2. SYNOPSIS

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Title of Study: 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. Clinicaltrials.gov ID NCT01734850.

Co-ordinating Investigator: PPD

Study Center(s): 2 centers

UCLA Center for Clinical AIDS Research & Education (CARE), Los Angeles, CA, USA

Quest Clinical Research, San Francisco, CA, USA

Publication (reference, if any):

- Burke BP, Boyd MP, Impey H, Breton LR, Bartlett JS, Symonds GP, Hütter G (2014). CCR5 as a natural and modulated target for inhibition of HIV. *Viruses*. 2014; 6(1): 54-68.
- Burke BP, Levin BR, Zhang J, Sahakyan A, Boyer J, Carroll MV, Colón JC, Keech N, Rezek V, Bristol G, Eggers E, Cortado R, Boyd MP, Impey H, Shimizu S, Lowe EL, Ringpis GE, Kim SG, Vatakis DN, Breton LR, Bartlett JS, Chen IS, Kitchen SG, An DS, Symonds GP (2015). Engineering cellular resistance to HIV-1 infection in vivo using a dual therapeutic lentiviral vector. *Mol Ther Nucleic Acids*; 4: e236.
- Peterson CW, Haworth KG, Burke BP, Polacino P, Norman KK, Adair JE, Hu SL, Bartlett JS, Symonds GP, Kiem HP (2016). Multilineage polyclonal engraftment of Cal-1 gene-modified cells and in vivo selection after SHIV infection in a nonhuman primate model of AIDS. *Mol Ther Methods Clin Dev*; 3:16007.
- Symonds G, Bartlett JS, Kiem HP2, Tsie M, Breton L. Cell-Delivered Entry Inhibitors for HIV-1: CCR5 Downregulation and blocking virus/membrane fusion in defending the host cell population (2016). *AIDS Patient Care STDS*. 30(12): 545-550.
- Wolstein O, Boyd M, Millington M, Impey H, Boyer J, Howe A, Delebecque F, Cornetta K, Rothe M, Baum C, Nicolson T, Koldej R, Zhang J, Keech N, Camba Colón J, Breton L, Bartlett J, An DS, Chen IS, Burke B, Symonds GP (2014). Preclinical safety and efficacy of an anti-HIV-1 lentiviral vector containing a short hairpin RNA to CCR5 and the C46 fusion inhibitor. *Mol Ther Methods Clin Dev*. 2014;1:11. Published Feb 12.

<table>
<tr><td>Name of Sponsor
Calimmune Inc.</td><td rowspan="2">Individual Study Table Referring to Part IV Of the Dossier

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Page:</td><td colspan="2" rowspan="2">(For National Authority Use Only)</td></tr>
<tr><td>Drug Substance
Cal-1</td></tr>
<tr><td>Date of First Subject Enrollment: 08 April 2013</td><td>Date of Last Subject Completion: 27 November 2017</td><td colspan="2">Phase of Development:
Phase I/II</td></tr>
<tr><td colspan="4">Background and Rationale for the Study: Human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) is a disease that impairs immune function, primarily by decreasing CD4+ T lymphocytes. Progression can be contained by daily dosing with antiretroviral therapy (ART), but the side effects can be treatment-limiting, and the development of HIV drug resistance may require the clinician 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, whereby an anti-HIV gene(s) is introduced into mature and/or hematopoietic stem/progenitor cells (HSPC) to produce a population of white blood cells (WBC) that are protected from the pathogenic effects of HIV-1. This strategy may provide a continuous means of controlling HIV-1 after a single or infrequent dose(s). This study aimed to protect CD4+ T lymphocytes and 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 HIV-1 fusion inhibitor, C46 peptide.

Clinical studies, meta-analyses, and case reports support the rationale that CCR5 is an excellent target for gene therapy-mediated gene silencing. HIV-1 is well known to rapidly develop resistance to monotherapy, therefore, a second gene therapeutic agent, C46 peptide, which blocks entry of the virus, has also been added. The primary aim of this study is to assess the safety and feasibility of introducing the anti- HIV -1 gene construct LVsh5/C46 (termed Cal-1) into autologous CD4+ T-lymphocytes and hematopoietic stem/progenitor cells (HSPC), as well as the safety of non-myeloablative pre-conditioning with busulfan to improve engraftment of the gene-transduced HSPC.</td></tr>
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Hypotheses:

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 ART.

Secondary Hypotheses

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

- HSPC^{tn} will home to the bone marrow (BM), engraft, and provide a population of peripheral blood (PB) 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 BM, gut-associated lymphoid tissue (GALT), and PB, 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 PB

Objectives:

Primary Objective:

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 Cal-1 transduced CD34+hematopoietic stem/progenitor cell (HSPC^{tn}) engraftment

Secondary Objectives:

The secondary objectives of this study were to assess the difference in the following outcomes between the 3 treatment cohorts:

- The extent of HSPC^{tn} contribution to hematopoiesis and Cal-1 transduced CD4+ T lymphocytes (T^{tn}) survival by evaluation of Cal-1 marking and expression in PB at time points up to study completion or discontinuation
- The extent of HSPC^{tn} contribution to hematopoiesis and T^{tn} survival by evaluation of Cal-1 marking and expression in GALT
- The potential benefit of busulfan conditioning as determined by:
  - The extent of engraftment and differentiation of HSPC^{tn} over time by evaluation of Cal-1 marking and expression in PB subpopulations (monocytes, granulocytes, CD4+ and CD8+ lymphocytes)
  - The extent of HSPC^{tn} engraftment by evaluation of Cal-1 marking and expression in BM at Week 12, 24, 48 and early discontinuation.
- The potential efficacy of Cal-1 in controlling HIV-1 infection for each subject, as measured by:
  - Plasma HIV-1 ribonucleic acid (RNA) relative to the Pre-busulfan Assessment visit
  - Plasma HIV-1 RNA over time
  - CD4+ T lymphocyte count, percentage, and CD4+/CD8+ T lymphocyte ratio relative to the Pre-busulfan Assessment visit
  - CD4+ T lymphocyte count, percentage, and CD4+/CD8+ T lymphocyte ratio over time
  - Time to commencement of ART

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Other secondary objectives for this study included:

- Assessment of impact on lymphocyte development for each subject in PB relative to the pre-infusion baseline over time, as measured by:
  - Changes in thymopoiesis (absolute and percentage [%] of total recent thymic emigrants [RTEs])
  - Changes in CD4+ T cells maturation subsets (absolute and % of naïve, central memory, transitional memory, effector memory and terminally differentiated effector cells)
  - Impact on chronic inflammation for each subject as measured by changes in immune activation markers (human leukocyte antigen-antigen D-related [HLA-DR] and CD38) and the immune exhaustion marker (PD-1) on total number and % of CD4+ T cells and their maturation subsets
- Monitoring for a tropism shift from R5 to dual/mixed or X4 at any time point post-infusion

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Methodology:

This was an adaptive design, open-label, pilot study in HIV-1 infected adults who had a history of previous treatment with ART but were no longer receiving ART. Up to 12 subjects were planned to be enrolled with up to 4 subjects per 3 possible treatment cohorts. Adaptive enrollment of 3 cohorts was used to evaluate busulfan as a pre-infusion conditioning agent to optimize engraftment, subsequent expression, and efficacy of Cal-1 in the progression of HIV-1 infection.

Cohort 1 enrolled subjects, who underwent CD4+ T lymphocyte apheresis and CD34+ HSPC mobilization with Granulocyte-colony stimulating factor (G-CSF) over 5 days, apheresis, and $HSPC^{tn}$ as well as T^{tn} infusion without any busulfan preconditioning. At Week 12 post-infusion for the third subject in Cohort 1, adverse events (AEs), as well as indications of Cal-1 engraftment (marking within BM, PB, and GALT) was assessed by an independent Data Safety Monitoring Board (DSMB). After DSMB approval, Cohort 2 underwent similar procedures with the exception of pre-infusion conditioning with a single dose of 4 mg/kg busulfan on Day -2 before $HSPC^{tn}/T^{tn}$ infusion. Similarly, at Week 12 post infusion for the third subject in Cohort 2, the DSMB compared AEs and indications of Cal-1 marking in Cohort 2 with that of Cohort 1 to assess the effects of busulfan on the early outcomes. The DSMB also assessed whether there was a favorable risk/benefit for busulfan and if a higher dose (8 or 6 mg/kg busulfan) could be administered to Cohort 3 subjects to potentially improve engraftment and Cal-1expression. Upon DSMB approval, busulfan dosing in Cohort 3 comprised 2 IV doses of busulfan, one dose on Day -4 (3 mg/kg) and a second dose on Day -2 (dose based on area under the curve [AUC] exposure data from Day -4 busulfan dosing with total target 2-day exposure of 8,000 µmolar/min AUC). Cohort 3 subjects also received a single dose of plerixafor (0.24 mg/kg) on Day 4 of G-CSF mobilization.

After infusion subjects were followed up for safety and efficacy of the Cal-1 infusion and busulfan conditioning for up to 48 weeks (4 years). Routine subject monitoring was performed weekly for the first 2 weeks and then every second week until 2 months post-infusion, and then monthly to Week 48. After this time, subjects who had resumed ART and demonstrated viral load suppression were transferred into long-term follow up. After infusion, subjects were to remain off ART unless confirmed CD4+ T lymphocyte counts, CD4+ T lymphocyte percentage, or plasma HIV RNA values reached the safety limits, or the subject decided to re-commence ART regardless of pre-specified laboratory parameters. Post-busulfan follow-up was performed more frequently for at least 6 weeks post-infusion. More frequent or extended follow-up was conducted if clinically indicated. Safety and efficacy monitoring included collection of safety and efficacy blood samples as well as collection of BM aspirates and sigmoid colon gastrointestinal mucosal samples for assessment of GALT at Weeks 12, 24, and 48 post-infusion (at Week 12 only for Cohort 1 subjects).

Number of Subjects:
Planned**:** Up to 12
Analyzed: 12

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Diagnosis and Main Criteria for Eligibility:
Eligible patients were aged 18 to 65 years, had confirmed HIV-1 infection, documented effective previous ART shown to suppress HIV RNA to ≤ 50 copies/ml but not taking ART for ≥ 6 weeks before screening, and plasma HIV-1 RNA ≥ 3,200 copies/ml and ≤ 160,000 at final screening visit. Patients were not eligible if they had clinically significant abnormal hematology or biochemistry parameters, CXCR-4-tropic HIV-1, evidence of hepatitis B or C, West Nile Virus, HTLV-1, or tuberculosis infection, or documented history of CD4+ T cell count ≤ 250 cells/mm^3. Patients with any previous or current AIDS-defining illness, including AIDS-related dementia, except for Kaposi's sarcoma confined to the skin were not eligible. Patients with steroid-dependent asthma in the past 5 years, history of seizure, or clinical history of hematologic diseases such as leukemia, myelodysplasia, myeloproliferative disease, thromboembolic disease, sickle cell disorder, thrombocytopenia, or leukopenia were not eligible. Patients currently taking or planning on taking systemic immunosuppressive or immunomodulatory medications during the study were ineligible. Patients who required warfarin, aspirin, or other non-steroidal anti-inflammatory drugs (NSAIDs) or any medication that could affect platelet or coagulation function during the mobilization/apheresis phase and for six weeks after busulfan dosing, were not eligible.

Test Product, Dose and Mode of Administration, Batch Number:
Cal-1 (LVsh5/C46)-transduced CD34+ HSPC ($HSPC^{tn}$) and Cal-1 (LVsh5/C46)-transduced CD4+ T lymphocytes (T^{tn}). A minimum of 2.0 x 10^6 $HSPC^{tn}$ and 0.5-20 x 10^9 T^{tn} with transduction efficiency of ≥ 10% and ≤ 5 copies of the transgene per cell to be administered in 50 ml IV infusion.

Batch number: not applicable.

Duration of Treatment:
Mobilization and aphereses: 10 days (~5 weeks before infusion)
Conditioning: 2 to 4 days before infusion
Infusion: single dose of $HSPC^{tn}$ and single dose of T^{tn} on 1 day
Follow-up period after infusion: 48 weeks (1 year)
(provisional follow-up extension: additional year of follow-up at the end of the study)

Reference Therapy, Dose and Mode of Administration, Batch Number:
Not applicable.

Endpoints - Criteria for Evaluation:

Safety Endpoints

Primary safety endpoints

- Incidence, nature, and severity of adverse events (AEs) and serious adverse events (SAEs)
- Clinical laboratory assessments (hematology, chemistry, lymphocyte phenotype, HIV-1 RNA)
- Abnormal vital signs (pulse rate, systolic and diastolic blood pressure, temperature and oxygen saturation)
- Abnormal physical examination
- Cal-1 integration analysis (including re-test)
- Presence of replication-competent retrovirus (RCL)
- Concomitant medications
- C46 immunogenicity (humoral and cellular response)

Secondary safety endpoint

- HIV-1 R5/X4 tropism

Feasibility Endpoints

- Analysis of the T^{tn} and $HSPC^{tn}$ release criteria and characterization
  - Number of T^{tn} and $HSPC^{tn}$ manufacturing procedures successfully completed (ie, compliance with all release criteria)
  - T^{tn} and $HSPC^{tn}$ purity
  - T^{tn} and $HSPC^{tn}$ viability
  - T^{tn} and $HSPC^{tn}$ transduction efficiency or vector copy number (VCN)
  - Expression of various surface markers on T^{tn} cells of the final cell products (FCPs) and colony-forming ability of $HSPC^{tn}$
  - Number of target cells harvested

Efficacy Endpoints

Primary efficacy endpoints

There were no primary efficacy endpoints for the study.

Secondary efficacy endpoints

- Measures of Cal-1 marking/expression in peripheral blood
- Measures of Cal-1 marking/expression in GALT
- Area under the curve (AUC) busulfan compared with the peak marking/expression data and marking/expression data at Week 48
- Measures of Cal-1 marking/expression in BM at Week 12
- Log10 HIV-1 RNA
- Changes in Log10 HIV-1 RNA from baseline using one-sample t-tests or non-parametric equivalents, as appropriate
- CD4+ T lymphocyte count, CD4+ percentage, and CD4:CD8 ratio

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- Changes in CD4+ T lymphocyte count from baseline using one-sample t-tests or non-parametric equivalents as appropriate
- Time to commencement of ART
- Absolute and % total recent thymic emigrants (RTEs)
- Absolute and % CD4+ T cells and maturation subsets
- Inflammatory markers: human leukocyte antigen-antigen D-related (HLA-DR), CD38, and the immune exhaustion marker, PD-1

<u>Exploratory Endpoints</u>

- Incidence of CCR5Δ32 mutation in patient population
- Development of potential resistance to Cal-1
- Characterization of Ubiquitin C (UbC) promoter splice site deletions
- Analysis of cellular immunity
- Analysis of immune repertoire
- Detection of latent HIV DNA

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Statistical Methods:

Sample size was not based on formal power considerations. In terms of safety data, the study was well powered to detect only SAEs that occurred with a reasonably high probability (10%-20%). The study had at least an 80% probability of observing ≥ 1 SAE if the true rate of these events in a subject was 12.6% or higher for 12 subjects, or ≥ 16.4% if 9 or more subjects. Within any cohort of 4 subjects, the study was estimated to have 80% probability of detecting events with a true rate of 33.1% per subject. Therefore, up to 12 subjects were to be recruited in the study.

All efficacy summary analyses were based on the safety population. Descriptive summaries were presented with parameters to be correlated listed by subject and by cohort. In addition to descriptive statistics, HIV-1 RNA and CD4+ T cell counts were compared with baseline values using one-sample t-tests or non-parametric equivalents. Pearson's product-moment correlation and Spearman's correlation coefficient were produced for all correlation analyses.

Statistical methods for the safety analyses were primarily descriptive in nature. Each cohort was summarized separately and combined overall. No formal statistical comparisons were made.

Safety endpoints include AEs, clinical laboratory assessments (hematology, chemistry, lymphocyte phenotype, HIV-1 RNA), abnormal vital signs (pulse rate, systolic and diastolic blood pressure, temperature and oxygen saturation), abnormal physical examination, Cal-1 marking/expression data, (peripheral blood, BM and GALT) and integration analysis (including re-test), RCL, HIV-1 tropism, the use of concomitant medications, and C46 immunogenicity (humoral and cellular response).

Safety endpoints were analyzed using the safety population and were based on actual treatment received.

SUMMARY OF RESULTS AND CONCLUSIONS

Subject disposition:
A total of 13 subjects were enrolled in the study, underwent mobilization, apheresis, and T^{tn} and $HSPC^{tn}$ cell manufacturing. One subject did not continue in the study after the CD34+ HSPC apheresis. Before infusion, subjects in Cohort 2 and 3 received busulfan conditioning and 12 subjects were infused with T^{tn} and $HSPC^{tn}$ FCPs. There were 4 subjects treated in each of Cohorts 1, 2, and 3. Of the 12 subjects infused, 4 completed the study to Week 48 off ART and 8 re-commenced ART and were classified as discontinued, to assist in safety and efficacy assessment. These subjects were asked to continue follow up as per protocol until Week 48.

There were a total of 157 protocol deviations, with 150 deviations in the enrolled population. Of these deviations, the vast majority (147/157; 93.6%) were minor and 3 were major. Major deviations included specimen handling errors, omitting the collection of protocol-specified laboratory samples, and GCP violations involving missing PI signatures on forms used to reconsent subjects on updated protocol versions. The major deviations did not necessitate the exclusion of any subjects from the study analyses.

Demography and baseline characteristics:
All treated subjects were males and the majority were Caucasian (11/12; 92%); 1 subject was of African-American race and 1 subject was of Hispanic ethnicity. The median subject age was 47.0 years. Subjects in each cohort were well-matched for HIV history (lowest historical CD4+ count and highest HIV plasma viral load), although Cohort 3 had the lowest mean CD4+ count prior to entry and the highest viral load. Overall, the mean ± SD lowest historical CD4+ T cell count was 494 ± 162 cells/µl and a highest mean ± SD historical HIV viral load was 134,581 ± 225,300 copies/ml.

EFFICACY RESULTS:

Primary Efficacy Results
There were no primary efficacy endpoint assessments/measures.

Secondary Efficacy Results
Cal-1 marking/expression in peripheral blood

Most of the subjects infused with T^{tn} and $HSPC^{tn}$ had Cal-1 DNA detected in PB. One subject in Cohort 1 had over 1% marking at Week 4 post infusion but this marking was not sustained; all Cohort 2 subjects had >1% Cal-1 marking detected at early time points, but marking was not sustained at Week 48. Two subjects in Cohort 3 showed a higher level of Cal-1 marking in terms of peak values and time period (PPD and PPD), but marking was not sustained. Detection of the expression of Cal-1 RNA for C46 and sh5 in PB occurred at much lower frequencies than DNA Cal-1 marking. However, for Subjects PPD and PPD, from cohort 3, relatively higher expression of C46 and sh5 was observed, although transient. The higher marking and expression seen in cohort 3 may have been associated with the higher dosing (2 doses) of busulfan used.

Most subjects infused with T^{tn} and $HSPC^{tn}$ had low levels of Cal-1 DNA detected in granulocyte subsets isolated from PB samples and subjects in Cohort 3 showed the greatest extent of marking compared with Cohorts 1 and 2. Peak marking in the granulocyte sub-population occurred early (but not sustained after Week 12) and was transient. The purity of the isolated granulocytes was inconsistent and low in some cases. Marking in granulocytes was also correlated with busulfan dose, with peak marking greatest in Cohort 3 subjects. Detection and quantification of C46 and sh5 in granulocytes occurred at much lower frequencies than Cal-1 marking and did not correlate with the extent of marking.

Similar findings were also observed in monocytes and CD4+ T lymphocytes, where peak marking was observed at early time points only (before Week 12) and not sustained. In these PB subsets, the highest levels of marking and expression of C46 and sh5 were observed in Cohort 3 subjects. CD4+ T lymphocyte marking and expression was greatest in Cohort 3 subjects PPD and PPD. Although, the population purity varied greatly between samples, the CD4+ T lymphocyte population purity of peak marking and expression at Week 4 for these two subjects was 79.2% and 91%, respectively.

CD8+ T lymphocytes also had detectable Cal-1 marking and expression, however, levels of marking and expression were generally lower in all cohorts and there was less of a trend for increased marking in Cohort 3 compared with CD4+ T lymphocytes.

T^{tn} and $HSPC^{tn}$ dose versus marking & expression

Within each cohort, there was no clear correlation between peak or Week 48 Cal-1 marking versus T^{tn} dose in PB or CD4+ T lymphocytes. As expected, no correlations were observed between peak Cal-1 marking or C46 or sh5 RNA expression versus T^{tn} dose in other PB subsets.

There was a positive correlation in Cohort 3 subjects between $HSPC^{tn}$ dose and peak Cal-1 marking and expression of C46 and sh5 in PB and granulocytes, where higher cell doses had been administered. This correlation was also observed in Cohort 3 subjects for Week 48 Cal-1 marking in granulocytes but not PB. Correlations of $HSPC^{tn}$ dose with Cal-1 expression at Week 48 in PB, granulocytes, and other PB subsets were not completed as there was no detectable expression of Cal-1 at Week 48. Correlations between peak Cal-1 marking and dose of $HSPC^{tn}$ in monocytes, granulocytes, as well as CD4+ and CD8+ lymphocytes were inconsistent and generally low.

Cal-1 marking/expression in GALT

No significant marking was observed in the GALT samples for all cohorts. No correlations were observed between dose of T^{tn} administered and Cal-1 peak marking in GALT in any subject. Although strong correlation was observed between T^{tn} dose and Week 48 Cal-1 marking in GALT 25-35 cm sigmoid colon samples in Cohort 3 subjects, the overall low level of marking suggests that any correlation was not expected to be of clinical importance. There were no clear correlations between dose of $HSPC^{tn}$ administered and Cal-1 peak marking in GALT 10-15 cm samples or GALT 25-35 cm samples in any subjects. However, there was some correlation between $HSPC^{tn}$ dose in Cohort 3 for Week 48 Cal-1 marking in GALT 10-15 cm samples but not 25-35 cm samples.

Cal-1 marking/expression in bone marrow

Very low levels of Cal-1 marking and expression were observed in BM collected at the Week 12 study visit and some subjects had undetectable BM marking and expression at Week 12. As a result of the low level of Cal-1 marking, there were no clear correlations between $HSPC^{tn}$ dose administered and peak Cal-1 marking in BM in any of the study cohorts.

Effect of busulfan on Cal-1marking and expression

Increased busulfan AUC was correlated with increased peak Cal-1 marking in PB, particularly for subjects in Cohort 2 (R = 1.0 for both Pearson and Spearman correlations) and to a lesser extent in Cohort 3 (R = 0.5 and 0.4 for Pearson and Spearman correlations, respectively). Busulfan AUC was also correlated with increased expression of Cal-1 C46 and sh5 in Cohort 3 subjects only (C46 expression: R = 0.6 for both Pearson and Spearman correlations; sh5 expression: R = 0.6 and 0.4 for Pearson and Spearman correlations, respectively). There was a correlation between busulfan AUC and Cal-1 marking at Week 48 for Cohort 3 subjects (R = 0.7 and 0.9 for Pearson and Spearman correlations, respectively).

Increased busulfan AUC was also associated with increased peak Cal-1 marking in granulocytes for subjects in Cohort 2 (R = 0.7 and 0.8 for Pearson and Spearman correlations, respectively) and Cohort 3 (R = 0.5 and 0.8 for Pearson and Spearman correlations, respectively), but marking levels were very low.

There was no correlation between increasing busulfan AUC and increased Cal-1 C46 or sh5 expression because Cal-1 expression was not detected in granulocytes.

There was also a correlation between increased busulfan AUC and Cal-1 marking at Week 48 in all PB subsets for subjects in Cohort 2 and 3. In monocytes, the busulfan AUC versus Week 48 Cal-1 marking Pearson and Spearman correlations for Cohort 2 were R = 0.8 and 0.9, respectively, and for Cohort 3 were R = 0.7 and R = 0.8, respectively. In granulocytes, the busulfan AUC versus Week 48 Cal-1 marking Pearson and Spearman correlations for Cohort 2 were R = 0.7 and 0.9, respectively, and for Cohort 3 were R = 0.8 and R = 0.8, respectively. In CD8+ T-lymphocytes, the busulfan AUC versus Week 48 Cal-1 marking Pearson and Spearman correlations for Cohort 2 were R = 0.8 and 0.8, respectively, and for Cohort 3 were R = 0.7 and R = 0.9, respectively. In CD4+ T-lymphocytes, the busulfan AUC versus Week 48 Cal-1 marking Pearson and Spearman correlations for Cohort 2 were R = 0.9 and R = 1.0, respectively, and for Cohort 3 were R = 0.7 and R = 0.8, respectively. There was little or no correlation between increasing busulfan AUC and increased Cal-1 C46 or sh5 expression because Cal-1 expression was not detected in most PB subtypes.

Effect of T^{tn} and $HSPC^{tn}$on HIV-1 RNA (viral load)

There was no effect of infusion of T^{tn} and $HSPC^{tn}$ on HIV RNA plasma levels. Subject viral set points appeared to be maintained and recommencement of ART was shown to suppress viral load. Comparison of the nadir viral load after cell infusion or at Week 48 (or last value before recommencing ART) with Screening, Pre-Apheresis, or Pre-Infusion Visit HIV-RNA levels were not statistically significant when viral load was measured with the Roche or Abbott HIV-1 RNA assay.

Effect of T^{tn} and $HSPC^{tn}$ on CD4+ T cell counts, CD4+T cell percentages, and CD4:CD8 ratios

There was an observed decline in CD4+ absolute counts over time, across all cohorts. This decline was variable, and dependent on the baseline comparator value used (ie, Screening Visit, Pre-Apheresis Visit, or Pre-infusion/Pre-Busulfan Visit). Overall, there was a statistically significant decline in mean/median absolute CD4+ counts at Week 48 (or the last value before restarting ART) when the CD4+ count post-infusion was compared with the Screening Visit (-247.4 mm^3 mean change in CD4+, P=0.0013, t-test; -250.8 mm^3 median change in CD4+, P=0.0034 signed rank test) or the Pre-Apheresis Visit (-205.1 mm^3 mean change in CD4+, P=0.0256, t-test; -209.5 mm^3 median change in CD4+, P=0.0342, signed rank test).

As anticipated, absolute CD4+ T lymphocyte counts were reduced following apheresis, but otherwise remained stable over time in Cohort 1 subjects, who were not treated with busulfan and had lower apheresis volumes. CD4+ counts were somewhat lower over time in Cohort 2 subjects, who received 1 dose of busulfan and had larger apheresis volumes compared with Cohort 1 subjects. There was a greater effect on CD4+ counts in Cohort 3 subjects who received two mobilizing agents (G-CSF and plerixafor), 2 doses of busulfan, and larger apheresis volumes compared with Cohort 1 & 2 subjects. Conversely, percentages of CD4+ cells across all cohorts were relatively stable.

Absolute CD8+ T lymphocyte counts were also reduced following apheresis, but generally CD8+ counts and percentages remained stable over time across all cohorts. CD4:CD8 cell ratios were stable across all cohorts.

Time to commencement of ART

There was no effect of the infused Cal-1 transduced cells on the time to commence ART. HIV-1 RNA viral load and CD4 counts were mostly stable after FCP infusion among the subjects in each cohort and treatment with T^{tn} and $HSPC^{tn}$ did not interfere with the effectiveness of the ART, as observed by the decrease in viral load after restart of ART in T^{tn}- and $HSPC^{tn}$-treated subjects.

Restart of ART was recommended for subjects if their CD4+ T lymphocyte counts, and/or CD4+ T cell percentages, or plasma HIV RNA values reached the safety limits. Other criteria to restart ART included subject's decision, regardless of pre-specified laboratory parameters. For all subjects who commenced

ART before Week 48, the decision to restart ART was irrespective of clinical indications or protocol-defined parameters (ie, CD4+ count and/or viral load values, per the clinical study protocol [CSP]). Subjects usually restarted ART based on the Investigator's advice or at the request of the subject. Thus, the rationale for restarting treatment was not consistent for each subject.

Absolute and total recent thymic emigrants – thymopoiesis

Flow cytometry analysis of CD4, CD31, CD45RA, and CD62L were performed to determine the absolute number and percentage of CD4+ cells that were CD45RA+/CD62L+/CD31+, as a measure of recent thymic emigrants (RTEs). Based on the CD45RA+/CD62L+/CD31+ phenotype, the percentage of RTEs was relatively stable in subjects over the course of the study.

Lymphocyte maturation & inflammatory status

An inverse correlation between CD4 RTEs and CD4+ cell expression of the immune activation marker, HLA-DR, was observed. Subjects in Cohort 2 and 3 with low thymic output displayed an aged phenotype (high number of effector memory [EM] and effector memory RA+ [EMRA] CD4+ T cells). This finding was consistent before and after FCP infusion and did not change as a result of treatment.

As expected, low thymic output correlated with markers of inflammation (eg, strong negative correlation between percentage of RTEs [thymic output] and CD4+/HLA-DR+ cells). However, this finding was also consistent after FCP infusion and did not change as a result of treatment. The change in RTEs was highly variable and the percentage of RTEs did not change as a result of autologous stem cell transplantation and did not correlate with viral load. Although, as expected, percentage RTEs did correlate with inflammatory phenotype, it was not influenced by therapy.

The distribution of naïve (CD3+/CD4+/CD27+/CD45RA+/CCR7+), central memory (CD3+/CD4+/CD27+/CD45RA-/CCR7+), transitional memory (CD3+/CD4+/CD27+/CD45RA-/CCR7-), effector memory (CD3+/CD4+/CD27-/CD45RA-/CCR7-), and terminally differentiated effector cells (CD3+/CD4+/CD27-/CD45RA+/CCR7-) for subjects were relatively stable throughout the study. Levels of PD-1 (marker of inflammation) expression were variable but several of the subjects in Cohorts 2 and 3 had increased PD-1 expression at Day 0, possibly in association with busulfan exposure. There also may have been an association between the inflammatory signature and the inability to detect long-lived Cal-1-modified cells *in vivo*.

SAFETY RESULTS:

Primary Safety Results

Feasibility

A total of 13 subjects underwent mobilization and apheresis to collect cells for manufacturing procedures to prepare Cal-1 transduced cells; before infusion, subjects in Cohort 2 and 3 received busulfan conditioning. One subject did not continue in the study after the CD34+ HSPC apheresis. The dose of T^{tn} and $HSPC^{tn}$ infused for each of the 12 subjects was highly variable across the 3 cohorts. The mean ± standard deviation (SD) total number of T^{tn} infused was $39.47 \times 10^8 \pm 32.99 \times 10^8$ cells (min: 11×10^8; max: 123.7×10^8). The number (mean ± SD) of $HSPC^{tn}$ /kg body weight infused was $5.034 \times 10^6 \pm 4.48 \times 10^6$ cells/kg (min: 0.35×10^6/kg; max: 12.5×10^6/kg). Improvement of the cell processing procedures yielded higher numbers of T^{tn} for Cohorts 2 and 3. The use of plerixafor as a second mobilizing agent, in addition to G-CSF and the 2 large-volume aphereses in Cohort 3 subjects, was associated with an increase in the number of $HSPC^{tn}$ infused compared with subjects in Cohort 1 or 2, who did not receive plerixafor.

The transduction efficiency of the Cal-1 vector in T^{tn} was variable between subjects and within cohorts. Using fluorescence-activated cell sorting (FACS) analysis, the transduction efficiency of CD4+ cells ranged from 21% to 60% in Cohort 1, 13% to 39% in Cohort 2, and 39% to 84% in Cohort 3. Using

polymerase chain reaction (PCR), the transduction efficiency ranged from 0.49 to 1.35 VCN/cell for Cohort 1, 0.21 to 1.42 VCN/cell for Cohort 2, and 0.57 to 1.84 VCN/cell for Cohort 3. The transduction efficiency in HSPC^{tn} was also variable between subjects and within cohorts. FACS analysis showed the transduction efficiency of CD34+ cells ranged from 51% to 76% in Cohort 1, 12% to 53% in Cohort 2, and 21% to 49% in Cohort 3. Transduction efficiency was lower in HSPC^{tn} compared with T^{tn} when PCR was used to assess transduction efficiency. HSPC^{tn} VCN ranged from 0.08 to 1.16 VCN/cell for Cohort 1, 0.17 to 0.35 VCN/cell for Cohort 2, and 0.07 to 0.24 VCN/cell for Cohort 3.

In terms of the feasibility of cell manufacturing, a total of 133 manufacturing deviations were reported for preparation of T^{tn} and HSPC^{tn}; the majority of deviations (97.7%) were considered minor and there were only 3 deviations (3.3%) assessed as major. The 3 major manufacturing procedure deviations were classified as "out of specification" deviations and the cell products were not released. Two subjects required an additional apheresis procedure and cell manufacturing before they received their FCP infusion. One subject discontinued from the study after the FCP did not meet viability release criteria. This subject did not undergo repeat mobilization and apheresis procedures and was not infused with any FCPs.

Summary of adverse events

- All of the 13 subjects enrolled in the study reported at least 1 AE.
- Overall, 324 AEs were reported, with subjects in Cohort 3 experiencing the majority of AEs (163/324; 50.3%), followed by subjects in Cohort 2 (117/324; 36.1%).
- Only 1 subject (PPD; Cohort 3) reported a SAE during the study, which was unrelated to study drug or study procedures. There were no deaths during the study and there were no study discontinuations that were related to study drug.
- The majority of AEs were related to study procedures rather than study drug (Cal-1). There were 53/324 AEs (16.4%) related to Cal-1 in 11/13 (84.6%) subjects and there were 13/324 (4.0%) AEs related to both Cal-1 and busulfan in 2/13 (15.4%) subjects (where causality of the AE could not be assigned to either Cal-1 or busulfan alone).
- There were 188/324 AEs (58.0%) related to study procedures in 13/13 (100%) subjects. Similar numbers of study procedure-related AEs were reported in Cohort 2 (92/188 AEs; 48.9%) and Cohort 3 (90/188 AEs; 47.9%) subjects. These study procedure-related AEs were most frequently due to busulfan conditioning (163/188; 86.7%).
- There were 2 Cal-1-related AEs graded as severe (Grade [Gr] 3) reported in one subject (PPD; Cohort 3) and included 1 AE of night sweats and 1 AE of nausea. Both events resolved without sequelae. There were no Gr 4 Cal-1 related AEs. There was one Cal-1-related AE (increased joint pain; Gr 1) experienced by Subject PPD, which was ongoing at Week 48 and required concomitant medication.
- There were 11/324 (3.4%) Grade 4 (potentially life-threatening) AEs and 31/324 (9.6%) Grade 3 (severe) AEs in 7/13 (53.8%) and 8/13 (61.5%) subjects, respectively, reported during the study. The majority of AEs (201/324; 62.0%) were graded as mild. The higher-grade AEs were primarily related to busulfan conditioning.
- The most frequent AEs experienced by subjects were neutropenia (9/13; 69.2%), thrombocytopenia (8/13; 61.5%), fatigue (8/13; 61.5%), back pain (8/13; 61.5%), and leukopenia (7/13; 53.8%) and were under the system organ class (SOC) of "Blood and lymphatic system disorders" (141/324; 43.5%). Most of these AEs (163/324; 50.3%) were related to busulfan conditioning.

Summary of clinical laboratory assessments

A total of 744 abnormal (out of normal range) hematology parameters were reported during the study. The most frequent abnormalities included low white blood cells (89 reports in 11 subjects), low platelet

counts (82 reports in 10 subjects), low absolute neutrophil counts [ANC] (79 reports in 12 subjects), low RBC counts (60 reports in 10 subjects) and high percentage lymphocytes (53 reports in 12 subjects).

There were 22 AEs of leukopenia reported in 8 subjects, 59 AEs of thrombocytopenia reported in 9 subjects, and 59 AEs of neutropenia reported in 9 subjects. The leukopenia observed in the subjects in Cohort 2 and 3 reflected the neutropenia (low ANC) also reported in these subjects. There was also one AE of leukocytosis in a Cohort 3 subject. The vast majority of hematology AEs occurred in Cohort 2 and 3 subjects and were related to busulfan conditioning, G-CSF, and apheresis. There were 10 hematology AEs that were not related to Cal-1 or study procedures. As expected, all cohorts showed a peak in ANC values prior to infusion, at the time of G-CSF administration. Following infusion, subjects in Cohort 2 and 3 who had received busulfan conditioning, developed neutropenia, which (in some cases) was treated with G-CSF therapy. Although no delayed hematopoietic recovery in terms of ANC was observed, ANC recovery was slower than expected. As expected, all subjects in Cohort 2 & 3 (who received busulfan conditioning) developed thrombocytopenia. One subject in Cohort 1 (PPD had an isolated low platelet count at Week 48 of uncertain etiology, but then recovered (subject did not receive busulfan). One subject in Cohort 2 (PPD) and two subjects in Cohort 3 (PPD & PPD) received a platelet transfusion due to thrombocytopenia associated with busulfan conditioning.

A total of 298 abnormal (out of normal range) biochemistry parameters were reported after subjects initiated study procedures (ie, CD4+ apheresis). The most frequent abnormalities included increased serum glucose (87 reports in 13 subjects [non-fasting]), increased aspartate aminotransferase (AST) (47 reports in 8 subjects), increased creatine kinase (46 reports in 9 subjects), low creatinine (19 reports in 3 subjects), and increased alanine aminotransferase (ALT; 18 reports in 6 subjects). There were 2 biochemistry AEs in one subject (PPD, Cohort 3), 1 event of increased ALT and 1 event of increased AST (both were Gr 2; not related). Both events resolved without sequelae.

See efficacy results section of synopsis entitled "Effect of T^{tn} and $HSPC^{tn}$ on CD4+ T cell counts, CD4+T cell percentages, and CD4:CD8 ratios" for a summary of clinical laboratory lymphocyte results. There was one reported laboratory AE of CD4 lymphocytes decreased (Subject PPD; Cohort 3), which was Gr 1 (mild) and "very likely" related to the apheresis procedure. The AE resolved without sequelae.

See efficacy results section of synopsis entitled "Effect of T^{tn} and $HSPC^{tn}$ on HIV-1 RNA (viral load)" for a summary of clinical laboratory HIV-1 RNA results.

Abnormal vital signs

A total of 136 abnormal vital signs parameters were reported during the study. The most frequent abnormalities included decreased temperature (79 reports in 10 subjects), decreased pulse rate (34 reports in 7 subjects), and increased diastolic blood pressure (12 reports in 6 subjects). There were no abnormalities in oxygen saturation for any subject.

There were 3 AEs of pyrexia and 1 AE of low grade fever reported in 4 subjects (3 subjects in Cohort 3, 1 subject in Cohort 1), however, no events of increased body temperature were recorded during assessment of vital signs. Of the 3 pyrexia AEs, 2 events were Gr 2 (1 AE was "very likely" and 1 AE was "probably" related to Cal-1 study drug administration) and 1 event was Gr 1 ("not related"). The 1 AE of low grade fever (Gr 1) was "probably" related to Cal-1 and busulfan. All AEs resolved without sequelae.

Abnormal physical examination findings

A total of 119 abnormal, not clinically significant (NCS) and 18 abnormal, clinically significant (CS) physical examination findings were reported during the study. The most frequent CS physical examination abnormalities included dermatologic findings (12 CS findings in 3 subjects), general appearance findings (4 CS findings in 1 subject), and head, eyes, ears, nose & throat findings (2 CS findings in 2 subjects).

Of the 18 abnormal, CS physical examination findings, 15 findings were reported as AEs in 3 subjects. There were 2 AEs of rash reported in Subject PPD, both were Gr 1, one event was "very likely" related to Bactrim® prophylaxis, the other event was "possibly" related to G-CSF. There was 1 AE of PPD in Subject PPD, Gr 1 and "very likely" related to busulfan. There was also 1 AE of head injury in Subject PPD, Gr 2, and was unrelated. All events resolved without sequelae.

Cal-1 integration analysis

No integration analysis was required for post-infusion samples, as Cal-1 peak marking was <1% in PB at Weeks 12, 24, and 48. As per the CSP, a Cal-1 marking level of ≥1% in peripheral blood was required to determine if the integration assay result could be interpreted as a true predominant integration site. Therefore, the assay was only to be performed if Cal-1 marking was ≥ 1% at the corresponding time point required.

Presence of replication-competent retrovirus (RCL)

No RCL was detected in any subject at any of the time points tested and integration analyses were not required because Cal-1 marking was < 1% in PB at Weeks 12, 24, and 48.

Concomitant medications

The most frequently used medications during the study included ondansetron (for busulfan-related nausea), valaciclovir hydrochloride (for post-busulfan antiviral prophylaxis), G-CSF (study-related procedures), and Bactrim® (infection prophylaxis).

C46 immunogenicity (humoral and cellular response)

No cellular C46 responses (ie, cell-mediated) against the C46 peptide expressed by Cal-1-transduced cells were detected. However, humoral (antibody) responses to C46 were mixed (transient increases in C46 titer or C46 antibody was already observed at baseline) and of uncertain significance.

Secondary Safety Results

HIV-1 R5/X4 tropism

All subjects retained tropism for the R5 receptor and no shift to or tropism for X4 or dual-mixed receptor was detected in the samples analyzed for enrolled subjects

EXPLORATORY RESULTS

Incidence of CCR5Δ32 mutation in patient population

Exploratory analyses to assess the incidence of HIV-1 CCR5Δ32 mutation after infusion was not completed as there was no significant impact of T^{tn} and $HSPC^{tn}$ infusion on viral load.

Development of potential resistance to Cal-1

Exploratory analysis of the development of Cal-1 resistance was not performed due to the low levels of Cal-1 marking observed.

Characterization of UbC promoter splice site deletions

With respect to the characterization of the UbC promotor splice site deletion in Cal-1 transduced cells for T^{tn} and $HSPC^{tn}$ FCP, T^{tn} showed a higher percentage of the spliced variant, while $HSPC^{tn}$ showed higher levels of the unspliced variants.

Analysis of cellular immunity

Exploratory analysis of cellular immunity was not performed due to the low levels of Cal-1 marking observed.

Analysis of immune repertoire

Exploratory evaluation of the immune repertoire (T cell receptor gene diversity) was not conducted because less than 1% of PB lymphocyte marking was achieved.

Detection of latent HIV DNA

Name of Sponsor Calimmune Inc.	**Individual Study Table Referring to Part IV Of the Dossier**	***(For National Authority Use Only)***
Drug Substance Cal-1	Volume: Page:	

Testing for integrated HIV DNA was performed on a selected number of samples, however, the majority of tested samples were below the level of detection for the assay. For 2 subjects (PPD and PPD), quantifiable levels of integrated HIV DNA were detected but there was no demonstrable impact of Cal-1 on the proviral DNA burden.

CONCLUSIONS:

The results of this gene therapy study showed that the delivery of $HSPC^{tn}$ and T^{tn} was feasible, well-tolerated, and the AE profile was as expected, based on the use of busulfan as a non-myeloablative conditioning agent in the majority of subjects. Notably, no Cal-1 or study procedure-related SAEs were observed and low- to moderate-dose busulfan could be safely used in this population of patients with HIV-1 infection.

Overall, the potential impact of $HSPC^{tn}$ and T^{tn} on the control of HIV replication could not be sufficiently assessed because of the low level of marking and expression of Cal-1 transduced cells in PB. No effect of $HSPC^{tn}$ and T^{tn} on HIV viral load was seen and no significant contribution of the infused Cal-1-tranduced cells on CD4+ T cell counts was observed. However, there was some evidence that busulfan dose was correlated with higher levels of Cal-1 marking in granulocytes, although marking levels were very low. Potential reasons for the lack of long-term survival of Cal-1-transduced cells include viremia and the associated inflammatory environment. The relatively low transduction efficiency of FCPs and the high frequency of PD-1-expressing cells on the day of infusion also may have contributed to the low level of Cal-1-transduced cell survival.

Date of Report:
22-MAR-2019