AN ADAPTIVE PHASE I/II STUDY OF THE SAFETY OF CD4+ T LYMPHOCYTES AND CD34+ HEMATOPOIETIC STEM/PROGENITOR CELLS TRANSDUCED WITH LVsh5/C46, A DUAL ANTI-HIV GENE TRANSFER CONSTRUCT, WITH AND WITHOUT CONDITIONING WITH BUSULFAN IN HIV-1 INFECTED ADULTS PREVIOUSLY EXPOSED TO ART

CLINICAL PROTOCOL CAL-USA-11

Sponsor*: Calimmune, Inc.

* Calimmune is a global organization that operates as Calimmune Australia Pty Ltd: Level 8, Lowy Packer Building 405 Liverpool Street, Darlinghurst NSW 2010, Sydney, Australia and Calimmune, Inc: 5151 E. Broadway Blvd., Suite 1020, Tucson, AZ 85711, USA. The term "Sponsor" is used throughout the protocol to represent both these legal entities, collectively known as Calimmune.

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Integration analysis	PPD	PPD	PPD
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Note: If the name, address or contact details of any of the above representatives changes during the course of the study, this section will be updated, without the requirement for a protocol amendment.

PROTOCOL SIGNATURE PAGE

SPONSOR REPRESENTATIVE

Name: PPD Signature: PPD Date: PPD

Title: PPD

PRINCIPAL (SITE) INVESTIGATOR

I have read this protocol and agree that it contains all necessary details for carrying out this study. I agree to conduct this clinical study in accordance with the design and specific provisions of this protocol. No changes will be made to the study protocol without the prior written approval of the Sponsor and the Institutional Ethics Committee. I understand that the study will not be started without the prior written approval of my Institutional Ethics and Biosafety Committees.

I understand and accept my responsibilities to ensure the adequate experience and training of all individuals responsible to me who assist in the conduct of this study. I will provide them copies of the protocol and all other pertinent information, and discuss this material with them to ensure that they are fully informed regarding the study drug, the conduct of the study, and the associated obligations of confidentiality.

Name: ..... Signature: ..... Date: .....

Institution name:.....

Address:.....

.....

Telephone:.....

STATEMENT OF COMPLIANCE

This document is a protocol for a clinical research study. This study will be conducted under U.S Food & Drug Administration IND regulations (21 CFR Part 312). The study will be conducted in compliance with this protocol, Good Clinical Practice, and applicable regulatory requirements and/or conditions of the applicable governing ethics committee(s) approval.

GLOSSARY OF ABBREVIATIONS

AE	Adverse event
ALT (SGPT)	Alanine aminotransferase
ANC	Absolute neutrophil count
AST (SGOT)	Aspartate aminotransferase
ART	Antiretroviral Therapy
BD	Twice daily
BP	Blood pressure
Cal-1	LVsh5/C46, a Dual Anti-HIV Gene Transfer Construct
CRO	Contract Research Organization
CRF	Case report form
DNA	Deoxyribonucleic acid
DSMB	Data Safety Monitoring Board
FBC	Full blood count
FDA	Food and Drug Administration
GALT	Gut-associated lymphoid tissue
GCP	Good clinical practice
GGT	Gamma-glutamyl transferase
GMP	Good manufacturing practice
G-CSF	Granulocyte colony-stimulating factor
HIV-1	Human immunodeficiency virus type 1
HSPC	CD34+ Hematopoietic stem/progenitor cells
$HSPC^{tn}$	Cal-1 transduced CD34+ Hematopoietic stem/progenitor cells
IB	Investigator's brochure
ICH	International Conference on Harmonization
IND	Investigational new drug
LLN	Lower limit of normal
MedDRA	Medical Dictionary for Regulatory Activities
MRC	Medical Review Committee
NSAID	Non-steroidal anti-inflammatory drugs
PBMC	Peripheral blood mononuclear cells
PICF	Participant informed consent form
QD	Once daily
SAE	Serious adverse event
SOP	Standard operating procedure
SUSAR	Serious and Unexpected Suspected Adverse Reaction
T^{tn}	Cal-1 transduced CD4+ T lymphocytes
ULN	Upper limit of normal
WBC	White blood count

SYNOPSIS

Title: An adaptive Phase I/II study of the safety of CD4+ T lymphocytes and CD34+ hematopoietic stem/progenitor cells transduced with LVsh5/C46, a dual anti-HIV gene transfer construct, with and without conditioning with busulfan in HIV-1 infected adults previously exposed to ART.

BB-IND number: CCI

RAC number: *1110-1130*

Public Registration: ClinicalTrials.gov (NCT01734850)

Sponsor: Calimmune, Inc.

Indication: Adult individuals with HIV-1 infection, who have previously received ART, but are not currently on any antiretroviral medication.

Participating country(s): USA

Anticipated number of sites: 2

Anticipated number of subjects: up to 12

BACKGROUND

LVsh5/C46 (hereafter referred to as Cal-1) is a dual therapeutic, self-inactivating lentiviral vector that inhibits two processes required for HIV-1 infection: 1) Binding of the virus to the cellular CCR5 co-receptor and 2) Fusion of the virus with the host cell. The vector encodes for both a short hairpin RNA against the HIV-1 co-receptor CCR5 (sh5) (*An et al, 2006)* and a HIV-1 fusion inhibitor, C46, (*Egelhofer et al, 2004*). The two active anti-HIV-1 agents in the vector (sh5 and C46) have been shown individually to be effective in inhibiting HIV-1 replication and decreasing HIV-1 induced cytopathicity in HIV-1 challenge tissue culture experiments, and there are strong pre-clinical data in animal models that C46 is very efficient at inhibiting HIV-1 replication and its pathogenic effects (*Egelhofer et al, 2004; Zahn et al, 2008; Younan et al 2013*). Combined they are more effective at inhibiting HIV-1. Once integrated into the target cell, sh5 driven by the H1 promoter, produces a small interfering RNA (siRNA) that reduces the level of CCR5 on the cell surface. The expression of the C46 component, that is derived from the HIV-1 envelope glycoprotein gp41, is driven by the ubiquitin C promoter. C46 will act as a membrane-anchored C-peptide that inhibits fusion of the HIV-1 envelope to the host cell membrane. In a Phase I clinical trial, C46 was shown to be safe with persistent detection of gene-marked CD4+ T lymphocytes in HIV+ subjects (*van Lunzen et al, 2007*).

CCR5 is the major co-receptor for HIV-1. Studies on a naturally occurring human mutation in the CCR5 gene, known as 'delta-32 (Δ32) CCR5' have shown that HIV-1 is unable to establish infection in homozygous individuals and disease progression is significantly slower in heterozygotes (*Ioannidis et al 2001; O'Brien and Nelson 2004*). This protective effect has been shown to be transferable: In 2007, an allogenic bone marrow transplant was performed using Δ32

mutant CCR5 hematopoietic stem/progenitor cells (HSPC) into a wild type CCR5 HIV-1 positive individual. The transplant was reported as 'functionally curative for HIV' (*Hütter et al, 2009*).

The rationale for the proposed clinical trial is that Cal-1 introduced into HSPC and CD4+ T lymphocytes will protect these cells and their progeny cells from HIV-1 infection and its pathogenic sequelae. This protective effect is predicted to act to lower HIV-1 RNA and increase CD4+ T lymphocyte counts, thereby decreasing or delaying (partially or completely) the need for antiretroviral drug therapy.

This is a phase I/II human clinical study of Cal-1. Prior clinical studies in the area of HIV-1 and gene therapy have pointed to the therapeutic potential of the genetic modification of HSPC and CD4+ T lymphocytes (*Aiuti et al 2002; Kang et al 2002; Malech et al 2004; Barese et al 2004; Ott et al 2006; Rossi et al 2007; van Lunzen et al 2007; Mitsuyasu et al 2009; Gaspar et al 2009; Aiuti et al 2009; Cartier et al 2009; Boztug et al 2010; Cavazzana-Calvo et al 2010; Symonds et al 2010*).

OVERVIEW OF STUDY DESIGN

This is an adaptive design study to evaluate the safety and feasibility of Cal-1 transduced HSPC and CD4+ T lymphocytes in HIV-1 infected subjects who have previously been exposed to ART but are not currently taking any antiretroviral agent. Three cohorts of at least 3 subjects each will be enrolled:

Cohort 1: No busulfan conditioning

Cohort 2: Conditioning with a single dose of 4mg/kg busulfan.

Cohort 3: Conditioning with two doses of busulfan, with first dose of 4, 3 or 2 mg/kg and second dose based on real-time pharmacokinetic-guided dosing to reach targeted drug exposure (AUC).

Progressive enrollment of Cohort 1, then Cohort 2 and then Cohort 3 is dependent on the recommendation of an independent Data Safety Monitoring Board (DSMB).

The primary objective is to evaluate the safety and feasibility of using Cal-1 transduced HSPC ($HSPC^{tn}$) and CD4+ T lymphocytes (T^{tn}), including the use of various doses of busulfan to increase $HSPC^{tn}$ engraftment. The trial will also assess the level of gene-marked cells and any impact on HIV-1 RNA and CD4+ T lymphocyte count, as well as other exploratory parameters.

Subjects will have two sets of cell collection procedures (aphereses) performed; first a single 5L apheresis to collect unstimulated CD4+ T lymphocytes, followed by a second set of two, larger volume (15L) aphereses to collect CD34+ HSPC on days 4 and 5 of mobilization. The mononuclear cell populations obtained from each of these two collection procedures will be separated into purified CD4+ T lymphocytes and CD34+ HSPC, respectively and processed (transduced, cultured and cryopreserved) separately.

The primary endpoint for all subjects will be at 48 weeks post-infusion, after which they may continue follow-up for up to one additional year in the Follow-up Extension phase. All subjects will transfer to a separate long-term follow-up protocol that will monitor each subject for potential long term adverse effects of Cal-1. Secondary data points are collected up to study completion or until early withdrawal or secondary analysis endpoint criteria is met (discontinuation), whichever occurs first. Subjects should commence ART at any time during the study if CD4+ T lymphocyte counts are confirmed to decline < 350 cells/µl (mm^3) or to less than 1/3 of the CD4+ T cell % at the Pre-busulfan Assessment visit, if plasma HIV RNA exceeds 250,000 copies/µl or > 20-fold increase from the Pre-busulfan Assessment visit, or in event of pregnancy (as defined in **Section 6.2.4**).

During the post-infusion study phase, periodic evaluations, as described in the Schedule of Events, are designed to assess safety and feasibility, as well as preliminary evidence of efficacy.

OBJECTIVES

PRIMARY OBJECTIVE

To evaluate the safety and feasibility of the introduction of Cal-1 gene-transduced, hematopoietic cell populations (T^{tn} and $HSPC^{tn}$) with or without intravenous busulfan conditioning at a range of doses in HIV-1-infected adults who have previously been on ART.

SECONDARY OBJECTIVES

To assess;

1. The extent of $HSPC^{tn}$ contribution to hematopoiesis and T^{tn} survival by evaluation of Cal-1 marking and expression in peripheral blood at time points up to study completion or discontinuation.
2. The extent of $HSPC^{tn}$ contribution to hematopoiesis and T^{tn} survival by evaluation of Cal-1 marking and expression in gut-associated lymphoid tissue (GALT).
3. The potential benefit of busulfan conditioning as determined by;
    a. The extent of engraftment and differentiation of $HSPC^{tn}$ over time by evaluation of Cal-1 marking and expression in peripheral blood subpopulations (monocytes, granulocytes, CD4+ and CD8+ lymphocytes)
    b. The extent of $HSPC^{tn}$ engraftment by evaluation of Cal-1 marking and expression in bone marrow at Week 12
4. The potential efficacy of Cal-1 in controlling HIV-1 infection for each subject, as measured by;
    a. Plasma HIV-1 RNA relative to the Pre-busulfan Assessment visit
    b. Plasma HIV-1 RNA over time
    c. CD4+ T lymphocyte count, percentage and CD4+/CD8+ T lymphocyte ratio relative to the Pre-busulfan Assessment visit
    d. CD4+ T lymphocyte count, percentage and CD4+/CD8+ T lymphocyte ratio over time

e. Time to commencement of antiretroviral therapy (as defined in **Section 6.2.4**)

The endpoint for these secondary analyses is study completion, withdrawal, or until discontinuation due to secondary analysis endpoint criteria (as defined in **Sections 8** and **9**).

5. Impact on lymphocyte development for each subject, as measured by changes in thymopoiesis and maturation markers in peripheral blood relative to pre-infusion baseline, and impact on chronic inflammation for each subject as measured by changes in inflammation markers in peripheral blood relative to pre-infusion baseline over time.
6. A tropism shift from R5 to dual/mixed or X4 at any time point.

STUDY POPULATION

Up to 12 subjects will be enrolled; at least 3 subjects per cohort. The 3 cohorts are defined according to the dose of busulfan (Busulfex®) to be administered for pre-infusion reduced intensity conditioning. The doses of busulfan are chosen to have a non-myeloablative conditioning effect. Two different busulfan dose ranges are being assessed in order to identify the most effective dose that can safely facilitate engraftment and maximize the contribution of Cal-1 transduced CD34+ HSPCs to hematopoiesis. Each cohort will receive Cal-1 transduced HSPC and CD4+ T lymphocytes.

The study is enrolling subjects who have previously been treated with antiretroviral therapy (ART), but are no longer receiving it. This target population will allow assessment of baseline characteristics of HIV-1 quantity, tropism and phenotype, as well as changes in HIV-1 RNA and CD4+ T lymphocyte counts without the requirement for a structured treatment interruption. In addition, each subject will serve as is/her own control for HIV-1 RNA and CD4+ T lymphocyte count comparisons pre- and post- cellular infusions. The screening eligibility criteria require that a subject be off ART for at least 6 weeks prior to screening. In terms of CD4+ T lymphocyte count, subjects must not have a documented historical CD4+ T lymphocyte count of < 250 cells/µl (mm^3) and a minimum count at screening of ≥ 500 cells/µl (mm^3). This is to provide safe limits for subjects who are anticipated to continue without ART, to enable evaluation of the effect of Cal-1 on HIV-1 RNA and CD4+ T lymphocyte counts.

STATISTICAL METHODS

This is a phase I/II study, and the sample size has not been based on formal power considerations. No recruitment limit is set for any of the participating sites, with the only restriction being consecutive enrollment that does not exceed 4 subjects per cohort. Subjects for the next cohort will not receive busulfan or be infused until the DSMB recommendations are received.

The primary analysis of safety will be conducted when all recruited subjects have completed 48 weeks follow-up or have ceased trial follow-up before that time.

Subject baseline demographic and clinical information will be summarized by treatment group as well as overall. There will be no formal comparison of the treatment groups in terms of baseline factors (i.e., no p-values). Any subjects discontinuing (i.e. starting antiretroviral or prohibited

concomitant medication treatment), or withdrawing (e.g. withdrawing consent to trial follow-up, lost to follow-up etc.) will be listed. The time to starting antiretroviral treatment will be summarized using Kaplan-Meier plots.

Safety and feasibility analyses

Numbers of individual adverse events, in numbers of subjects, will be summarized by severity and treatment, both overall and by nominal study week. Adverse events will also be summarized and aggregated by body system for clinical events. The worst severity of each clinical and laboratory adverse event for each subject will be summarized by treatment. All grade 3 and 4 adverse events, and any other events that lead to subject discontinuation, will be listed by treatment, with duration and resolution.

Feasibility measures will include analysis of the T^{tn} and $HSPC^{tn}$ release criteria and characterization. CCI [redacted]

Efficacy analyses

Log_{10} HIV-1 RNA concentration, lymphocytes (CD4+ T lymphocyte count, CD4+ %, CD4:CD8 ratio), measures of Cal-1 marking/expression and lymphocyte development (thymopoiesis, maturation and inflammation) will be plotted over time for each individual subject, and will be summarized for each nominal study week by treatment and overall. Changes in log_{10} plasma HIV-1 RNA and CD4+ T lymphocyte count from baseline will be formally compared in all subjects using one-sample t-tests or non-parametric equivalents as appropriate.

Exploratory analyses of log_{10} plasma HIV-1 RNA, lymphocytes (CD4+ T lymphocyte count, CD4+ %, CD4:CD8 ratio), measures of Cal-1 marking/expression and lymphocyte development (thymopoiesis, maturation and inflammation) will use repeated measures ANOVA techniques to explore changes from baseline in these endpoints over time, and any differences between treatment groups. These analyses are not well powered, and it is accepted they will be exploratory in nature and interpreted cautiously.

Preliminary analyses

There are two planned DSMB safety analyses and one preliminary analysis for safety and secondary endpoints at Week 24. The independent DSMB will conduct a complete review of data to Week 12 for at least 3 subjects in the preceding Cohort before subjects in Cohorts 2 and 3 receive any busulfan and are infused. At each analysis, all subjects' safety data will be summarized including all available data up to the time the analysis is conducted. Measures of Cal-1 marking/expression will also be summarized. There will be no formal statistical stopping rules, rather the study will continue on the basis of there being no evidence of sufficient safety concerns to warrant study cessation. The occurrence of bone marrow failure (defined as a protracted

neutropenia (ANC < $0.5x10^3/\mu L$ (mm^3)) and/or thrombocytopenia (platelets < $25x10^3/\mu L$ (mm^3)) after Week 12 post-infusion), the occurrence of grade 4 laboratory abnormalities, unanticipated incidence, severity or frequency of AE/SAEs, or failure of T^{tn}/$HSPC^{tn}$ manufacturing processes may be events that could present sufficient safety concerns to warrant study cessation. Efficacy indicators, such as the degree of Cal-1 marking in peripheral blood, GALT and bone marrow will also be assessed.

DOSAGE & ADMINISTRATION

There will be two apheresis stages; the first (1 x 5L) to obtain CD4+ T lymphocytes and the second (2 x 15L) to obtain CD34+ HSPC following mobilization with G-CSF (Neupogen®) and plerixafor (Mozobil®). The 6 day interval between the first (CD4+) apheresis and commencement of G-CSF is to allow hematopoiesis to return to steady state prior to the next procedure. A 5 week (+/-7 days) interval between the last apheresis and infusion is in place to give sufficient time for the manufacturing steps of transduction, culture and release testing to be completed as well as to allow the subject's bone marrow compartment to return to steady state following the period of mobilization. The Cal-1 modified HSPC and CD4+ T lymphocytes will be prepared (cultured and transduced) separately and cryopreserved at the completion of the transduction and culture period.

The following number of cells in the final Cal-1 modified infusion products are anticipated (the actual doses being dependent on the numbers of cells harvested by apheresis and yields from the laboratory purification, transduction and culture procedures for each individual subject):

- Minimum $2.0x10^6$ cryogenically preserved $HSPC^{tn}$/kg of body weight with a transduction efficiency of ≥ 10% and ≤ 5 copies of the transgene per cell in an approximate volume of up to 50ml.
- $0.5\text{-}20x10^9$ total cryogenically preserved T^{tn} with a transduction efficiency of ≥ 10% and ≤ 5 copies of the transgene per cell in a maximum volume of 200ml.

Each cell type will be thawed and infused following completion of pre-infusion assessments. The cellular products will be administered consecutively as intravenous infusions. Subjects will be monitored for 2 hours after each infusion for any acute adverse effects.

A back-up apheresis product containing $1.2x10^6$ unmanipulated CD34+ cells/kg will be available for use in the event of delayed hematopoietic recovery and/or bone marrow failure (both as defined above).

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SCHEDULE OF EVENTS – PRE-INFUSION

	Weeks relative to CD4+ Apheresis						Days relative to preceding visit (or relative to Day 0)								
	Can be up to 3 months prior to Screening 1		≤ 14 to ≥ 4	≥ 2 (weeks after Screen 1)	≤ 2	0	+6	+1	+1	+1	+1	(≤ -11)	(-4)[6]	(-2)[6]	+35 ±7 days
ASSESSMENTS	Consent	Pre-screening (optional)	Screening 1	Screening 2	Pre-apheresis[20]	CD4+ Apheresis	G-CSF 1	G-CSF 2	G-CSF 3	G-CSF 4 & Plerixafor CD34+ Apheresis	G-CSF 5 CD34+ Apheresis	Pre-busulfan Assessment	Cohort 3: Busulfan Administration	Cohort 2 & 3: Busulfan Administration	Day 0
Consent	x														
Inclusion/exclusion criteria			x	x											
Demographics & medical history		x	x												
HIV-related history		x	x												
Concomitant medications & therapies			x	x		x	x	x	x	x	x	x	x	x	x
Adverse events				x		x	x	x	x	x	x	x	x	x	x
Physical exam[1]			x[1]				x					x[1]			x
Vital signs[2]			x			x	x				x	x	x[2]	x[2]	x[2]
Weight						x	x				x	x			x
Venous access assessment		x	x												
Chest x-ray			x												
Apheresis[3]						x				x	x				
G-CSF 10µg/kg[4]							x	x	x	x	x				
Plerixafor 0.24mg/kg[5]									x[5]	x					
Busulfan administration[6]													x	x	
Infusion of Transduced Cell Products[7]															x
LABORATORY															
Complete blood count with differential[9]			x	x	x[20]	x	x		x[9]	x[9]	x	x			x
Biochemistry[10]			x	x	x[20]		x				x	x			x
Plasma HIV-1 RNA[18]		x	x	x		x	x				x	x			x
Lymphocyte phenotype (CD3+/CD4+/CD8+)		x	x	x		x	x					x			x
Lymphocyte development studies[11]						x									x
CD34+ enumeration (peripheral blood)									x	x	x				
Pregnancy test[12]			x				x[11]					x[11]			x[11]
Pre-screening for co-infection[13a]		x													
Screening for co-infection[13b]			x												
HIV-1 tropism			x												
Busulfan pharmacokinetic (PK) monitoring[17]													x	x	
Cal-1 marking/expression analysis – PB[14]												x			
Cal-1 marking/expression analysis – Subsets[14]												x			
Cal-1 integration site analysis												x			
Specimen storage[15]												x			
Pre-emptive HLA typing[22]												x			

SCHEDULE OF EVENTS – POST-INFUSION

TIME POINT (DAYS | WEEKS) / WINDOW PERIOD (DAYS) / ASSESSMENTS	Days post infusion							Weeks post infusion													
	Every 3 ±1	(Wk 1) 7 ±2	Every 3 ±1	(Wk 2) 14 ±2	Every 3 ±1	(Wk 4) 28 ±2	Every 3 ±1	6 ±3	8 ±3	12 ±7	16 ±3	20 ±3	24 ±7	28 ±3	32 ±3	36 ±3	40 ±3	44 ±3	48 ±7		+4 ±14
	Busulfan F/U[16]		Busulfan F/U[16]		Busulfan F/U[16]		Busulfan F/U[16]			DSMB analysis			Preliminary analysis						End of study	Early discontinuation / withdrawal[19]	Follow-up Extention[21]
Concomitant medications & therapies	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Adverse events	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Physical examination[1]		x		x		x		x	x	x[1]	x	x	x[1]	x	x	x	x	x	x[1]	x[1]	x
Vital signs[2]	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Weight						x				x			x						x	x	
GALT biopsy[8]										x			x						x	x[7]	
Bone marrow aspirate[8]										x			x						x	x[8]	
LABORATORY																					
Complete blood count with differential[9, 16]	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Biochemistry[10]		x		x		x		x	x	x	x	x	x	x	x	x	x	x	x	x	
Plasma HIV-1 RNA[18]		x		x		x		x	x	x	x	x	x	x	x	x	x	x	x	x	x
Lymphocyte phenotype (CD3+/CD4+/CD8+)		x		x		x		x	x	x	x	x	x	x	x	x	x	x	x	x	x
Lymphocyte development studies[11]										x			x						x	x	
Pregnancy test[12]										x			x						x	x	
HIV-1 tropism										x			x						x	x	
Cal-1 marking/expression analysis – PB[14]		x		x		x		x		x			x		x		x		x	x	x
Cal-1 marking/expression analysis – Subsets[14]						x				x			x						x	x[19]	
Cal-1 integration site analysis										x			x						x	x	
Specimen storage[15]						x				x			x						x	x[19]	

FOOTNOTES

[1] **Physical examination:** Full examinations as indicated. Targeted examination at all other visits.

[2] **Vital signs:** Vital signs routinely will involve pulse, blood pressure and temperature measurements, except at busulfan and cell infusion (Day 0) visits, when blood oxygen saturation will also be monitored pre- and post-administration of busulfan or cells.

[3] **Apheresis:** CD4+ apheresis will be 5L and the CD34+ aphereses will be 15L volumes.

[4] **G-CSF daily (x 5):**10μg/kg QD, commencing day +6, after CD4+ apheresis. The day 4 and 5 dose is to be administered in the morning, prior to visit assessments and apheresis.

[5] **Plerixafor (x1):** Single dose of 0.24 mg/kg on Day 4 G-CSF mobilisation. The dose of plerixafor (Mozobil®) is to be administered after apheresis that day, and as late as possible in the day (at least 9 hours, but preferably not more than 16 hours prior to the scheduled time of Day 5 apheresis). A second (optional) dose of plerixafor is allowed on the afternoon/evening of Day 3 of G-CSF mobilization, if experience with a single dose is not sufficient to achieve desired levels of CD34+ cell harvest.

[6] **Busulfan:** The dose of busulfan to be administered is dependent on the cohort to which the subject has been enrolled. Dose is to be based on the subject's height and current weight (as measured at the Pre-busulfan Assessment visit). Day-4 is for Cohort 3 only. Cohort 2 will receive a single dose of 4mg/kg IV busulfan on Day -2 and cohort 3 will receive two doses of IV busulfan over 3 Days (Days -4 and -2). Cohort 1 will not receive any busulfan, so this visit is not applicable to this cohort. Busulfan dosing is always scheduled to complete 2 days prior to the planned cell infusion day (Day 0).

[7] **Infusion of Transduced Cell Product:** Both $CD34^+$ and $CD4^+$ transduced cell products will be thawed bed-side for immediate infusion. Prior to infusion, viability and sterility will be assessed. For cohorts 2 and 3, the cell infusions will be 2 days after busulfan administration.

[8] **GALT Biopsy:** Gut Associated Lymphoid Tissue Biopsy will be performed to assess Cal-1 marking/expression. A window period of ± 7 days from the time of the visit is permitted for the GALT biopsy. This procedure will be performed earlier, as part of the Early Discontinuation/Withdrawal visit, if a subject discontinues prior to the Week 12 visit. If the subject discontinues or withdraws from the study *within 8 weeks* of the Week 12 or 24 visits (i.e. having already recently undergone the scheduled biopsy), additional GALT biopsies will be included as part of the Early Discontinuation/Withdrawal visit at the discretion of the Principal Investigator.

Bone Marrow Aspirate: For Cal-1 marking/expression analysis. A window period of ± 7 days from the time of the visit is permitted for the bone marrow aspirate. This procedure will be performed earlier, as part of the Early Discontinuation/Withdrawal visit, if a subject discontinues prior to Week 12 visit. If the subject discontinues or withdraws from the study *within 8 weeks* of the Week 12 or 24 visits (i.e. having already recently undergone the scheduled aspirate), an additional bone marrow aspirate will be included as part of the Early Discontinuation/Withdrawal visit at the discretion of the Principal Investigator.

[9] **Complete blood count**: Hemoglobin, hematocrit, red blood cell count, white blood cell count including differential and platelet count. Local CBC (in addition to Central Laboratory CBC) will be drawn on both G-CSF 4 and G-CSF 4 & Plerixafor CD34+ Apheresis days for safety before proceeding to the next G-CSF dose.

[10] **Biochemistry**: Non fasting serum biochemistry including electrolytes (bicarbonate, calcium, chloride, magnesium, potassium, sodium), glucose, urea, creatinine/creatinine clearance, creatine phosphokinase (CPK), liver function tests (albumin only, total, direct and indirect bilirubin, alkaline phosphatase, GGT, AST/SGOT, ALT/SGPT).

[11] **Lymphocyte Development Studies:** Extensive phenotypic analysis will be performed to quantify number and percentage of various populations of CD4+ lymphocytes in peripheral blood as a measure of thymopoiesis, T-cell maturation, and inflammatory status.

[12] **Pregnancy test:** serum β-HCG testing of serum from women of reproductive potential, except at commencement of G-CSF, busulfan and cell infusion, when urine test is performed.

[13] **a) Pre-screening:** Optional Lymphocyte phenotype, HIV-1 RNA, hepatitis B antibody and surface antigen (confirmed by HBV DNA if positive), hepatitis C serology (confirmed by HCV RNA if positive). The venous assessment can also be done at this time (or any time after study consent and Screening 1).

b) Screening: Hepatitis B antibody and surface antigen (confirmed by HBV DNA if positive), hepatitis C serology (confirmed by HCV RNA if positive), West Nile virus RNA, QuantiFERON®-TB Gold/IGRA for tuberculosis, HTLV-1/2 and syphilis serology will be performed at screening 1 as part of safety and eligibility checks. Any subject who screens positive for syphilis will not be excluded from the study, but must have commenced effective treatment prior to the first apheresis procedure. If a subject completes hepatitis B and/or C co-infection at a pre-screening visit, this testing is repeated again at Screening 1.

[14] **Cal-1 marking/expression analysis – PB and subsets:** DNA and RNA will be isolated from peripheral blood (PB) and Subsets consisting of purified peripheral blood monocytes, granulocytes, CD4+ and CD8+ lymphocytes.

[15] **Specimen Storage:** Plasma, serum and cryopreserved PBMC. DNA and RNA remaining after scheduled Cal-1 marking/expression and integration analyses will also be retained as part of the stored specimens. CCI [redacted]

[16] **Busulfan F/U:** For subjects in Cohorts 2 and 3, the busulfan follow up regimen will consist of local CBC/differential (manual differential preferred), vital signs and review for AEs and con-medication changes at 2-4 day intervals out to Week 6 post infusion. More frequent or extended follow up may be performed as per PI discretion.

[17] **Busulfan PK monitoring:** For subjects in Cohorts 2 and 3, blood concentration of busulfan will be measured at pre- and post-infusion time points as described in Appendix IV.

[18] **Plasma HIV-1 RNA:** A second EDTA plasma sample will be collected and prepared at every time point, except pre-screening, for the purposes of batch analysis with the Abbott m2000 assay.

[19] **Early Discontinuation/Withdrawal visit:** Blood for the subsets & specimen storage will only be drawn if it has been > 8 weeks since the last subsets blood draw and is anticipated to be > 8 weeks until the next scheduled subsets.

[20] **Pre-apheresis local labs:** Recent CBC/differential and platelets and electrolyte panel is required by apheresis centers for optimization of apheresis collection settings. If the Screening 2 visit is performed ≤ 2 weeks prior to the CD4+ apheresis visit, this visit will not be required. This visit will also be required in event of a repeat manufacturing procedures (refer to **Section 5.5**).

[21] **Follow-up Extension visits:** Follow-up visits will continue for any subject who has not commenced ART prior to Week 48, or who has resumed ART but has not achieved a HIV-1 RNA level of < 50 copies/mL by Week 48. Any subject who commenced ART and has demonstrated HIV suppression will complete the study at Week 48.

[22] **Pre-emptive HLA typing:** To be performed prior to busulfan administration, as standard-of-care, according to site collection and laboratory protocols.

1. INTRODUCTION

1.1 Background

It is estimated that 34 million individuals are currently infected with HIV. HIV/AIDS is a disease that impairs immune function, primarily by decreasing CD4+ T lymphocytes. The progression can be contained by daily dosing with antiretroviral therapy (ART) but there are side effects that can be treatment limiting, and the development of HIV drug resistance can force the physician to modify the ART regimen. There are no effective vaccines currently available for HIV. An alternative approach that could provide a path to a curative therapy is the use of cell-derived gene therapy in which an anti-HIV gene(s) is introduced into mature and/or hematopoietic stem/progenitor cells (HSPC) to produce a population of white blood cells that are protected from the pathogenic effects of HIV-1. This may provide a continuous means of controlling HIV-1 after a single or infrequent dose(s).

The proposed clinical trial is built upon the results of *in vitro* laboratory studies of Cal-1 and the experience in other gene therapy trials for the treatment of HIV-1 and other immunological or hematopoietic conditions (*Aiuti et al 2002; Kang et al 2002; Malech et al 2004; Barese et al 2004; Ott et al 2006; Rossi et al 2007; van Lunzen et al 2007; Mitsuyasu et al 2009; Gaspar et al 2009; Aiuti et al 2009; Cartier et al 2009; Boztug et al 2010; Cavazzana-Calvo et al 2010; Symonds et al 2010*).

1.1.1 Non-clinical studies

Pre-clinical safety and efficacy studies have been carried out on human primary cells containing the CD4+ and CD34+ target cells. Safety/toxicity studies assessing and analyzing differences between Cal-1 transduced and control cells include:

- apoptosis assessment (caspase assays on human primary PBMC);
- proliferation studies (in primary human PBMC);
- cell progenitor skewing assessment (methylcellulose colony progenitor assays on CD34+ cells);
- inflammation studies (via IFNγ, TNFα and IL-6 ELISA);
- assessment of recombination and replication competent lentivirus (RCL) formation of Cal-1.

None of the above safety/toxicity studies indicated any toxicity risk.

Efficacy studies analyzing differences between Cal-1 transduced and control cells include:

- MOLT4/CCR5 (immortalized T-cell line) HIV-1 challenge assays with a variety of laboratory strains;

- PBMC HIV-1 challenge assays with a variety of HIV-1 laboratory and primary isolate strains;
- Real Time quantitative RT-qPCR assays (RNA expression) using PBMC to assess C46 expression.
- Flow cytometric analyses (FACS) using PBMC and T-cell lines to assess the protein expression levels of CCR5 and C46.
- Ex vivo efficacy study using hu-NSG (BLT) mice. Splenocytes isolated from BLT mice generated with Cal-1 transduced CD34+ HSPC were isolated and challenged ex vivo with either R5 tropic HIV (BaL) or X4 tropic HIV (NL4-3). These cells were found to be protected from both R5- and X4-tropic HIV-1 infection.

In vivo studies

Safety and efficacy studies have been carried out using animal models.

- *In vivo* safety and toxicity in humanized murine model. The study did not detect any safety or tolerability issues for the human fetal liver CD34+ cells transduced with Cal-1 in NSG murine test system.
- *In vivo* efficacy in humanized BLT mice. Animals treated with Cal-1 transduced human CD34+ HSPC were challenged with high dose of R5-tropic BaL HIV-1. Treated animals displayed a profound reduction in HIV infection, as well as stable marking of Cal-1 expression.
- In addition, safety and efficacy study in non-human primates, is currently underway.

The efficacy results indicate that an MOI between 1 and 5 leads to a copy number between 1 and 2 of the therapeutic target and expression of the therapeutic sequences (as determined by quantitative real time PCR for C46). Moreover, HIV-1 challenge assays demonstrate protection against a range of laboratory and primary isolates.

1.1.2 Clinical studies

Gene therapy approaches to the treatment of HIV-1 have included a variety of viral and cellular targets. Although clinically significant efficacy has yet to be established, the feasibility of gene therapy as a therapeutic approach was established in a phase II clinical trial involving 74 gene therapy recipients in two countries (*Mitsuyasu et al 2009 and 2011*). The clinical potential of gene therapy as a treatment for HIV-1 has also been realized with the 'Berlin patient' who received an allogeneic bone marrow graft homozygous for the CCR5 Δ32 mutation in conjugation with myeloablation for leukemia treatment (discussed further below). Collectively, experience to date has shown the feasibility and clinical potential of the approach.

Our approach seeks to protect CD4+ T lymphocytes and/or CD34+ HSPC progeny, from HIV-1 by reducing the expression of the HIV-1 co-receptor CCR5 and blocking fusion of HIV-1 to

the cell membrane through expression of the C46 peptide. Clinical data available to date on these two targets is discussed below.

CCR5

Genetic modulation of CCR5 for HIV-1 therapy has a strong theoretical basis. Individuals with naturally occurring mutations in CCR5 (CCR5Δ32) are protected from HIV-1 infection and disease progression. Heterozygous individuals with only a 50% reduction of CCR5 cell surface expression have substantially reduced disease progression rates (*Ioannidis et al 2001; O'Brien and Nelson 2004*). A recent report has shown that allogeneic transplantation of CCR5Δ32 HSPC eliminated detectable levels of HIV-1 and may be curative (*Hütter et al 2009; Allers et al 2011*). As treatment for acute myeloid leukemia, the HIV+ patient underwent myeloablative conditioning and received a graft containing $2.3x10^6$ CD34+ cells (with the Δ32 mutation) per kg of body weight, followed by a second transplant of $2.1x10^6$ CD34+ cells per kg from the same donor approximately 1 year later. Prior to the transplant, sequence analysis identified that the patient carried HIV variants with predominantly CCR5 co-receptor tropism and only 2.9% CXCR4 and dual-tropic variants. ART was discontinued on the day of the initial transplant and HIV-1 RNA and proviral DNA remained undetected in peripheral blood, bone marrow and rectal mucosa 20 months post-transplant (*Hütter et al 2009*), and no replication-competent virus could be cultured from PBMCs (*Yukl et al 2013*). 5 years post-transplantation, the patient remained off ART, CD4+ T-cell count had increased and plateaued within the normal range and HIV-1 RNA and DNA had remained undetectable in plasma and PBMCs respectively (*Allers et al 2011).* The absence of recrudescent HIV replication and waning HIV-specific immune responses five years after withdrawal of treatment has been reported (*Yukl et al 2013*).

In 2010, Sangamo Biosciences Inc. commenced a Phase I clinical trial of CCR5 Zinc Finger Nuclease (ZFN)-modified autologous CD4+ T lymphocytes in HIV-1 infected subjects (*clinicaltrials.gov, NCT01044654*). This study is ongoing and while there have been presentations at meetings, results have not yet been published. Pre-clinical *in vitro* and mouse studies have demonstrated generation of CD4+ T lymphocytes that are resistant to HIV-1 infection by disruption of endogenous CCR5 through engineered zinc-finger nucleases (CCR5 ZFNs) delivered by the adenoviral vector Ad5/35 (*Perez et al 2008; Holt et al 2010*). NOG mice that received primary human CD4+ T lymphocytes transduced with the Ad5/35 CCR5 ZFNs showed a significantly lower plasma HIV-1 viremia compared to the control mice after 50 days and a 3-fold enrichment of the CCR5 ZFN-modified human CD4+ T lymphocytes after 30 days. These results suggested that the infusion of *ex vivo* expanded CCR5 ZFN-modified autologous CD4+ T lymphocytes may be a safe and viable approach to the treatment of HIV-1 infection.

The first approved drug on the market targeting CCR5, Maraviroc®, (*Dorr et al 2005*), is a small molecule inhibitor that antagonizes CCR5 and prevents HIV-1 entry into cells; it was

approved by the US FDA in 2007 for treatment-experienced patients and later in 2009 for treatment-naïve patients.

C46

HIV-1 fusion inhibitors either inhibit the first step of membrane fusion; the insertion of the viral fusion peptide into the target cell membrane, or prevent the subsequent six-helix bundle formation. The N and C peptides belong to the latter group and are derived from the highly conserved amino acid sequences of the N and C heptad repeats of gp41, respectively. Several C peptides have been described, the most prominent family member being T20 (C36, enfuvirtide or Fuzeon®), which was the first entry inhibitor approved for clinical treatment of HIV-1 infection (*Cervia and Smith 2003*). T20 is a synthetically produced soluble peptide of 36 amino acids representing a portion of the natural sequence of the gp41 HR2 of HIV-1 (*Wild et al, 1994*). In clinical Phase III trials, an optimal dose of 100 mg enfuvirtide (T20) twice daily resulted in a clearly reduced viral load (two logs) and an increase in the patients' CD4 cell count (*Kilby et al 1998*). However, the peptide is not orally bioavailable and thus has to be injected subcutaneously twice per day. It also has a very short serum half-life of only 2-4 hours, which is the reason for the high peptide dose, leading to a therapy cost of approximately $20,000 per patient per year. Another major problem of enfuvirtide treatment is the rapid emergence of resistant virus variants with monotherapy (*Rimsky et al 1998*). Another C-peptide, Sifuvirtide, designed on the basis of the three-dimensional structure of the HIV-1 gp41, was tested recently in a phase IIb clinical trial (reviewed in *Yu et al 2013*).

Several novel C peptides are also active against viruses that are resistant to enfuvirtide. These C peptides are elongated at the amino terminus and interact with a highly conserved hydrophobic groove at the C-terminus of the central N heptad repeat coiled-coil structure (T-1249, C46).

There is strong pre-clinical data in tissue culture and animal models that C46 is very efficient at inhibiting HIV-1 replication and its pathogenic effects (*Egelhofer et al 2004; Zahn et al 2008, Younan et al 2013*). C46 expressed on transduced CD4+ T lymphocytes were introduced into HIV+ subjects in a Phase I clinical trial and was shown to be safe with ongoing detection of gene-marked cells (*van Lunzen et al 2007*). In a Phase I clinical trial of 10 advanced HIV+ subjects who received T lymphocytes transduced with a retroviral vector that expressed the entry-inhibiting peptide, maC46, gene-modified cells could be detected in the peripheral blood, lymph nodes and bone marrow throughout a 1 year follow up period, with marking levels correlating to the transduced cell dose (*van Lunzen et al 2007*). After CD8+ depletion, the remaining cells were cultured, transduced and expanded over a maximum total period of 13 days before cryopreservation for later infusion. The 10 subjects received an infusion of between $3x10^9$ to $17x10^9$ CD8-depleted T lymphocytes, with an average of $1x10^9$ of these containing the gene vector.

As the gene modification coded for production of a receptor on the cell surface, subjects were monitored for a possible immune response against the gene-modified cells. Titers of antibodies against maC46 remained stable or even declined during the first months after infusion, and 2 subjects who were maC46 sero-negative pre-infusion did not seroconvert post-infusion, indicating that a humoral immune response against the maC46 peptide produced by the gene-modified T lymphocytes was unlikely (*van Lunzen et al 2007*).

Busulfan

Despite relatively high numbers of CD34+ HSPC and their efficient *ex vivo* transduction, when the cells are re-introduced into the subject, the number of HSPC[tn] that home to, and engraft in, the bone marrow (as measured by peripheral blood marking) appears to be generally low. The reasons for this are not well understood, but one obstacle may be the space available in the stem cell compartment (the bone marrow) for the engraftment of the new, modified HSPC. Thus creating an environment that enhances the ability of the modified cells to engraft and significantly contribute to the hematopoietic regeneration, either by a natural survival advantage (as occurs with some genetic immunodeficiencies) or through myeloablation, may be a crucial element in successful engraftment and expression of gene-modified CD34+ HSPC.

Busulfan is indicated for use in conditioning regimens prior to allogeneic hematopoietic stem/progenitor cell reconstitution for the treatment of hematologic malignancies and increasingly used in non-malignant conditions, such as autoimmune disease (*Burt et al 200*8). For myeloablation of adults, busulfan (16 doses of 0.8mg/kg) is often used in conjunction with cyclophosphamide. A moderate dose of 8 to 10mg/kg busulfan with fludarabine is used to achieve reduced intensity conditioning and low dose busulfan (3.2mg/kg) with fludarabine is used in non-myeloablative transplantation (*Alyea et al 2005; Lim et al 2010; Shi et al 2013*). The use of single dose busulfan as a conditioning agent prior to infusion of genetically modified autologous HSPC has not been established clinically, however there are precedents for the use of conditioning regimens within the area of gene therapy.

Low dose busulfan administered in the context of gene transfer for ADA-SCID has shown transient myelosuppression without organ toxicity (*Aiuti et al 2002*). Since 2000, a total of 15 pediatric patients with ADA-SCID have received ADA genetically modified CD34+ HSPC following non-myeloablative preconditioning with 2mg/kg/day busulfan delivered intravenously at 3 and 2 days prior to infusion (*Gaspar et al 2009; Aiuti et al 2002; Aiuti et al 2009*). To provide further selective advantage for the ADA-transduced cells, PEG-ADA enzyme replacement therapy was not given after infusion of the gene modified CD34+ HSPC. The study participants were aged between 0.6 to 5.6 years of age, and published data out to a median of 4 years demonstrates that the gene therapy delivered in conjunction with the reduced intensity conditioning regimen is a safe and effective treatment for ADA-SCID affected children and that the use of the non-myeloablative conditioning (along with withdrawal of PEG-ADA) was crucial in achieving clinically significant outcomes (*Aiuti et al 2009*). In a recent

study, 10 ADA-SCID pediatric patients, aged between 15 months and 20 years received intravenous infusion of gene-modified autologous bone marrow CD34+ cells. Four subjects received the modified cells without conditioning, while 6 subjects were administered between 1.9-4.9mg/kg busulfan on two successive days before infusion of the gene-corrected cells. The study demonstrated that the group receiving busulfan had no clinical toxicities related to the conditioning, and that busulfan led to substantial levels of gene-corrected T-lymphocytes expressing ADA enzyme activity (*Candotti et al 2012*).

In 2 subjects with chronic granulomatous disease (CGD) who received non-myeloablative busulfan conditioning prior to transplantation of genetically modified CD34+ cells, Ott and colleagues reported sustained engraftment of functionally corrected cells that produced therapeutically relevant levels of superoxide (*Ott et al 2006*). In this study, liposomal busulfan at a dose of 4mg/kg was administered intravenously every 12 hours at 3 and 2 days prior to the transplant. The dose and conditioning regimen was reportedly well tolerated, with no serious adverse events. A period of myelosuppression with absolute neutrophil counts below 500 cells/µl and CD4+ T lymphocyte counts below 200 cells/µl was observed in both subjects for out to 32 days post transplantation. Both cell counts gradually recovered within 2 to 3 months to normal values (P1, 476 CD4+ cells/ml; P2, 313 CD4+ cells/ml) observed before G-CSF mobilization and busulfan conditioning (*Ott et al 2006*). Previous attempts to correct human CGD by gene therapy in the absence of bone marrow ablation have been unsuccessful (*Malech et al 2004; Barese et al 2004*). In a brief report, an 8.5 years old X-linked CGD child received busulfan conditioning of 8.8 mg/kg intravenously, and CD34+ gene marked *ex-vivo*. The patient experienced a short period of myelosuppression, followed by hematopoietic reconstitution and gene marking (*Bianchi et al 2009*). In a recent report, Malech and colleagues have treated 3 X-linked CGD adult patients with a higher, but still not ablative dose of busulfan (10mg/kg total dose) followed by autologous CD34+ transduced *ex vivo* with a retroviral vector encoding $gp91^{phox}$. The busulfan conditioning was well tolerated by all patients and it was reported to greatly improve initial marking and resulted in persistence of marking in 2 of the 3 patients (*Kang et al 2010*).

The successful use of busulfan as a conditioning agent in the experimental transplant of genetically modified CD34+ cells for other genetic abnormalities such as Wiskott-Aldrich syndrome (*Boztug et al 2010*), X-linked adrenoleukodystrophy (ALD) (*Cartier et al 2009*) and β-Thalassemia (*Cavazzana-Calvo et al 2010*) has also been reported. Boztug and colleagues administered a busulfan dose of 4mg/kg per day 3 and 2 days prior before infusion. Transient myelosuppression and partial alopecia were observed as busulfan-associated side effects (*Boztug et al 2010*).

More intensive conditioning regimens for transplantation of genetically modified CD34+ cells have also been recently reported. A myeloablative regimen of cyclophosphamide and busulfan was justified by Cartier and colleagues for use in the treatment of ALD because the genetically

modified CD34+ cells were not anticipated to have any selective growth advantage. In the 2 pediatric subjects, sustained hematopoietic recovery was reported 13-15 days post infusion (*Cartier et al* 2009). Likewise, with the aim to eliminate diseased hematopoietic stem cells in β-Thalassemia, Cavazzana-Calvo and colleagues administered intravenous Busulfex® (3.2mg/kg/day for 4 days) without the addition of cyclophosphamide before infusion of $3.9x10^6$ cryopreserved genetically modified CD34+ cells per kg (*Cavazzana-Calvo et al 2010*). This intensive pre-transplant conditioning appears to have been well tolerated, with uneventful hematopoietic reconstitution reported. In both studies, the degree of pre-transplant conditioning appears to have been a crucial factor in the clinical outcomes observed.

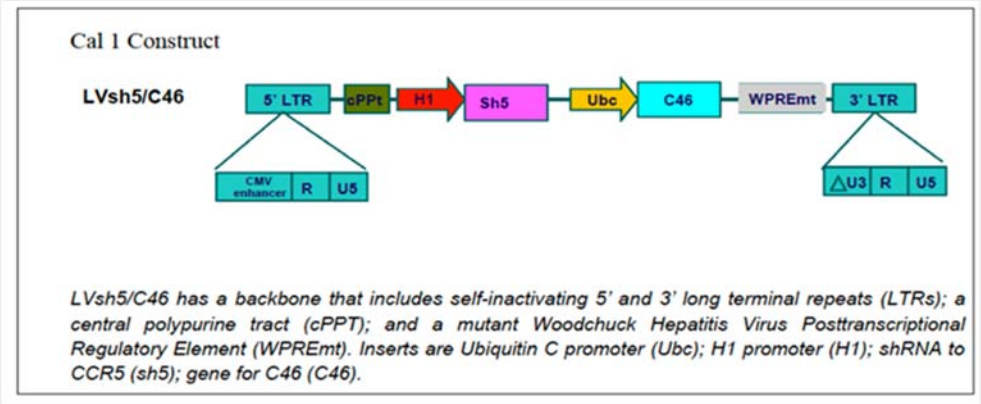
Approximately 100 HIV+ patients with lymphomas have been treated with autologous peripheral stem cell transplant using various conditioning regimens that have included the alkylating agents busulfan, cyclophosphamide, carmustine and/or melphalan (*Michieli et al 2011*). A multicenter AIDS Malignancy Consortium trial treated 20 patients who had recurrent and extensively chemotherapy pretreated HIV-associated non-Hodgkin's lymphoma or Hodgkin's lymphoma with a dose-reduced busulfan and cyclophosphamide preparative regimen with autologous stem cell transplantation (*Spitzer et al 2008*). The majority of subjects received a total dose of 11.2mg/kg intravenous busulfan over 3.5 days (14 doses of 0.8mg/kg every 6 hours) followed by 120mg/kg cyclophosphamide over 2 days for subjects 50 years of age or older. Baseline viral load ranged between 50 to 5712 copies/ml and CD4+ T-cell counts between 53 and 574 cells/µl. CD4+ T-cell counts and viral load were measured at 2 and 3 weeks and at months 1, 2, 3, 6, 9, and 12 post-transplant. The conditioning regimen did not appear to have a consistent effect on these measures of HIV activity and appeared to be well tolerated in the patient group (*Spitzer et al 2008*). One serious adverse event related to the conditioning regimen was reported in a subject who developed post-transplant hepatic veno-occlusive disease at day +12 that resulted in death due to multi-organ failure at day +33. Non-myeloablative conditioning with cyclophosphamide and fudarabine has been successful in conditioning 2 HIV+ patients for allogeneic CD34+ cell transplantation, including 1 subject who received gene-modification with a HIV-resistant vector carrying a dominant-negative mutant Rev (*Kang et al 2002*).

1.2 Rationale

Our approach aims to protect CD4+ T lymphocytes and/or CD34+ HSPC progeny from HIV-1 by reducing the expression of the HIV-1 co-receptor CCR5 and blocking fusion of HIV-1 to the cell membrane through expression of the C46 peptide. Clinical studies, meta analyses, and case reports described above support the rationale that CCR5 is an excellent target for gene therapy-mediated gene silencing. It is well known that HIV-1 can rapidly develop resistance to monotherapy. We therefore are also using a second gene therapeutic agent, a HIV-1 fusion inhibitor termed C46 that blocks entry of the virus. The vector termed Cal-1 encodes both a shRNA to CCR5 (termed sh5) (*Qin et al 2003; An et al 2006; An et al 2007; Shimizu et al 2009;*

Liang et al 2010; Shimizu et al 2010) and the C46 peptide (*Egelhofer et al 2004; Perez et al 2005; Schambach et al 2006; van Lunzen et al 2007; Zahn et al 2008; Trobridge et al 2009; Kimpel et al 2010)*, within a single self-inactivating lentiviral vector.

The short hairpin RNA against the HIV-1 co-receptor CCR5 (sh5) is driven by the H1 promoter, and the HIV-1 fusion inhibitor, C46 is driven by the ubiquitin C promoter. Once integrated into the target cell, sh5 produces an siRNA that reduces the expression of CCR5 on the cell surface. The additional C46 component codes for production of a membrane-anchored C-peptide derived from the HIV-1 envelope glycoprotein gp41 (*Zahn et al 2008*). The peptide is expressed on the surface of the transduced cell and acts as a HIV-1 fusion inhibitor.



LVsh5/C46 has a backbone that includes self-inactivating 5' and 3' long terminal repeats (LTRs); a central polypurine tract (cPPT); and a mutant Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPREmt). Inserts are Ubiquitin C promoter (Ubc); H1 promoter (H1); shRNA to CCR5 (sh5); gene for C46 (C46).

Because the progression of HIV infection depletes CD4+ T cells, the T^{tn} treatment is included in addition to the $HSPC^{tn}$ to rapidly replenish the immune system with disease-resistant T cells and provide a short to medium term effect. CD4+ memory/effector T cells migrate mostly through the peripheral tissues and have been observed to persist *in vivo* for several years and have the capability for self-renewal (*Perez et al 2008; Sallusto et al 1999*). Early clinical data in the field of gene therapy for genetically or acquired immunodeficiency and metastatic melanoma show that delivery of genetically modified CD4+ T lymphocytes is safe and the modified cells can have a survival advantage and sustained engraftment (*Deeks et al 2002; Frecha et al 2010; Humeau et al 2004; June 2011; Kreeger 2010; Levine et al 2002; Levine et al 2006; Levine 2008; Macpherson et al 2005; Mitsuyasu et al 2000; Morgan et al 2005; Ranga et al 1998; Podsakoff 2005; Tebas 2010; van Lunzen et al 2007; Muul et al 2003; Morgan et al 2006; Edelstein et al 2007; Scholler et al 2012*) and suggests potential for a prolonged clinical effect.

We have conducted preclinical experiments that show the harvested CD4+ T lymphocytes and mobilized/harvested CD34+ HSPC can be cultured *ex vivo* and effectively transduced with Cal-1 to yield sufficient numbers of viable, sterile cell populations for intravenous infusion. Once transduced with Cal-1, CD4+ T lymphocytes (T^{tn}) and the CD34+ HSPC ($HSPC^{tn}$) progeny will be protected from HIV-1 infection and its pathogenic sequelae. This protective effect may act to lower HIV-1 RNA and increase CD4+ T lymphocyte counts in the individual, thereby decreasing or delaying (partially or completely) the need for antiretroviral therapy.

2. HYPOTHESES

2.1 Primary Hypothesis

In subjects with HIV-1 infection that have previously received treatment with antiretroviral agents, delivery of $HSPC^{tn}$ and T^{tn} has the potential to be a safe and feasible means to reduce HIV-1 RNA and increase CD4+ T lymphocyte cell counts (as compared to a Pre-Busulfan Assessment visit baseline) in the absence of other antiretroviral therapy.

2.2 Secondary Hypotheses

In subjects with HIV-1 infection that have previously received treatment with antiretroviral agents:

- $HSPC^{tn}$ will home to the bone marrow, engraft and provide a population of peripheral blood hematopoietic cells protected from HIV-1.
- Intravenous busulfan will facilitate re-engraftment of $HSPC^{tn}$ in the bone marrow, resulting in increased levels of Cal-1 marking in the bone marrow, GALT and peripheral blood, compared to the non-busulfan cohort.
- T^{tn} will be protected from the pathogenic effects of HIV-1 and provide a short to medium term replenishment of CD4+ T lymphocytes in the peripheral blood.

3. OBJECTIVES AND OUTCOME MEASURES/ENDPOINTS

This protocol is a first in human study of Cal-1 modified CD4+ and CD34+ hematopoietic cells, and is designed to detect and characterize acute, sub-acute and medium term safety considerations and toxicities of Cal-1 and the associated delivery procedures. Additionally, the following data will be assessed to provide evidence required to consider larger scale phase II evaluation of Cal-1:

- Cal-1 marking/expression by PCR in peripheral blood, bone marrow and GALT to assess the distribution, percentage contribution and expansion of $HSPC^{tn}$ and T^{tn}.
- Assessment of lymphocyte development through analysis of thymopoiesis and maturation markers in peripheral blood to determine if thymic production is active.
- Assessment of chronic inflammatory status through analysis of inflammation markers on peripheral blood lymphocytes.
- Plasma HIV-1 RNA and lymphocyte phenotyping (CD4+ T lymphocyte count, percentage, and CD4+:CD8+ ratio) to assess the potential impact on HIV-1.
- Development of potential resistance by a tropism shift from R5 to dual/mixed or X4.

3.1 Primary Objective and Outcome Measures

To evaluate in HIV-1 infected adults who have previously been on ART:

- The safety and feasibility of the introduction of Cal-1, gene-transduced, hematopoietic cell populations.

- The safety of intravenous busulfan as a means to improve HSPC^{tn} engraftment.

Safety outcomes up to study completion will be measured according to the safety evaluations described in section 7.3. Feasibility measures will include the number of T^{tn} and HSPC^{tn} manufacturing procedures successfully completed (i.e. complying with all release criteria), CD4+ and CD34+ purity, expression of various surface markers, and the number of target cells harvested.

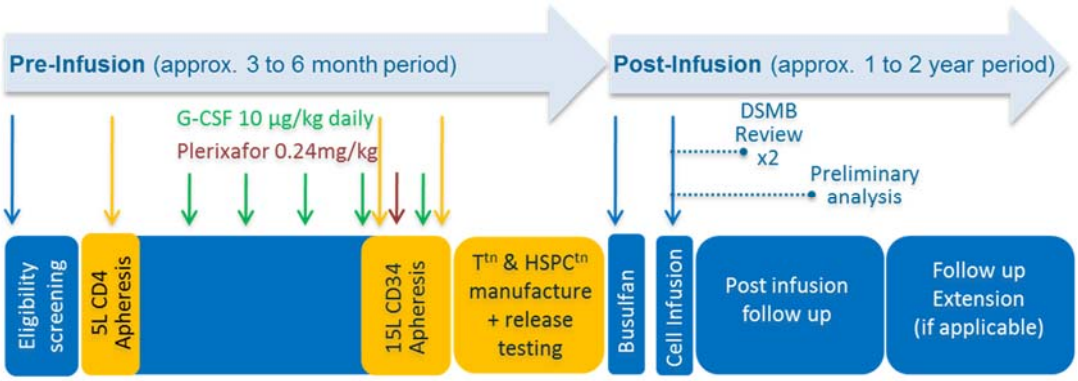
3.2 Secondary Objectives and Outcome Measures

To assess the difference between the 3 treatment cohorts in:

1. The extent of HSPC^{tn} contribution and T^{tn} survival by evaluation of Cal-1 marking and expression in peripheral blood at time points up to study completion or discontinuation.
2. The extent of HSPC^{tn} contribution and T^{tn} survival by evaluation of Cal-1 marking and expression in gut-associated lymphoid tissue (GALT).
3. The potential benefit of busulfan conditioning as determined by;
    a. The extent of engraftment and differentiation of HSPC^{tn} over time by evaluation of Cal-1 marking and expression in peripheral blood subpopulations (monocytes, granulocytes, CD4+ and CD8+ lymphocytes)
    b. The extent of HSPC^{tn} engraftment by evaluation of Cal-1 marking and expression in bone marrow at Week 12
4. The potential efficacy of Cal-1 in controlling HIV-1 infection for each subject, as measured by:
    a. Plasma HIV-1 RNA relative to the Pre-busulfan Assessment visit
    b. Plasma HIV-1 RNA over time
    c. CD4+ T lymphocyte count, percentage and CD4+/CD8+ T lymphocyte ratio relative to the Pre-busulfan Assessment visit
    d. CD4+ T lymphocyte count, percentage and CD4+/CD8+ T lymphocyte ratio over time
    e. Time to commencement of antiretroviral therapy (as defined in **Section 6.3.5**)

    The endpoint for these analyses is study completion, withdrawal, or until discontinuation due to secondary analysis endpoint criteria (as defined in sections 8 and 9).
5. Impact on lymphocyte development for each subject, as measured by changes in thymopoiesis, maturation and inflammation markers in peripheral blood relative to pre-infusion baseline over time.
6. A tropism shift from R5 to dual/mixed or X4 at any time point post-infusion.

4. OVERVIEW OF STUDY DESIGN



4.1 Overview

This is an adaptive design, open-label pilot study to evaluate the safety and feasibility, and preliminary indications of efficacy of Cal-1 in HIV-1 infected adults who have a history of previous treatment with ART but are no longer receiving ART. It is planned that up to 12 subjects will be enrolled, at least 3 subjects per each of 3 possible cohorts. One of the critical factors affecting potential efficacy is engraftment of the gene-modified HSPC in the bone marrow. Through the adaptive enrollment of 3 cohorts, the study aims to evaluate the safety and tolerability of busulfan as a pre-infusion conditioning agent to optimize engraftment, subsequent expression and efficacy in affording protection against progression of HIV-1 infection.

Cohort 1 will enroll subjects, who will undergo CD4+ T lymphocyte apheresis and CD34+ HSPC mobilization, apheresis and infusion procedures without any busulfan conditioning. At Week 12 post-infusion for the third subject in Cohort 1, acute and sub-acute adverse effects, as well as indications of Cal-1 engraftment (marking within bone marrow, peripheral blood and GALT) will be assessed by an independent Data Safety Monitoring Board (DSMB). With DSMB approval the study will then continue with Cohort 2, who will undergo identical procedures with the exception of pre-infusion conditioning with a single dose of 4mg/kg busulfan 2 days prior to infusion. Similarly, at 12 weeks post infusion for the third subject in Cohort 2, the DSMB will compare the acute and sub-acute adverse events and indications of Cal-1 engraftment of Cohort 2 against Cohort 1 to assess the effects of busulfan on the early outcomes and if the data suggest:

- there is favorable benefit relative to risk for the use of busulfan; and/or
- a higher dose (8 or 6 mg/kg busulfan) may result in further benefits with respect to potential engraftment and peripheral expression

The DSMB may recommend a number of possible adjustments with respect to the dosing with busulfan in Cohort 3:

- continue non-conditioning protocol without the use of busulfan
- continue to use a single 4mg/kg dose of busulfan at Day -2

- proceed using a dose (8, 6 or 4 mg/kg busulfan) administered as two doses of 4, 3 or 2 mg/kg QD completing 2 days prior to infusion

4.2 Design Rationale

4.2.1 Pre-infusion Period

Because of the logistical considerations with scheduling the pre-infusion procedures, subjects may sign consent and have pre-screening performed up to 3 months prior to the first procedure of the Screening 1 visit. Additionally, concurrent screening of potential subjects is permitted within the limitation described in **Section 12.1**.

Two screening visits are performed to allow confirmation of the potential subject's plasma HIV-1 RNA and CD4+ T lymphocyte count.

The first apheresis procedure is for the collection of CD4+ T lymphocytes. Subjects will undergo a 5L apheresis following completion of all other assessments scheduled to occur at that study visit. It is anticipated that apheresis will yield 2.5 to $5x10^8$ CD4+ T lymphocytes in total. The apheresis volume may be adjusted during the study to yield the desired cell number.

Subjects will receive 10µg/kg QD G-CSF (Neupogen®) as a subcutaneous injection over 5 consecutive days, starting 6 days after the CD4+ apheresis. The 6 day period between the lymphocyte apheresis and the first dose of G-CSF is to allow hematopoiesis to return to steady state before mobilization commences.

Regarding the second set of apheresis procedures. previous clinical trial data has shown that large volume apheresis (20L) on 2 consecutive days yielded a mean infused CD34+ cell dose of $9x10^6$ CD34+ cells/kg body weight, but also resulted in a protracted period of lymphodepletion (*Mitsuyasu et al 2009*). Thus far, a single 20L apheresis has yielded a mean of $2.2x10^6$ CD34+ HSPC^{tn}/kg (Cohorts 1 and 2). In Cohort 3, subjects will undergo two 15 L aphereses. It is estimated that this volume of apheresis, on Days 4 and 5 of mobilisation, will yield adequate CD34+ HSPCs to consistently manufacture the minimum desired HSPC^{tn} dose of $2.0x10^6$ cells/kg, without significant depletion of other peripheral blood components (*Anderlini et al 1996*).

In the event that the manufactured yield of HSPC^{tn} using this CD34+ mobilisation regimen and 2x15 L aphereses is $<2.0x10^6$ HSPC^{tn}/kg, the existing HSPC^{tn} product will be stored and the subject given the option for repeat CD34+ mobilisation and apheresis (refer to **Section 5.5**).

Additionally, if the HSPC^{tn} yield using the above CD34+ mobilisation/apheresis regimen is $<2.0x10^6$ HSPC^{tn}/kg, this will trigger a review of the mobilisation regimen by the study MRC. The review will consider an extra 0.24 mg/kg dose of plerixafor to be administered by subcutaneous injection on Day 3 of G-CSF dosing (as well as Day 4) (refer to **Section 6.3.2**).

Urine pregnancy tests are scheduled to be performed before the commencement of G-CSF and again before busulfan and infusion to confirm that a female subject of child-bearing potential has not become pregnant during the pre-infusion study phase.

The 5 week (+/- 7 days) interval between the final CD34+ apheresis and infusion is to allow time for:

- All manufacturing steps of transduction, culture and release testing to be completed.
- The subject's bone marrow to restore to steady state following the period of CD34+ cell mobilization and apheresis.

The use of intravenous busulfan prior to infusion of the HSPC^{tn} is as a reduced intensity regimen designed to have a non-myeloablative conditioning effect. Based on the available clinical and pre-clinical information, the following doses of Busulfex® were chosen for this study:

- 4mg/kg single dose, which represents approximately one-third of the total myeloablative dose, and is comparable with published experience that reports enhancement of engraftment of gene modified CD34+ cells with minimal toxicity and no long term adverse effects.
- 6 to 8mg/kg (3 to 4mg/kg QD for 2 days), which is a moderate dose equivalent to that previously administered to CGD patients *(Ott et al 2006*).

Different doses are being assessed to potentially identify the minimum effective dose to safely facilitate engraftment without being myeloablative. Pharmacokinetic analysis will be performed on plasma samples (refer to **Appendix IV** for details). Progression to each cohort is dependent on the recommendation of the DSMB, based on safety and secondary efficacy data from cohorts 1 and 2.

During the Pre-busulfan Assessment visit, all subjects in Cohort 3 will have pre-emptive HLA genotyping and/or serotyping from peripheral blood samples collected according to the site's internal standard-of-care protocol(s). This is to facilitate a rapid transition to standard-of-care clinical treatment(s) – including possible allogeneic transplantation – in the unlikely event of sustained bone marrow failure that is unresponsive to treatment with G-CSF and/or infusion of the back-up, unmodified $1.2x10^6$ CD34+ cells/kg apheresis product.

4.2.2 Post-infusion Follow-up

Routine subject monitoring will be performed weekly for the first 2 weeks and then every second week until 2 months post-infusion, extending to 4-weekly intervals to Week 48. After this time, subjects may continue follow up for up to one additional year (refer to **Section 4.2.3**). During the post-infusion period, subjects in each cohort will remain off ART unless confirmed CD4+ T lymphocyte counts, CD4+ T lymphocyte percentage, or plasma HIV RNA values reach the safety limits, or other criteria, specified in **Section 6.2.4**.

Post-busulfan follow-up will be performed more frequently (per the post-infusion schedule of events) for at least 6 weeks post-infusion. More frequent or extended follow up may be conducted if clinically indicated (per **Section 7.2.1** Conditioning Follow Up).

Cal-1 marking/expression data obtained from peripheral blood mononuclear cells will provide information on the survival of Cal-1 containing cells over time, but it will not be possible to differentiate if these are derived from the $HSPC^{tn}$ or T^{tn} populations. Analysis of CD8+ lymphocyte, monocyte and granulocyte fractions will be informative as to the contribution from $HSPC^{tn}$. Additionally, bone marrow aspirates at 12, 24 and 48 weeks' post-infusion (each ± 1 week) will enable assessment of the extent of engraftment of Cal-1-containing cells in the bone marrow. This will be used to evaluate the potential benefits and risks of a reduced intensity busulfan conditioning regimen. A maximum bone marrow volume of 10ml will be collected. The whole bone marrow aspirate will be for Cal-1 marking and expression analysis.

A substantial proportion of the body's total CD4+ T lymphocytes reside in the gastrointestinal mucosa (*Allers et al 2011*). A rectal gut-associated lymphoid tissue (GALT) biopsy will be obtained at 12, 24 and 48 weeks' (each ± 1 week) post-infusion in order to obtain a number of small tissue specimens for the purposes of Cal-1 marking and expression level analysis.

All enrolled subjects will be followed for a maximum period of 48 weeks post-infusion. If a subject should withdraw consent, they will be asked to continue in long term follow-up.

Any subject who meets any one of the discontinuation criteria will continue study visits per the schedule of events. In the event a subject discontinues or meets a secondary analysis endpoint criteria prior to Week 12, the GALT biopsy and bone marrow aspirate will be performed, if possible, at the time of discontinuation, or the next earliest time point. If a subject discontinues post-Week 12, an additional GALT biopsy and bone marrow aspirate will be performed if possible, providing eight weeks has elapsed since the last biopsy/aspirate.

4.2.3 Follow-up Extension

At Week 48, subjects who remain off ART will continue to have HIV and general health monitoring at monthly intervals (± 2 weeks) until confirmed CD4+ T lymphocyte counts, CD4+ percentage of total lymphocytes, or plasma viral load values reach pre-specified safety limits, or other criteria, as described in **Section 6.3.5**. Subjects will continue to be monitored for up to one year, or until ART is commenced and HIV-1 RNA levels of < 50 copies/mL are achieved (whichever occurs first).

Subjects who commenced ART prior to Week 48, but have not achieved HIV-1 RNA levels of < 50 copies/mL prior to Week 48 will also be followed until HIV suppression is achieved.

4.2.4 Long Term Follow-up

All enrolled subjects will automatically transfer into long term follow-up. The long term follow-up protocol is a separate protocol that provides assessments at defined intervals for the purposes

of determining possible long-term adverse events. The first long term follow-up visit is due 1.5 years post-infusion and will continue at 6-monthly (± 1 month) intervals to 5 years post infusion and then annually thereafter to 15 years post-infusion. The specific procedures and requirements of long term follow-up are described in a separate protocol.

All subjects will be informed of the long-term follow-up requirements at the time of consenting to this study. A separate and detailed consent form for long-term follow-up will be presented to all enrolled subjects at the time of completion of this study, or any time prior to the first scheduled long term follow-up visit.

5. TRIAL POPULATION

5.1 General Considerations

The minimum 6-week period off ART prior to Screening allows time to establish a "viral set point". The historical, Screening 1, and Screening 2 CD4+ T lymphocyte count limits are determined to provide a safe limit for subjects to remain off ART for the complete pre- and post-infusion period.

The DSMB will review data through Week 12 for the first 3 subjects in Cohorts 1 and 2 to decide upon on the busulfan dose for Cohorts 2 and 3, respectively. To facilitate timely recruitment, but allow adequate time for the DSMB decision, subjects for the next cohort can be identified and screening may commence after the third subject in the preceding cohort reaches the Week 12 post-infusion time point. Busulfan will not be administered until the DSMB recommendation is received.

5.2 Inclusion Criteria

Eligible subjects will undergo screening assessments at two time points: Screening 1 to establish initial eligibility and Screening 2 to confirm eligible HIV RNA and CD4+ T lymphocyte levels, prior to the planned commencement of the first apheresis procedure.

Potential subjects must satisfy all of the inclusion and exclusion criteria to be enrolled in the study and proceed with the CD4+ apheresis visit.

In-A. Prior to any study-related procedures, signed informed consent indicating that they understand the purpose, risks and procedures required for the study and are willing to participate in the study

In-B. Individuals aged 18 to 65 years of age (inclusive) at time of consent

In-C. Documented HIV-1 infection $\geq$ 6 months prior to Screening 1

In-D. Previous treatment with antiretroviral agents that had a demonstrated suppressive effect (defined as plasma HIV RNA $\leq$ 50 copies/ml)

In-E. A documented virologically-effective ART regimen option, as determined by the Investigator, taking into account prior ART experience and HIV geno/phenotyping analyses

In-F. Not taking antiretroviral therapy for ≥ 6 weeks prior to Screening 1, for one or more of the following reasons:
i) Concerns over short-term or long-term toxicities associated with antiretroviral agents, or
ii) Treatment fatigue from the daily regimen of life-long therapy

In-G. Plasma HIV-1 viral RNA ≥ 5,000 copies/mL ± 0.2 $\log_{10}$ and ≤ 100,000 copies/ml ± 0.2 $\log_{10}$ at Screening 1 and Screening 2 (i.e., ≥ 3,200 copies/mL and ≤ 160,000 copies/mL)

In-H. CD4+ T lymphocyte count ≥ 500 cells/µl (mm^3) at Screening 1 and Screening 2

5.3 Exclusion Criteria

Potential subjects will not be eligible if they meet any of the following criteria:

Ex-A. Abnormal hematology at Screening 1:
Absolute neutrophil count (ANC) < 1.5 x 10^3/µL
Platelet count < 100 x 10^3/µL
Hemoglobin < 10 g/dL

Ex-B. Abnormal biochemistry at Screening 1:
Alanine aminotransferase (ALT) > 2.5 x ULN
Total bilirubin > 1.5 x ULN
Serum creatinine > 1.5 x ULN

Ex-C. Detection of any CXCR4-tropic HIV-1 at Screening 1

Ex-D. Evidence of co-infection with hepatitis B virus, hepatitis C virus, West Nile Virus, or HTLV-1 as detected at Screening 1

Ex-E. Evidence of active TB infection determined by positive QuantiFERON®-TB Gold/IGRA test result and clinical confirmation at Screening 1

Ex-F. ART or other antiretroviral therapy within 6 weeks of Screening 1 or any time during the pre-infusion period

Ex-G. Documented history of CD4+ T lymphocyte count < 250 cells/µl (mm^3)

Ex-H. Any previous or current AIDS-defining illnesses (CDC Category C), including AIDS-related dementia, with the exception of Kaposi's sarcoma confined to the skin

Ex-I. History of malignancy or systemic chemotherapy within the last 5 years (i.e., subjects with prior malignancy must be disease-free for 5 years), except curatively-treated basal

cell carcinoma, cutaneous squamous cell carcinoma, or cervical or anal intra-epithelial neoplasia

Ex-J. History of steroid-dependent asthma in the past 5 years

Ex-K. History of seizure

Ex-L. Any clinical history of hematologic diseases including leukemia, myelodysplasia, myeloproliferative disease, thromboembolic disease, sickle cell disorder, thrombocytopenia or leukopenia

Ex-M. Class II-IV heart failure, according to the New York Heart Association classification

Ex-N. Inadequate venous access for apheresis, as assessed prior to, or at Screening 1

Ex-O. Current or planned systemic immunosuppressive or immunomodulatory medication

Ex-P. Taking warfarin, aspirin, NSAIDS or any medication that is likely to affect platelet function or other aspects of blood coagulation, and unable to safely cease this medication during the periods of mobilisation/apheresis (19 days) and busulfan follow-up (up to 6 weeks)

Ex-Q. Participation in any study involving any investigational drug or medical device within 30 days prior to Screening 1

Ex-R. Receipt of a vaccine for HIV-1 or any gene transfer product at any time

Ex-S. Prior treatment with recombinant G-CSF or busulfan or other stem-cell mobilizing or modulating agent within the previous 4 weeks

Ex-T. Known hypersensitivity to busulfan, G-CSF (Neupogen™) or *E. coli*-derived proteins

Ex-U. Subjects who will not accept transfusions of blood products

Ex-V. Pregnant or breast-feeding at any time between Screening 1 and cell infusion (Day 0)

Ex-W. History of alcohol or drug abuse within the 12 months prior to Screening 1

Ex-X. Inability to understand and provide informed consent

5.4 Re-screening

Non-qualifying Screening 1 test results that in the opinion of the investigator are not representative of the subject's medical history or can possibly be attributed to a transient effect (for example, poor hydration on the day of the visit or short term mild infection) can be repeated, with the agreement of the Sponsor.

A subject may withdraw or disqualify during the pre-infusion period and remain eligible to re-screen only if, in the investigator's opinion, the disqualifying result, though confirmed, was transient and not medically significant, and they have not commenced apheresis. In this event, the subject may repeat the screening process. The subject must sign a new consent form, be

assigned a new unique study ID and repeat all screening assessments to confirm they meet all eligibility criteria.

5.5 Provisions for Repeating Apheresis or Mobilization/Apheresis

In the event that a subject has commenced apheresis or mobilization and study procedures cannot proceed for any reason (for example, acute infection/illness, change in personal circumstances, transduced cell product(s) that fail to meet release specifications, minimum HSPC^{tn} dose not achieved, or failure of storage/transport conditions), the subject will have an option to repeat the applicable mobilization and/or apheresis procedures if within 14 weeks from Screening 1 and > 4 weeks between doses of G-CSF.

Any manufacturing failure will be communicated to the site Investigator and Study Co-ordinator, who will notify the subject. If the subject wishes to undergo a second round of apheresis or mobilization/apheresis, and the Investigator(s) agrees, the subject will be asked to provide consent using a supplemental information and consent specific to the required repeat procedures.

Whether CD4+ apheresis alone and/or CD34+ mobilisation/apheresis are repeated will depend upon the nature of the process failure. The following considerations apply:

- only the cellular product affected by the process failure will need to be repeated (i.e. if T^{tn} manufacturing was successful, only CD34+ mobilisation/apheresis will need to be repeated, and vice versa);
- repeat CD34+ mobilisation should be scheduled at least four weeks from the date of the last G-CSF dose;
- if repeat CD34+ mobilisation/apheresis only is required, the mobilisation regimen may be modified to include an extra dose of 0.24 mg/kg plerixafor on Day 3 of G-CSF, subject to MRC recommendation (refer to **Sections 6.3.2**);
- the back-up rescue apheresis product of unmodified 1.2x10^6 CD34+ cells/kg will not be prepared if successfully prepared at the first attempt;

Participants who do not return for repeat procedures will be discontinued and followed-up as described in **Section 10** (Adverse event reporting).

6. INVESTIGATIONAL AND CONCOMITANT TREATMENTS

6.1 Investigational Product

The investigational product is defined as Cal-1 (LVsh5/C46) transduced CD34+ HSPC (HSPC^{tn}) and Cal-1 (LVsh5/C46) transduced CD4+ T lymphocytes (T^{tn}).

6.1.1 Physical Description (preparation, handling, packaging, labeling & storage)

The final T^{tn} and HSPC^{tn} dosage forms will be labeled with the following information:

- Name, address and telephone number of the manufacturer;
- Description of the content [Cal-1 transduced CD34+ HSPC ($HSPC^{tn}$)] or [Cal-1 transduced CD4+ T lymphocytes (T^{tn})], dose (total cell number), volume, route of intended administration;
- The subject's unique study identification number, initials and date of birth;
- Study reference code (CAL-USA-11);
- The phrase "For clinical trial use only" or similar wording;
- The required storage conditions;
- Date of manufacturing and Expiry date.

The investigational products will be released with a Certificate of Analysis, which will include the results of release testing and other product identification criteria as determined by local regulations. Directions for handling and administration are provided in Appendix III.

In addition, the back-up autologous CD34+ apheresis product containing $1.2x10^6$ CD34+ cells/kg will be cryopreserved prior to the commencement of the $HSPC^{tn}$ manufacturing procedures.

6.1.1.1. CD4+ T lymphocytes (T^{tn})

Following the apheresis, the collected lymphocytes will be transferred to a dedicated processing facility. The cells will undergo an enrichment process to collect the CD4+ cell component, then will be transduced with Cal-1 and cultured for a total period of 14 days.

CCI [redacted]

The final T^{tn} cellular product will be required to meet defined release criteria to proceed with infusion.

6.1.1.2. CD34+ ($HSPC^{tn}$)

For each of the aphereses, the collected cells will be transferred to a dedicated processing facility. Prior to any *ex vivo* manipulations on the first CD34+ harvest, an aliquot of apheresis product containing $1.2x10^6$ CD34+ cells/kg (of subject body weight) will be cryopreserved as a back-up (refer to **Section 6.2**). The remainder of this first CD34+ apheresis product will then undergo a positive selection process to collect the CD34+ component, then will be stored overnight, pooled with the purified CD34+ component from the second CD34+ harvest, before transduction with Cal-1 and culture for a total period of 3 days.

CCI [redacted]

The final HSPC^{tn} cellular product will be required to meet defined release criteria to and a minimum dose of 2.0x10^6 CD34+ cells/kg before proceeding with infusion (Day 0).

6.1.2 Treatment Allocation

All subjects will receive an autologous transplant of Cal-1 modified CD4+ (T^{tn}) and CD34+ (HSPC^{tn}) cells. The dose will be dependent on the yields from the apheresis collections and laboratory purification, transduction and culture procedures for each individual subject.

Recruitment to each cohort is sequential, with at least 3 subjects recruited consecutively to each cohort.

In the event that either the T^{tn} or HSPC^{tn} fails to meet the release criteria, or any other event occurs that prevents the successful preparation or release of either of these cellular products, infusion will not proceed. The subject will have an option to repeat the applicable apheresis or mobilization/apheresis procedure, subject to provision of informed consent (refer to **Section 5.5**).

6.1.3 Dosage and Administration

The following number of cells in the final Cal-1 modified infusion products are anticipated:

- Minimum 2.0x10^6 cryogenically preserved HSPC^{tn}/kg of body weight with a transduction efficiency of ≥ 10% and ≤ 5 copies of the transgene per cell in an approximate volume of up to 50mL.
- 0.5-20x10^9 total cryogenically preserved T^{tn} with a transduction efficiency of ≥ 10% and ≤ 5 copies of the transgene per cell in a maximum volume of 200mL.

After pre-infusion assessments, the infusion products will be administered as rapid intravenous infusions in accordance with a standardized administration and handling directions (see **Appendix III**). To minimize cellular toxicity, re-infusion of the cryopreserved cells will occur immediately upon thawing. A minimum interval of 2 hours will be observed between administration of infusions of the modified cells to allow monitoring of vital signs and indications of any acute adverse reaction in the subject. The subject will continue to be monitored for 2 hours following the last infusion.

6.1.4 Product Quality Incidents

The following situations will be considered a product quality incident and will result in an investigation by the Sponsor's Quality Assurance group to determine the root-cause and if any change to the laboratory or clinical procedures is warranted:

- Any occasion of inability to complete the manufacturing or release testing of a subject's $HSPC^{tn}$ and/or T^{tn} cell product.
- Failure to achieve required specifications for any release test of the $HSPC^{tn}$ and/or T^{tn} cell product.
- An unexpected event detrimentally affecting either the cellular product or packaging that occurs despite observance of the standard operating procedures for preparation or release of the $HSPC^{tn}$ and/or T^{tn} cell product.
- Any acute adverse event that the investigator suspects may have a causal relationship to the $HSPC^{tn}$ and/or T^{tn} cell product infused.

A report will be issued to the investigator and the Sponsor with the results of the investigation and recommendations. The Sponsor has procedures and forms for the reporting, investigation and follow up of all incidents relating to product quality.

6.2 Back-up Apheresis Product

Prior to any *ex vivo* manipulations on the first CD34+ harvest, an aliquot of unmanipulated apheresis product containing $1.2x10^6$ CD34+ cells/kg (of subject body weight) will be cryopreserved.

This back-up product is intended for autologous use only in the event that a subject experiences delayed hematopoietic recovery and/or bone marrow failure (as defined in **Section 6.3.3**). Hematopoietic recovery will be assessed at Week 12. If recovery is complete, the back-up apheresis product will be destroyed or released for research purposes, according to the subject's consent.

6.3 Concomitant Medication and Therapies

6.3.1 G-CSF (Neupogen®)

Subjects will receive G-CSF (Neupogen®; filgrastim) 10µg/kg QD by subcutaneous injection for 5 days, commencing 6 days after the first apheresis. The dose is to be calculated based on the subject's weight as measured on the first day of G-CSF. Administration of the first G-CSF dose should be the final procedure of the visit, however the fourth and fifth dose must be administered on the morning prior to the CD34+ HSPC apheresis.

The Day 4 G-CSF dose will be supplemented by a single dose of plerixafor (see **Section 6.3.2** below).The G-CSF dosing schedule should not be interrupted for reasons other than a total WBC ≥ 75,000 x 10^9/L, grade 3 or 4 musculoskeletal pain, headache or arthralgia unresponsive to analgesia, severe hypersensitivity reaction, other known rare serious side effects including splenic rupture, capillary leak syndrome or respiratory distress syndrome, or for any unexpected SAE that occurs during the G-CSF mobilization schedule, regardless of suspect cause.

In the case of a missed dose of G-CSF, apheresis may proceed if not more than 1 dose was missed out of the 5 day dosing schedule.

All AEs and concomitant medication(s) and treatment dates and doses must be recorded.

In the event of a subject repeating the mobilization, a minimum of 4 weeks must have elapsed since the previous final dose of G-CSF/apheresis.

6.3.2 Plerixafor (Mozobil®)

Subjects will receive a single dose of plerixafor (Mozobil®) 0.24mg/kg (based on the same body weight used to calculate the G-CSF dose) by subcutaneous injection on Day 4 of G-CSF (Neupogen®) dosing. The dose is to be administered after completion of apheresis procedures on that day, and as late as possible to ensure a period of at least 9 hours, but preferably not more than 16 hours to the next (and final) scheduled CD34+ apheresis.

All subjects receiving plerixafor should be observed for 30 minutes post-injection before discharge. Appropriate anti-emetics or anti-diarrhoeal medications should be prescribed by the Investigator as required to manage possible gastrointestinal symptoms.

There is scope for increasing the total dose of plerixafor. In the event that the $HSPC^{tn}$ yield from any subject in this or any Calimmune clinical trial using the original aforementioned CD34+ mobilisation/apheresis regimen (i.e. 5 x 10µg/kg/day G-CSF, plus 1 x 0.24mg/kg plerixafor on Day 4, with 2 x 15L CD34+ aphereses) is $< 2.0x10^6$ $HSPC^{tn}$/kg, a review of the mobilisation regimen will be undertaken by the study MRC. This review will consider an extra 0.24mg/kg dose of plerixafor (based on the same body weight use to calculate the G-CSF dose) to be administered by subcutaneous injection on Day 3 of G-CSF dosing (as well as Day 4).

All AEs and concomitant medication(s) and treatment dates and doses must be recorded.

6.3.3 Busulfan (Busulfex®)

Busulfan is a potent cytotoxic drug and should be administered under the supervision of a qualified physician who is experienced in allogeneic HSPC transplantation and the use of cancer chemotherapeutic drugs. The dose is to be based on the subject's weight as determined at the pre-busulfan assessment visit and the dose prepared, administered and monitored according to standardized directions (see Appendix IV).

A single intravenous dose of Busulfex® will be administered to Cohort 2, while Cohort 3 will receive two doses of Busulfex® over 3 days (Day -4 and Day -2). Cohort 1 does not receive any Busulfex® conditioning.

Busulfex® will be delivered as an intravenous infusion completing two days prior to the scheduled infusion of autologous $HSPC^{tn}$ and T^{tn} (Day 0). Busulfan conditioning will *not* proceed if the minimum $HSPC^{tn}$ dose of $2.0x10^6$/kg is not achieved.

If, for any reason, the cell infusions cannot proceed as planned, busulfan conditioning should be postponed and rescheduled for as soon as possible. A delay of up to 2 weeks will not be considered a protocol deviation.

Dosing for Cohort 2 will be a single 4mg/kg dose on Day -2.

As recommended by the DSMB on June 24, 2015, dosing for Cohort 3 will commence at a total dose of approximately 6mg/kg and will be determined specifically based upon real-time, pharmacokinetic-guided dosing to achieve a 2-day total busulfan exposure of 8000 µMol/L•min area-under-the-curve (AUC). The first dose will be administered starting at 3mg/kg on Day -4, the second dose on Day -2. The second dose will be calculated based on subject-specific, real-time pharmacokinetic results collected on Day -4 to achieve a target busulfan exposure of 8,000 µMol/L•min AUC. The administration of busulfan using real-time, AUC-guided dosing provides additional safety measures for monitoring total busulfan exposure to a subject. The target of 8,000 µMol/L•min AUC is recommended by the DSMB as a reduced intensity regimen based on the oncology population (the closest patient population to this study's population).

There is scope to reduce the dose within Cohort 3 if any subject experiences delayed hematopoietic recovery, as defined below.

Delayed hematopoietic recovery is defined as an ANC < $0.5x10^3$/µL (mm^3) and/or platelet count of < $25x10^3$/µL (mm^3) after 42 days post-infusion. Bone marrow failure is defined as a protracted neutropenia (ANC < $0.5x10^3$/µL (mm^3)) and/or thrombocytopenia (platelets < $25x10^3$/µL (mm^3)) after Week 12 post-infusion).

The sponsor will notify the investigators in writing if delayed hematopoietic recovery occurs in any subject and if (in Cohort 3) the Busulfex® dose is to be reduced, or if bone marrow failure occurs and there is a temporary hold on administration of Busulfex® and subject enrolment pending DSMB safety assessment.

All subjects will have had pre-emptive HLA genotyping and serotyping performed according to site standard-of-care protocol(s) at the pre-busulfan assessment visit as a precautionary diagnostic measure in case sustained bone marrow failure warrants consideration of allogeneic bone marrow transplantation.

6.3.4 Post-busulfan Infection Prophylaxis

The following antimicrobial prophylaxis regimen will be administered to all subjects in Cohorts 2 and 3 who receive busulfan:

- Valacyclovir 500mg PO QD, commencing the same day as busulfan and continuing until Week 6 or until the ANC > $1x10^3$/µL (mm^3) on 2 consecutive visits.
- Trimethoprim-sulfamethoxazole (TMP-SMX), 1 single strength tablet, PO QD (atovaquone 1500mg PO QD will be used for TMP-SMX allergic or intolerant subjects),

commencing if a subject's CD4+ T cells are <200/ml until the CD4+ count has risen > 200 cells/ml on at least 2 occasions.

- Fluconazole 200mg PO QD, commencing only if ANC < $0.5x10^3/\mu L$ (mm^3), continuing until ANC > $1x10^3/\mu L$ (mm^3) on 2 consecutive visits.

6.3.5 Antiretroviral Therapy

Subjects will be recommended to re-commence ART if one or more of the following post-infusion events occur:

- CD4+ T lymphocyte counts decline below 350 cells/µl (mm^3) confirmed by retest within 14 days ±7 days; and/or
- A decline in the CD4+ T lymphocyte percentage greater than 1/3 of the value at Pre-busulfan Assessment visit, confirmed by retest within 14 days ±7 days; or
- Plasma HIV RNA exceeds 250,000 copies/ml or > 20-fold increase from Pre-busulfan Assessment visit, whichever is less. Confirmed by retest within 1 month ± 7 days. Retest will be performed using the Abbott RealTi*m*e m2000 HIV-1 assay. For the purposes of comparative evaluation, <u>all</u> time points to the date the safety limit is observed, must be analyzed with the Abbott assay. Because of the possibility of cellular transduction with Cal-1 giving a falsely elevated result with the Roche assay, the decision to resume ART should be based on the Abbott assay confirmatory data (refer to **Section 7.5** for details); or
- Subject decision to re-commence ART regardless of pre-specified laboratory parameters; or
- Pregnancy in a female subject.

In this event, or any other clinical indication, the investigator or primary care physician is to advise recommencement of ART. The ART regimen that the subject commences should be as determined to be the most clinically appropriate for each individual, taking onto account their past history of compliance, tolerance and resistance as demonstrated through standard of care HIV-1 geno/phenotyping results. In the event of pregnancy, ART should be commenced in accordance with the current DHHS Guidelines (http://aidsinfo.nih.gov/guidelines).

Subjects who commence ART will be discontinued from secondary analysis and follow up should continue as described in **Section 8**.

6.3.6 Other on-study concomitant medication and therapies

Anti-platelet agents, anticoagulants or medications with known anticoagulant effects will not be allowed for at least 1 week prior to starting G-CSF, during G-CSF, and for at least 1 week after G-CSF dosing is completed (total period 19 days), and for the period starting with busulfan

administration until demonstrated hematopoietic recovery (an expected period of up to 6 weeks).

Subjects in Cohorts 2 and 3 should not be taking contraindicated medications for the periods of time as indicated in **Appendix IV**.

In accordance with the eligibility criteria, subjects are not permitted use of systemic immunosuppressive/immunomodulatory therapy at study entry or at any time during the study period. Refer to **Appendix II** for the list of limited and prohibited immunosuppressive and immunomodulatory agents. If an adverse event necessitates this treatment, the subject will be discontinued and follow up should be performed as described in **Section 8**.

Any diagnostic, therapeutic or surgical procedure performed during the trial period, should be recorded including the date, indication, description of the procedure(s), and any clinical findings.

7. STUDY EVALUATIONS

7.1 Pre Infusion Phase

7.1.1 Consent

Written, informed, consent of potential subjects may be obtained up to 3 months prior to a potential date for Screening 1. This is to allow evaluation of potential subjects through collection and review of general medical and HIV history, prior to formally commencing the screening process.

7.1.2 Pre-screening

After provision of written, informed consent, potential subjects may undergo a pre-screening period, where data on the subject's general health and HIV-related history can be obtained from past and/or current primary health care providers, and the potential subject's suitability for screening evaluated. This pre-screening stage includes an optional provision to conduct laboratory tests to confirm the potential subject's current HIV and co-infection status. These tests are routinely performed as part of standard clinical practice in the care of a HIV-infected individual (HIV RNA, lymphocyte phenotype, and/or hepatitis B and C). If the subject is suitable to proceed, all tests would be repeated at the Screening 1 visit.

7.1.3 Screening 1

Within 3 months of written, informed consent, and preferably after completion of the optional pre-screening evaluations, potential subjects will undergo the screening procedures specified below. If Screening 1 cannot be scheduled within 3 calendar months of consent, the subject must be re-consented to proceed with Screening 1:

- Demographics, including details of gender, date of birth, age, race.

- Documenting of medical and surgical history, including concurrent and recurrent illnesses (with detailed herpes simplex infection history), allergies (with detailed antibiotic allergy history), current and relevant recent past concomitant medications and therapies (including vaccinations). Collection of this information is anticipated to continue from consent throughout the screening period. Medical history review to confirm inclusion/exclusion criteria must be completed prior to commencement of the first (CD4+ T lymphocyte) apheresis procedure.
- HIV history requires date of diagnosis, CD4+ T lymphocyte history and previous ART regimen(s) including the reason(s) for discontinuation. Subject self-reported history alone is not accepted as adequate documentation and independent medical records, such as laboratory reports, clinic or hospital progress notes and/or referral letters pre-dating consideration for this study must be available at the site as source documentation. Collection of this information is anticipated to continue through the screening period and must be completed prior to commencement of the first (CD4+ T lymphocyte) apheresis procedure.
- Complete physical examination
- Vital signs (temperature, blood pressure and pulse)
- Chest x-ray
- Assessment of venous access to ensure suitability to the apheresis procedures. This can be performed at any time between Consent and Screening 1
- Clinical laboratory testing for the following:
    - Complete blood count with differential and platelet count
    - Biochemistry (fasting not required)
    - Pregnancy testing (serum β-HCG) for women of childbearing potential only
    - HIV-1 RNA in plasma
    - Lymphocyte phenotyping (CD3+/CD4+/CD8+)
    - HIV-1 R5/X4 tropism
    - Screening for co-infection with hepatitis B antibody and surface antigen, hepatitis C serology, West Nile virus RNA, tuberculosis QuantiFERON®-TB Gold/IGRA, HTLV-1/2 serology, and syphilis serology

7.1.4 Screening 2

Screening 2 is to be conducted at least 2 weeks after Screening 1. The following assessments will be performed at the second screening visit:

- Assessment for new/changed adverse events
- Assessment for new/changed concomitant medications and therapies
- Clinical laboratory testing for the following:
    - Complete blood count with differential and platelet count
    - Biochemistry (fasting not required)
    - HIV-1 RNA in plasma
    - Lymphocyte phenotyping (CD3+/CD4+/CD8+)

Medical and HIV-related history data can continue to be collected during this time to ensure qualification of eligibility criteria.

7.1.5 CD4+ T lymphocyte Apheresis

Subject's screening procedures must be complete and eligibility confirmed prior to proceeding to this apheresis visit. Recent CBC/differential with platelet count and serum electrolytes is required to optimize apheresis collection settings. If the Screening 2 study visit is performed ≥ 2 weeks prior to this apheresis visit, the study site is responsible for ensuring these laboratory tests are performed within this timeframe.

Subjects will attend the study center for visit assessments and apheresis to collect peripheral CD4+ T lymphocytes. Prior to apheresis, the following assessments will be performed:

- Vitals signs (temperature, blood pressure and pulse)
- Weight (shoes and outer clothing removed)
- Assessment for new/changed adverse events
- Assessment for new/changed concomitant medications & therapies
- Clinical laboratory testing for the following:
    - Complete blood count with differential and platelet count.
    - HIV-1 RNA in plasma
    - Lymphocyte phenotyping (CD3+/CD4+/CD8+)
    - Lymphocyte Development Studies

The subject's weight should be reported to the manufacturing laboratory to enable calculation of the anticipated T^{tn} dose.

Subjects will undergo a 5L apheresis to collect an anticipated 2.5 to 5 x 10^8 total CD4+ T lymphocytes CCI [redacted]. An optimum target inflow rate and product volume collection rate

should be used to achieve maximal lymphocyte cell numbers. The apheresis volume may be adjusted during the study to yield the desired cell number.

Apheresis product will be transported to a dedicated laboratory processing facility for purification, transduction and culture. Universal safety precautions will be followed at all times and personnel will be trained in applicable Good Manufacturing Practice (GMP) standards.

If a repeat apheresis is required (in accordance with provisions described in **Section 5.5**), the same visit assessments and collection specifications will be followed. Any repeat apheresis visit must be confirmed with the Calimmune manufacturing laboratory

7.1.6 CD34+ Cell Mobilization

G-CSF dosing begins exactly six days after the first (CD4+) apheresis, with no window period permitted. This 6 day interval between CD4+ T lymphocyte apheresis and the commencement of G-CSF is to allow the subject's hematopoiesis to return to steady state before mobilization commences, and is precisely scheduled to accommodate the manufacturing timelines for both T^{tn} and $HSPC^{tn}$ products. The following assessments will be performed on the first day of G-CSF, prior to administration of the first dose of G-CSF:

- Targeted physical exam
- Vitals signs (temperature, blood pressure and pulse)
- Weight (shoes and outer clothing removed)
- Assessment for new/changed adverse events
- Assessment for new/changed concomitant medications & therapies
- Urine pregnancy test (for women of childbearing potential only)
- Clinical laboratory testing for the following:
    - Complete blood count with differential and platelet count
    - Biochemistry (fasting not required)
    - HIV-1 RNA in plasma
    - Lymphocyte phenotyping (CD3+/CD4+/CD8+).
- Prescription and administration of first dose of G-CSF as the final procedure for this visit

G-CSF administration is to continue on consecutive days for a total of 5 days as described in **Section 6.3.1** and the **Schedule of Events**, with a single dose of 0.24mg/kg plerixafor (refer to **Section 6.3.2)**, and CBC/differential measurement required on Day 4 of CD34+ mobilisation. Peripheral blood CD34+ enumeration will be performed on Days 3, 4 and 5.

In addition to administration of the daily dose of G-CSF, information on adverse events (AEs) and new or changed concomitant medications and therapies is to be collected and recorded daily.

7.1.7 CD34+ HSPC Aphereses

Subjects will undergo two consecutive aphereses on Days 4 and 5 of the CD34+ mobilization to collect peripheral CD34+ HSPC. G-CSF doses are to be administered early on these days, prior to any other visit procedures or assessments.

Prior to apheresis, the following assessments will be performed:

- Vitals signs (temperature, blood pressure and pulse)
- Weight (shoes and outer clothing removed)
- Assessment for new/changed adverse events
- Assessment for new/changed concomitant medications & therapies
- Clinical laboratory testing for the following:
    - Complete blood count with differential and platelet count
    - CD34+ enumeration (peripheral blood)

Additionally, on the day of the final apheresis (Day 5), the following laboratory testing will also be performed on blood samples collected prior to commencement of the apheresis:

- biochemistry and LFT panel (fasting not required);
- plasma viral load.

After completion of the first apheresis (Day 4), the subject will receive a dose of plerixafor, as described in **Section 6.3.2**.

The subject's weight should be reported to the manufacturing laboratory to enable calculation of the anticipated HSPC^{tn} dose.

CCI [redacted]

Collection parameters are defined in **Appendix V**. An optimum target inflow rate and product volume collection rate should be used to achieve maximal cell numbers, yet minimise both red cell and platelet contamination, over a run time of 4 to 6 hours (approximately).

The harvested apheresis product will be transported each day to a dedicated laboratory processing facility for purification, transduction and culture. Universal safety precautions will be followed at all times and personnel will be trained in applicable Good Manufacturing Practice (GMP) standards.

If a repeat CD34+ mobilization/apheresis is required (in accordance with provisions described in **Section 5.5**), the same mobilization schedule and specifications described in **Section 7.1.6** will be followed. Any repeat mobilization is to be scheduled at least 4 weeks after the last dose of G-CSF in the first attempt, and dates must be confirmed with the Calimmune manufacturing laboratory.

7.1.8 Administration of Busulfan- Cohorts 2 & 3 only

Refer to **Appendix IV** for the busulfan preparation and administration procedures, including monitoring and PK data collection.

Cohort 2 will receive a single dose 4mg/kg in the morning on Day -2. Cohort 3 will receive 4, 3 or 2 mg/kg in the morning on both Day -4 and Day -2. The second dose will be calculated to achieve a target 8,000 μMol/L•min AUC, based on the pharmacokinetic data from the first dose.

This visit can only proceed after confirmation has been received that the T^{tn} and $HSPC^{tn}$ products have met release testing criteria and the products are on site, and available for infusion. If, for any reason, the cell infusions cannot proceed as planned, busulfan conditioning should be postponed and rescheduled for as soon as possible.

Subjects will attend a study visit up to 7 days prior to the planned commencement of busulfan in order to assess their general health, vital signs, and weight. The required busulfan dose is to be prepared according to this weight. The following assessments are to be performed:

- Targeted physical examination
- Weight (shoes and outer clothing removed)
- Vital signs (blood oxygen saturation to be monitored in addition to temperature, blood pressure and pulse) pre- and post-infusion
- Assessment for new or changed adverse events
- Assessment for new or changed concomitant medications & therapies
- Urine pregnancy test (for women of childbearing potential only)
- Clinical laboratory testing for the following:
    - Complete blood count with differential and platelet count
    - Biochemistry (fasting not required)
    - HIV-1 RNA in plasma
    - Lymphocyte phenotyping (CD3+/CD4+/CD8+)
    - Cal-1 marking/expression analysis – PB
    - Cal-1 marking/expression analysis – Subsets
    - Cal-1 integration site analysis

- Lymphocyte Development Studies
- Specimen storage sample (plasma, serum and cryopreserved PBMC)

Pre-emptive HLA genotyping and/or serotyping from peripheral blood samples will be collected at this visit according to the site's internal standard-of-care protocol(s).

Information on adverse events (AEs) and new or changed concomitant medications and therapies is to be collected and recorded.

Females of child-bearing potential should have a urine pregnancy test performed prior to administration of busulfan. No other procedures are performed at this visit.

7.1.9 Cell Infusion (Day 0)

The 5 week interval (± 1 week) between the final CD34+ HSPC apheresis and the day of infusion (Day 0) is to allow for the subject's bone marrow to restore to steady state following the period of stimulation and for all manufacturing steps of transduction, culture, and release testing to be completed. The 7 day window period allows for flexibility to accommodate the subject's and clinic schedules.

The cryopreserved $HSPC^{tn}$ and T^{tn} products will be thawed after the pre-infusion clinical assessments are complete, immediately prior to planned infusion.

The following pre-infusion assessments are to be performed:

- Complete physical examination
- Weight (shoes and outer clothing removed)
- Vital signs (blood oxygen saturation to be monitored in addition to temperature, blood pressure and pulse) pre and post infusion
- Assessment for new or changed adverse events
- Assessment for new or changed concomitant medications & therapies
- Urine pregnancy test (for women of childbearing potential only)
- Clinical laboratory testing for the following:
  - Complete blood count with differential and platelet count
  - Biochemistry (fasting not required)
  - HIV-1 RNA in plasma
  - Lymphocyte phenotyping (CD3+/CD4+/CD8+)

Following completion of pre-infusion assessments, each cell type will be thawed and administered as described in **Appendix III**. The cellular products will be administered consecutively as rapid intravenous infusions. The subject's vital signs (temperature, blood

pressure, pulse, and oxygen saturation) will be monitored for 2 hours after each infusion for any acute adverse effects. The total process is anticipated to take between 4 to 5 hours.

The infusion visit may be postponed only if busulfan conditioning has not been performed. Such a delay will not be considered a protocol deviation unless greater than 2 weeks.

7.2 Post Infusion Phase

7.2.1 Busulfan Follow-up

Only for Cohort 2 and 3 subjects.

Subjects will be asked to return to the clinic every 3 days ± 1 day until Week 6 (or more frequently per PI discretion), and the following assessments performed:

- Vital signs (temperature, blood pressure and pulse)
- Assessment for new or changed adverse events
- Assessment for new or changed concomitant medications & therapies
- Local STAT complete blood count with differential and platelet count. Manual WBC/differential required.
- A symptom-directed physical exam is to be performed in the event of any significant or grade 3 or 4 clinical or laboratory finding

CBC/differential results must be reviewed prior to the subject leaving the clinic if a temperature of ≥ 100.5 F (38.0°C) is recorded. An ANC < $0.5x10^3/\mu L$ (mm^3), with corresponding temperature of ≥ 100.5 F (38.0°C) is a medical emergency, and an investigator must be notified for immediate medical evaluation of the subject and admission (as soon as reasonably possible) to an accredited transplant facility (such as those with Foundation for the Accreditation of Cellular Therapy (FACT) accreditation).

More frequent or extended follow up may be conducted if clinically indicated.

7.2.2 Weeks 1, 2, & 6

The following assessments are to be performed:

- Targeted physical examination
- Vital signs (temperature, blood pressure and pulse). *A local STAT CBC/differential must be ordered if temperature is ≥ 100.5°F (38.0°C), and the results reviewed prior to the subject leaving the clinic. An ANC < $0.5x10^3/\mu L$ (mm^3), with corresponding temperature of ≥ 100.5°F (38.0°C) is a medical emergency, and an investigator must be notified for immediate medical evaluation of the subject and admission (as soon as reasonably possible) to an accredited transplant facility*

(such as those with Foundation for the Accreditation of Cellular Therapy (FACT) accreditation).

- Assessment for new or changed adverse events
- Assessment for new or changed concomitant medications & therapies
- Clinical laboratory testing for the following:
    - Complete blood count with differential and platelet count
    - Biochemistry (fasting or non-fasting)
    - HIV-1 RNA in plasma
    - Lymphocyte phenotyping (CD3+/CD4+/CD8+)
    - Cal-1 marking/expression analysis – PB

7.2.3 Week 4

The following assessments are to be performed:

- Targeted physical examination
- Vitals signs (temperature, blood pressure and pulse). *A local STAT CBC/differential must be ordered if temperature is ≥100.5°F (38.0°C) and the results reviewed prior to the subject leaving the clinic. An ANC < 0.5x10^3/µL (mm^3), with corresponding temperature of ≥ 100.5°F (38.0°C) is a medical emergency, and an investigator must be notified for immediate medical evaluation of the subject and admission (as soon as reasonably possible) to an accredited transplant facility (such as those with Foundation for the Accreditation of Cellular Therapy (FACT) accreditation).*
- Weight (shoes and outer clothing removed)
- Assessment for new or changed adverse events
- Assessment for new or changed concomitant medications & therapies
- Clinical laboratory testing for the following:
    - Complete blood count with differential and platelet count
    - Biochemistry (fasting not required)
    - HIV-1 RNA in plasma
    - Lymphocyte phenotyping (CD3+/CD4+/CD8+)
    - Cal-1 marking/expression analysis – PB
    - Cal-1 marking/expression analysis – Subsets
    - Specimen storage sample (plasma, serum and cryopreserved PBMC)

7.2.4 Weeks 8, 16, 20, 28, 36 & 44

The following assessments are to be performed:

- Targeted physical examination
- Vital signs (temperature, blood pressure and pulse).
- Assessment for new or changed adverse events
- Assessment for new or changed concomitant medications & therapies
- Clinical laboratory testing for the following:
    - Complete blood count with differential and platelet count
    - Biochemistry (fasting or non-fasting)
    - HIV-1 RNA in plasma
    - Lymphocyte phenotyping (CD3+/CD4+/CD8+)

7.2.5 Weeks 12, 24 & 48 (End of Study)

The following assessments are to be performed on Weeks 12, 24 and 48 (End of Study):

- Complete physical examination
- Vitals signs (temperature, blood pressure and pulse)
- Weight (shoes and outer clothing removed)
- Assessment for new or changed adverse events
- Assessment for new or changed concomitant medications & therapies.
- Clinical laboratory testing for the following:
    - Complete blood count with differential and platelet count
    - Biochemistry (fasting not required)
    - HIV-1 RNA in plasma
    - Lymphocyte phenotyping (CD3+/CD4+/CD8+)
    - Pregnancy testing (serum β-HCG) for women of childbearing potential
    - HIV-1 R5/X4 tropism
    - Cal-1 marking/expression analysis – PB
    - Cal-1 marking/expression analysis – Subsets
    - Cal-1 integration site analysis
    - Lymphocyte Development Studies
    - Specimen storage sample (plasma, serum and cryopreserved PBMC)
- Bone marrow aspirate for Cal-1 marking/expression analysis

- Gut associated lymphoid tissue (GALT) biopsy for Cal-1 marking/expression analysis

Bone marrow aspirate for Cal-1 marking/expression analysis must be performed within 1 week of the scheduled visit when all other clinical and laboratory assessments are performed. This procedure may also be performed as part of the Early Discontinuation/ Withdrawal visit if the subject discontinues or meets secondary analysis endpoint criteria). Two approximate 5ml pulls of the soft marrow drawn from the center of the hip bone will be performed.

Gut associated lymphoid tissue (GALT) biopsy for Cal-1 marking/expression analysis must be performed within 1 week of the scheduled visit when all other clinical and laboratory assessments are performed. This procedure may also be performed as part of the Early Discontinuation/Withdrawal visit). 8 endoscopic biopsies will be collect from each of 2 sites within the sigmoid colon - at 10-15cm and 25-35cm from the anal verge of the sigmoid colon (i.e. 16 biopsies in total).

If the subject discontinues or withdraws from the study *within 8 weeks* of the Week 12 or 24 visits (i.e. having already recently undergone the scheduled bone marrow aspirate and GALT biopsies), additional bone marrow aspirate and GALT biopsies will be included as part of the Early Discontinuation/Withdrawal visit at the discretion of the Principal Investigator.

7.2.6 Weeks 32 & 40

The following post-infusion assessments are to be performed:

- Targeted physical examination
- Vitals signs (temperature, blood pressure and pulse)
- Assessment for new or changed adverse events
- Assessment for new or changed concomitant medications & therapies
- Clinical laboratory testing for the following:
    - Complete blood count with differential and platelet count
    - Biochemistry (fasting not required)
    - HIV-1 RNA in plasma
    - Lymphocyte phenotyping (CD3+/CD4+/CD8+)
    - Cal-1 marking/expression analysis – PB

7.2.7 Follow-up Extension

The following assessments are to be performed in subjects who, at Week 48, remain off ART or have not achieved HIV RNA-1 levels of < 50 copies/mL if ART was previously commenced:

- Targeted physical examination

- Vitals signs (temperature, blood pressure and pulse)
- Assessment for new or changed adverse events
- Assessment for new or changed concomitant medications & therapies
- Clinical laboratory testing for the following:
    - Complete blood count with differential and platelet count
    - HIV-1 RNA in plasma
    - Lymphocyte phenotyping (CD3+/CD4+/CD8+)
    - Cal-1 marking/expression analysis – PB

7.3 Safety Evaluations

The investigator must review laboratory reports, document this review and record any clinically relevant results or changes during the study as an adverse event. Where ever possible the attributed cause for the laboratory abnormality should be recorded as the AE term, not the abnormal result itself. Laboratory evaluations include:

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

Biochemistry: Bicarbonate, calcium, chloride, magnesium, potassium, and sodium electrolytes; glucose; urea; creatinine (including MDRD estimated creatinine clearance); creatine phosphokinase (CPK); and albumin, total, direct and indirect bilirubin, alkaline phosphatase, GGT, AST/SGOT and ALT/SGPT liver function tests.

Fasting is not required, but status must be noted at the time of collection.

Lymphocyte phenotype (T-cell count): Flow cytometry analysis of CD3, CD4 and CD8 cells reported as number of cells/µl (mm^3) whole blood, percentage, and CD4:CD8 ratio.

Pregnancy test (urine & serum): Serum β-HCG testing will be performed at the time points designated in the Schedule of Events for all women of child-bearing potential. A urine pregnancy test must be performed on site, before any other study-related procedures are performed at commencement of mobilization, administration of busulfan and cell infusion. Additional urine pregnancy tests may be performed, as considered necessary by the investigator at any time during the study (for example, prior to busulfan, if it is to be administered). In the event of a positive urine pregnancy test, study procedures must be postponed (if possible) pending result of a confirmatory serum β-HCG test. A positive serum β-HCG test result will lead to screen-failure or discontinuation of the subject from the study.

All confirmed pregnancies must be reported using the pregnancy reporting procedures described in section 10.2.

HIV-1 R5/X4 tropism: HIV-1 enters human cells in a process that comprises several steps, including the binding of the viral gp120 protein to the cellular receptor protein CD4 and a co-receptor protein, usually one of the two chemokine receptors CCR5 (R5) and CXCR4 (X4). In addition to the purely X4- and R5-tropic viruses, there are also "dual-mixed" (D/M) strains, that are able to use both co-receptors.

Sequencing and bioinformatics analysis will determine the HIV-1 tropism from HIV RNA in plasma samples. Enhanced sensitivity assays are capable of detecting as low as 0.3% of X4 viral species.

The test will be performed at various time points, as indicated in the Schedule of Events.

Cal-1 integration analysis ('insertional mutagenesis'): Integration site analyses will be performed by linear amplification–mediated PCR (LAM PCR) (*Schmidt et al* 2007) which is currently the most sensitive PCR-based method available to detect the presence or emergence of any predominant integration site(s). The test will be performed on DNA extracted from peripheral blood at various time points, as indicated in the Schedule of Events. A Cal-1 marking level of ≥ 1% in peripheral blood is required to determine if the LAM-PCR result can be interpreted as a true predominant integration site, therefore the assay will only be performed if Cal-1 marking is ≥ 1% at the corresponding time point.

In the event that a predominant integration site(s) is detected, persistence will be confirmed by performing another analysis for clonality no more than 3 months later. A confirmed pattern of predominance will be further investigated to determine the nucleotide sequence adjacent to the site of vector integration, and this sequence compared to known sequences in the human genome database to identify if it is a known oncogene.

Significance of oligo- or mono-clonality must be evaluated in association with clinical laboratory data (CBC/differential) and observations (physical exam) relevant to that point in time. A predominant integration site in the absence of any abnormal clinical observations is to be recorded as an AE and continue to be followed for clinical significance. In the event that a hematopoietic malignancy or other abnormality develops, the event is to be reported as an SAE. Refer to section 10 for more information on adverse event reporting.

C46 immunogenicity studies: An immune response against the expressed C46 peptide is a theoretical risk, and testing for humoral and cell-mediated immune responses will be performed using ACD plasma and viably preserved PBMC aliquots from the stored specimens at pre-infusion Week 12, Week 24 and Week 48 time points. Testing will be batched either by cohort or by time point and will be performed regardless of clinical presentation. Batch sizes and frequency will be tailored to minimize inter-assay variability, and optimal assay capacity/throughput.

CCI [redacted]

Physical Exam (PE): Full PE will be performed at selected visits [Screening 1, Pre-busulfan Assessment, Week 12, Week 24, end of study (Week 48) or early discontinuation/withdrawal]. All other visits include a 'targeted examination' to include follow up examination of previously reported abnormalities and/or investigation of specific symptoms consistent with other clinical, laboratory or subject-reported observations.

Pelvic/genitourinary, anal/rectal and detailed neurologic system examinations should be performed only if considered clinically required by the Investigator.

Vital signs: Temperature, pulse and blood pressure will be obtained after the subject has been at rest for at least 5 minutes and prior to blood collection. Additionally, at the busulfan and cell infusion visits, pulse oximetry for oxygen saturation measurement will be measured (See **Sections 7.1.8** and **7.1.9**)

Weight: Subject should be weighed wearing minimal clothing (i.e., outer coats or bulky items of clothing removed) and without shoes. Subjects should be asked to urinate before being weighed.

Chest x-ray: Posterior-Anterior (PA) and lateral views

Replication Competent Lentivirus (RCL): Tests for the presence of RCL will be performed by VSV-G PCR using subject peripheral blood DNA samples obtained pre-infusion and post-infusion Weeks 12, 24 and 48. Samples of PBMC stored cryogenically or DNA extracted from peripheral blood may also be used to test for RCL at additional time points in the event of an adverse event that has suspect causality with Cal-1. These stored samples are collected at various time points as indicated in the Schedule of Events.

If a positive VSV-G PCR signal is obtained, an additional peripheral blood DNA sample will be obtained as soon as possible for the purposes of confirming the result. A confirmed positive VSV-G signal will be reported as an SAE (per SAE reporting requirements in section 10.1.3) and blood collected for further investigation.

7.4 Feasibility Evaluations

Feasibility measures will include analysis of the T^{tn} and $HSPC^{tn}$ release and characterization criteria as defined in section 6.1.

7.5 Efficacy Evaluations

HIV-1 RNA: An FDA approved plasma HIV-1 RNA assay with a wide quantitative range will be used for real time monitoring of HIV-1 viral load monitoring.

Gene transfer using a lentiviral vector has been associated with a molecular cross-reaction with the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 RNA PCR assay *(De Ravin et al, 2014)* and such cross-reactivity confirmed by Calimmune laboratory studies to also occur with Cal-1. For this reason, except for pre-screening, an EDTA plasma sample will be stored at each time point that a real time HIV-1 RNA analysis is performed. This stored sample will be used for batch analysis with the Abbott m2000 HIV-1 assay (quantitative range 40 to 10,000,000 copies/mL). The Abbott assay does not show cross-reactivity with Cal-1.

Batching will be by order of receipt unless otherwise indicated (according to safety limit retesting as described in **Section 6.2.4**). The Abbott m2000 assay will not be used to determine eligibility of subjects, and screen-failed subject samples will not be analyzed.

Cal-1 marking/expression: will be analyzed by quantitative PCR for Cal-1 DNA (WPRE region) and RT-PCR for both C46 and sh5 RNA in the following cell types:

- Peripheral blood (PB)
- Peripheral blood subpopulations (Monocytes, granulocytes, CD4+ & CD8+ lymphocytes)
- Bone marrow aspirate
- Gut associated lymphoid tissue (GALT)

Lymphocyte phenotype (standard T-cell count): Flow cytometry analysis of CD3, CD4 and CD8 cells will be reported as number of cells/µl (mm^3) whole blood, percentage, and CD4:CD8 ratio.

Lymphocyte Development Studies - Thymopoiesis: Thymic function is known to decline with HIV-1 infection and disease progression. CCI [redacted]

Lymphocyte Development Studies – Maturation: Flow cytometry analysis of CCI [redacted]

Lymphocyte Development Studies - Inflammatory status: Flow cytometry analysis of the immune activation markers, CCI [redacted]

CCI [redacted]

CCI [redacted]

7.6 Exploratory Evaluations

CCI [redacted]

7.7 Specimen storage

Samples of cryopreserved PBMC, ACD plasma and SST serum will be frozen at various time points as indicated in the Schedule of Events. Additionally, any surplus DNA and/or RNA after Cal-1 marking/expression and integration analysis is completed, will also be stored. These samples will be archived for the duration of the study and the required long term follow up period. CCI [redacted]

8. SUBJECT WITHDRAWAL/DISCONTINUATION

Participants have the right to withdraw from the trial at any time for any reason without affecting their right to follow-up or standard of care treatment which they would otherwise receive.

If a subject withdraws or discontinues from participation during the pre-infusion phase, no follow up will be performed other than for adverse events, as applicable. Adverse events that warrant discontinuation during this phase will be followed per standard of care and their outcome documented. SAEs will be followed as described in **Section 10.1.3**.

If withdrawal or discontinuation was after the aphereses, but prior to busulfan administration, the transduction and culture processes will be completed for cells collected, and stored for a maximum period of 6 months. After this time, if it is confirmed that the subject will not proceed, the T^{tn} and $HSPC^{tn}$ cell products will be destroyed.

Reasons a subject must withdraw during the pre-infusion phase include, but are not limited to:

- Serious adverse event relating to apheresis, mobilization or any other cause that prevents completion of the scheduled study procedures
- Adverse event that causes the subject to no longer be able to comply with the study requirements
- Death
- Pregnancy
- Subject request (withdrawal of consent)
- Protocol violation relating to eligibility
- Non-compliance with the G-CSF dosing schedule (≥ 2 doses missed)
- Any event that prevents the successful infusion of both T^{tn} and $HSPC^{tn}$ cellular products.
- Trial termination by the Sponsor

Reasons a subject may withdraw during the post-infusion phase include, but are not limited to:

- Adverse event that causes the subject to no longer be able to comply with the study requirements
- Pregnancy
- Death
- Subject request (withdrawal of consent)
- Trial termination by the Sponsor

If possible, subjects who meet any one of the post-infusion withdrawal criteria will be asked to attend an Early Discontinuation/Withdrawal visit and then continue safety follow up per the schedule of events until Week 48. In the event that a subject meets any of the secondary analysis endpoint criteria (discontinuation as defined in **Section 9**), they will be asked to attend an Early Discontinuation/Withdrawal visit and then continue per the schedule of events until Week 48 or until HIV suppression is demonstrated. If a discontinuation/secondary analysis endpoint is prior to Week 12, the bone marrow aspirate and GALT biopsies should be performed, if possible, as part of the Early Discontinuation/Withdrawal visit.

The following assessments are to be performed as part of the Early Discontinuation/Withdrawal visit:

- Complete physical examination
- Vitals signs (temperature, blood pressure and pulse)
- Weight (shoes and outer clothing removed)
- Assessment for new or changed adverse events
- Assessment for new or changed concomitant medications & therapies.
- Clinical laboratory testing for the following:
    - Complete blood count with differential and platelet count
    - Biochemistry (fasting not required)
    - HIV-1 RNA in plasma
    - Lymphocyte phenotyping (CD3+/CD4+/CD8+)
    - Pregnancy testing (serum β-HCG) for women of childbearing potential
    - HIV-1 R5/X4 tropism
    - Cal-1 marking/expression analysis – PB
    - Cal-1 integration site analysis
    - Lymphocyte Development Studies

- Cal-1 marking/expression analysis – Subsets (conditions apply - see footnote 19 to Schedule of Events)
- Specimen storage sample - plasma, serum and cryopreserved PBMC (conditions apply - see footnote 19 to Schedule of Events)

As outlined in **Section 7.2.5**, an additional bone marrow aspirate and GALT biopsy may be performed at the discretion of the Principal Investigator and the consent of the subject if the subject discontinues or withdraws from the study *within 8 weeks* of the Week 12 or 24 visits (i.e. having already recently undergone the scheduled bone marrow aspirate and GALT biopsies).

For the purposes of data analysis, the date and reason for discontinuation or secondary analysis endpoint criteria will be captured in the source documentation and CRF.

In the event that a subject decides to withdraw consent, a reason does not have to be provided. However, every attempt should be made to counsel the subject on the implication to the study and their ongoing safety monitoring if they choose to withdraw. He/she should be asked to attend long term follow up. The outcome of such discussions should be documented.

A subject will be considered lost to follow-up if contact cannot be resumed after reasonable and documented attempts by the investigator. A minimum of 3 attempts are required over a reasonable time frame, with both the subject directly and the alternate contacts provided at study entry. At least 1 attempt must be in writing before a subject can be declared lost to follow up. Should a subject re-initiate contact after being declared lost to follow up, the subject can be re-instated and re-commence safety follow up visits at the next scheduled time point.

Participants who withdraw or discontinue early from the trial after infusion (Day 0) will not be replaced. An excessive rate of withdrawals/discontinuations can render the trial non-interpretable; therefore, every care should be taken by the investigator during the subject selection and consenting process to ensure suitable subjects are selected.

9. STATISTICAL METHODS

This is a Phase I/II study, and the sample size has not been based on formal power considerations. No recruitment limit is set for any of the participating sites, with the only restriction being consecutive enrollment that does not exceed 4 subjects per cohort. Subjects for the next cohort will not be treated (i.e. commence busulfan) until the DSMB recommendations are received.

Safety

In terms of safety data, pooling all subjects across the three arms, the study will have at least an 80% probability of observing one or more serious adverse events if the true rate of these events in a subject is 12.6% or higher for 12 subjects, or ≥ 16.4% if 9 or more subjects. Pooling the busulfan subjects will give at least an 80% probability of observing one or more serious adverse events that occur with a true rate of 18.2% per subject for n=8, and 23.5% for n=6. Within any arm of four

subjects, the study will have 80% probability of detecting events with a true rate of 33.1% per subject.

Hence the study will be well powered to detect only serious adverse events that occur with a reasonably high probability. The study will not be well powered to detect events that are rarer than about 10-20% per subject.

Feasibility

Feasibility measures will include analysis of the T^{tn} and $HSPC^{tn}$ release criteria and characterization. Correlations between safety and secondary efficacy data with feasibility measures will be exploratory and made where a causal relationship of an adverse event to the investigational product or study procedure is classified as possible, probable or very likely. It is accepted that these analyses will have limited power.

Key efficacy variables

Pooling all subjects, and assuming that the within subject variability corresponds to a standard deviation of 1.0 logs, the study will have 80% power to detect a mean change in Log_{10} HIV-1 RNA of 0.81 logs (2-alpha = 5%) for 12 subjects, , or ≥ 0.94 logs if 9 or more subjects. For changes in CD4+ T lymphocyte count across all subjects, assuming the within subject variability corresponds to a standard deviation of 250 cells/μL (mm^3) (Australian HIV Observational Database – data on file), the study will have 80% power to detect a mean change in CD4+ T lymphocyte count of 205 cells/μL (mm^3) for 12 subjects, or ≥ 235 cells//μL (mm^3) if 9 or more subjects.

CCI [redacted]

Secondary analysis endpoints

Early endpoints for secondary analysis (discontinuation) include:

- Commencement of ART for any reason
- Commencement of systemic immunosuppressive therapy/immunomodulatory therapy. Refer to **Appendix II** for the list of limited and prohibited immunosuppressive and immunomodulatory agents.

9.1 Statistical Analyses

The primary analysis will be conducted when all recruited subjects have completed 48 weeks follow-up or have ceased trial follow-up before that time.

Primary analyses will include all subjects who received both cell infusions. Subjects who consented to the trial, but subsequently were not infused with the investigational product, will be excluded. If any subjects have partial or incomplete infusions, then sensitivity analyses will also be performed excluding these subjects.

Subject baseline demographic and clinical information will be summarized by treatment received and overall. There will be no formal comparison of the treatment groups in terms of baseline factors (i.e. no *p*-values).

Treatment received will be summarized, as will duration of subject follow-up. Any subjects discontinuing (i.e. starting antiretroviral or prohibited concomitant medication treatment), withdrawing (e.g. withdrawing consent to trial follow-up, lost to trial follow-up etc.) will be listed. The time to starting antiretroviral treatment will be summarized using Kaplan-Meier plots.

Safety

The severity of clinical adverse events and laboratory safety parameter adverse events will be graded according to DAIDS criteria. The original term used in the case report form (CRF) to document adverse events will be coded using the current Medical Dictionary for Regulatory Activities (MedDRA).

Numbers of individual adverse events, in numbers of subjects, will be summarized by severity and treatment, both overall and by nominal study week. Adverse events will also be summarized and aggregated by body system for clinical events.

The worst severity of each clinical and laboratory adverse event, for each subject, will be summarized by treatment.

All grade 3 and 4 adverse events, and any other events that lead to a subject ceasing trial follow-up, will be listed by treatment, with duration and resolution.

Feasibility

Feasibility measures such as the number of T^{tn} and $HSPC^{tn}$ manufacturing procedures successfully completed (i.e. complying with all release criteria); number of gene modified cells, as affected by CD4+ and CD34+ purity, transduction efficiency, and viability; and the number of target cells harvested will be summarized overall.

CCI

Associations with continuous safety and secondary efficacy data, such as laboratory parameters, will use repeated measures regression analyses. Associations with binary events will compare

the feasibility measures in those patients with and without events using linear regression methods or non-parametric equivalents as appropriate.

Efficacy endpoints

Log_{10} HIV-1 RNA, lymphocytes (CD4+ T lymphocyte count, CD4+ %, CD4:CD8 ratio), measures of Cal-1 marking/expression and lymphocyte development will be plotted over time for each individual subject during the period(s) of time they remain off ART, and will be summarized for each nominal study week by treatment and overall. Batch analysis data from the Abbott RealTi*m*e m2000 assay will be used for these analysis.

Changes in Log_{10} HIV-1 RNA and CD4+ T lymphocyte count from baseline will be formally compared in all subjects using one-sample t-tests or non-parametric equivalents as appropriate.

CCI [redacted]

9.2 Preliminary Analyses

There are two planned safety analyses and one preliminary analysis.

The first safety analysis will be when three subjects in Cohort 1 (all receiving T^{tn} and $HSPC^{tn}$ with no busulfan) have completed 12 weeks follow-up. The second safety analysis will be timed for when three subjects in Cohort 2 (all receiving T^{tn} and $HSPC^{tn}$ with single dose of busulfan) have completed 12 weeks follow-up. The independent DSMB will conduct a complete review of safety data at each of these analyses before administration of study treatment in the following cohort of subjects is commenced.

At each safety analysis, all subjects' safety data will be summarized including all available data up to the time the analysis is conducted. Measures of Cal-1 marking/expression will also be summarized. There will be no formal statistical stopping rules; rather the study will continue on the basis that the medical review Committee determines that there is no evidence of safety concerns to necessitate study cessation. Reasons for not continuing with the next planned cohort may include the occurrence of grade 4 elevation of transaminases (>10.0 x ULN ALT and AST) or thrombocytopenia (<25,000/mm^3 or $25x10^9$/L), unanticipated incidence, severity or frequency of AE/SAEs, and outcome of T^{tn}/$HSPC^{tn}$ manufacturing processes. Efficacy indicators, such as the degree of Cal-1 marking in peripheral blood, GALT and bone marrow will also be assessed.

The preliminary analysis will include safety data and secondary endpoints when all subjects have completed 24 weeks follow-up.

10. ADVERSE EVENT REPORTING

Timely, accurate, and complete reporting and analysis of safety information is crucial for the protection of subjects, investigators, and the Sponsor, and are mandated by regulatory agencies worldwide. The Sponsor or its contracted designee(s) has established Standard Operating Procedures (SOPs) in conformity with applicable regulatory requirements to ensure appropriate reporting of safety information.

10.1 Adverse Event Definitions and Criteria

An adverse event (AE) is any untoward medical occurrence in a clinical study subject administered a medicinal (investigational or non-investigational) product. An AE does not necessarily have a causal relationship with the treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal finding), symptom, or disease temporally associated with the use of a medicinal (investigational or non-investigational) product, whether or not related to that medicinal (investigational or non-investigational) product (Definition per International Conference on Harmonization [ICH]). For the purposes of this study, this definition also includes the study specified procedures.

An AE also includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition, or abnormal results of diagnostic procedures, including laboratory test abnormalities.

AE reporting starts on the day of the Screening 1 visit (with the first study-related procedure) and ends at study completion. In the event of withdrawal of consent, the subject will be asked to continue in long term follow up to allow ongoing collection of AE and safety information.

All AEs, regardless of seriousness, severity, or presumed relationship to the investigational product or study procedures, must be recorded using medical terminology in the source document and the CRF. Whenever possible, diagnoses may be recorded when signs and symptoms are due to a common etiology (e.g., cough, runny nose, sneezing, sore throat, and head congestion should be reported as “upper respiratory infection”). Investigators must record their opinion concerning the relationship of adverse events to study therapy or procedures in the source document. All measures required for adverse event management must be recorded in the source document.

Analysis for emergence of any predominant site of integration of Cal-1 in peripheral blood is routinely conducted throughout the post-infusion study period (as per the schedule of events). Clonal expansion of any cell with a particular integration site could be an early indication of malignancy secondary to the insertion of Cal-1. In the event that a predominant integration site(s) is detected, clinical significance must be evaluated in association with laboratory data (CBC/differential) and observations (physical exam) relevant to that point in time. A predominant integration site in the absence of any abnormal clinical observations is to be recorded as an AE and continue to be followed for clinical significance. In the event that a

hematopoietic malignancy or other abnormality develops, the event is to be reported as an SAE (see section 10.1.4).

10.1.1 Adverse Event Severity Criteria

Adverse events are to be graded into 4 potential severities: mild, moderate, severe or potentially life threatening, according to the Division of AIDS Table for Grading the Severity of Adult Adverse Events (see Appendix I). If the need arises to grade a specific clinical or laboratory adverse event that is not identified in the DAIDS AAE grading table, the "Estimating Severity Grade" is to apply.

If the severity of an adverse event could fall under either one of two grades (e.g., the severity could be either grade 1 or grade 2), select the higher of the two grades.

10.1.2 Adverse Event Causality/Association

An adverse event is considered associated with the use of the investigational product, concomitant medication or study procedure if the attribution is possible, probable, or very likely by the definitions below:

Not related: An adverse event that is not considered to be associated or attributed to the use of the investigational product or procedure.

Doubtful: An adverse event for which an alternative explanation is more likely, e.g., concomitant drug(s), concomitant disease(s), or the relationship in time suggests that a causal relationship is unlikely.

Possible: An adverse event that might be due to the use of the investigational product or procedure. An alternative explanation, e.g., concomitant drug(s), concomitant disease(s), is inconclusive. The relationship in time is reasonable; therefore, the causal relationship cannot be excluded.

Probable: An adverse event that might be due to the use of the investigational product or procedure. The relationship in time is suggestive. An alternative explanation is less likely, e.g., concomitant drug(s), concomitant disease(s).

Very likely: An adverse event that is listed as a possible adverse reaction in the applicable product reference safety information and cannot be reasonably explained by an alternative explanation, e.g., concomitant drug(s), concomitant disease(s). The relationship in time is very suggestive.

For the investigational product, the expectedness of an adverse event will be determined by whether or not it is described in the Investigator's Brochure. For the protocol required marketed products such as G-CSF (Neupogen®), plerixafor (Mozobil®) and busulfan (Busulfex®), the expectedness of an adverse event will be determined by whether or not it is listed in the approved label for the product. For study required procedures, the expectedness of an adverse

event will be determined by known risks as described in the subject information and consent form and according to the investigator's judgment.

10.1.3 Serious Adverse Event

A serious adverse event (SAE) as defined by ICH/GCP is any untoward medical occurrence that at any dose meets any of the following conditions:

- Results in death
- Is life-threatening (the subject was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe or if left untreated)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect

Medical and scientific judgment should be exercised in deciding whether it would be appropriate to report an event that does not meet the above criteria, but may be an important medical event. These situations should also be considered as serious and be reported as an SAE. For gene transfer studies, additional events to be reported as SAEs include, but are not limited to:

- Any malignancy, with the exception of basal cell carcinoma, squamous cell carcinoma or carcinoma of the cervix in situ
- A predominant integration site as detected by the Cal-1 integration assessment assay in association with abnormalities in clinical laboratory data or physical examination
- A confirmed positive VSV-G PCR signal for detection of Replication Competent Lentivirus.

All SAEs occurring during this study must be reported by the investigational staff to the appropriate Sponsor contact person within 24 hours of their knowledge of the event. Contact details for SAE reporting are provided on the contacts page of this protocol. The appropriate SAE reporting form template must be used.

SAEs that have not resolved by the end of the study, or that have not resolved upon discontinuation of the subject's participation in the study, must be followed until any of the following occurs:

- The event resolves
- The event stabilizes
- The event returns to baseline, if a baseline value is available

- The event can be attributed to agents other than the study drug or to factors unrelated to study conduct
- It becomes unlikely that any additional information can be obtained (subject or healthcare practitioner refusal to provide additional information, lost to follow-up after demonstration of due diligence with follow-up efforts)

Any event requiring hospitalization that occurs during the course of a subject's participation in this clinical study must be reported as a serious adverse event, except hospitalizations for the following reasons:

- Social reasons in absence of an adverse event
- Admissions that do not require an overnight stay (for example, admission for a day procedure or for observation for less than a 24 hour period).
- Planned surgery or procedure before entry into the study (must be documented in the source document and CRF)

In the event of a subject death secondary to any malignancy, or to any hematopoietic, immunologic or neurologic abnormality, the investigator must follow up to try to obtain post mortem tissue samples for the purposes of analysis for Cal-1. Subjects will be informed of this requirement and asked to consider their wishes in this regard. A subject is not obliged to agree to autopsy in order to participate in the study and consent for autopsy will be documented separately. Tissue samples to be requested include:

- Any malignant or benign tumor
- Bone marrow
- Lymph node

All tissue samples obtained will be processed to extract DNA for the purposes of PCR analysis for Cal-1 marking, integration analysis and RCL.

10.1.4 Serious & Unexpected Suspected Adverse Reaction (SUSAR)

An unexpected serious suspected adverse reaction is one for which the nature or severity is not consistent with the applicable reference safety information, and thus requires expedited reporting to authorities and all participating investigators as required according to local guidelines and regulations. Medical and scientific judgment should however be exercised in deciding whether expedited reporting is also appropriate in situations other than those listed above. For example, important medical events may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject, may require intervention to prevent an SAE or may be of wider scientific or medical interest due to suspect causality.

In the event of a SUSAR, storage samples from the subject may undergo analysis for RCL, integration analysis and marking/expression testing. Additional blood and/or tissue samples may be requested to enable further evaluation.

SUSARs that represent a serious threat to public health will be reported within 48 hours. Fatal and life threatening SUSARs will be reported as soon as possible, and not later than 7 calendar days from the date the event report was initially received from the reporting investigator. All other SUSARs and safety issues warranting expedited reporting will be reported no later than 15 calendar days from the date the event report was initially received from the reporting investigator. In each case, relevant follow up and outcome information will be sought and submitted as soon as possible. The Sponsor (or contracted designee) assumes responsibility for appropriate reporting of adverse events to the regulatory authorities. The Sponsor will also report all SUSARs to the investigator. The investigator (or Sponsor where required) must report these events to the appropriate Institutional Ethics Committee (IEC) and Institutional Biosafety Committee (IBC) that approved the protocol unless otherwise required and documented by the IEC and IBC.

10.2 Notification of pregnancy

Female subjects of child-bearing potential, as well as male subjects with partners of child-bearing potential must practice an acceptable method of effective birth control while in the study. Pregnancy occurring in either a female study subject or the partner of a male subject in the trial must be reported to the Sponsor by the investigational staff within 24 hours of their knowledge of the event using the appropriate pregnancy notification form. The study subject and/or female partner should be counseled, and the risks of continuing the pregnancy discussed, as well as the possible effects on the fetus.

Any study subject who becomes pregnant during the study will be discontinued from secondary analysis, but asked to continue in follow up to allow collection of adverse event and pregnancy outcome information. If pregnancy occurs prior to Week 12, the scheduled bone marrow aspirate and GALT biopsy procedures will not be performed. The investigator is to assess if a pregnant subject should continue with the scheduled blood collections. This assessment is to be documented in the subject's medical record/clinical source document.

Follow-up information regarding any postnatal sequelae in the infant will be required. Abnormal pregnancy outcomes are considered SAEs and must be reported using the SAE notification form.

Contact details for notification of pregnancy are provided on the contact information page of this protocol.

11. SPECIAL CONSIDERATIONS

11.1 Study-Specific Design Considerations

The safety profile of Cal-1 has not been established, subjects may therefore be placing themselves at risk of unexpected acute, sub-acute and/or long term adverse events by participating in this study.

To participate in this study, potential subjects must have made a prior decision to cease ART due to concerns over short-term or long-term toxicities associated with antiretroviral agents, or treatment fatigue from the daily regimen of life-long therapy. Efficacy of Cal-1 has not been established and subjects could fail to achieve optimal virologic control during the course of the study. To mitigate potential risk to the subject, only subjects with a documented virologically-effective ART regimen option will be enrolled and HIV-related criteria for the commencement of ART are specified in **Section 6.2.4**.

The potential risks that are apparent in the present study include:

- exposure to the investigational agent, with the potential for unknown acute, sub-acute and/or long term side effects;
- exposure to the alkylating bone morrow conditioning agent busulfan (Busulfex®) with the potential for both known and unknown side effects, including potential carcinogenicity;
- possibility of mishap during the cell processing procedures, resulting in inability to proceed with infusion of the modified cell product; and
- the inherent risks associated with venipuncture, apheresis and biopsies.

The study has been designed to balance these risks with potential benefit and improved understanding of Cal-1 and the associated procedures.

The use of the 3 cohorts in this study does not represent an ethical compromise because all subjects will receive the same post-infusion follow up and this study is designed to answer important questions about Cal-1, including the benefit/risk of using busulfan as a means to increasing bone marrow engraftment of the transduced HSPC^{tn} population as well as provide scientific guidance on using Cal-1 in an ART-intolerant subject population.

11.2 Ethical and Regulatory Information

This study is being conducted under an IND (CCI [redacted]) and has undergone public review by the Recombinant DNA Advisory Committee of the NIH Office of Biotechnology Activities. The study is registered and listed in clinicaltrials.gov (NCT01734850).

Before the start of the study, the investigator (or Sponsor where required) will provide the Institutional Ethics Committee (IEC) and Institutional Biosafety Committee (IBC) with current and complete copies of the following documents:

- Final protocol and, if applicable, amendments
- Sponsor-approved informed consent form (and any other written materials to be provided to the subjects, including any Sponsor-approved recruiting materials)
- Investigator's Brochure and, if applicable, amendments
- Any other documents that the IEC or IBC requests to fulfill its obligation

This study will be undertaken only after the IEC and IBC has given full approval of the submitted material, and the Sponsor has received a copy of this approval.

During the study, the investigator (or Sponsor where required) will send the following documents and updates to the IEC and IBC for their review and approval, where appropriate:

- Protocol amendments
- Revision(s) to informed consent form and any other written materials to be provided to subjects
- If applicable, new or revised subject recruiting materials approved by the Sponsor
- Investigator's Brochure amendments or new edition(s)
- Summaries of the status of the study at intervals stipulated in guidelines of the IEC and IBC (at least annually)
- Reports of adverse events that are serious, unlisted, and associated with the investigational drug
- New information that may adversely affect the safety of the subjects or the conduct of the study or represent a serious threat to public health
- Deviations from, or changes to, the protocol
- Notification if a new investigator is responsible for the study at the site
- Annual Safety Report and Line Listings, where applicable
- Recommendations of the independent Data safety Monitoring Board
- Any other requirements of the IEC and/or IBC

For all protocol amendments (excluding the ones that are purely administrative, with no consequences for subjects, data or trial conduct), the amendment and applicable informed consent form revisions must be submitted promptly to the IEC and IBC for review. Approval must be obtained in writing before implementation of the change(s).

If any study subject incurs an injury or health problem that is due to Cal-1 or participation in this study, the Sponsor will cover the costs of investigations and necessary treatment.

Regulatory agencies may be asked to adjudicate the likelihood of attribution to causality to Cal-1.

At the end of the study, the investigator (or Sponsor where required) will notify the IEC and IBC about the study completion.

Long term follow up is a requirement for all recipient of experimental gene transfer therapy and is an important means of determining long term ethical and medical implications. All subjects enrolled in a Cal-1 protocol will be informed of the long term follow up requirements at the time of initial consent.

11.3 Informed Consent

Informed consent is a process and the subject will be given sufficient time to read the informed consent form and the opportunity to ask questions and consider the answers. Before enrollment in the study, the investigator or an authorized and qualified member of the investigational staff must explain to potential subjects the aims, methods, reasonably anticipated benefits, and potential hazards or discomforts of the study, and the obligations that participation in the study and long term follow up may entail. This process of consent should be documented in the source document and completion of the process documented by means of each subject personally dating their own signature on the study consent form. The consent form must be signed before any study-related procedure is performed, and may be signed up to 3 months prior to the Screening 1 study visit. The consent form that is used must have prior written approval by the reviewing IEC. The informed consent should be in accordance with principles that originated in the Declaration of Helsinki, current ICH and GCP guidelines, applicable regulatory requirements, and Sponsor policy.

Potential study subjects will be told that alternative treatments are available if they refuse to take part and that such refusal will not prejudice future treatment. They will be informed that choosing not to participate will not affect the care the subject would otherwise receive for the treatment of his or her HIV infection.

Potential study subjects will be informed that their participation is voluntary and that they may withdraw consent to participate at any time. If they choose to withdraw, subjects will be asked to continue in follow up to enable ongoing monitoring of their health for any long term effects of the investigational treatment and contribution to safety data. This includes follow up to at least Week 48 and the separate long term follow up study.

All subjects will be asked to nominate a minimum of one alternative contact to help ensure contact is maintained with the subject throughout the study and the long term follow up period. By signing the informed consent form, the subject is authorizing such access, and agrees to allow a member of the site study staff to contact the subject and/or their nominated contact(s) for the purpose of the study and for follow up for at least 15 years post infusion.

After having obtained written consent, a copy of the signed informed consent form must be given to the subject.

If the subject is unable to read or write, an impartial witness should be present for the entire informed consent process (which includes reading and explaining all written information) and should personally date and sign the informed consent form after the oral consent of the subject is obtained.

Finally, potential study subjects will be told that the investigator will maintain a subject identification register for the purposes of long-term follow-up and that their records may be accessed by health authorities and authorized Sponsor staff without violating the confidentiality of the subject, to the extent permitted by the applicable law(s) or regulations.

11.4 Long Term Follow-up

Current requirements are for all gene transfer recipients to be followed for 15 years post administration of the gene transfer agent to allow safety monitoring and evaluation of recipients for potential long term associated adverse events.

As part of the Cal-1 program, all clinical trial subjects will roll over into a separate long term follow up protocol. This enables completion of the immediate objectives of the clinical trial and provides a streamlined process for all subjects to enter into an established long term follow up protocol. Long term follow up will commence 1.5 years post infusion (Day 0) and will continue at 6-monthly (± 1 month) intervals to 5 years post infusion and then annually thereafter.

The long term follow up obligations for both Sponsor and subject will be explained at study entry, however a more detailed separate long term follow up consent form will also be provided at the time of completion (or early withdrawal) of this study.

11.5 Privacy and Confidentiality

The collection and processing of personal and health data from subjects enrolled in this study will be limited to those data that are necessary to investigate the safety, efficacy, quality, and utility of the investigational study drug, and will be managed in accordance with U.S. Health Insurance Portability and Accountability Act (HIPAA) regulations.

Each subject will be assigned a unique identification code at the time of study screening, and this code will be used throughout the study and follow up period to identify subject-specific data. As it will be important in long-term follow-up to identify the protocol subjects were initially enrolled to, the unique subject identification numbering system will contain references to the original protocol and site. Additionally, initials and date of birth of each subject will be collected to ensure the correct identification of laboratory data and archived blood and/or tissue samples to individual subjects. These data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations. Appropriate technical and organizational measures to protect the personal

data against unauthorized disclosures or access, accidental or unlawful destruction, or accidental loss or alteration must be put in place. Sponsor personnel whose responsibilities require access to personal data agree to keep the identity of study subjects confidential.

The informed consent obtained from the subject includes explicit consent for the processing of personal data and for the investigator to allow direct access to his or her original medical records for study-related monitoring, audit, IEC/IBC review, and possible regulatory inspection. This consent also addresses the transfer of the data to other entities and to other countries and use for publication.

The unique study identification code will be able to be linked back to an individual subject identity only through the subject identification and enrollment log maintained by the investigator. Stored samples will only be used for the described exploratory analysis or investigation into a specific subject adverse event or other safety concern. If no further safety investigations have been required, the subject has the right to request their samples are destroyed after a minimum storage period of 15 years has expired.

The subject has the right to request, through the investigator, access to his or her personal data and the right to request rectification of any data that are not correct or complete. Reasonable steps will be taken to respond to such a request, taking into consideration the nature of the request, the conditions of the study, and the applicable laws and regulations.

12. ADMINISTRATIVE CONSIDERATIONS

12.1 Subject Screening and Enrollment

The investigator will be required to maintain a log of pre-screening and screening activity, which provides de-identified details (initials and date of birth) for all potential subjects who were seen to determine suitability and eligibility for the study.

All screened subjects (i.e. those subjects who signed consent and for whom a unique study identification number was assigned) will be recorded on a subject identification and enrollment log that permits easy identification of each subject during and after the study. This log will be treated as confidential, with the original held at the study site and no copy made for any purpose.

The T^{tn} and $HSPC^{tn}$ manufacturing is specific to each individual study subject, and only one subject's dosage forms can be manufactured at a time. Consequently, screening failures can have a significant detrimental effect on the manufacturing schedules for all participating subjects and for the study timelines over all. In order to minimize potential negative impact, concurrent screening of potential subjects is permitted to the extent that the total number of subjects in screening does not exceed the total number of available places within each cohort (i.e. a maximum of 4). Once a subject/subjects are determined to meet the Screening 1 eligibility criteria, they will progress to Screening 2 in order to confirm HIV RNA and CD4+ lymphocyte counts continue to be within the required range for eligibility. If there are multiple subjects who

have completed Screening 1, their order of progression to Screening 2 will be determined by the Sponsor, taking into consideration their medical history, Screening 1 laboratory results, and factors relating to their overall appropriateness for the study.

12.2 Source Documentation

Source data comprises all information, original record of clinical findings, observations or other activities in a clinical trial, necessary for the reconstruction and evaluation of the trial. The author of an entry or record in the source documents should be identifiable. Any specific source data requirements will be reviewed with the investigator and investigational staff before the study and will be described in the monitoring plan (or equivalent document).

12.2.1 Documentation of Eligibility

At a minimum, source documentation must be available to confirm the study subject's identification and eligibility. Documentation concerning previous ART regimens should include the start and stop dates, with documented reasons for cessation. The reasons for stopping ART are to be in accordance with the special considerations discussed in Section 11.1. The investigator must document what he/she considers to be a virologically-effective ART regimen option for each subject, including the basis for the selection.

Historical CD4+ T lymphocyte records must be complete enough to give reasonable evidence that the CD4+ T lymphocyte count has been ≥ 250 cell/µl since HIV-1 diagnosis (with the exception of the period immediately following acute infection).

12.2.2 On-Study Documentation

At a minimum, the type and level of detail of source data for a study subject should be consistent with that commonly recorded at the site as a basis for standard medical care. Additionally, the source documentation must include study identification, dates of visits, study procedures performed, administration of the transduced cell product, results of safety and efficacy parameters as required by the protocol, record and follow up of all adverse events, concomitant medication, and date of study completion or reason for discontinuation (if applicable). A study subject is not obliged to provide a reason for their decision to withdraw (withdrawal of consent) from the study, however it is expected that the decision and due diligence in inquiry and follow up be documented.

12.3 Case Report Form Completion

A Case Report Form (CRF) is provided for each individual study subject in electronic format.

The study CRF is the primary data collection instrument for the study and the study data will be transcribed by study personnel from the source documents onto the CRF. All data recorded on the CRF must be substantiated by the source documents at the site. All missing data must be explained.

Worksheets may be used for the capture of some data to facilitate completion of the CRF. Any such worksheets will become part of the source documentation for that subject. All data must be entered into the CRFs in English.

Designated site personnel must complete the CRF as soon as possible after a study subject's visit, and the forms should be available for review at the next scheduled monitoring visit.

All CRF entries, corrections, and alterations must be made by the investigator or other authorized study-site personnel, as indicated on the sites delegation of responsibilities and signature log. Data queries will be generated by either the monitor or data management as necessary. The investigator or an authorized member of the investigational staff must adjust the CRF (if applicable) and complete the query. All changes to entered data must have a documented audit trail that allows identification of when and who made the data change.

By signing the study completion page the investigator is attesting that all data recorded in the CRF is a complete and accurate record of the subject's pre-study medical history and on-study observations and results.

Data from exploratory analyses will not be captured in the study CRF.

12.4 Monitoring and Data Quality Assurance

Monitoring will be performed by the Sponsor or contracted designee. The first monitoring visit will be performed as soon as possible, and not more than 2 weeks after screening of the first subject. Thereafter all screened subjects will be monitored to confirm eligibility prior to commencement of apheresis. At the completion of the screening to infusion phase of the study, routine monitoring will be performed on a regular basis until the last subject last visit is achieved and all data monitored. 100% source data verification will be performed to verify the accuracy and completeness of all data collected on the case report forms (CRF). On-site visits will be documented with a signed site visit log kept at the site.

The site is required to maintain source documents that support subject eligibility and all data reported in the CRF. Direct access to source documentation (medical records) must be allowed for the purpose of verifying that the data recorded in the CRF are consistent with the original source data. If electronic records are maintained at the investigational site, compliance with CFR Part 11 and monitor access must be confirmed prior to study initiation at the site. The Sponsor expects that, during monitoring visits, the relevant investigational staff will be available, the source documentation will be accessible, and a suitable environment will be provided for review of study-related documents. The monitor will meet with the investigator and other applicable investigational staff on a regular basis during the study to provide feedback on the study conduct.

Additional steps to be taken to ensure the accuracy and reliability of data include; the selection of qualified investigators and appropriate study center(s), training of the investigator and

associated personnel on the protocol and CRF completion before the study commences and throughout the study period as required per staff or protocol changes, and written instructions for collection, preparation, and shipment of blood, urine and tissue samples.

12.5 Record Retention

In compliance with the ICH/GCP guidelines, the investigator/institution will maintain all CRFs and all source documents that support the data collected from each study subject, as well as all study documents as specified in ICH/GCP Section 8, Essential Documents for the Conduct of a Clinical Trial, and by the applicable regulatory requirement(s).

Essential documents must be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents will be retained for a longer period if required by the applicable regulations or by an agreement with the Sponsor. The investigator/institution will take measures to prevent accidental or premature destruction of these documents. Under no circumstance shall the investigator relocate or dispose of any study documents before having obtained written approval from the Sponsor.

If it becomes necessary for the Sponsor or the applicable regulatory authority to review any documentation relating to this study, the investigator/institution must permit access.

12.6 Regulatory Review and Approval Process

This protocol was submitted for review at the December 12-14, 2011 meeting of the National Institutes of Health (NIH) Recombinant DNA Advisory Committee (RAC), and will be conducted under an IND submitted to the FDA Center for Biologics Evaluation and Research (CBER).

Pending approval of the protocol by RAC and CBER, submission will be made to the applicable Institutional Biosafety Committees (IBC) and Institutional Ethics Committees (IEC) for the selected sites. The protocol will not be initiated until all of the above federal and institutional approvals have been received.

Amendments will be provided to RAC and CBER and will not be implemented until approval is received from both IBC and IEC at all selected sites.

12.7 Trial Committees

12.7.1 Medical Review Committee (MRC)

A medical review committee will be established to ensure that the safety of subjects participating in the study is not compromised. The MRC will include, but will not be limited to, at least two expert physicians in the area of HIV medicine and/or hematology. The MRC will include members from the Sponsor and external consultants. The MRC will monitor the

safety of the study by reviewing AE/SAE, clinical, and laboratory data in a regular and timely manner. Any grade three or four toxicities will be comprehensively reviewed and the MRC will be required to make formal recommendations. Specific recommendations concerning the dose of busulfan will be made by the MRC following assessment of post-conditioning hematopoietic recovery. Delayed hematopoietic recovery is defined as an ANC < $0.5x10^3/\mu L$ (mm^3) and/or platelet count of < $25x10^3/\mu L$ (mm^3) after 42 days post-infusion, and observation of this adverse event in any subject will prompt a reduction in the busulfan dose, as described in section 6.2.2. The MRC will communicate any occurrence of delayed hematopoietic recovery to the DSMB in real-time.

The MRC will assess the hematopoietic recovery of every subject, and following completion of week 12 make formal recommendation if the back-up apheresis product is no longer required. With the agreement of the Principal Investigator, these samples may then be destroyed, or donated to research, in accordance with the subject's wishes at time of study consent.

The MRC will also be responsible for the review and/or input into the case narratives for SUSARs, including adjudication of possible causality to Cal-1 or study procedures.

Details of the composition, roles, responsibilities, and relationship with the DSMB are documented in the MRC charter.

12.7.2 Data Safety Monitoring Board (DSMB)

An independent DSMB will be commissioned for this study to evaluate the risk/benefit of busulfan and to review all safety and selected efficacy (such as the degree of Cal-1 marking in peripheral blood, GALT and bone marrow) data from cohorts 1 and 2 prior to proceeding with the next planned cohort. Progression to each cohort is dependent on the positive recommendation of the DSMB. Details of the composition, roles, responsibilities and timelines will be documented in the DSMB charter.

There are no formal statistical stopping rules, however the DSMB will have the responsibility for independently evaluating safety on the bases of there being no evidence of safety concern(s) to warrant study cessation. The occurrence of bone marrow failure (defined as a protracted neutropenia (ANC < $0.5x10^3/\mu L$ (mm^3)) and/or thrombocytopenia (platelets < $25x10^3/\mu L$ (mm^3)) after Week 12 post-infusion) will trigger a temporary hold on the administration of busulfan and subject enrolment pending a safety assessment by the DSMB. The occurrence of grade 4 laboratory abnormalities, unanticipated incidence, severity or frequency of AE/SAEs, or failure of T^{tn}/$HSPC^{tn}$ manufacturing processes may also be events that could present sufficient safety concerns to warrant study cessation. Following completion of the safety data review, the DSMB may also recommend that the study be modified to improve monitoring of safety, additional subjects be enrolled to assess safety, or the study be terminated for safety reasons.

In addition to scheduled review of Cohort data, the DSMB chairperson will receive a copy of all MRC recommendations and all SAE reports that have possible suspect causality to Cal-1 and/or the study procedures in real time (i.e. at the same time as the MRC). The Chairperson is responsible for determining if a special meeting of the DSMB is required to review any event in further detail and make a recommendation between cohorts. Recommendations will be submitted to the Sponsor and authorities as required, according to local guidelines and regulations.

12.8 Protocol Amendments

Neither the investigator nor the Sponsor will modify this protocol without a formal amendment. All protocol amendments must be issued by the Sponsor, and signed and dated by the investigator. Except when necessary to eliminate immediate hazards to the subjects, amendments must not be implemented without prior IEC/IRB approval.

Documentation of amendment approval by the investigator and IEC/IRB must be provided to the Sponsor or its designee. When the change(s) involves only logistic or administrative aspects of the study, the IRB (and IEC where required) only needs to be notified.

During the course of the study, in situations where a departure from the protocol is unavoidable, the investigator or other physician in attendance will contact the appropriate Sponsor representative (refer to protocol contact information page). Except in emergency situations, this contact should be made before implementing any departure from the protocol. In all cases, contact with the Sponsor must be made as soon as possible to discuss the situation and agree on an appropriate course of action. It is the responsibility of the investigator to document in the source documents any departure from the protocol, the circumstances requiring it, the action taken and the outcome. No waivers or exceptions to the study entry procedures or requirements will be granted.

12.9 Audits

The Sponsor or assigned designees may visit the site at any time during or after completion of the study to conduct an audit of the study in compliance with the protocol and regulatory guidelines. These audits will require access to all study records, including source documents, for inspection and comparison with the CRFs. The investigator and staff are responsible for being present and available for consultation during scheduled site audit visits conducted by the Sponsor or its designees.

Similar auditing procedures may also be conducted by agents of any regulatory body. The investigator should immediately notify the Sponsor if they have been contacted by a regulatory agency concerning an upcoming inspection.

12.10 Disclosure of Financial Interests

12.10.1 Sources of Funding and Disclosure of Financial Interest

This study is financed by Calimmune, Inc. Calimmune, Inc. is financially supported through private and corporate investment, and research grants. This study is partially funded by the California Institute of Regenerative Medicine. These funding sources had no role in the design of this protocol and will not have any role the conduct, analyses, interpretation of the data, or preparation of the study report.

All principal and sub investigators and members of all trial committees (per section 12.7) will be required to complete a written declaration of potential financial interest, disclosing if they, their spouse, dependents, or other institution(s) they are affiliated with, have or receive from Calimmune, Inc.;

- Honoraria or other form of material compensation which exceeds USD25,000
- Grant support for institutional or personal research projects which exceeds USD25,000
- Other research or business arrangement
- Consultancy
- Employment
- Significant equity interest in the form of stock &/or employee stock options to the value of USD50,000 or more.
- Any proprietary or financial interest in Cal-1, such as patent, trademark, copyright, royalty or licensing agreement
- A financial arrangement for the conduct of Cal-1 clinical trials that provides explicitly greater payment for other compensation for a favorable outcome or decision

12.10.2 Subject Payment and Compensation

Study subjects will not be compensated for their participation in this study, except for reasonable travel-related expenses, which will be described in the participant information and consent form and approved by the IEC/IRB.

12.11 Use of Information and Publication

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. The Sponsor will comply with the requirements for publication of trial results.

The results of this trial may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to the Sponsor prior to submission. This allows the Sponsor to protect proprietary information and to provide comments based on information from other studies that may not yet be available to the investigator.

In accordance with standard editorial and ethical practice, the Sponsor will only support publication of multicenter trials in their entirety and not as individual center data. In this case, a coordinating investigator will be designated by mutual agreement.

Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors (ICMJE) authorship requirements. Any formal publication of the trial in which input of Sponsor personnel exceeded that of conventional monitoring will be considered as a joint publication by the investigator and the appropriate Sponsor personnel.

Data derived from analysis of stored blood and cell specimens on individual subjects will not be provided to trial investigators, except where explicitly stipulated in a trial protocol (e.g. if the result is an enrollment criterion) or related to investigation into an adverse event. Exceptions may be granted (e.g. if data would be linked to non subject-specific safety issues). The aggregate results of any research conducted using stored blood and cell specimens will be available in accordance with the effective Sponsor policy on trial data publication.

Any inventions and resulting patents, improvements and/or know-how originating from the use of the stored blood and cell specimens will become and remain the exclusive and unburdened property of the Sponsor, except where agreed otherwise.

All information concerning Cal-1 supplied by the Sponsor to the investigators and not previously published is considered confidential and remains the sole property of the Sponsor. The investigators agree to use this information only to accomplish this study and not for any other purpose without the Sponsor’s written consent.

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APPENDIX I

DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT ADVERSE EVENTS (PUBLISH DATE DECEMBER, 2004; CLARIFICATION AUGUST 2009)

http://rsc.tech-res.com/safetyandpharmacovigilance/gradingtables.aspx

General Instructions

Estimating Severity Grade for Parameters Not Specifically Identified

If the need arises to grade a clinical AE that is not identified in the DAIDS AE grading table, use the category "Estimating Severity Grade" located below.

Determining Severity Grade for Parameters "between Grades"

If the severity of an AE could fall under either one of two grades (e.g., the severity of an AE could be either Grade 2 or Grade 3), select the higher of the two grades for the AE.

Values Below Grade 1

Any laboratory value that is between either the LLN or ULN and Grade 1 should not be graded.

Definitions

Basic Self-care Functions	Activities such as bathing, dressing, toileting, transfer/movement, continence, and feeding.
LLN	Lower limit of normal
Medical Intervention	Use of pharmacologic or biologic agent(s) for treatment of an AE.
NA	Not Applicable
Operative Intervention	Surgical OR other invasive mechanical procedures.
ULN	Upper limit of normal
Usual Social & Functional Activities	Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, pursuing a hobby, etc.

CLINICAL				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
ESTIMATING SEVERITY GRADE				
Clinical adverse event NOT identified elsewhere in this DAIDS AE grading table	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Symptoms causing inability to perform basic self-care functions OR Medical or operative intervention indicated to prevent permanent impairment, persistent disability

GRADE 5: FOR ANY AE WHERE THE OUTCOME IS DEATH, THE SEVERITY OF THE AE IS CLASSIFIED AS GRADE 5.

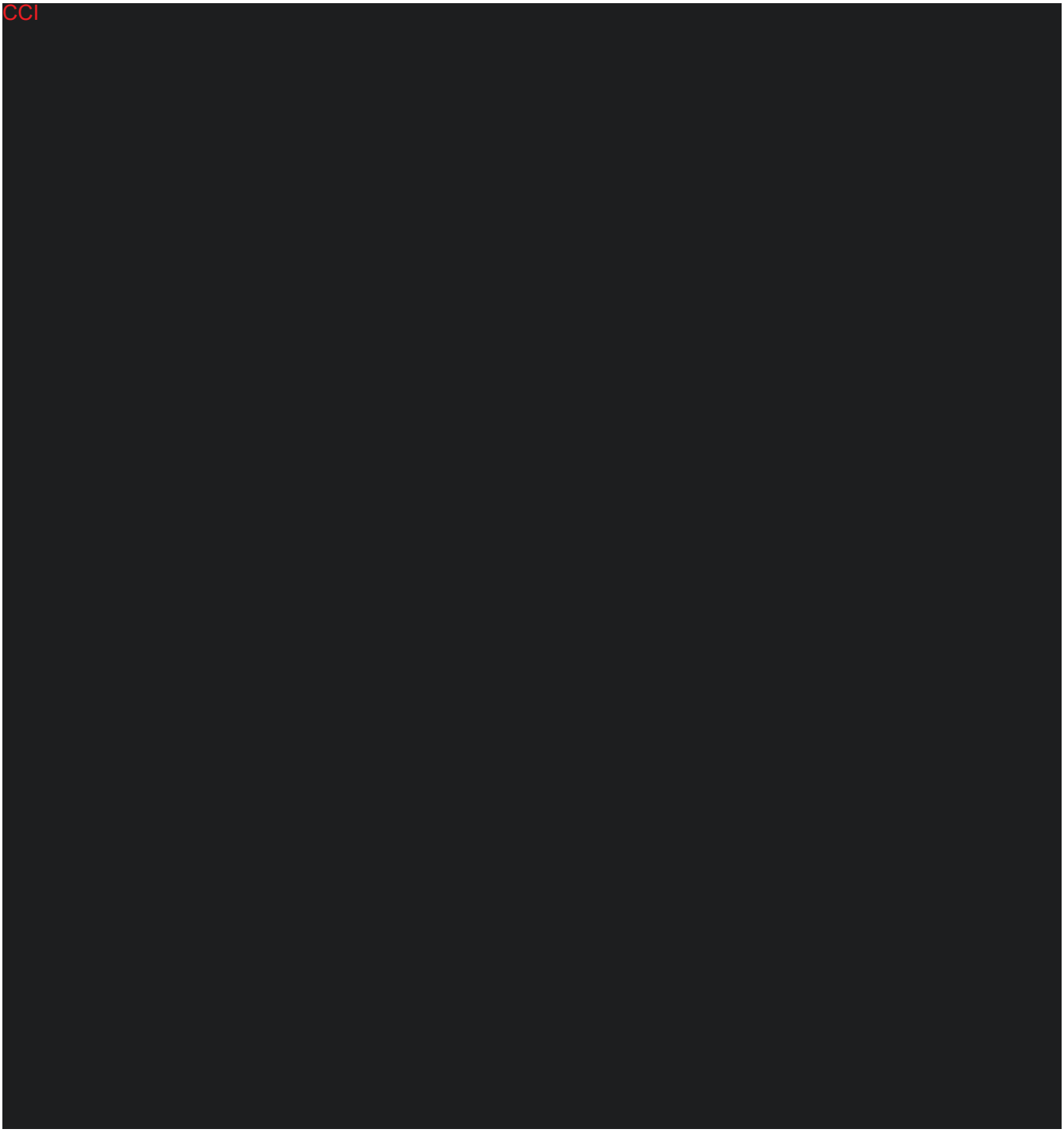
APPENDIX II

PROHIBITED IMMUNOSUPPRESSION / IMMUNOMODULATION

- Azathioprine
- Cyclosporin A
- Cyclophosphamide
- Systemic Glucocorticosteroid therapy cumulative dose ≥ 210mg prednisolone, or equivalent, over a two-week period (e.g. oral prednisolone ≥ 15mg/day for 14 days or more).
- Leflunamide
- Methotrexate
- Mycophenolate (mofetil or sodium)
- All biological immunomodulatory agents (e.g. etanercept, infliximab, adalimumab, anakinra, natalizumab, etc.)
- All immunostimulants (e.g. IL-2, Interferon-α or –β, other cytokine therapies)

APPENDIX III

CCI



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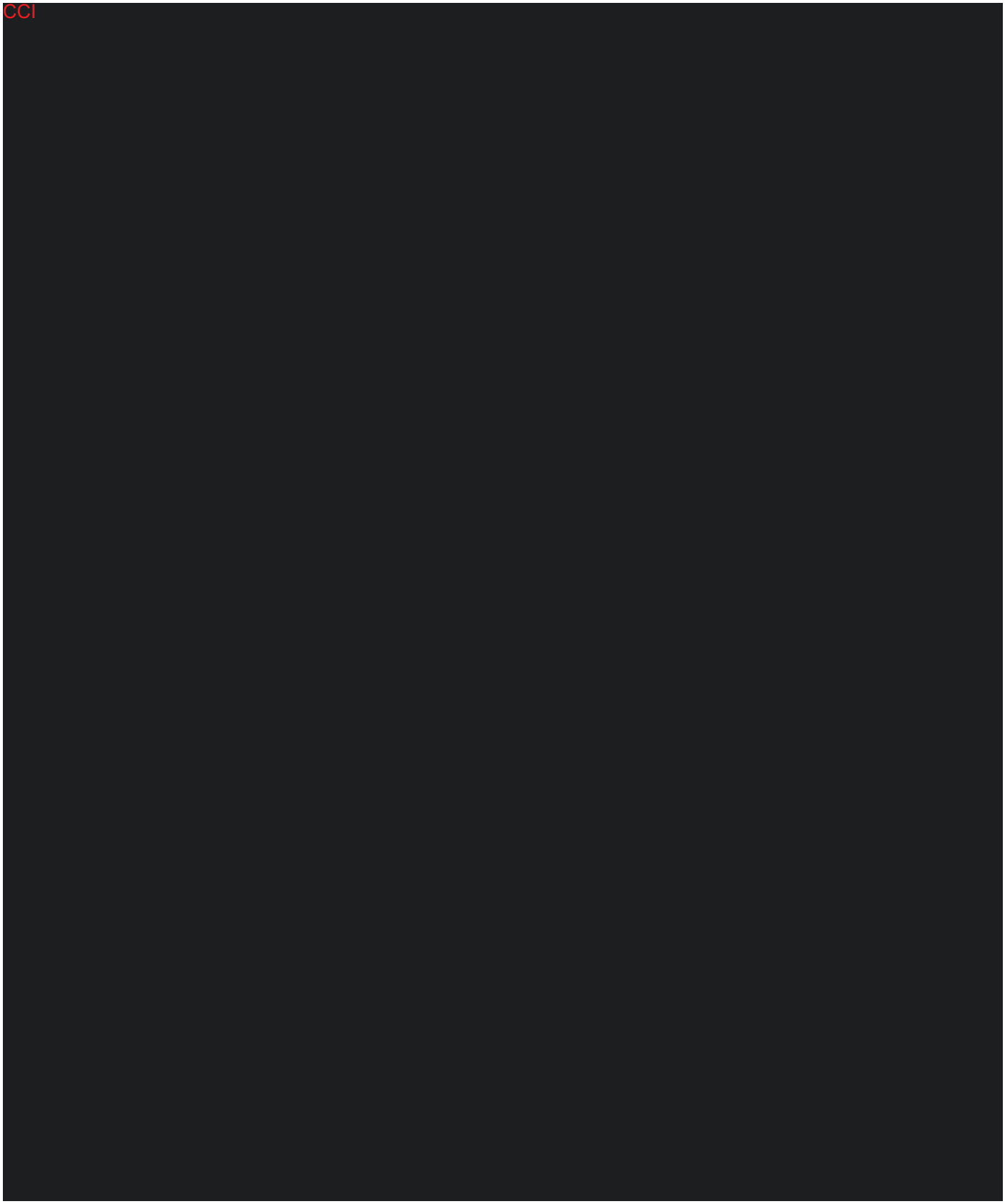
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APPENDIX IV

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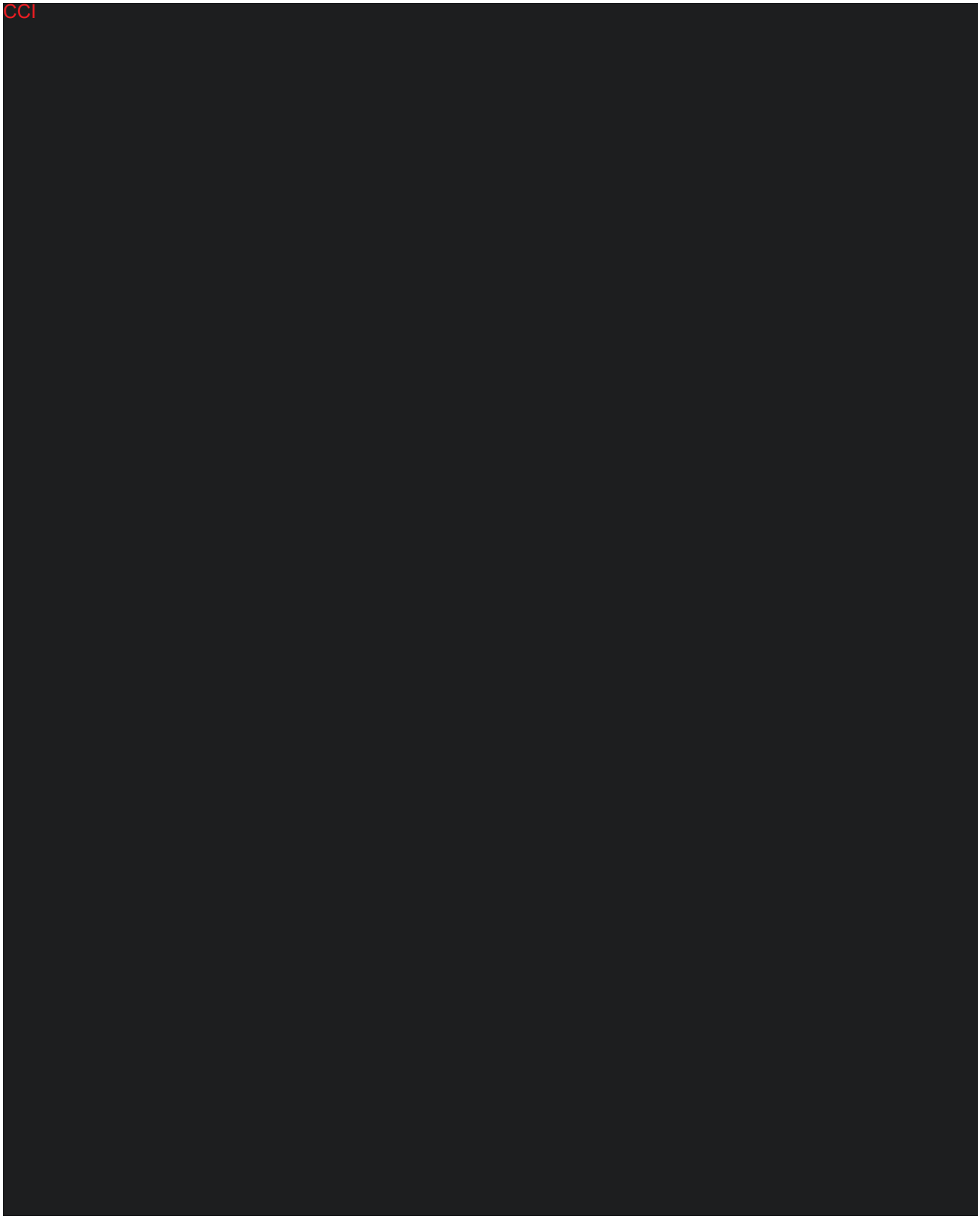

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APPENDIX V

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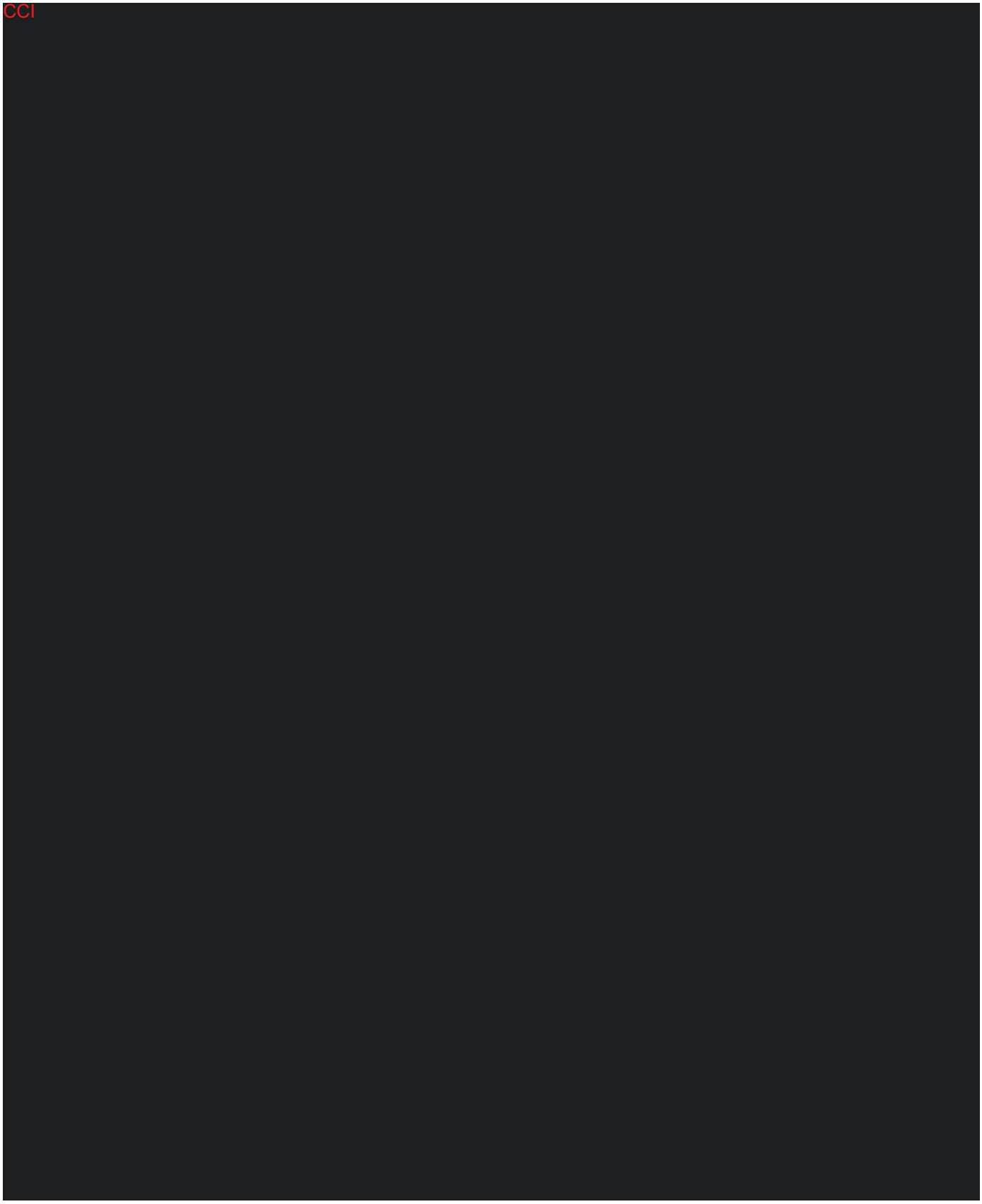


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