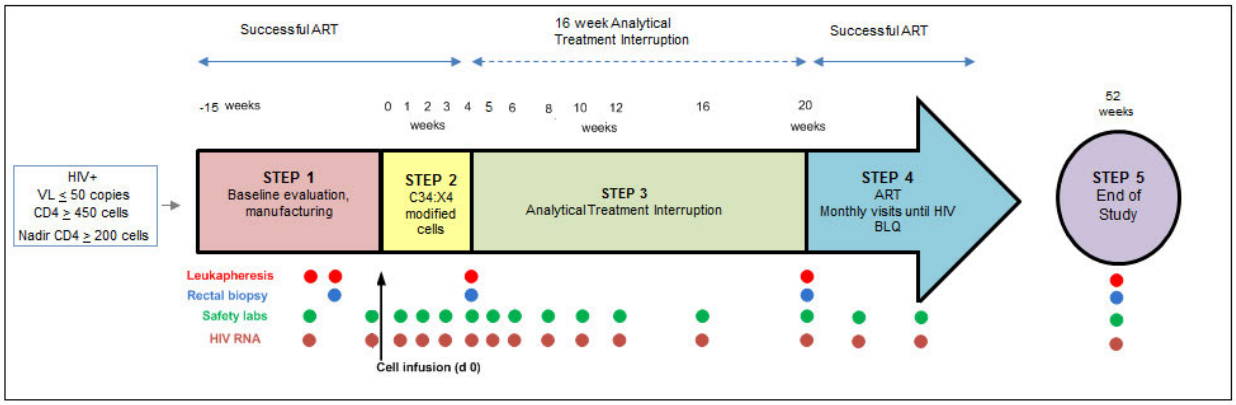
- Subject judged by the investigator to be at significant risk of failing to comply with the provisions of the protocol as to cause harm to self or seriously interfere with the validity of the study results.
- Decision to stop further study visits by the Data Safety Monitoring Board (DSMB), or an independent review board or regulatory review committee.
- At the discretion of the IRB, Office for Clinical Research (OCR), Food and Drug Administration (FDA), NIH/NIAID, DAIDS, investigator, or pharmaceutical supporter.

5.0 STUDY DRUGS

5.1 Description of Study Drugs

The study drug in this protocol is autologous CD4+ T cells genetically modified by lentiviral vector expressing the C34-peptide conjugated to the CXCR4 N-terminus. Study treatment is defined as infusion of subject specific autologous CD4+ T cells genetically modified by lentiviral vector expressing the C34-peptide conjugated to the CXCR4 N-terminus.

5.2 Treatment Regimen



Step 1

- Subject is screened, undergoes the first and second leukapheresis and rectal biopsy, and undergoes safety evaluations before dosing.
- The University of Pennsylvania's Clinical Cell and Vaccine Production Facility (CVPF) manufactures autologous CD4 T-cells genetically modified by lentiviral vector expressing the C34-peptide conjugated to the CXCR4 N-terminus.

Step 2

- All participants will receive a single infusion of C34-CXCR4-modified CD4+ T-cells at one of 3 dose levels. The first 3 subjects will receive dose level 1 of 0.8-1x10^9 transduced CD4+T-cells. Provided no dose limiting toxicity (DLT) is seen at the first dose level at the completion of the Day 21 study visit of the 3rd subject, the next 3 subjects will receive infusion at the second dose level of 2.4-3x10^9 transduced CD4+ T-cells. If no DLT occurs at that dose by the completion of the Day 21 study visit of the 6th overall subject infused, the final 3 subjects will receive the third dose level of 0.8-1x10^{10} transduced CD4+ T-cells. In the event of a DLT (grade 3 or higher unexpected, related AE) recruitment will be paused pending DSMB decision.

- At the end of Step 2 all participants will undergo mini-leukapheresis and an optional rectal biopsy.

Step 3

- HAART interruption for 16 weeks (or less if patients viral load is sustained ≥100,000 or CD4 count ≤350).
- All participants undergo a mini-leukapheresis and an optional rectal biopsy at the end of the Step 3.

Step 4

- All participants will be advised to resume antiretroviral therapy and be followed until plasma HIV RNA fall below the limit of detection.

  Note: For subjects with viral loads ≤1000 copies/ml at the end of 16 weeks, re-initiation of antiretroviral therapy will be recommended. However, resumption of ART medications will be left to the discretion of the subject and their physician. If a subject declines to restart antiretroviral medications, the subject will be followed in Step 3 with monthly visits until ART is resumed, in an effort to fully capture the impact of the intervention. These monthly visits should capture all procedures outlined in the Week 16 visit of the schedule of events. The study team will discuss re-initiation of ART with the subject at each visit. The subject may choose to restart ART at any time, and thus would move to Step 4 of the study when antiretroviral medication is resumed. All procedures outlined in the ATI Discontinuation visit should be performed just prior to restarting antiretroviral medications (Step 4), if possible.

Step 5

- All participants will undergo leukapheresis and an optional rectal biopsy 52 weeks post infusion and then be enrolled onto a separate long-term follow-up study as required by regulatory authorities.

5.3 Preparation of Autologous CD4+ T genetically modified with C34-CXCR4

The manufacture and release of C34-CXCR4 modified CD4 T-cells will be performed by the Clinical Cell and Vaccine Production (CVPF) at the University of Pennsylvania. CD4+ lymphocytes will be isolated from leukapheresis product using negative selection for monocytes and CD8+ T-cells. The resulting enriched CD4+ T-cells are activated with anti-CD3/CD28 mAb coated magnetic beads and transduced with lentiviral vector expressing an HR2, C34-peptide conjugated to the CXCR4 N-terminus. The cell expansion process will initiate in a static tissue culture bag. Cells will be transferred to a WAVE bioreactor if needed for additional expansion. At the end of the culture, cells will be depleted of the magnetic beads, washed, concentrated using the Haemonetics Cell Saver system, and then formulated in infusible cryopreservation media. Each dose will be packed and cryopreserved in infusion bag(s) in a volume dependent on the total cell number which is a function of the transduction efficiency; the minimum volume will be 10mL per bag.

The cell product will be ready to be released after 14 days post-formulation and cryopreservation. Modified cells are not released from the CVPF until release criteria for the infused cells (e.g., cell purity, sterility, transduction efficiency, viability, cell number) are met.

Note: In cases where a product may need to be remanufactured, use of frozen apheresis as starting material is permitted. However, the apheresis may not be frozen longer than 6 months prior to use in manufacturing.

Additionally, several vials of cells will be cryopreserved and retained as sentinel vials, for performing an endotoxin gel clot and viability count at the time of the infusion. Remaining vials will be used to conduct the "for information only (FIO)" functional assays. All cryopreserved cells will be stored in a monitored freezer at ≤ -130°C.

5.4 Administration of Study Drugs

5.4.1 Administration of autologous T-cells genetically modified with C34-CXCR4

Procedure for Study Agent Handling and Preparation

The autologous T-cells genetically modified by lentiviral vector expressing the C34-peptide conjugated to the CXCR4 N-terminus will not be released from the manufacturer (University of Pennsylvania Clinical Cell and Vaccine Production Facility, CVPF) until the release criteria for the infused cells (e.g., cell purity, sterility, identity, HIV-1 gag level) are met. On the day of T-cell infusion, the genetically modified T-cells will be transported on dry ice from CVPF to the subject's bedside in the Center for Human Phenomic Science (CHPS) according to CVPF SOP for thaw and infusion. Each bag of cryopreserved genetically modified CD4+ T-cells will contain approximately 1 x 10^9 to 1 x 10^{10} cells at a concentration of approximately 1 x 10^8 cells/mL of infusible cryomedia.

Package and Labeling

The infusion bag will be affixed with a label containing information regarding the dose, the method of manipulation, the vector and the following statements "FOR AUTOLOGOUS USE ONLY" And "Caution: New Drug- Limited by Federal Law to Investigational Use". In addition the label will have at least two unique identifiers.

Premedication

Side effects following T-cell infusions include transient fever, chills, and/or nausea. It is recommended that the subject be pre-medicated with acetaminophen (650 mg) by mouth and diphenhydramine hydrochloride by mouth or IV, prior to the infusion of *C34-CXCR4-modified CD4+ T-cells*. If Benadryl is contraindicated, an H2-blocker such as ranitidine will be administered. These medications may be repeated every six hours as needed. A course of non-steroidal anti-inflammatory medication may be prescribed if the subject continues to have fever not relieved by acetaminophen. It is recommended that subjects not receive systemic corticosteroids such as hydrocortisone, prednisone, prednisolone (Solu-Medrol) or dexamethasone (Decadron) at any time, except in the case of a life-threatening emergency, since this may have an adverse effect on T-cells. If corticosteroids are required for an acute infusional reaction, an initial dose of hydrocortisone (100 mg) is recommended.

Administration of genetically modified T-cells

The cells will be thawed by trained personnel using a water bath maintained between 36°C to 38°C. The bag will be gently massaged until the cells have just thawed. There should be

no frozen clumps left in the container at the time of infusion. If the cell product appears to have a damaged or leaking bag, or otherwise appears to be compromised, it should not be infused, and should be returned to the CVPF. Prior to the infusion, two individuals from the study staff will independently verify all information in the presence of the subject to confirm that the information is correctly matched to the subject.

A licensed nurse, in the presence of the Principal Investigator or Sub-Investigator, will infuse the cells into the subject within approximately 10-15 minutes after thaw to ensure that the cells are still cold. The genetically modified T-cells will be administered to subjects by intravenous infusion at a flow rate of approximately 10-20 mL per minute. There should be no frozen clumps left in the bag at the time of the infusion. The cells will be infused into an intravenous catheter, either through a peripheral vein (preferred) or central vein. A macrodrip intravenous tubing will be used to infuse the cells by gravity (i.e., no infusion pump). The macrodrip intravenous tubing will be connected to a "Y" adapter with one end of the adapter spiked to the product bag(s) and the other to a normal saline solution bag. A **leukoreduction filter must not be used for the infusion of the cell product**. At the end of infusion, the bag will be rinsed twice with saline to maximize removal of cells from the bag.

Subjects will be monitored before the infusion and for 2 hours after the infusion of the transduced T-cells. Vital signs (temperature, pulse/heart rate, blood pressure, and respiratory rate) and pulse oximetry will be obtained and recorded within 10 minutes before the infusion and within 15 minutes after the infusion. Thereafter, vital signs will be measured every 15 minutes (+/ - 5 min) after the infusion for the first hour, and then every 30 minutes (+/- 5 minutes) for the next hour. A crash cart must be available for emergency situations. Emergency medical equipment will be available during the infusion in case the subject has an allergic response, severe hypotensive crisis, or any other reaction to the infusion. The subject will be discharged when medically stable and in accordance with hospital policy.

Product Return/Disposition

C34-CXCR4 CD4+-modified T-cells may require return to the CVPF for a variety of reasons, including but not limited to: 1) Mislabeled product; 2) Condition of patient prohibits infusion/injection, and 3) Subject refuses infusion/injection. Any unused product that has not yet been thawed will be returned for disposal as per CVPF SOP.

Any unused product will be disposed of according to local institutional procedures for the disposal of hazardous medical waste/blood product. Final disposition of the investigational product will also be documented.

Research Tests

A blood sample will be drawn before the infusion of *C34-CXCR4 CD4+ T-cells* and at 20 min (±5 min) and 2 hours (±5 min) post-infusion. It is recommended that post-infusion blood draws on Day 0 be obtained from the arm not used for infusion.

Reactions following infusion

The following reactions are possible during and/or after infusion:

Febrile reactions

In the unlikely event that the subject develops sepsis or systemic bacteremia following T-cell infusion, appropriate cultures and medical management should be initiated. If a

contaminated T-cell product is suspected, the product can be retested for sterility using archived samples that are stored in the CVPF.

Flu-like Symptoms

- Fever/arthralgia/myalgia: The onset of these symptoms usually occurs 2 to 4 hours after the cell product administration. Administration of acetaminophen or non-steroidal anti-inflammatory agents (NSAIDS) is effective in controlling and preventing these symptoms.

- Rigors/chills: These symptoms may occur during and/or after cell infusion administration. The agent of choice to improve severe chills or rigors is an opioid such as dilaudid. If subjects develop rigors consistently after cell product administration, prophylactic administration of dilaudid may prevent these reactions.

Hemodynamic Effects

- Edema: Adequate hydration is necessary to ensure renal perfusion, but over hydration should be avoided. Subjects should be encouraged to drink electrolyte-containing fluids such as sport drinks and soups. If diuretics are to be used, they should be used with caution to avoid decreasing intravascular volume. If the participants develop hypotension, IV fluids and observation in an acute hospital bed is advisable.

- Hypotension: Organ dysfunction, oliguria, and increases in BUN and creatinine are almost always reversible upon discontinuation of cell product and 24 to 48 hours of supportive treatment. On rare occasions, low doses of dopamine and fluid support may be warranted. Educating the subject to slowly rise from a supine to a sitting position and then to a standing position can prevent orthostatic hypotension. Quick evaluation in the ER if symptoms occur is advisable.

Dermatologic Reactions

Dry skin, pruritus, erythema, and sloughing commonly occur following cell infusions. No interruption of therapy is usually needed. Antihistamines, water or oil-based lotions, and oatmeal baths may help control the symptoms. Diphenhydramine hydrochloride (Benadryl) should be given before the administration of the T-cell product to prevent this from happening.

Cytokine release syndrome

Cytokine release syndrome is caused by a release of inflammatory cytokines such as IL2, IFN-γ, and TNFα. This type of reaction is common in cancer immunotherapy where antibodies bind T-cells that release large amounts of cytokines, or cause rapid destruction of tumor/target cells. This causes a systemic inflammatory response similar to sepsis and includes fever, nausea, chills, rash or flushing, rigors, hypotension, tachycardia, headache, rash, throat tightness, and dyspnea. Capillary leak with fluid retention is worsened by hydration commonly given to treat hypotension. The syndrome can be associated with pulmonary infiltrates, pulmonary edema, arrhythmias and cardiac arrest. The syndrome is also associated with an elevation in LFTs, d-dimers, LDH, CR, uric acid, and phosphorus from immune-mediated cytolysis of targeted cells, with release of intracellular contents as well as a possible bystander effect (on neighboring, nontargeted cells). We have not had this type of reaction in any of the HIV positive subjects treated to date at the University of Pennsylvania.

5.5 Concomitant Medications

Below are lists of selected concomitant medications. These lists are only current as of the date of this protocol. Therefore, whenever a concomitant medication or study agent is initiated or a dose changed, investigators must review the concomitant medications' and study agents' most recent package inserts, or updated information from DAIDS to obtain the most current information on drug interactions, contraindications, and precautions.

5.5.1 Recommended Medications

Each subject will receive acetaminophen (650 mg) and diphenhydramine hydrochloride (25-50 mg) between 30 minutes to approximately 1 hour before cell infusion. If Benadryl is contraindicated, an H2-blocker, such as ranitidine, will be administered.

5.5.2 Prohibited Medications

Aspirin, dipyridamole, warfarin, or any other medication likely to affect platelet function or other aspects of blood coagulation is prohibited during the 2-week period prior to leukapheresis. Oral corticosteroids, hydroxyurea, and immunomodulating agents are prohibited until completion of study follow-up. A brief course (< 1 week) of oral corticosteroids is allowed during Steps 2 and 3.

Routine vaccines are also prohibited prior to completion of study follow-up unless medically indicated.

NOTE: It is recommended that enrolled subjects should not receive their influenza vaccine during the ATI (Step 3) as it may cause a transient increase in viral load in some subjects. If medically indicated while on study, attempts should be made to receive influenza vaccine in the window between screening (Weeks -15 to -11) and Day 28. Influenza vaccines may also be administered at any time after the subject restarts antiretroviral medications (Step 4).

5.6 Withdrawal of HAART Medication

Subjects will discontinue HAART regimens at week 4 post-infusion in consultation with primary care providers. NNRTIs (e.g. Rescriptor, Sustiva, Viramune and rilpivirine) have been reported to have longer half lives than other ARV classes. Therefore, based on the preference of the subject in consultation with the referring physician, subjects on an NNRTI containing regimen should either (a) discontinue the NNRTI 7 days prior to discontinuation of the other antiretrovirals in their regimens or (b) initiate a potent PI-based regimen (with discontinuation of the NNRTI) without altering the nucleosides of the regimen for the last two weeks of HAART prior to simultaneous interruption of all antiretrovirals.The time of medication interruption will be noted in medication records and CRFs.

6.0 CLINICAL AND LABORATORY EVALUATIONS- STUDY PROCEDURES

6.1 Schedule of Events

	STEP 1 Manufacturing				STEP 2 C34-CXCR4 / ART							STEP 3 ATI							Prior to Restart of ART	STEP 4 ART		STEP 5 Discont. Visit
Procedure	Screen (~Wk -15 to -11)	Apheresis 1 (~Wk -9 to -7)	Apheresis 2 and rectal biopsy (~Wk -5 to -3)	Safety Eval. (within 14 days (+/-3 days) prior to Dosing)	*C34-CXCR4 Modified CD4+ T-cells* Day 0	24 hrs	48 hrs	Day 7 ±3	Day 14 ±3	Day 21 ±3	Day 28 ±3	Step 3-1 Wk 5 ±3	Step 3-2 Wk 6 ±3	Step 3-3 Wk 8 ±7	Step 3-4 Wk 10 ±7	Step 3-5 Wk 12 ±7	Step 3-6 Wk 16 ±7	Steps 3-7 through 3-13 Monthly (every 30 days ±7) [22]	ATI Discontinuation Visit [21, 22, 23]	Steps 4-1 through 4-7 Monthly (every 30 days ±7) until viral load below detection	Month 6 RCL Visit[24]	Wk 52 (±14 days)
CLINICAL PROCEDURES																						
Inclusion Exclusion	X[8]																					
Informed Consent	X																					
Medical History	X																					
Concomitant medications	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical Exam/Karnofsky performance status [15]	X			X							X					X			X			
Targeted Physical exam					X	X	X	X	X	X		X	X	X	X		X	X		X	X	X
Leukapheresis Screening	X																					
Adverse events		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
EKG	X					X																
CLINICAL LABORATORY TESTS																						
Hep B and C (2ml serum)	X[9]																					
Serum Pregnancy Test (1ml SST)	X			X																		
Pregnancy Test (urine)					X																	
Chemistry Panel/LFTs (5ml SST)	X			X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Cholesterol, LDL, HDL, triglycerides				X															X			
CBC w diff / platelets (4ml lav) [11]	X			X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urinalysis	X			X		X	X	X	X	X	X			X					X	X	X	
CD4+ T-cell counts [11,12,]	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Viral Load (10 ml serum) (HIV-1 RNA PCR assay) [13]	X			X	X[3]	X	X	X	X	X	X[13]	X	X	X	X	X	X	X	X	X	X	X
ART Resistance Test (10ml lav)																			X[7]			
Antiretroviral Drug Levels [20]												X	X	X	X	X	X	X	X			
INTERVENTION																						
Leukapheresis[18]		X	X[19]								X[19]								X[19]			X[19]
T-cell infusion					X																	
Rectal Biopsy[5]			X[5]								X								X			X

	STEP 1 Manufacturing				STEP 2 C34-CXCR4 / ART							STEP 3 ATI							Prior to Restart of ART	STEP 4 ART		STEP 5 Discont. Visit
Procedure	Screen (~Wk -15 to -11)	Apheresis 1 (~Wk -9 to -7)	Apheresis 2 and rectal biopsy (~Wk -5 to -3)	Safety Eval. (within 14 days (+/-3 days) prior to Dosing)	*C34-CXCR4 Modified CD4+ T-cells* Day 0	24 hrs	48 hrs	Day 7 ±3	Day 14 ±3	Day 21 ±3	Day 28 ±3	Step 3-1 Wk 5 ±3	Step 3-2 Wk 6 ±3	Step 3-3 Wk 8 ±7	Step 3-4 Wk 10 ±7	Step 3-5 Wk 12 ±7	Step 3-6 Wk 16 ±7	Steps 3-7 through 3-13 Monthly (every 30 days ±7)[22]	ATI Discontinuation Visit [21, 22, 23]	Steps 4-1 through 4-7 Monthly (every 30 days ±7) until viral load below detection	Month 6 RCL Visit[24]	Wk 52 (±14 days)
RESEARCH TESTS																						
Peripheral Blood – Purple Top (10cc)			X	X	X[3]	X	X	X	X	X	X[4]	X	X	X	X	X	X	X	X[4]	X	X	X[4]
PBMC																						
Immune Analysis			X								X									X[17]		X
C34-CXCR4 detection (flow cytometry)					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
HIV Reservoir Assays[1]			X								X								X			X
Transcriptional profiling			X								X	X	X	X	X	X	X	X	X	X	X	X
DNA																						
C34-CXCR4 detection (qPCR)					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
VSV-G				X							X			X		X					X[16]	X
HIV Reservoir Assays[1]			X								X								X			X
Integration analysis			X					X			X								X			X
Plasma																						
HIV Coreceptor usage (Tropism)	X[6]												X[14]						X[14]			
Serum –Red top (~10cc)				X	X[3]	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X[16]	X
Cytokine Profile					X	X	X	X	X	X	X											
Pre-existing Antibody detection[10]				X							X			X							X[16]	X
Rectal Biopsy Assays[2]																						
HIV Reservoir Assays			X								X								X			X
C34-CXCR4 detection (flow cytometry)			X								X								X			X
C34-CXCR4 detection (qPCR)			X								X								X			X
Total Blood Volume (ml)	48	55	85	43	94	43	38	43	43	43	43	43	47	43	43	83	83	89	83	83	83	83
~Total Blood Volume (tbl)	3.2	3.7	5.7	3	6.4	3	2.6	3	3	3	3	3	3.2	3	3	5.6	5.6	6	5.6	5.6	5.6	5.6

1. Peripheral Blood processed at TCSL and transferred to appropriate labs for analysis; Remaining PBMC to be archived (TCSL or Riley Lab). In the event something unexpected occurs during the subject's participation in the protocol, the research team may request an additional blood draw be performed to collect additional blood samples for research analysis such as viral sequencing, antiviral resistance testing, etc. This is being done with the intention of evaluating the likely effects from the investigational product. The maximum amount of extra blood that will be collected will be 3 tablespoons of blood twice in one week.
2. Rectal biopsy processed at UPenn HIC and analyzed or transferred to appropriate labs for analysis (TCSL for qPCR). Any remaining MMC to be archived (TCSL or Riley Lab).
3. Blood draw for research samples and viral load to be performed on Day 0 before T-cell infusion and at 20 min (±5 min) and 2 hours (±5 min) post-infusion. It is recommended that post-infusion blood draws on Day 0 be obtained from the arm not used for infusion.
4. If the apheresis is collected at this time-point, additional peripheral blood (purple top tubes) should still be drawn for use in the C34-CXCR4 detection; the apheresis products can be used for other research analysis. Serum collection (red top tube) is still required where indicated in Schedule of Events.
5. Optional rectal biopsies will be performed during Step 1, at end of the Step 2 and just prior to restarting HAART/end of Step 3 if possible. Rectal biopsy will also be performed during the Step 5 window. Windows for rectal biopsies are as follows: Baseline (Apheresis 2 study visit) should be done between Week -5 and Week-3. Step 2 rectal biopsy (Day 28 visit) should occur within 2 weeks prior to Analytical Treatment Interruption/entry into Step 3, and the Step 3 biopsy (Week 20 visit) should be done within 2 weeks prior to re-starting antiretroviral therapy. If a subject must restart HAART early, best efforts should be made to schedule a rectal biopsy prior to the reinstatement of HAART. In no cases should the restarting of HAART for safety reasons be delayed for the purpose of obtaining a rectal biopsy.
6. HIV co-receptor usage assay at screening is a tropism DNA assay recommended for patients with undetectable viral loads. Draw 4 mL of whole blood into an EDTA (lavender-top) test tube; DO NOT CENTRIFUGE; **place** tube and test request form in specimen bag; freeze sample immediately at -20˚C; ship to Quest. Note: This tube should be separate from blood designated for TCSL.
7. For subjects restarting ART before week 20 due to viral rebound, ART resistance samples and all other required research/clinical lab samples will be collected on the last visit before ART re-initiation.
8. Consent may be obtained at any time prior to research procedures being performed.
9. **Hepatitis B Surface Antigen (HBsAg):** Subjects must have documentation of a negative HBsAg test within 6 months prior to screening. Subjects who have not previously had the test or subjects who were negative more than 60 days prior will have a test at screening for Step 1.

   HCV Antibody: Subjects must have documentation of a negative HCV antibody test within 6 months prior to screening for step 1. Subjects who have not previously had the test or subjects who were negative more than 60 days prior to screening will have a HCV antibody test at screening for step 1. If the HCV antibody is positive, the subject may return for a HCV RNA test. If the HCV RNA test is negative, the subject may be enrolled in the study.

   HCV RNA: If the HCV antibody is positive, a screening HCV RNA by any RT-PCR or bDNA assay must be performed at screening by a local laboratory with a CLIA certification or its equivalent. Eligibility will be determined based on the screening value. The test is not required if documentation of a negative result of a HCV RNA test performed within 6 months prior to screening is provided.
10. 2-3ml frozen serum to be delivered to Hoxie lab (from TCSL) for pre-existing antibody detection.
11. CBC with diff/platelets and CD4+ T cell counts should come from the same 4 mL lavender tube.

12. If a subject experiences a decrease in CD4+ T-cell count to ≤350 cells/mm^3, the test will be repeated the following week. If the CD4+ T-cell counts stay equal to or below 350 cells/mm^3, the subject will be advised to reinstitute HAART. The HAART will not be provided by the study. If the CD4+ decrease is not sustained the subject will continue in the study.
13. If prior to initiating treatment interruption the subject experiences an increase in viral load from undetectable to greater than or equal to 5,000 copies/ml, a viral load test will be repeated once a week for up to two additional weeks or until the viral load is below 5,000 copies/ml (whichever occurs first). If the viral load increase is sustained over this 3-week period, this event will be reported as an SAE and will be managed as medically appropriate, and the subject will not undergo ATI. If during ATI the subject experiences a viral load ≥100,000 copies/ml, this test will be repeated every week for up to 3 weeks. If the subject experiences a sustained viral load ≥100,000 copies/ml consistently over a period of 3 weeks, the subject will be advised to restart HAART. The HAART will not be provided by the study. If the viral load is not sustained (i.e. as indicated by a single test < 100,000 copies/ml), the subject will be advised not to reinstate HAART, and continue the study as planned, until week 16 after the treatment interruption to allow for evaluation of the subject's viral load set point.
14. HIV co-receptor usage assay at Weeks 6 and 20 is a tropism assay recommended for patients with viral load ≥ 1,000 copies/mL. Using 2 lavender-top (EDTA) tubes, draw enough whole blood to provide at least 3 mL plasma; transfer to a plastic screw-cap tube; centrifuge immediately at 1,000 to 1,200g for 10 to 15 minutes (Don't leave samples in centrifuge after spinning.); place tube(s) and test request form in specimen bag; freeze sample immediately at -20˚C; ship to contracted laboratory. For subjects restarting ART before week 20, the coreceptor tropism samples will be collected at the last visit before ART re-initiation.
15. Complete Physical Exam with Karnofsky performance status should be obtained at least every 8 weeks, unless symptoms require more frequently.
16. Test for VSV-G (RCL) and pre-existing antibody should be performed at the 6 month visit. These tests are not required again until the Week 52 visit.
17. First monthly visit only
18. Apheresis 1 is a full apheresis (~10L). Apheresis 2, if not needed for cell processing is a mini-apheresis (~5L). Mini-apheresis (~5L) to be done at the end of Steps 2 and 3. Full ~10L apheresis should be performed at the end of Step 5. Final apheresis should be >16 weeks after subject's viral load becomes undetectable.
19. Apheresis product (if not used for cell manufacturing) will be processed by TCSL. Cells from apheresis product will be processed and used for assays including, but not limited to, immune analysis, and to extract DNA for integration analysis. C34-CXCR4 detection should be done using peripheral blood draw. Remaining cells should be viably frozen in TCSL freezing media to be archived.
20. To monitor compliance to ATI, random testing of blood and/or urine for antiretroviral drug levels can be done during Step 3 at the discretion of the Primary Investigator. This is not required at each Step 3 visit.
21. If a subject will restart antiretroviral therapy prior to completing 16 week of treatment interruption, the ATI Discontinuation Study Visit should be performed prior to restarting antiretroviral medication, and all procedures outlined for that visit should be captured prior to restarting ART, if possible
22. For subjects extending the treatment interruption beyond a total of 16 weeks, subjects should be seen every 30 days (±7 days) with visits capturing the procedures outlined in the Step 3 Monthly visit. Before restarting ART, all procedures outlined in the Schedule of Events at the ATI Discontinuation Study Visit should be captured prior to restarting ART, if possible.
23. It is possible that the ATI discontinuation visit may be combined with another study visit. In this situation, all procedures captured in both visits should be performed, however, they do not need to be completed in duplicate.

24. The month 6 post-infusion visit is a required visit, even if the subjects has already reached undetectable viral load as part of Step 4. If a subject is still in Step 3 at the 6 month post-infusion time point, this visit may be combined with a Step 3 monthly visit. If this month 6 visit is being combined with a Step 3 Monthly or Step 4 Monthly visit, all procedures captured in the schedule of events for both visits should be performed, however they do not need to be completed in duplicate.

6.2 Study Visit Procedures

STEP 1

6.2.1 Screening Evaluations Visit (~Week -15 to -11)

Before an individual undergoes screening, informed consent must be obtained by means of a written subject information document. The information will be provided in the language of the subject's choice and written so that the subject can easily understand it. The subject will be given sufficient time to read the document and to ask questions before deciding to participate. If the person would like to participate, he or she must indicate this by signing the document. The document must also be dated with the date of signature. The subject will receive a copy of the signed and dated informed consent document. All potential subjects screened for eligibility will be listed on a subject screening and enrollment log. This log will include documentation of reasons for ineligibility and for non-participation of eligible subjects.

The following screening evaluations to determine eligibility must be completed within 15 weeks of dosing (Step 2) unless a protocol exception has been granted:

- Obtain a signed subject informed consent form- may be performed at any time prior to research procedures being performed
- Review inclusion/exclusion criteria
- History and physical exam (see section 6.3)
- Karnofsky performance status
- Concomitant medications (see section 6.3)
- EKG
- Leukapheresis screening (assess for adequate iv access)
- Hepatitis B surface antigen and hepatitis C antibody (see section 6.3)
- Serum pregnancy test (see section 6.3)
- Chemistry/LFTs (see section 6.3)
- CBC with differential and platelets
- Urinalysis
- CD4+ T-cell counts
- Viral load (HIV-1 RNA ultrasensitive PCR assay)
- A documented CD4+ nadir and documented HIV viral load set point

Once required screening tests have been completed and the subject has been determined eligible by the physician-investigator, provide the documents listed below to:

Protocol Monitor and Sponsor Project Manager
[redacted]

Documents required:

- Completed Eligibility Checklist
- Redacted copies of any source documentation to confirm eligibility
- Redacted copies of signed subject informed consent and HIPAA Authorization

Upon informed consent completion and receipt of screening and eligibility documentation, the Sponsor Protocol Monitor will review and provide documentation that the monitoring visit for

eligibility has been completed. This documentation must be received prior to apheresis and cell product manufacturing.

A Subject Screening and Enrollment Log will be maintained by the site.

6.2.2 Apheresis 1 Visit (~Week -9 to -7 prior to dosing)

6.2.2.1 Leukapheresis Procedure

For subjects who have successfully passed screening and who wish to continue to participate in the study, a visit date for a ~10L leukapheresis will be scheduled. This will be done at the University of Pennsylvania Apheresis Unit according to standard clinical procedures. From a single ~10L volume leukapheresis, at least 5 x 10^9 white blood cells will be harvested to manufacture autologous CD4+ T genetically modified by lentiviral vector expressing the C34 peptide conjugated to the CXCR4 N-terminus. At the time of leukapheresis, PBMCs for baseline immunologic studies (CD4+ and CD8+ ICS) will also be obtained.

At this visit, subjects will have vital signs (temperature, respiration, pulse and blood pressure) recorded at baseline and as per hospital practice until the procedure has been completed and the vital signs are found to be satisfactory and stable. Blood for research is also obtained. Subjects are monitored for adverse events. In addition to the screening testing requirements provided in the protocol, blood from all apheresis donors undergoes testing according to Apheresis Unit SOP.

Leukapheresis takes ~2 to 3 hours. This procedure removes only the white blood cells as blood flows out of the body through a needle inserted into one arm and returns to the body through a second needle inserted in the other arm. The cells are separated into blood components. The white cells are collected and the rest of the blood components (including the red blood cells) are returned. There are risks associated with leukapheresis; however, all precautions are taken to avoid complications. The leukapheresis procedure can occasionally cause local infections, pain or bleeding from the needle stick, bruising of the skin, or inflammation or irritation of the vein (phlebitis). There is a possibility that due to technical failure (breakage in equipment) the subject's blood circuit through the leukapheresis machine may be interrupted resulting in blood loss. If this should occur, the blood loss would not be more than that occurring during donation of whole blood and the procedure will be stopped. Anemia may occur. With the infusion of ACD during leukapheresis which can lower the calcium, the subject may experience tingling around the mouth, hands or feet, or slight twitching of muscles. Any signs and symptoms of citrate toxicity due to apheresis (such as perioral paresthesia and muscle cramps) will be treated according to the COH Donor/Apheresis Center procedure. However, these symptoms can be reduced by slowing down the rate of infusion of the blood being returned or by calcium administration. Subjects may also experience chilling, nausea, vomiting, lightheadedness, or migraine headache, which can be treated with appropriate measures, including warm compresses and slowing or stopping the procedure.

A sample aliquot from the leukapheresis product (leukapheresis 1 or 2) will be used for baseline viral and immune response analysis. In the event that the manufacturing of the cellular product fails using the first leukapheresis, then this second leukapheresis will be used for a second manufacturing run. In this case, this second leukapheresis will be a larger scale (≥10L) run. If the second leukapheresis is not needed for manufacturing, a small scale apheresis (approximately a 5L volume) will be performed instead to serve as a cell bank for baseline controls for immunological evaluations. If a mini-apheresis is collected at this timepoint, no

additional research blood will be drawn; the mini-apheresis products will be used for research analysis. Excess leukapheresis product will be archived for the look-back purposes mentioned above. A 3rd and 4th mini-apheresis (~5L) will be performed to obtain cells for research purposes (week 4 and 20). A 5th leukapheresis will be performed at the end of study (week 52).

6.2.3 Rectal Biopsy Procedure and Visit

All subjects enrolled in the study are asked to undergo four optional rectal mucosal biopsies pre and post gene-modified T-cell infusion to evaluate the GALT for viral burden and the presence of gene-modified T-cells. These data will be compared to concurrent data of viral load obtained from peripheral blood. Refer to the Schedule of Events, for the timing of these procedures (Apheresis 2 Visit, Day 28 Visit, Week 20 Visit, and Week 52 Visit).

The biopsy procedure takes approximately 30 minutes to complete, is performed in the outpatient setting, and does not require anesthesia. A trained gastroenterologist at the GI clinic will perform the rectal biopsy. Biopsy tissue will be collected in medium and delivered to the UPenn Human Immunology Core (HIC) laboratory for preparation of mucosal mononuclear cells (MMCs) and analysis; aliquots of MMCs will be transferred to the TCSL for storage, analysis, and distribution to other labs (HIV reservoir). The primary risks associated with rectal mucosal biopsies include mild rectal irritation and urgency and limited rectal bleeding for 2-3 days following the procedure. Infection and bowel perforation are extremely rare complications that may require antibiotics and/or surgical repair. Study volunteers will be followed in clinic for any complications. Even if a participant consents to these optional rectal biopsies, they may decide at any point to have no further biopsy procedures. Prophylactic antibiotics should be given to patients who would routinely be given such treatment prior to invasive procedures (e.g., cardiac valvular disease). Subjects should not have taken antithrombotic medication (except ASA and NSAIDS) for 5 days prior to the procedure. If a participant is taking these medications for medical reasons, biopsies should not be collected and the medications should not be interrupted.

Subjects should be instructed that they should not have rectal sex and/or insert any foreign object or substance into the rectum for 5 days after biopsy samples have been collected.

At one week following the second rectal biopsy, the study team will contact the subject by phone call to follow up and monitor possible adverse effects related to the procedure such as bleeding, infection, or discomfort.

The first rectal biopsy is scheduled during the Apheresis 2 Visit (~Week -5 to -3 prior to dosing):

- Leukapheresis
- Assessment of adverse events related to rectal biopsy
- Research Blood
  NOTE: if the mini-apheresis is collected at this timepoint, no additional research blood is drawn; the mini-apheresis product to be used for research analysis.
- Rectal Biopsy - to be performed approximately between Week -5 and Week -3

6.2.4 Safety Evaluations (within 14 days (+/- 3 days) of T-cell Infusion)

The following entry evaluations must be completed within 14 days (+/-3 days) of entering into step 2. Subjects must have all pre-dosing evaluations completed and enter Step 2 within 15 weeks (~100 days) after entry into Step 1 (see study schema **Figure 1**). Results for hemoglobin, ANC, platelets, creatinine, AST, ALT, and pregnancy test must be known prior to

dosing and must continue to meet the protocol defined limits for inclusion into the study. If any of the test results are outside the limits used to determine eligibility, they may be repeated x 1. If any test result continues to be outside the protocol defined limits, the subject may not be infused and will terminate from the study.

- Concomitant medications
- Complete Physical exam and Karnofsky performance status
- Assessment of adverse events
- Pregnancy test -serum
- Chemistry/LFTs
- Cholesterol/LDL, HDL, triglycerides
- CBC with differential and platelets
- Urinalysis
- CD4+ T-cell count
- Viral load (HIV-1 RNA ultrasensitive PCR assay)
- Research blood

STEP 2

6.2.5 Infusion of C34-CXCR4 modified CD4+ T-cells (Day 0)

All assessments and tests to be performed prior to infusion of C34-CXCR4 modified CD4+ T-cells and results of pregnancy test must be known

- Concomitant medications
- Targeted physical Exam (assessments directed at causes of complaints or adverse events)
- Assessment of adverse events
- Pregnancy test (urine)
- CD4+ T-cell count
- Blood draw for research samples and viral load to be performed before T-cell infusion and at 20 minutes (±5 min) and 2 hours (±5 min) post-infusion.

If for any reason a subject is not able to enter Step 2 (infusion of CD4+ modified T-cells) within 15 weeks of enrollment, contact the regulatory sponsor to discuss granting a protocol exception. However, all subjects must be enrolled in Step 2 within 16 weeks of enrollment (enrollment is defined as the date eligibility is confirmed by the Principal Investigator).

6.2.6 Post Infusion Visits

Post Infusion visits will take place per the Schedule of Events in Section 6.1.

6.3 Protocol Definitions and Evaluations

All clinical and laboratory information required by this protocol is to be present in the source documents. All evaluations are to be recorded on the CRF and keyed into the database unless otherwise specified.

6.3.1 Medical History and Physical Examination

Medical History. The medical history must include all diagnoses identified by the ACTG criteria for clinical events and other diagnoses. The medical history must include all diagnoses and

surgical procedures of major organ systems. Surgical procedures must be entered into all source documents only. Any allergies to any medications and their formulations must be documented using Medra guidelines.

Medication History. All prescription and nonprescription medication, vitamins, herbal, and nutritional supplements, taken by the subject within 90 days prior to study enrollment will be recorded at the screening visit. A complete antiretroviral medication history including start and stop dates will be recorded in the medical records and on the appropriate CRF.

Concomitant Medications. At screening, all prescription and nonprescription medications, vitamins, herbal and nutritional supplements, taken by the subject within 90 days prior to study enrollment will be recorded in the medical record and on the appropriate CRF. At each study visit, any additions, deletions, or changes of these medications will be documented. All study-mandated pre-medications taken by the subject during the study will be recorded in the medical records and on the appropriate CRF.

Karnofsky Performance. Karnofsky Performance Score will be assessed at screening using the Karnofsky Performance Status Scale presented by using the DAIDS Clinical Trial Toxicity Criteria of November 2014.

Complete Physical Exam. This physical exam is performed by a physician or nurse practitioner listed on the 1572 form for this study. A complete physical examination will be conducted at the screening visit, safety evaluation, and at least every 8 weeks, unless symptoms require more frequently. This examination must include at a minimum an examination of the skin, head, mouth, and neck; auscultation of the chest; cardiac exam; abdominal exam; and examination of the lower extremities for edema. The complete physical exam will also include signs and symptoms, diagnoses, Karnofsky Performance Status and vital signs (temperature, pulse, and blood pressure).

Targeted Physical Exam. A targeted physical exam can be performed by a licensed professional delegated this task (Physician, Nurse Practitioner, Physician Assistant, Registered Nurse, etc). A targeted PE consists of vital signs and assessment of the subject including symptom complaints and adverse event (AE) assessment.

-If a subject reports no AEs or symptoms then no additional components of the physical exam will be performed

-If a subject reports any AEs or symptoms then targeted components of the physical exam will be performed to further evaluate causes of those AEs and/or symptoms. If a nurse is completing the targeted PE and notes a finding, a physician will evaluate the subject. The physician may then complete a complete physical exam if indicated.

Height: Height will be measured and recorded at the screening visit only.

Diagnoses. Record all diagnoses identified by the Medra criteria for clinical events and other diseases.

Study Intervention Modifications. Record all study intervention modifications, subject-initiated and/or protocol-mandated modifications, and inadvertent and deliberate interruptions at each visit. Record any permanent discontinuation of treatment.

Clinical Adverse Event. Sites must refer to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), Version 2.0, November 2014, which can be found on the Regulatory Support Center Web site: http://rsc.tech-res.com/docs/default-source/safety/daids_ae_grading_table_v2_nov2014.pdf

6.3.2 Clinical Laboratory Evaluations

All laboratory results collected at screening and enrollment must be recorded on the CRFs.

Documentation of HIV-1. HIV-1 infection, as documented by any licensed ELISA test kit and confirmed by Western blot at screening. HIV-1 culture, HIV-1 antigen, plasma HIV-1 RNA, or a second antibody test by a method other than ELISA is acceptable as an alternative confirmatory test.

Plasma HIV-1 RNA. Screening HIV-1 RNA must be performed as indicated in the Schedule of Events. Eligibility will be determined based on the screening value. The screening HIV-1 RNA must be done with an Ultrasensitive HIV-1 PCR assay at a laboratory with a CLIA certification or equivalent. This assay will be used to assess the anti-viral effect of the investigational cell therapy by comparing the viral set point $\log_{10}$ HIV-1 RNA level (defined as the median of values available before the initiation of antiretroviral therapy) and the set point after the intervention, during the analytical treatment interruption as part of the secondary objectives. The new viral set point will be defined as the average of two consecutive viral load measurements of $\log_{10}$ HIV-1 RNA levels after dosing and just prior to the reinitiation of antiretroviral therapy or at weeks 20 and 24 after the administration of the modified CD4 positive T-cells (weeks 12 and 16 of the analytical treatment interruption), whichever comes first.

Hepatitis B Surface Antigen (HBsAg). Subjects must have documentation of a negative HBsAg test within 6 months prior to study enrollment. Subjects who have not previously had the test or subjects who were negative more than 60 days prior will have a test at screening for Step 1.

HCV Antibody. Subjects must have documentation of a negative HCV antibody test within 6 months prior to screening for Step 1. Subjects who have not previously had the test or subjects who were negative more than 60 days prior to screening will have a HCV antibody test at screening for step 1. If the HCV antibody is positive, the subject may return for a HCV RNA test. If the HCV RNA test is negative, the subject may be enrolled in the study.

HCV RNA. If the HCV antibody is positive, a screening HCV RNA by any RT-PCR or bDNA assay must be performed at screening to Step 1 by a local laboratory with a CLIA certification or its equivalent. Eligibility will be determined based on the screening value. The test is not required if documentation of a negative result of a HCV RNA test performed within 6 months prior to screening is provided.

Complete blood count (CBC)/differential. Hemoglobin, hematocrit, platelet count, red cell count, and white cell count with differential count.

Liver Function Tests/Chemistries. Bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, sodium, potassium, chloride, carbon dioxide, urea nitrogen, creatinine, total protein, albumin, calcium, and phosphorus.

Total cholesterol, LDL, HDL, triglycerides. Periodic measurements will be made to monitor patients for HIV treatment related metabolic abnormalities that may pose safety concerns.

Pregnancy Test. For women with reproductive potential (see definition in section 4.3.3): Serum or urine β-HCG (urine test must have a sensitivity of 25-50 mIU/mL).

Absolute CD4 T-cell counts. Absolute CD4+ T-cells count, and CD3, CD4, and CD8 percentage and absolute counts must be obtained from a laboratory with a CLIA certification or equivalent.

Urinalysis or Urine Dip Stick Analysis will include measurements of pH, glucose, protein and ketones and assessment by microscopy if urinalysis is abnormal (i.e., WBC, RBC, epithelial cells, casts, and crystals).

ART Resistance Testing for those subjects that fail to complete the 16 week analytical treatment interruption (ATI) due to viral rebound. A PCR/sequencing test will be performed to ensure that subjects resume ART using a regimen to which their HIV is most likely susceptible. GenoSURE PRIme and GenoSURE MG are examples of acceptable ART resistance tests.

6.3.3 Research Laboratory Evaluations

C34-CXCR4 Detection assay. This flow cytometry assay employing an anti-C34 monoclonal antibody will be used to detect T-cells expressing the C34-CXCR4 protein. This assay will be used for release of manufactured T-cells from the CVPF, and also for monitoring the persistence of C34-CXCR4 modified T-cells in subjects' peripheral blood and gut mucosa as part of the secondary objectives. As a supportive assay, qPCR for C34-CXCR4 will be conducted to track persistence of modified cells post-infusion. Subjects will also be assessed for the presence of C34-CXCR4-modified cells in peripheral blood prior to and after initiating the analytical treatment interruption using these assays.

Immunologic Studies. These include activation of T-cells and intracellular cytokine staining and /or CD107 degranulation of T-cells in response to HIV, EBV, Flu and CMV peptides. The intracellular cytokine staining will be done on frozen/thawed cells. The specific assays to be done include staining of CD4+ and CD8+ cells for the following cytokines: interferon-gamma, interleukin-2, and tumor-necrosis factor-alpha. The stimulating HIV antigens will be pools of peptides spanning HIV gag, env, and nef. The peptides will match the HIV clade B consensus sequence and consist of 15-mers with 11aa overlaps.

Virologic Studies. These studies are designed to measure the size of the HIV reservoir using the available methodologies

The effects of C34-CXCR4-modified CD4 T-cells on the HIV reservoir will be measured in subjects with undetectable viral loads at the end of the 16 week analytical treatment interruption using the following assays:

1) Number of latently-infected resting CD4 T-cells quantified using a limiting dilution co-culture assay (Comparing Step 1 to Step 2).
2) Frequency of integrated provirus as measured by Alu-PCR (Comparing Step 1 to Step 2). (Liszewski, Yu et al. 2009)

Storage of PBMC, serum, plasma. Samples will be stored for future use to assess the kinetics of the response and/or other research questions and for future virology and immunology testing.

Samples will be collected and processed by the Translational and Correlative Studies Laboratory (TCSL) at the University of Pennsylvania prior to archiving in the laboratory of James L. Riley.

Rectal Biopsies. All subjects enrolled in the study are asked to undergo four optional rectal mucosal biopsies. The biopsy procedure takes approximately 30 minutes to complete, is performed in the outpatient setting and does not require anesthesia. A trained gastroenterologist at the UPenn GI clinic will perform each rectal biopsy. Please see Section 6.2.3 for additional details.

***VSV-G DNA*.** VSV-G DNA in PBMC will be measured by DNA-TaqMan PCR assay using primers and a probe that is specific for the VSV-G sequence. This assay will have specificity equal to or greater than 50 copies per µg of genomic DNA, in accordance with FDA Guidance.

Transcriptional Profiling. These studies are designed to define the transcriptional program and differentiation state of C34-CXCR4 modified CD4 T cells in subjects before, during and after treatment interruption.

7.0 CLINICAL MANAGEMENT ISSUES

7.1 Toxicity

- Infusion related adverse events may develop as described in Section 5.4.2. The premedication helps to mitigate the symptoms; if they develop, the symptoms resolve spontaneously within 1-2 days and the clinical investigator will follow the subject until these events resolve.

- If a subject experiences a new grade 3 or 4 treatment related toxicity (ACTG table for grading severity of adult adverse experiences) that is confirmed by further tests, and fails to normalize or return to baseline within 1 month, the subject will be followed until the toxicity resolves or until no further improvement is anticipated by the investigator.

- Cytokine Release Syndrome (CRS). CRS is managed in the same fashion as septic shock following infection with gram negative bacteria, with the exception that antibiotics are not required. Capillary leak with fluid retention is worsened by hydration commonly given to treat hypotension. The syndrome can be associated with pulmonary infiltrates, pulmonary edema, arrhythmias and cardiac arrest. The syndrome is also associated with an elevation in LFTs, d-dimers, LDH, Creatinine, uric acid, and phosphorus from immune-mediated cytolysis of targeted cells, with release of intracellular contents as well as a possible bystander effect (on neighboring, nontargeted cells). Prophylactic measures to prevent CRS include correction of overhydration/volume overload before infusion with diuresis if necessary and pre-medication. Patients are pre-medicated with acetaminophen and an antihistamine before T-cell infusion. As a routine, after infusion the vital signs (including pulse oximetry) will be monitored pre- and post-infusion per section 5.4.1. In the event of CRS, patients with rigors will be treated symptomatically with hot pack, blankets, and Dilaudid (0.5 mg IVP prn). An MD will be called immediately if subject experiences a decrease in blood pressure. If a subject experiences

hypotension, this will be managed by administering intravenous fluids in boluses as needed to maintain an adequate blood pressure. Should the hypotension not respond to intravenous fluid boluses, admission to the CHPS or inpatient hospital will be performed to provide the appropriate level of care. Should hypoxia or dyspnea develop that results in oxygen desaturation as detected by standard pulse oximetry, the patient will be administered nasal cannula oxygen and have a chest X-ray performed as deemed appropriate by the investigator. The use of ACE inhibitors and angiotensin-II receptor blockade may have benefits for CRS. Corticosteroids have been reported to block systemic effects of T-cell toxicity.

If a subject develops CRS, all standard of care will be offered to assure subject wellbeing including treatment with steroids. According to the protocol, the serum cytokine levels will be tested from subjects' blood draws pre-infusion (baseline), and 20min and 2h post-infusion; however, in case of a CRS, blood will be collected as soon as possible and sent to the TCSL for real time cytokine analysis. Our experience to date on other research protocols treating oncologic subjects with CAR modified T-cells has indicated that CRS is associated with high levels of serum IL-6 among other cytokines. Consequently, some of our subjects developing CRS have been offered Tocilizumab as treatment for CRS following laboratory confirmation of elevated levels of serum IL-6 post-infusion. This approach has proved successful; however it is at the discretion of the clinical PI as to its usage.

If a subject develops CRS prior to initiating treatment interruption the subject will not undergo ATI.

- Sustained (confirmed) virologic failure without an alternative explanation:

  Before ATI: If prior to initiating treatment interruption the subject experiences an increase in viral load from undetectable to greater than or equal to 5000 copies/ml, a viral load test will be repeated once a week for two additional weeks. If the viral load increase is sustained over this period, this event will be reported as an SAE and will be managed as medically appropriate, and the subject will not undergo ATI.

  During ATI: If the subject experiences a viral load ≥100,000 copies/ml, this test will be repeated every week for up to 3 weeks. If the subject experiences a sustained viral load to ≥100,000 copies/ml over a period of 3 weeks, it will be recommended to the subject that HAART should be reinstated. The HAART will not be provided by the study. If the viral load is not sustained (i.e. as indicated by a single test <100,000 copies/ml), the subject will be advised not to reinstate HAART until week 16 after the treatment interruption to allow for evaluation of the subject's viral load set point.

- Sustained (confirmed) decrease in CD4+ T-cell count:

  If a subject experiences a decrease in CD4+ T-cell count to ≤350 cells/mm^3, the test will be repeated the following week. If the CD4+ T-cell counts stay equal to or below 350 cells/mm^3, the subject will be advised to reinstitute HAART. The HAART will not be provided by the study. If the CD4+ decrease is not sustained the subject will continue in the study.

- Monitoring for C34-CXCR4-Mediated Clonal Outgrowth
  Patients will be monitored for total lymphocyte count at each study visit. If a patient experiences a total lymphocyte count greater than 10,000, then their lymphocytes will be analyzed for monoclonality by Vβ TCR analysis and for C34-CXCR4 marking by flow cytometry and/or PCR. If monoclonality is observed and the cells are confirmed to be modified by C34-CXCR4, then best attempts will be made to determine the etiology of the cancer and the relationship to the treatment (C34-CXCR4-T cells). In parallel, the patient will be referred to a hematologist for a leukemia/lymphoma work-up.

- Monitoring for aberrant T cell trafficking
  Patients will be monitored for any new incidence of pulmonary, cardiovascular, or gastrointestinal disorders (considered to be atypical) following the infusion of autologous C34-CXCR4 gene-modified cells. The C34-CXCR4 transgene that will be expressed in autologous CD4 T cells under control of the lentivirus promoter does retain competence to mediate migration of cells (i.e., chemotaxis) in response to the chemokine ligand (CXCL12) for CXCR4. Thus, any new clinical problem in which organ infiltration by lymphocytes could be taking place will be thoroughly investigated.

7.2 Criteria for pausing or stopping the study

The study will be paused if:

- There are excessive or unexpected toxicities associated with the protocol. Specifically, we will pause the study and re-evaluate the approach if it is determined that two or more of the subjects have one of the following: ≥ grade 3 toxicity determined by using the DAIDS Clinical Trial Toxicity Criteria of November 2014. SAEs will be reported to the DSMB in accordance to the DSMB Charter.

- A patient experiences an absolute lymphocyte count greater than 10,000 during visits spaced 1 week apart, until the evaluation of the nature of the lymphocyte increase is determined. If it is found to be unrelated to the study treatment, then the study will resume.

- If a positive VSV-G DNA signal is detected and confirmed by a second PCR test (taken from a fresh blood sample). The study will be paused and no new patients will be infused with the study drug until the patient who has the positive test is evaluated for RCL. If an RCL is confirmed the study will be stopped. If an RCL is not confirmed, the study can be restarted after the source of the positive signal in the VSV-G DNA test is understood by the investigators

The study will be stopped if:

- The Investigator, Study Funder, Sponsor or any independent review board or regulatory body decides for any reason that subject safety may be compromised by continuing the study.

- The Sponsor or Study Funder decides to discontinue the development of the intervention to be used in this study.

- An analysis of clonal outgrowth of T-cells determines that it is a result of C34-CXCR4 lentiviral-mediated oncogenesis.

- Any subject develops an increase from baseline in viral load to > 5000 and is sustained for at least 3 weeks during Step 2 of the protocol, and has no alternative explanation (such as non-compliance) other than the study treatment.

- Any subject develops a decrease in CD4 T-cell count of 350 or below which is sustained for at least 2 weeks during Step 2 of the protocol, and has no alternative explanation (such as non-compliance) other than the study treatment.

Futility assessment has been included in the responsibilities of the DSMB. Futility will be assessed at each DSMB meeting and will be evaluated using guidelines stated in the DSMB charter.

If stopping rules are invoked, accrual of additional subjects to the study will be precluded.

7.3 Special Procedures for Subjects Developing Cancer or Dying

For subjects who develop cancer and who have previously given their consent, an attempt will be made to obtain a sample of the biopsy of the cancerous tissue and a blood sample will be obtained for evaluation.

For subjects who die and who have previously given their consent, the subject's family will be asked to consent to an autopsy procedure in order to obtain appropriate tissue samples for evaluation during a scheduled autopsy.

7.4 Pregnancies

It is not known whether the study intervention carries increased risks for pregnant women or their unborn children. Therefore, pregnant women cannot participate in the study. Women should be informed through the informed consent process that if, while in the study, they become pregnant or miss a menstrual period or for other reasons suspect that they might be pregnant, they should immediately notify the investigator who should then notify the regulatory sponsor. If the subject is confirmed to be pregnant, the investigator will immediately discontinue the subject from the study treatment. Counseling will also be provided to the women to help them decide how to proceed.

To ensure patient safety, each pregnancy occurring while the patient is on study treatment must be reported to the protocol sponsor within 24 hours of learning of its occurrence. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications.

Pregnancy should be recorded on a Clinical Trial Pregnancy Form and reported by the investigator to the protocol sponsor. Pregnancy follow-up should be recorded on the same form and should include an assessment of the possible relationship to the study drug for any pregnancy outcome. Any SAE experienced during pregnancy must be reported on the SAE Report form.

Pregnancy outcomes must be collected for the female partners of any males who took study treatment in this trial. Consent to report information regarding these pregnancy outcomes should be obtained from the mother.

Pregnancies will also be reported to the Antiretroviral Pregnancy Registry.

7.5 Long-Term Follow-up

Long-term follow-up for this study will be conducted under a separate long-term follow-up protocol. This destination protocol describes long term follow up of patients for up to 15 years in accordance with guidelines set forth by the FDA. At the time of informed consent for this protocol, subjects will be made aware of the long term follow-up requirement.

7.6 RCL testing, patient monitoring, and procedure for dealing with positive test results in subjects

RCL may be generated during the C34-CXCR4-T cell manufacturing phase or subsequently after introduction of vector transduced cells into the patient. However, an RCL resulting from the production phase is highly unlikely since elements are incorporated in the design of the vector system that minimize vector recombination and generation of RCL. Furthermore, the vector used to transduce the product undergoes sensitive assays for detection of RCL before it can be released to a patient. Nevertheless, generation of an RCL following infusion of the vector product remains a theoretical possibility. For ongoing patient monitoring for RCL, PBMC samples obtained from subjects at 3, 6, and 12 months post-treatment will be tested for VSV-G DNA sequences.

If a positive VSV-G DNA signal is obtained, the PI will be informed and the patient will be scheduled for a retest. If the second test is positive, the patient with the confirmed positive VSV-G signal will be scheduled for apheresis and a biological RCL will be performed on the apheresed product. If the biological RCL is positive, the study will be stopped. If the test is negative, infusions for all patients can resume.

8.0 STATISTICAL CONSIDERATIONS

8.1 General Design Issues

This is a single cohort, open-label pilot study of the safety and antiviral activity of a single infusion of autologous CD4+ T-cells in HIV-infected subjects. Participants will have well controlled viral replication on HAART. Participants will be HIV-1-positive men and women $\geq$ 18 years of age with HIV-1-RNA levels ≤50 copies/ml for at least 2 years who have peripheral CD4+ T-cell counts ≥450 cells/mm^3 and a documented CD4 nadir of not lower than 200 cells/mm^3, and who have a recorded viral load set point prior to starting therapy. Following a leukapheresis at Step 1 (study enrollment), eligible subjects will enroll into Step 2 of the study and receive one infusion of thawed, autologous T-cells genetically modified by lentiviral vector expressing the C34-peptide conjugated to the CXCR4 N-terminus. All subjects will undergo a 16 week analytical treatment interruption starting at week 4 (Step 3).

At enrollment to Step 1, subjects will undergo 2 leukaphereses and an optional rectal biopsy. Within ~15 weeks after enrollment to Step 1, subjects will enter Step 2 and receive one infusion

of thawed autologous CD4+ T-cells. The cells will be administered at entry to Step 2. Intensive follow-up will continue up to 12 months.

8.2 Primary Objective Analysis

The primary objective of this study is to evaluate the safety and tolerability of a single infusion of 0.8-x10^9-1 x 10^{10} autologous CD4+ T-cells Genetically Modified to express C34-CXCR4 in HIV-1-positive subjects. The primary analysis of safety will be conducted after the completion of all subjects' Step 2 Day 28 visits and follow-up. Primary safety analysis will be conducted, and monitoring for delayed adverse events that may be associated with the genetic modification will be continued through long term follow-up. The primary analysis of safety will summarize the distribution of the number of subjects who experience treatment related adverse events (AE). A summary of all reported signs/symptoms and laboratories at any grade will be produced. For each type of event, 95% confidence intervals will also be produced where possible.

Each dose level is designed to enroll 3 subjects, with a maximum of 9 subjects total for the study across all dose levels. With 3 similarly dosed evaluable subjects in, we can be 95% confident that the true adverse event rate is less than 71% in the dose level if no adverse events are observed at that dose. The **Table 8.2-1** below shows exact 95% confidence intervals for possible number of observed safety outcomes in three, six, and nine subjects. For example, if no adverse events are observed in a group of six evaluable subjects, we can be 95% confident that the true adverse event rate is less than 45.9%. The true adverse event rate is no more than 33.6% if we observe no adverse events with nine evaluable subjects.

Table 8.2-1: Exact 95% Confidence Interval
Shown are upper and lower limits of the 95% CI

# Observed Adverse Events	N=3	N=6	N=9
0	(0.0%, 70.8%)	(0.0%, 45.9%)	(0.0, 33.6)
1	(0.8%, 90.6%)	(0.4%, 64.1%)	(0.3, 48.2)
2	(9.4%, 99.2%)	(4.3%, 77.7%)	(2.8, 60.0)
3	(29.2%, 100.0%)	(11.8%, 88.2%)	(7.5, 70.1)
4	–	(22.3%, 95.7%)	(13.7,78.8)
5	–	(35.9%, 99.6%)	(21.2, 86.3)
6	–	(54.1%, 100.0%)	(29.9, 92.5)
7	–	–	(40.0, 97.2)
8	–	–	(51.8, 99.7)
9	–	–	(66.4, 1.0)

Table 8.2-2 provides the probability of failing to observe an adverse event in a sample size of three evaluable subjects, for various underlying true adverse event rates. For example, if the true underlying AE rate is 60%, there is only a 6% chance of observing no events in 3 subjects. Thus, with three evaluable subjects, we can be reasonably assured of observing an adverse event that occurs in the population at a rate of at least 60%.

Table 8.2-2: Probability of Failing to Observe an Adverse Event			
True Adverse Event Rate	**N=3**	**N=6**	**N=9**
5%	86%	74%	63%
10%	73%	53%	39%
20%	51%	26%	13%
30%	34%	12%	4%
40%	22%	5%	1%
50%	13%	2%	0%
60%	6%	0%	0%
70%	3%	0%	0%

8.3 Secondary Objectives Analysis

Secondary objectives include evaluation of enrichment and persistence of C34-CXCR4-modified autologous T-cells, and the effects of C34-CXCR4-modified CD4 cells on CD4 T-cells counts, HIV specific immunity, viral load, and immunogenicity of C34-CXCR4-modified cells. Of particular interest, are any appreciable differences observed between dose levels of C34-CXCR4-modified CD4 cells.

There is limited statistical power to evaluate efficacy and related biological endpoints. Therefore, analysis will be primarily descriptive. Descriptive statistics including mean, standard deviation, median, and range will be provided for all secondary endpoints. Correlation between duration of engraftment of modified cells and viremia, and CD4 counts will be calculated as Pearson's correlation coefficient or Spearman's correlation if the normality assumption does not hold. The within-subject changes before and after infusion will be tested by a paired t-test. When evaluating within-subject change, an evaluable sample size of three, subjects will provide 80% power at a two-sided significance level of 5%, to identify an effect size of 3.3 in the unit of standard deviations of differences for normally distributed outcomes using the paired t-test. The pattern of persistence of transgene over time will be summarized and compared between subjects with differing levels of viremia based on a repeated measures one-way analysis of variance model.

To evaluate the effects of C34-CXCR4-modified CD4 cells on CD4+ T-cell count, summary statistics for time points at baseline and after the infusion will be shown. We will summarize the changes between baseline CD4+ T-cell count (defined as the average of the Step 1 and 2 values) and the average of two consecutive CD4+ T-cell count values after dosing at 8 and 12 weeks or just prior to initiation of a new drug regimen, or at weeks 16 and 20.

To evaluate the effects of C34-CXCR4-modified CD4 cells on viral load, we will summarize the changes in $\log_{10}$ HIV-1 RNA level from set point before the initiation of antiretroviral therapy to average viral load of weeks 12 and 16 after initiation of treatment interruption (week 16 and 20 of the study). To evaluate the time and the frequency to recrudescence to >200 copies/ml viral load, summary statistics at different time points after the analytical treatment interruption will be described.

To examine the persistence of C34-CXCR4-modified autologous T-cells in peripheral blood and gut mucosae, and evaluate the preferential expansion of modified cells during the analytical treatment interruption, we will summarize the proportion of circulating CD4+ T-cells that are

modified by C34-CXCR4 throughout the duration of the study and attempt to correlate with viremia values during the analytical treatment interruption.

To evaluate the effects on immune function, we will compare the percent of CD4+ and CD8+ T-cells that secrete cytokines as a response to stimulation by HIV-specific or other viral antigens (Flu, EBV, CMV) at baseline (defined as the average of the Step 1 and 2 values) and after the infusion of genetically modified CD4+ T-cells. Summary statistics for time points at baseline and after the infusion will be shown. Additionally, proliferative capacity will also be assessed.

The assay for CTL Phenotyping will employ a 6-hour *in vitro* stimulation of unfractionated patient PBMCs using antigens as described above. Specifically, the CTL Phenotyping assay will examine the following external cellular markers: CD3, CD4, CD8 (T cell identification), CD45RO, CD27, CCR7 (memory subset identification), CD107a, (marker of lytic degranulation and cytotoxic potential). The CTL Phenotyping assay will additionally analyze the following intracellular markers: Interferon Gamma (Th1 biasing cytokine), Tumor Necrosis Factor Alpha, Perforin (proteins involved in lytic degranulation and cytotoxic potential).

The ability to proliferate in response to a specific antigen is a central part of immune assessment. We will utilize carboxyfluorescein diacetate succinimidyl ester (CFSE), and surface staining for CD4 and CD8 to monitor T cell proliferative responses to antigen-specific stimulation.

Statistics
The immune response comparisons will be made using descriptive statistics and graphical methods for both phases of the study. Immune response measures will be correlated with dose level/treatment arm and participant outcome (i.e., adverse events) and with each other as well. Descriptive statistics and simple scatter plots will be generated to review the continuous immune response data. In addition, for continuous immune response values, the actual and % change in the level of each of the biomarkers from baseline to post-baseline time points will be explored within each dose level/arm using Wilcoxon signed rank tests, and paired sample t-tests. All categorical variables will be analyzed using chi-square tests or Fisher's exact test (the latter if warranted). Confidence intervals for both continuous and categorical data will be provided as well, as appropriate. For all translational endpoints, any notable statistical result will be viewed as an impetus for further study rather than as a definitive finding in and of itself.

To evaluate immunogenicity of C34-CXCR4-modified CD4 T-cells, the ability of modified cells to persist, protect CD4 cell decay and control viremia as described above will be important indicators. Additionally, the development of a humoral anti-C34-CXCR4 response post-infusion will also be monitored as a marker of immunogenicity.

8.4 Sample Size and Accrual

This is a pilot study with one cohort that completes infusions of C34-CXCR4-modified CD4 cells (Step 2) and undergoes an analytical treatment interruption (Step 3). In order to have an evaluable sample size, any subject who prematurely discontinues the study prior to the infusion will be replaced with another subject. Subjects who discontinue ATI prior to completion of the 16-week ATI duration will not be replaced.

Accrual is anticipated to take up to 72 weeks.

9.0 DATA COLLECTION, MONITORING, AND ADVERSE EVENT REPORTING

9.1 Records to Be Kept

Electronic CRFs will be provided for each subject. Subjects must not be identified by name on any eCRFs. Subjects will be identified by the subject identification number.

9.2 Role of Data Management

Instructions concerning the recording of study data on eCRFs will be provided by the sponsor (eCRF Completion Guidelines).

It is the responsibility of the principal investigator to assure the quality of computerized data for the study. This role extends to generation of the final study databases.

9.3 Clinical Site Monitoring and Record Availability

This study will be monitored according to the Sponsor Data and Safety Monitoring Plan.

Interim Monitoring Visits will be conducted during the course of the study. The Monitors will assure that submitted data are accurate and in agreement with source documentation; verify that investigational products are properly stored and accounted for; verify that subject consent for study participation has been properly obtained and documented; confirm that research subjects entered into the study meet inclusion and exclusion criteria; and assure that all essential documentation required by Good Clinical Practices (GCP) guidelines are appropriately filed. At the end of the study, Monitors will conduct a close-out visit and will advise on storage of study records and disposition of unused investigational products.

The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities and has adequate space to conduct the monitoring visit.

<u>Independent Data and Safety Monitoring Board (DSMB)</u>
An Independent DSMB comprised of at least three individuals including physicians with experience in HIV infection and/or gene transfer therapy will be assembled and will work under a charter specifically developed for safety oversight of this study. They will provide advice to the Sponsor. The DSMB will evaluate subject-subject safety as specified in the DSMB Charter.

The initial responsibility of the DSMB will be to approve the initiation of this clinical trial. Thereafter, the DSMB will meet approximately every 6 months. If necessary, additional meeting of the DSMB may be held if safety issues arise in between scheduled meetings.

Meetings will also be convened in the event of any DLT, or any grade 3 or higher serious adverse event that is possibly related to the study drug and unexpected. These meetings can be initiated by the Sponsor, PI of the study, the protocol team, or the DAIDS Medical Officer to address specific safety issues and/or pausing of dosing and/or stopping the study.

If a Grade 4 event or death is observed that is determined to be at least possibly related to study treatment (based on the core team assessment), accrual will be halted and the DSMB will convene to review the study data.

A sponsor representative will share the outcome of the DSMB meeting with the PI via email for submission to the IRB and other local regulatory review committees per institutional requirements.

9.4 Safety and Adverse Events

9.4.1 Definitions

Adverse Event
An ***adverse event*** (AE) is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. Intercurrent illnesses or injuries should be regarded as adverse events.

Serious Adverse Event
Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay:
- leads to a persistent or significant disability or incapacity or substantial disruption of the ability to conduct normal life functions
- a congenital anomaly or birth defect
- an important medical event

Note that hospitalizations that meet the following criteria should not be reported as serious adverse events:

- Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, such as preplanned study visits and preplanned hospitalizations for study procedures or treatment administration
- Elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the informed consent
- Social reasons and respite care in the absence of any deterioration in the patient's general condition

Note that treatment on an emergency outpatient basis that does not result in hospital admission and involves an event not fulfilling any of the definitions of a SAE given above is not a serious adverse event.

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the patient, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

Unexpected adverse events

An adverse event is considered unexpected if the event severity and/or frequency is not described in the investigator brochure or protocol. As this investigational product is first in humans the severity/frequency of events are not yet known, an investigator brochure is not available.

Related adverse events

An adverse event is considered related to participation in the research if there is a reasonable possibility that an event was caused by an investigational product, intervention, or research-required procedures. For the purposes of this study, "reasonable possibility" means there is evidence to suggest a causal relationship.

Dose Limiting Toxicity (DLT)

Dose limiting toxicity is a grade 3 or higher new AE within 21 days which is at least possibly related to study treatment and not manageable or reversible. A formal DLT assessment will be performed prior to dose level advancement or expansion.

Adverse Event Reporting Period

Adverse events will be collected and reported from the time of informed consent to the completion of the scheduled study visits following the administration of study treatment.

If a subject is taken off study within 30 days of receiving C34-CXCR4-modified autologous T-cells, all SAEs experienced within 30 days after the T-cell infusion should be reported to the sponsor. Any SAEs experienced after this 30 day period should be reported to the sponsor if the investigator suspects a causal relationship to the study treatment.

Preexisting Condition/General Physical Examination Findings

A preexisting condition is one that is present at the start of the study. At screening, any clinically significant abnormality should be recorded as a preexisting condition on the medical history eCRF. During the course of the study, a preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens. Preexisting conditions that improve should also be recorded appropriately.

Abnormal Laboratory Values

A clinical laboratory abnormality should be documented as an adverse event if any one of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; (e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.)

Laboratory abnormalities that meet the criteria for Adverse Events should be followed until they have returned to normal or an adequate explanation of the abnormality is found. When an abnormal laboratory or test result corresponds to a sign/symptom of an already reported adverse event, it is not necessary to separately record the lab/test result as an additional event. Laboratory abnormalities that do not meet the definition of an adverse event, should not be

reported as adverse events. A Grade 3 or 4 event (severe) as per the DAIDS AE Grading Table Version 2.0 does not automatically indicate a SAE unless it meets the definition of serious defined above and/or as per investigator's discretion. Whenever possible, a diagnosis, rather than a symptom should be provided (i.e. anemia instead of low hemoglobin).

9.4.2 Recording of Adverse Events

Safety will be assessed by monitoring and recording potential adverse effects of the treatment using the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0, November 2014. This table can be found at:
http://rsc.tech-res.com/docs/default-source/safety/daids_ae_grading_table_v2_nov2014.pdf

Patients will be monitored by medical histories, physical examinations, and blood studies to detect potential toxicities from the treatment. If grading does not exist for an adverse event, the severity of mild, moderate, severe, life-threatening, and death, corresponding to Grades 1-5, will be used whenever possible.

At each contact with the subject, the investigator must seek information on adverse events by non-directive questioning and, as appropriate, by examination. Adverse events also may be detected when they are volunteered by the subject during the screening process or between visits, or through physical examination, laboratory test, or other assessments. Information on all adverse events should be recorded in the source documentation. All clearly related signs, symptoms, and abnormal diagnostic procedure results should be recorded in the source document, though should be grouped under one diagnosis. To the extent possible, adverse events should be recorded as a diagnosis and symptoms used to make the diagnosis recorded within the diagnosis event. Do not list symptoms separately if a diagnosis can be assigned.

All adverse events occurring during the adverse event reporting period (defined in Section 9.4.1) must be recorded.

As much as possible, each adverse event should be evaluated to determine:

1. The severity grade (Grade 1-5)
2. Its duration (Start and end dates)
3. Its relationship to the study treatment- [Reasonable possibility that AE is related: No (unrelated/ not suspected) or Yes (a suspected adverse reaction)]. If yes (suspected)- is the event possibly, probably or definitely related to the investigational treatment?
4. Expectedness to study treatment- [Unexpected- if the event severity and/or frequency is not described in the investigator brochure (if applicable) or protocol].
5. Action taken with respect to study or investigational treatment (none, dose adjusted, temporarily interrupted, permanently discontinued, unknown, not applicable)
6. Whether medication or therapy taken (no concomitant medication/non-drug therapy, concomitant medication/non-drug therapy)
7. Whether it is serious, where a serious adverse event (SAE) is defined as in **Section 9.4.1**.

All adverse events should be treated appropriately. If a concomitant medication or non-drug therapy is given, this action should be recorded. Once an adverse event is detected, it should be followed until its resolution or until it is judged to be permanent, and assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the study treatment, the interventions required to treat it, and the outcome.

Serious adverse events that are still ongoing at the end of the adverse event reporting period must be followed to determine the final outcome. Any serious adverse event that occurs after the adverse event reporting period and is considered to be possibly related to the study treatment or study participation, should be recorded and reported.

9.4.3 Reporting of Serious Adverse Events

Every SAE, **regardless of suspected causality**, occurring during the adverse event reporting period defined in **Section 9.4.1** must be reported to the sponsor within 24 hours of learning of its occurrence. The original SAE notification may take place by email to meet the 24 hour reporting window. However within 3 business days of knowledge of the event, the investigator must submit a complete SAE form to the Sponsor along with any other diagnostic information that will assist the understanding of the event. The Investigator will keep a copy of this SAE Form on file at the study site.

Follow-up information on SAEs should be reported when updates are available, as a follow-up to the initial SAE form, and should include both the follow-up number and report date. New information on ongoing serious adverse events should be provided promptly to the sponsor. The follow-up information should describe whether the event has resolved or continues, if there are any changes in assessment, if and how it was treated, and whether the patient continued or withdrew from study participation.

Report serious adverse events by email to:

Attention: Sponsor Clinical Safety Manager or designee
[redacted]

At the time of the initial notification, the following information should be provided:

1. Study identifier
2. Subject number
3. A description of the event
4. Date of onset
5. Current status
6. Whether study treatment was discontinued
7. The reason the event is classified as serious
8. Investigator assessment of the association between the event and study treatment
9. Expectedness relative to investigational product(s)

9.4.4 Investigator Reporting: Local Regulatory Review Committees

Report events to local regulatory review committees per institutional requirements.

9.4.5 Sponsor reporting: Notifying the FDA

The study sponsor is required to report certain study events in an expedited fashion to the FDA. These written notifications of adverse events are referred to as IND safety reports. The following describes the safety reporting requirements by timeline for reporting an associated type of event:

- ***Within 7 calendar days***
  Any study event that is:
  - associated with the use of the study drug
  - unexpected, and

– fatal or life-threatening

- ***Within 15 calendar days***
  Any study event that is:
  – associated with the use of the study drug,
  – unexpected, and
  – serious, but not fatal or life-threatening
  -or-
  – a previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting (reporting within 15 calendar days from when event was deemed reportable).

  Any finding from tests in laboratory animals that:
  – suggest a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

Additional reporting requirements
Sponsors are also required to identify in IND safety reports all previous reports concerning similar adverse events and to analyze the significance of the current event in light of the previous reports.

9.4.6 Reporting to the IBC

Notify the Institutional Biosafety Committee of serious adverse events according to institutional requirements.

9.4.7 Reporting to the Division of AIDS

The DAIDS Medical Officer and Project Officer will receive copies of all serious adverse drug reactions (SADRs) and all expedited adverse events (EAEs) from the Sponsor. They should also receive study progress updates and be informed of all unanticipated problems involving risk to subjects or others, as well as quarterly reports of all adverse events (AEs) from the Sponsor.

9.5 Protocol Exceptions and Deviations

Exception
A one time, **intentional** action or process that departs from the IRB approved study protocol, intended for **one** occurrence. If the action disrupts the study progress, such that the study design or outcome (endpoints) may be compromised, or the action compromises the safety and welfare of study subjects, **advance** documented approval from the Regulatory Sponsor and other local regulatory review committees per institutional guidelines is required. Approval from the Regulatory Sponsor must be received prior to submission to local regulatory review committees for approval.

Deviation
A one time, **unintentional** action or process that departs from the IRB approved study protocol, involving one incident and **identified retrospectively**, after the event occurred. If the impact on the protocol disrupts the study design, may affect the outcome (endpoints) or compromises the safety and welfare of the subjects, the deviation must be reported to the Regulatory Sponsor within 10 business days of PI knowledge and to local regulatory review committees per institutional guidelines. Acknowledgement from the Regulatory Sponsor must be received prior to submission to local regulatory review committees.

Other deviations that do not meet the reporting criteria above should be documented in a memo to file or on a deviation log.

Include the following information on the Sponsor supplied exception/deviation form: protocol number, subject study number, description of protocol deviation or exception request and rationale. Ensure all completed exception/deviation forms are signed by the Principal Investigator (or sub-investigator) and submitted to the Sponsor Project Manager for review.

Attention: Sponsor Project Manager
[redacted]

Once approval of the exception request or acknowledgement of the deviation, has been granted by the Regulatory Sponsor, the exception or deviation will be submitted to all other applicable committees for review and approval as required per institutional guidelines.

10.0 HUMAN SUBJECTS

10.1 Institutional Review Board (IRB) Review and Informed Consent

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the IRB, IBC or ethics committee responsible for oversight of the study. A signed consent form will be obtained from the subject. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the subject and this fact will be documented in the subject's record.

10.2 Subject Confidentiality

All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified by coded number only to maintain subject confidentiality. All records will be kept locked. All computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring/auditing activities.

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

10.3 Source Documents

Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

10.4 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. All entries will be entered into an electronic data capture system (EDC) via Oracle. The Principal Investigator is responsible for assuring that the data entered into eCRF is complete, accurate, and that entry and updates are performed in a timely manner.

10.5 Records Retention

It is the investigator's responsibility to retain study essential documents for at least 2 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in their country or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period if required by an agreement with the sponsor. In such an instance, it is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

10.6 Study Discontinuation

The study may be discontinued at any time by the Sponsor, IRB, NIAID, DAIDS, the financial supporter, the FDA, OCR or other government agencies as part of their duties to ensure that research subjects are protected.

11.0 STUDY MONITORING, AUDITING, AND INSPECTING

11.1 Study Monitoring Plan

This study will be monitored according to the Sponsor Data and Safety Monitoring Plan.

Interim Monitoring Visits will be conducted during the course of the study. The Monitors will assure that submitted data are accurate and in agreement with source documentation; verify that investigational products are properly stored and accounted for, verify that subjects' consent for study participation has been properly obtained and documented, confirm that research subjects entered into the study meet inclusion and exclusion criteria, and assure that all essential documentation required by Good Clinical Practices (GCP) guidelines are appropriately filed.

At the end of the study, Monitors will conduct a close-out visit and will advise on storage of study records and disposition of unused investigational products.

The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

11.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, government regulatory bodies, and University compliance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance offices.

The Principal Investigator must notify the Sponsor in real-time if an audit/inspection notification is received.

12.0 ETHICAL CONSIDERATIONS

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject and by the investigator-designated research professional obtaining the consent.

12.1 Rationale for Exposure to Risks

As this is a Pilot study to establish safety and tolerability, there are no direct benefits expected for study participants. There are, however, some risks associated with C34-CXCR4 modified T-cells.

Risks associated with C34-CXCR4-modified cells include:

- Chills and fever
- Headache, myalgia, arthralgia
- Increase in blood pressure
- Low heart rate
- Allergic reaction (itching, swelling of the tongue)
- Seizures
- Nausea and vomiting
- Injection site reactions such as bruising, swelling, black and blue marks, fainting and/or infection at the site
- A decrease in hemoglobin and hematocrit (red blood cell number)
- Worsening of HIV infection (increase in HIV-1 viral load or decrease in T-cell count)
- Exclusion from future gene therapy or vaccine trials as a result of participation in this study.
- Possible restriction from future HIV trials based on the timing of HIV viral rebound in this study.
- Antibody formation that could lead to allergic reaction (skin rash, itching, fever)

There is a theoretical risk of blood cancer in gene therapy studies which use integrating vectors. However, to date, no malignancies have been identified with the lentiviral vector used in this study.

In an effort to minimize the above-mentioned risks, patients will be closely monitored, have frequent visits, and have supportive care. Acetaminophen and Diphenhydramine hydrochloride will be used to prevent side effects (flu-like symptoms) associated with infusion of the C34-CXCR4-modified cells.

Although there are risks to the individual subject, as outlined above, the majority of the side effects associated with autologous T-cell infusions are transient, spontaneously solved in 24-48 hours, or can be managed medically. There is a great potential for benefit to both general scientific understanding and knowledge, and to society on the whole. The individuals who participate in the completion of this study will potentially provide new and important information on the safety of controlling HIV through co-receptor modification. Because both quality of life and economic burden on the health care system would be improved by obviating the need for patients with HIV infection to take daily and life-long antiviral medication, one goal for the future is to build an HIV-reisistant immune system. This study will be a step along the path to reaching that goal, and will further support the concept of using a cell-based therapy to advance work towards improving HIV control/cure.

13.0 STUDY FINANCES

13.1 Funding Source

This study is financed through a U19 grant awarded by the NIH NIAID.

13.2 Conflict of Interest

All University of Pennsylvania Investigators will follow the University of Pennsylvania Policy on Conflicts of Interest Related to Research.

13.3 Subject Stipends or Payments

Subjects will be compensated for their time in participation of the study. Compensation will be paid via ClinCard (a secure, reloadable debit card). Compensation for the apheresis procedure is commensurate with that offered in other trials. The reimbursement will be $25 per study visit, with the following exceptions:

STEP 1
Completion of Apheresis 1.....$75
Completion of Apheresis 2.....$75
Completion of baseline rectal biopsy.....$75

STEP 2
Completion of Infusion $75
Completion of rectal biopsy/leukapheresis Day 28.....$75 each (total $150)

STEP 3
Completion of rectal biopsy/leukapheresis week 20.....$75 each (total $150)

STEP 5
Completion of rectal biopsy/leukapheresis Week 52.....$75 each (total $150)

Estimated Total compensation for the trial.....**$1150**

14.0 BIOHAZARD CONTAINMENT

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the Centers for Disease Control and Prevention and the National Institutes of Health.

All dangerous goods materials, including diagnostic specimens and infectious substances, must be transported according to the instructions detailed in the International Air Transport Association (IATA) Dangerous Goods Regulations.

15.0 PUBLICATION PLAN

Publication of the results of this trial will be governed by University of Pennsylvania policies. Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor. Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study.

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