Phase 1-2 Trial for Patients with Advanced Hematologic Malignancies undergoing Myeloablative Allogeneic HCT with a T-cell Depleted Graft with Infusion of Conventional T-cells and Regulatory T-cells

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PROTOCOL SYNOPSIS

TITLE	Phase 1-2 Trial for Patients with Advanced Hematologic Malignancies undergoing Myeloablative Allogeneic HCT with a T-cell Depleted Graft with Infusion of Conventional T-cells and Regulatory T-cells
STUDY PHASE	Phase 1-2
INDICATION	Subjects with hematologic malignancies eligible for myeloablative allogeneic HCT using human leukocyte antigen (HLA)-matched donor or 9/10 HLA-matched donor (related or unrelated)
INVESTIGATIONAL PRODUCT	Purified regulatory T-cells (T_{reg}) plus $CD34^+$ hematopoietic progenitor cells ("$CD34^+$ HSPC")
PRIMARY OBJECTIVE	1. To determine the efficacy, safety and feasibility of administration of several dose combinations of conventional T-cells (T_{con}) and regulatory T-cells (T_{reg}) in subjects undergoing allogeneic hematopoietic cell transplantation (HCT) with HLA-matched donor or 9/10 HLA-matched donors (related or unrelated) using a T-cell depleted graft [$CD34^+$ hematopoietic progenitor cells ("$CD34^+$ HSPC")], without immune-suppression. 2. To determine the maximum tolerated dose (MTD) of infused regulatory and conventional T-cells in the matched donor setting 3. Determine if concomitant single-agent immunosuppression is needed with fresh T_{reg} cells (phase 2 stage 1) 4. To determine 1-year GvHD-free relapse-free survival (GRFS) post-HCT (phase 2 stage 2)
SECONDARY OBJECTIVES	1. To determine the 1-year OS in subjects undergoing allogeneic HCT with matched donors. 2. To measure the incidence and severity of acute and chronic graft vs host disease (GvHD) 3. To measure incidence of serious infections
HYPOTHESIS	For subjects with hematologic malignancies undergoing allogeneic myeloablative (MA) HCT with a T-cell depleted graft, the infusion of naturally occurring regulatory T-cells with conventional T-cells (T-cell addback) in pre-defined doses and ratios will reduce the incidence of acute GvHD while augmenting the graft vs leukemia effect and improving immune reconstitution.

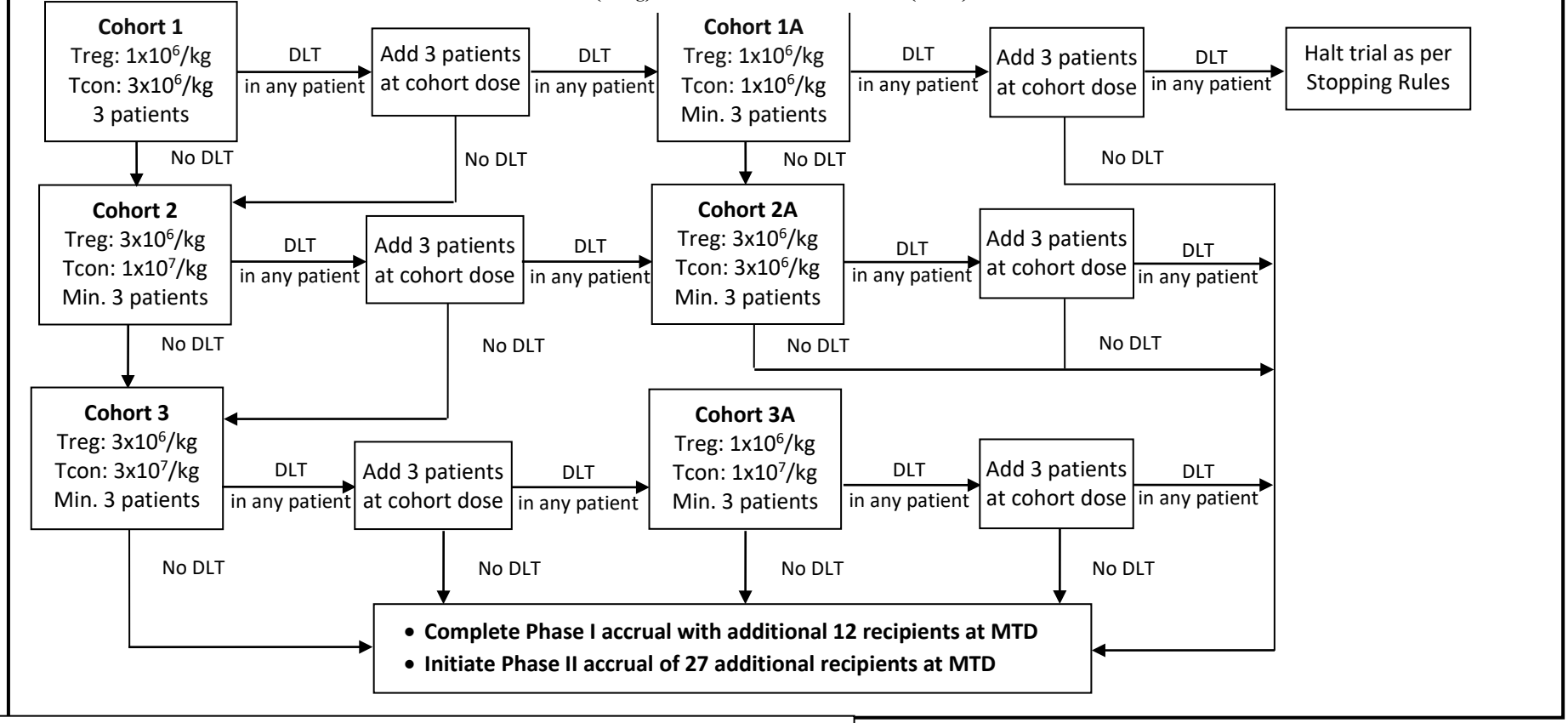
TREATMENT SUMMARY	Conditioning regimen will be determined by diagnosis: **Acute leukemia: high-risk CML; myelodysplastic syndrome; myeloproliferative disorders**: fTBI / VP16 / Cyclophosphamide OR Busulfan / Cyclophosphamide OR Thiotepa/ Busulfan/ Fludarabine **Lymphoma: non-Hodgkin; Hodgkin:** BCNU / VP16 / Cyclophosphamide
SUMMARY OF SUBJECT ELIGIBILITY CRITERIA	See Section 3 for complete eligibility criteria. Subjects with the following diseases are eligible: • Acute leukemia, primary refractory or beyond CR1, or minimal residual disease (MRD) positivity. • Chronic myelogenous leukemia (accelerated, blast or second chronic phase, or first chronic phase that and has not achieved a molecular remission after ≥ 3 prior therapies • High-risk leukemia with any of the following features: o Acute myeloid leukemia (AML) in CR1 as follows: ▪ Complex karyotype (≥ 3 clonal chromosomal abnormalities) ▪ Any of the following high-risk chromosomal abnormalities: • Monosomal karyotype (-5, 5q-, -7, 7q-) • t(11q23); t(9;11); inv(3); t(3;3) t(6;9); t(9;22) • Normal karyotype with FLT3-ITD mutation ▪ Other high-risk features as determined by molecular studies, or clinical presentation as assessed by a study investigator o Other high-risk leukemia in CR1, as determined by a study investigator • Myelodysplastic syndromes • Myeloproliferative disorders • Non-Hodgkin lymphoma with poor risk features not suitable for autologous HCT • Hodgkin lymphoma with poor risk features not suitable for autologous HCT • Age ≤ 73 years old • Availability of an HLA-matched donor or 9/10 HLA-matched donor (related or unrelated) defined by Class I (HLA–A and -B) serologic typing (or higher resolution) and Class II (HLA-DRB1) molecular typing. • No prior myeloablative therapy or hematopoietic cell transplantation; prior allo transplants are excluded

SAMPLE SIZE	Up to 88 subjects, consisting of: Phase 1 trial: 3 subjects per dose pair of regulatory T-cells and conventional T-cells (3 dose pairs to be studied). Total of 13 subjects. Phase 2 trial: adaptive Simon 2-stage design. Initial stage cohort: 24 subjects Second stage cohort: up to 51 subjects
STATISTICAL CONSIDERATIONS	This is a phase 1-2 dose finding trial, with 2 degrees of freedom in the definition of dose (numbers of T_{reg} and T_{con}, or, equivalently, the number of T_{con} and the ratio of T_{reg} to T_{con}). The study will nevertheless follow a traditional 3+3 model, as described in the diagram, to find the maximum tolerated dose (MTD) of T_{con} at the minimum necessary ratio of T_{reg} to T_{con}. The Phase 2 portion of the trial will consist of an admissible Simon 2-stage design (Simon, 1989). The 1st phase is designed to evaluate the need for immunosuppression (IS) by comparing IS + graft-engineered product to graft-engineered product alone. In the first stage, the trial investigates if the rate of GVHD is non-inferior for the graft-engineered product with and without IS. Define θ to be the difference in GVHD rates between the two arms: (1) – (2), so that large θ is promising for the graft-engineered product (2). To test for non-inferiority, we want to test H_0:$\theta = -\delta$ vs H_α:$\theta = 0$, where δ is a specified margin of non-inferiority. With 24 evaluable subjects this method has 80% power to test for a $\delta = 10\%$ margin of non-inferiority at $\alpha = 0.5$ (assuming a variance of 0.35). The 2nd phase will either use IS + graft-engineered product or graft-engineered product alone based on the first stage and evaluate 1-year GRFS as the primary endpoint. The null hypothesis that the true 1-year GRFS is 40% will be tested against a one-sided alternative. The probability of early termination under the null hypothesis is 64%. In the first stage, 17 patients will be accrued. If there are 7 or fewer successes in these 17 patients, the study will be stopped. Otherwise, 24 additional patients will be accrued for a total of 41. The null hypothesis will be rejected if 22 or more responses are observed in 41 patients. This design yields a type I error rate of 5% and power of 80% when the true 1-year GRFS is 60%.

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ADL	Activities of daily living
AE	Adverse event
BID	Twice daily
BSA	Body surface area
CBC	Complete blood count
CI	Confidence interval
CNS	Central nervous system
CRF	Case report/record form
CR	Complete response
CTCAE	Common Terminology Criteria for Adverse Events
CTF	Cell Therapy Facility
DLT	Dose-limiting toxicity
DSMB	Data safety monitoring board
ECG	Electrocardiogram
EFS	Event free survival
GI	Gastrointestinal
GvHD	Graft vs host disease
GRFS	GvHD-free, relapse-free survival
HCT	Hematopoietic cell transplantation
Hgb	Hemoglobin
HIV	Human immunodeficiency virus
HPF	High-power field
HTN	Hypertension
IRB	Institutional review board
IV	Intravenous
LLN	Lower limit of normal
MA	Myeloablative
MLR	Mixed lymphocyte reaction
OS	Overall survival
PLT	Platelet
PD	Progressive disease
PFS	Progression-free survival
PR	Partial response
QD	Once daily
RR	Response rate
SAE	Serious adverse event
SD	Stable disease
T_{con}	Conventional T-cells
T_{reg}	Regulatory T-cells
TTP	Time to progression
ULN	Upper limit of normal
UNK	Unknown
URD	Unrelated donor
WBC	White blood cell
WHO	World Health Organization

Figure 1. Phase 1 Dose Escalation Schema of Regulatory T-cells (T_{reg}) Conventional T-cells (T_{con})



Dose Limiting Toxicity (DLT):

- **Acute GvHD > grade 2**
- **Grade 4 neutropenia lasting to 28 days after HCT**
- **Grade 3 to 5 cytokine/release syndrome/acute infusion reaction**

1 OBJECTIVES

Primary Objectives

1. To determine the efficacy, safety, and feasibility of administration of several dose combinations of conventional T-cells (T_{con}) and regulatory T-cells (T_{reg}) in subjects undergoing allogeneic hematopoietic cell transplantation (HCT) with HLA-matched donor or 9/10 HLA-matched donor (related or unrelated), using a T-cell depleted graft [$CD34^+$ hematopoietic progenitor cells ("$CD34^+$ HSPC")], without immunosuppression (see Section 3.1 Recipient Inclusion Criteria for the protocol-specified definition of matched donor).
2. To determine the maximum tolerated dose of infused T_{reg} and T_{con} cells with myeloablative allogeneic HCT using matching donors
3. Determine if concomitant single-agent immunosuppression is needed with fresh T_{reg} cells (phase 2 stage 1)
4. To determine 1-year GvHD-free relapse-free survival (GRFS) post-HCT (phase 2 stage 2).

Secondary Objectives

1. To determine the 1-year overall survival in subjects undergoing allogeneic hematopoietic cell transplantation with matched donors.
2. To measure the incidence and severity chronic GvHD.
3. To measure incidence of serious infections

Exploratory Objective

1. To measure immune reconstitution parameters.

ClinicalTrials.gov outcomes are specified in Section 9.1.

2. BACKGROUND

The procedures for this study include the infusion of 3 phenotypically-distinct cell types obtained from the apheresis harvests. The cellular infusions may be referred to herein as treatment components. The treatment components used in Study IRB-21257 / BMT236 are described as:

- Conventional, unmanipulated, $CD3^+$ T-cells ("T_{con} cells" or "T_{con}"). These cells an unmanipulated aliquot of the apheresis harvests; are used in regular medical care; and

are not considered the investigational agent. The T_{con} cells will be administered to the patient-subject on Study Day 2.

- Purified $CD34^+$ hematopoietic progenitor cells ("$CD34^+$ HSPC"). The $CD34^+$ HSPC will be administered to the patient-subject on Study Day 0.
- Highly-purified regulatory T-cells ("T_{reg} cells" or T_{reg}) are defined as $CD4^+$ $CD25^+$ $CD127^-$ $Foxp3^+$ regulatory T-cells. The T_{reg} cells will be administered to the patient-subject on Study Day 0.

2.1 Background/Rationale/Pilot Data

Background/Rationale/Pilot Data

Patients with high risk hematologic malignancies such as leukemia and lymphoma beyond first remission or with refractory relapse are rarely cured with standard chemotherapy. Myeloablative (MA) allogeneic HCT is their only chance for long term survival with disease free survival ranging from 10 to 50% and non-relapse mortality ranging from 30 to 50%.[1,2] The early morbidity and mortality associated with aGvHD is a major factor limiting the success of HCT as is the long-term morbidity associated with chronic GvHD. The incidence of aGvHD following allogeneic HCT from an HLA-matched sibling donor (MSD) is 20 to 60% despite the use of various immunosuppressive agents such as tacrolimus, cyclosporine, methotrexate, mycophenolate, anti-thymocyte globulin and corticosteroids.[2,3,4,5] Accordingly, approximately one-third of patients who undergo allogeneic HCT using a MSD and a T-cell replete graft will develop chronic GvHD.[6,7,8]

T-cell Depletion of Allografts:

T-cell depletion (TCD) of donor grafts is an accepted method to prevent acute and chronic GvHD. There is extensive experience with TCD of marrow grafts and such techniques have involved physical adsorption of T-cells to protein ligands such as lectins, elutriation and immuonadsorption or immunodeletion with T-cell or lymphocyte-specific monoclonal antibodies. These methods have yielded results in marrow grafts ranging from 1.5 to 3.2$\log_{10}$ T-cell depletion. Much less is published regarding TCD of PBSC allografts. However, in light of the evidence that the risk of acute and/or chronic GvHD may be greater with PBSC grafts, strategies for TCD of PBSC allografts is of considerable interest. Using an immunoadsorption column or immunoaffinity techniques, investigators have performed TCD of GCSF-mobilized PBSC grafts and obtained T-cell log depletions ranging from 1 to 2.9 log depletion and with the incidence of grade 2 to 4 aGvHD ranging from 0 to 4%. When G-CSF mobilized grafts from haploidentical donors were utilized, an immunoaffinity system (CliniMACS) achieved a 4 to 5 log $CD3^+$ depletion and a median $CD3^+$ dose of 0.2 x 10^5/kg. The incidence of aGvHD was < 10% despite the use of haploidentical grafts and without use of any post-transplant GvHD prophylaxis.[9-14]

Consequences of TCD include impaired immune recovery and increased incidence of relapse. Persistent immune dysregulation is a known complication of allogeneic HCT

and can manifest as opportunistic infections especially in the setting of chronic GvHD. Peripheral blood T-lymphocyte counts do not return to normal levels until ~3 to 12 months after HCT and functional T-cell recovery may be even more protracted especially after TCD HCT.[9,13,15] Additionally, when marrow grafts are TCD in the MSD setting, the risk of leukemia relapse is significantly higher especially for advanced phase acute leukemia patients.[15]

2.2 Purified Regulatory T-cells

To ameliorate the impaired immune recovery and address the increased relapse incidence after TCD allografting, we will infuse conventional donor T-cells (T_{con}) or "T-cell addback" along with naturally occurring donor regulatory T-cells (T_{reg}). This allows assessment of the ability of T_{reg} to reduce the extent and severity of GvHD and to ascertain the maximum tolerable dose of donor T_{con} in relation to specific T_{reg} doses. To circumvent the potential acute GvHD associated with T-cell addback, the planned addition of T_{reg} at appropriate numbers and ratios compared to T_{con} will occur at a defined timepoint after hematopoietic cell infusion. The scientific rationale for pursuing T_{reg} in this setting arises from reports from several different laboratories demonstrating that highly purified T_{reg} are capable of controlling GvHD and improving immune reconstitution in the preclinical setting.[16,17,18,19] In a murine GvHD model induced by T_{con}, we have previously demonstrated that adoptive transfer of T_{reg} leads to abrogation of GvHD and accelerated donor lymphoid reconstitution of a diverse TCR-Vβ repertoire.[20] T_{reg} will be obtained by selection of donor T-cells expressing the surface phenotype $CD4^+CD25^+CD127^-$. This population has been shown to express high levels of intracellular $FoxP3^+$ which is currently the most reliable marker of T_{reg} activity based on *in vitro* assays and non-human animal models. These cells also actively suppress the mixed lymphocyte reaction. Preliminary studies in the setting of cord blood transplantation and haploidentical transplantation are highly supportive of this concept.[21-22]

Dose Escalation

A baseline cell dose of conventional T-cells of 1×10^6/kg will be used with escalation to the maximum tolerated dose up to 1×10^7/kg. As demonstrated in mouse models of allogeneic HCT and *in vitro* assays, T_{reg} can effectively suppress proliferation of alloreactive T-cells even when T_{con} are added at 10-fold greater doses if the T_{reg} are infused prior to the T_{con} which allows for these cells to home to the proper microenvironment and proliferate. The suppressive effect is most pronounced when T_{reg} are added prior to T_{con}. Mice infused with donor T_{reg} 48 hours before T_{con} infusion show little, if any, evidence of GvHD despite significant MHC disparities. This finding is replicated in the proposed transplant regimen. In addition, the ratio of T_{con}:T_{reg} will be staggered to establish and confirm the ability of T_{reg} to suppress alloreactivity at doses lower than the infused T_{con}.

In order to measure the impact of our intervention, at specified timepoints, before and after infusion of the planned dose of T_{reg} and T_{con}, we will measure immune reconstitution in the peripheral blood via immunophenotyping of B-cell; T-cell; and NK subsets. To assess the contribution of thymopoiesis to T-cell immune recovery after HCT, we will measure peripheral recent thymic emigrants by determining the number of

T-cell receptor excision circles generated during intrathymic TCR-α rearrangement using real-time PCR assays from the peripheral blood.

Phase 2 Simon 2-Stage

Based on the prior protocol modifications and the use of fresh T-regulatory cells with single-agent immunosuppression prophylaxis, a safe and feasible dose has been established for further research. Since 2 modifications were made during dose-finding and the initial phase 2 extension of the use of fresh donor T_{reg} and also the use of single-agent GvHD prophylaxis with either tacrolimus or sirolimus, this aspect of the phase 2 extension study has a Simon Two-Stage design, with the 1st stage designed to test if the incidence of GVHD is different between subjects who receive T_{reg} alone versus those who received single-agent prophylaxis and T_{reg}. A total of 24 subjects will be required (2 arms of 12 each). For the 2nd stage, the better strategy will be used in the phase 2 stage 2 extension: T_{reg} alone vs T_{reg} plus single-agent prophylaxis. In the event of non-inferiority of the T_{reg} alone arm, stage 2 will use this treatment strategy. A further 51 subjects would be enrolled in the Phase 2 Stage 2 extension, enough to determine if there is a significant increase in GFRS (60%) from historic rates (40%).

Overview

After obtaining the MTD is obtained from the phase 1 trial, we propose conducting a Simon 2-Stage phase 2 trial in which the 1st stage will determine if immunosuppression with graft product is required and the 2nd stage will determine the 1-year GRFS compared against historical control patients who underwent the same conditioning regimens but received a T-cell replete graft. This protocol will be eligible to patients with high-risk hematologic malignancies, that is, patients beyond CR1, CR1 with high-risk features or with refractory disease who would be otherwise eligible for MA allogeneic HCT.

3. PARTICIPANT SELECTION AND ENROLLMENT PROCEDURES

All eligibility criteria of transplant recipients are to be satisfied within 30 days of initiation of the recipient's conditioning regimen.

All eligibility criteria of transplant donors are to be satisfied within 40 days of the donor leukapheresis procedures.

For the purposes of this study, "registration" will be considered to be signing of the informed consent. Enrollment will be considered to be confirmation of eligibility per the following criteria. Initiation of study treatment will be the initiation of the conditioning regimen (infusion recipients) or mobilizing agents (cellular donors).

3.1 Recipient Inclusion Criteria

3.1.1 Patients with the following diseases that are histopathologically-confirmed are eligible

- Acute leukemia, primary refractory or beyond CR1, or minimal residual disease (MRD) positivity
- High-risk leukemia with any of the following features:
  - Acute myeloid leukemia (AML) in CR1 as follows:
    - Complex karyotype (≥ 3 clonal chromosomal abnormalities)
    - Any of the following high-risk chromosomal abnormalities:
      - Monosomal karyotype (-5; 5q-; -7; 7q-)
      - t(11q23); t(9;11); inv(3); t(3;3); t(6;9); t(9;22)
      - Normal karyotype with FLT3-ITD mutation
    - Other high-risk features as determined by molecular studies, or clinical presentation as assessed by a study investigator
  - Other high-risk leukemia in CR1, as determined by a study investigator
- Chronic myelogenous leukemia (accelerated, blast or second chronic phase or first chronic phase that and has not achieved a molecular remission after three or greater therapies)
- Myelodysplastic syndromes
- Myeloproliferative syndromes
- Non-Hodgkin lymphoma with poor risk features not suitable for autologous HCT
- Hodgkin lymphoma with poor risk features not suitable for autologous HCT

3.1.2 Age ≥ 18-years old, and for subjects in Cohort 1 only, ≤ 73 years of age
At the start of Cohort 2A and beyond, eligibility will be expanded to allow pediatric subjects age ≥ 13 years old.

3.1.3 Left ventricular ejection fraction (LVEF) ≥ 45%

3.1.4 Diffusing capacity of the lungs for carbon monoxide (DLCO) ≥ 50%

3.1.5 Calculated creatinine clearance ≥ 50 mL/min or creatinine < 2.0 mg/dL

3.1.6 SGPT and SGOT ≤ 7.5 x ULN

3.1.7 Total bilirubin ≤ 3 x ULN (patients with Gilbert's syndrome may be included at the discretion of the PI or where hemolysis has been excluded

3.1.8 Negative serum or urine beta-HCG test in females of childbearing potential

3.1.9 Availability of a 10/10 or 9/10 HLA-matched donor (related or unrelated) defined by Class I (HLA–A and -B) serologic typing (or higher resolution) and Class II (HLA-DRB1) molecular typing. If the donor is a 9/10 HLA-match, the mismatch may be a permissive or non-permissive allelic mismatch as assessed by an independent HLA and transplantation expert.

3.1.10 Karnofsky performance status ≥ 70%

3.2 Recipient Exclusion Criteria

3.2.1 Seropositive for any of the following:
HIV antibodies; hepatitis B surface antigen (sAg); hepatitis C antibodies
3.2.2 Prior myeloablative therapy or hematopoietic cell transplant
3.2.3 Candidate for autologous transplant
3.2.4 HIV-positive
3.2.5 Active uncontrolled bacterial, viral or fungal infection, defined as currently taking antimicrobial therapy and progression of clinical symptoms.
3.2.6 Uncontrolled CNS disease involvement
3.2.7 Pregnant or a lactating female
3.2.8 Positive serum or urine beta-HCG test in females of childbearing potential
3.2.9 Psychosocial circumstances that preclude the patient being able to go through transplant or participate responsibly in follow-up care
3.2.10 Recipients of prior allogeneic transplants

3.3 Donor Inclusion Criteria

3.3.1 Age ≥ 13 and ≤ 75 years of age

3.3.2 Karnofsky performance status of ≥ 70% defined by institutional standards or cleared by the NMDP for NMDP donors

3.3.3 Seronegative for HIV-1 RNA PCR; HIV 1 and HIV 2 ab (antibody); HTLV-1 and HTLV-2 ab; PCR+ or sAg (surface antigen) hepatitis B; or PCR or sAg negative for hepatitis C; negative for the *Treponema palladum* antibody *Syphillis* screen; and negative for HIV-1 and hepatitis C by nucleic acid testing (NAT) within 40 days of donor apheresis procedures.

In the case that *T palladum* antibody tests are positive, donors must:

- Be evaluated and show no evidence of syphilis infection of any stage by physical exam and history
- Have completed effective antibiotic therapy to treat syphilis
- Have a documented negative non-treponemal test (such as RPR) or in the case of a positive non-treponemal test must be evaluated by an infectious disease expert to evaluate for alternative causes of test positivity and confirm no evidence of active syphilitic disease

3.3.4 Must be a related or unrelated, HLA match or 9/10 HLA match to recipient.

3.3.5 Female donors of child-bearing potential must have a negative serum or urine beta HCG test

3.3.6 Capable of undergoing leukapheresis, have adequate venous access, and be willing to undergo insertion of a central catheter should leukapheresis via peripheral vein be inadequate

3.3.7 Agreeable to 2nd donation of PBPC (or bone marrow harvest) in the event of graft failure

3.3.8 The donor or legal guardian greater than 18 years of age, capable of signing an IRB approved consent form.

3.3.9 Meet criteria for donation as specified by standard NMDP guidelines for NMDP unrelated donors.

3.4 Donor Exclusion Criteria

3.4.1 Evidence of active infection

3.4.2 HIV-positive

3.4.3 Medical, physical, or psychological reason that would place the donor at increased risk for complications from growth factor or leukapheresis

3.4.4 Lactating female

3.5 Informed Consent Process

The BMT attending physician discusses the rationale, logistics, risks, and benefits of the study with the subject. Alternative therapies are discussed. The subject's bill of rights is reviewed. The subject is given a copy of the consent form to take home and read. Then a meeting is scheduled with a clinical nurse specialist or research nurse to review the rationale, logistics, treatment plan, risks, benefits and consent form. The subject's bill of rights is reviewed with an emphasis placed on voluntary participation. Subjects are given time to ask questions and do not need to sign the consent form until they are ready to proceed. In general, there is one hour devoted to the consent discussion with a clinical nurse specialist or research nurse educator. The BMT attending physician is available to discuss the study with the subject at any time. For pediatric subjects, all efforts will be made to have both parents sign. If one parent is unable to sign, the reason will be documented.

During the consent teaching, the physician, clinical nurse specialist or research nurse frequently asks questions of the subject to assess their understanding and a consent note is written documenting the subjects understanding. For subjects that are not fluent in English, the consent procedure is performed with an interpreter present (or via telephone), or a signer for those that are hearing impaired. Consents are translated into Spanish as needed.

Subjects are evaluated by a physician, nurse coordinator, or clinical nurse specialist and social worker. Any member of the team can request a psychiatric evaluation if a concern of competency to provide consent is raised.

4. TREATMENT PLAN

4.1 Phase 2 Stage 1 treatment allocation

Between Day -10 and Day -1, incoming subjects in Phase 2 Stage 1 will be assigned to GvHD prophylaxis or not. An unblinded study coordinator will ascertain the consecutive number of the subject and the arm assignment and convey this to the investigator or study representative.

4.2 Mobilization Therapy

Following screening and enrollment, the donor will receive mobilization therapy per institutional guideline treatment with daily G-CSF (filgrastim or equivalent). Recommended

dose is 16 µg/kg/day SQ (rounded off to the nearest vial size of either 300 or 480 µg). The Mobilization Phase starts on the first day of administration of G-CSF and continues until the final day of leukapheresis.

4.3 Cell Collection

All cellular components will be obtained from matched donors mobilized with G-CSF [filgrastim (Neupogen) or equivalent] and collected by apheresis at Stanford Hospital and Clinics (SHC) or certified National Marrow Donor Program (NMDP) collection centers as per current institutional allogeneic transplant protocols.

Leukapheresis will be performed on a continuous flow cell separator according to institutional standards and will start on Day +4 of G-CSF administration. Leukapheresis of the donor will start on Day -3 of infusion with a target of $\geq 3 \times 10^6$ $CD34^+$ cells/kg recipient body weight post-selection and a maximum dose of 10×10^6 $CD34^+$cells/kg. In order to have sufficient cells available for CD34 enrichment and T_{reg} sorting procedures, donors will undergo apheresis collections on 2 or more consecutive days; additional apheresis may be needed to ensure a sufficient T-cell count for above stated reasons; an additional mobilizing agent such as plerixafor (Mozobil) may be used under these circumstances at the discretion of the attending physician. Collections will occur on Day -3; Day -2; and potentially Day -1 of the CD34-enriched and T_{reg} product infusions (Day 0). The 1st day's apheresis collection will be scheduled for afternoon hours and the 2nd day's for early morning, thereby limiting the time from the end of the first collection to the infusion of the cellular products to less than 72 hours.

At the conclusion of each collection at Stanford, cellular products will be transferred to the cell processing facility. For collection occurring outside of Stanford, products can be transferred on the same day, with the previous days product stored at 4ºC or according to institutional practice but not frozen.

The first day's collection (Day -3) will be kept under refrigeration as a quarantined product in a continuously monitored, secure refrigerator in accordance with apheresis collection storage standards. Prior to being placed in storage, samples for cell count, flow cytometric analysis, and collection sterility testing will be aseptically removed by the cell processing facility staff and submitted to the designated group within the SHC Clinical Laboratory for assessment. The 2nd and 3rd days' collections (Day -2, Day -1) will be transferred to the cell processing facility upon completion of apheresis. The product will be sampled in the same manner for assessment but retained at room temperature for cell processing.

After collection, the cellular harvests will be transferred via validated transport methods to Orca BioSystems, Inc, facility at 3400 Business Dr (Suite 140) in Sacramento, CA (95820). All cellular processing subsequent to December 2019 will occur at this location pursuant to Orca BioSystems IND 018542, to which the IND for this protocol is cross-referenced.

4.4 Cell Processing

The donor leukapheresis collections will be the source of the 3 infusions products: conventional T-cells (T_{con}), regulatory T-cells (T_{reg}), and $CD34^+$ hematopoietic progenitor/stem cells. $CD34^+$ cell enrichment will be performed according to procedures given in the CliniMACS Users Operating Manual and institution SOPs. Collections from Day -3 and Day -2 will be pooled for CD34 enrichment as described in the SOP "Selection of $CD34^+$ Cells Using the CliniMACS System." Upon completion of the cell selection process, samples of the CD34-enriched product will be aseptically removed for release testing to

assess the cellular content, the cell doses and viability, sterility by Gram staining, and endotoxin content (table below), along with additional quality assessments including standard 14-day sterility cultures.

$CD34^+$ HSPC release criteria

Final Release Tests and Specifications for $CD34^+$ HSPC Final Formulated Drug Product

CD34 Post-selection Treatment Component Release Testing	Assay Type	Method	Specification
Purity	Viability	Dye exclusion (7AAD or Trypan Blue)	≥ 70% *
Purity / Identity	$CD34^+$ cells	Flow Cytometry	≥ 50% $CD34^+$ cells*
Potency	$CD3^+$ cell number	Flow Cytometry	≤ 5 x 10^4 cells/kg FIO
	$CD34^+$ cell number	Flow Cytometry	3 ± 1 x 10^6 cells/kg FIO
Safety	Sterility	Gram-stain	Negative *
Safety	Sterility	Bactec Method	Negative / no growth observed
Safety	Endotoxin	Endosafe-PTS Assay or Limulus amoebocyte assay (LAL)	5 EU/kg/hour *

* Release specification for $CD34^+$ HSPC Drug Product

FIO: For information only.

After cell selection and sampling, the CD34-enriched products will be securely stored at 2 to 8°C in a continuously monitored refrigerator until released for infusion. Product labeling will be compliant with ISBT 128 specifications.

T_{reg} release criteria

Release Tests and Specifications for Treg Cells Final Formulated Drug Product

T_{reg} Cells Drug Product Release Testing	Assay Type	Method	Specification
Identity / Potency	$FoxP3^+$ Cells	Flow Cytometry	≥ 80% *
Potency	Cell count	Automated Cell Count	To meet cohort dose* ($3 \pm 1 \times 10^6$ cells/kg for Cohort 2A)
Purity	Viability	Dye exclusion (7AAD or Trypan Blue)	≥ 70% *
Safety	Sterility	Gram Stain	Negative *
Safety	Sterility	14-day sterility	Negative
Safety	Endotoxin	Endosafe-PTS Assay or LAL	5 EU/kg/hour *

* Release specification for T_{reg} Drug Product

After processing by Orca BioSystems, Inc, the cellular components (drug products), will be transferred via validated transport methods to BMT-CTF, which is the "cellular pharmacy" for the Stanford University Medical Center. The BMT-CTF will dispense the cellular components to the respective subjects.

4.5 Infusion of $CD34^+$ Cells and T_{reg} cells

The selected $CD34^+$ cells will be infused fresh into the subject according to institutional guidelines. The day that the subject receives the donor CD34+ peripheral blood stem cells (PBSC) will be defined as Subject Day 0. Infusion of cell products to occur prior to cell expiration which will be determined by the cell lab.

T_{reg} cells meeting the release criteria will be infused on Day 0 after $CD34^+$ cell infusion. The cells will be infused through a central venous catheter or a peripheral IV of at least 18 gauge. T_{reg} cell infusion will take place over 5 minutes or less. It is anticipated that the volume of the cellular products will range from 5 mL to 45 mL.

After T_{reg} infusion, subjects will be monitored for vital signs every 30 minutes including pulse oximetry following infusion for at least 4 hours with evaluation every 30 minutes. Recipients will be monitored for infusional toxicity and treated as needed. Benadryl and hydrocortisone will be utilized should a reaction occur and epinephrine will be available at the bedside.

4.6 Conditioning Regimen

The conditioning regimen that will be used will be determined by subject diagnoses. Regimens will be assigned as follows:

ACUTE LEUKEMIA, CHRONIC MYELOGENOUS LEUKEMIA (HIGH RISK), MYELODYSPLASTIC SYNDROME AND MYELOPROLIFERATIVE DISORDERS will be offered 1 of the 3 following regimens (TBI-based or chemotherapy-based):

Day -8	Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Day 0	Day +1	Day +2
fTBI	fTBI	fTBI	fTBI	VP16	rest	Cy	Rest*	$CD34^+$ HSPC and T_{reg} infusion	rest	T_{con} infusion

Doses: **Fractionated total body irradiation (fTBI)**: 1320 cGy
VP16: 60 mg/kg x 1 dose
Cyclophosphamide(Cy): 60 mg/kg x 1 dose

OR

Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Day 0	Day +1	Day +2
Bu	Bu	Bu	Bu	Cy	Cy	Rest*	$CD34^+$ HSPC and T_{reg} infusion	rest	T_{con} infusion

Doses: **Busulfan**: 3.6 mg/kg Q24h
Cyclophosphamide: 60 mg/kg x 2 doses

OR

Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Day 0	Day +1	Day +2
Bu Flu	Bu Flu	Bu Flu	TTP	TTP	Rest*	$CD34^+$ HSPC and T_{reg} infusion	Rest	T con infusion

Doses: **Thiotepa**: 5 mg/kg x 2 doses
Busulfan: 3.2 mg/kg x 3 doses
Fludarabine: 50 mg/m^2 x 3 doses

* Two days of rest are allowed at the discretion of the treating physician.

NON-HODGKIN LYMPHOMA, HODGKIN LYMPHOMA

Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Day 0	Day +1	Day +2
Carmustine	rest	VP16	rest	Cy	Rest*	$CD34^+$ HSPC and T_{reg} infusion	rest	T_{con} infusion

Doses: **Carmustine**: 300 mg/m^2
VP16: 60 mg/kg x 1 dose
Cyclophosphamide: 100 mg/kg x 1 dose

4.7 Graft-vs-Host Disease Prophylaxis

For subjects who are determined to receive GvHD prophylaxis in the phase 2 stage 1 or phase 2 stage 2 extension, this will be administered corresponding to the specific conditioning regimen utilized and as follows:

For subjects receiving fTBI, VP-16 and Cy:

Subjects will start tacrolimus at Day +3 at an initial dose of 0.015 mg/kg/day intravenous infusion with a target of 4 to 8 ng/mL.

For subjects receiving Bu and Cy:

Subjects will start tacrolimus at Day +3 at an initial dose of 0.015 mg/kg/day intravenous infusion with a target of 4 to 8 ng/mL.

For subjects receiving carmustine, VP-16 and Cy:

Subjects will start tacrolimus at Day +3 at an initial dose of 0.015 mg/kg/day intravenous infusion with a target of 4 to 8 ng/mL.

For subjects receiving Thiotepa, Fludarabine and Busulfan:

Subjects will start tacrolimus at Day +3 at an initial dose of 0.015 mg/kg/day intravenous infusion with a target of 4 to 8 ng/mL.

For subjects with a 9/10 HLA matched non-permissive donor:

Subjects will receive two options for GVHD prophylaxis, depending on primary treating physician discretion:

Cyclophosphamide at Day +5 as a single infusion dose of 50mg/kg and Tacrolimus at Day +6 at an initial dose of 0.015 mg/kg/day intravenous infusion with a target of 4 to 8 ng/mL.

Or

Tacrolimus at day +3 at an initial dose of 0.015 mg/kg/day intravenous infusion with a target of 4 to 8 ng/mL. and at day +4 CellCept (Mycophenolate mofetil) 1,000 mg BID.

Should a subject become intolerant of their specific GvHD prophylaxis or other reasons to change are determined by the treating physician then prophylaxis can be altered at the discretion of the treating physician, with the recommendation that tacrolimus, sirolimus, or mycophenolate mofetil be utilized.

Should GvHD occur, the appropriate treatment schedule and dose will be initiated. Recipients who develop acute GvHD will be treated at the discretion of the treating physician.

4.8 Infusion of T_{reg} and T_{con} cells will proceed as follows:

On Day 0:

* Infusion of regulatory T-cells with dose based on assigned cohort

On Day 2

* Infusion of conventional T-cells with dose based on assigned cohort.

As shown in the Schema in Figure 1, box "Cohort 1a," dose escalation will start with:

- **T_{reg} dose = 1 x 10^6/kg** T_{reg} enriched by immunomagnetic selection of $CD25^+$ cells and further purified by flow cytometric cell sorting of the $CD4^+CD127^-$ population).
- **T_{con} dose = 1 x 10^6/kg** T_{con} administered as unfractionated mobilized peripheral blood cells containing specified $CD3^+$ cell dose

4.9 Cell Source

All cellular components will be obtained from matched donors mobilized with G-CSF [filgrastim (Neupogen) or equivalent] and collected by apheresis at Stanford Hospital and Clinics (SHC) or certified National Marrow Donor Program (NMDP) collection centers as per current institutional allogeneic transplant protocols. The clinical protocol requires 3 cellular products for infusion as indicated in Figure 2, below:

1) $CD34^+$ HSPC: The cell processing facility currently performs CD34 cell immunomagnetic selection using the CliniMACS (Miltenyi Biotec) system.

The $CD34^+$ HSPC will be infused on Day 0.

2) T_{reg}: The selection of T_{reg} will include a tandem selection procedure utilizing immunomagnetic selection on the CliniMACS Cell Selection System (Miltenyi Biotec) followed by flow cytometric cell sorting on an Influx high-speed cell sorter. T_{reg} will be enriched by immunomagnetic selection of the $CD25^+$ population and highly-purified to ≅ 90% $FoxP3^+$ cells by cell sorting of the $CD25^+$ cell fraction gated for selection of the $CD4^+CD127^-$ population. In our experience, cells recovered through this process are typically > 90% $FoxP3^+$ (range 86% to 99%) with a mean of 95%, $FoxP3^+$ cells in sorted T_{reg} performed to date. T_{reg} isolation will use the flow-through fraction from the CD34 selection as the cell source as depicted in Figure 2.

The T_{reg} product will be infused on Day 0 after the $CD34^+$ are infused. There will be a minimum of a 1-hour interval between the end of the $CD34^+$ product infusion and the start of the T_{reg} infusion.

3) T_{con}. This designation is used to indicate the infusion product containing the conventional T-cell dose. The T_{con} product will be obtained from the Day 2 or Day 3 leukapheresis product prior to pooling with the Day 1 product. Cell dose of the T_{con} infusion product will be based on the $CD3^+$ cell content in order to assess GvHD risk. The T_{con} product will be cryopreserved until the planned infusion on Day +2 post-HCT. Flow cytometry to measure initial CD3 and CD34 percentages of leukapheresis product will be performed in the BMT-CTF located in Stanford Hospital. Flow cytometry of selected $CD34^+$ products is performed by the SHC Clinical Laboratory Flow Cytometry service.

4.10 Supportive Care Guidelines

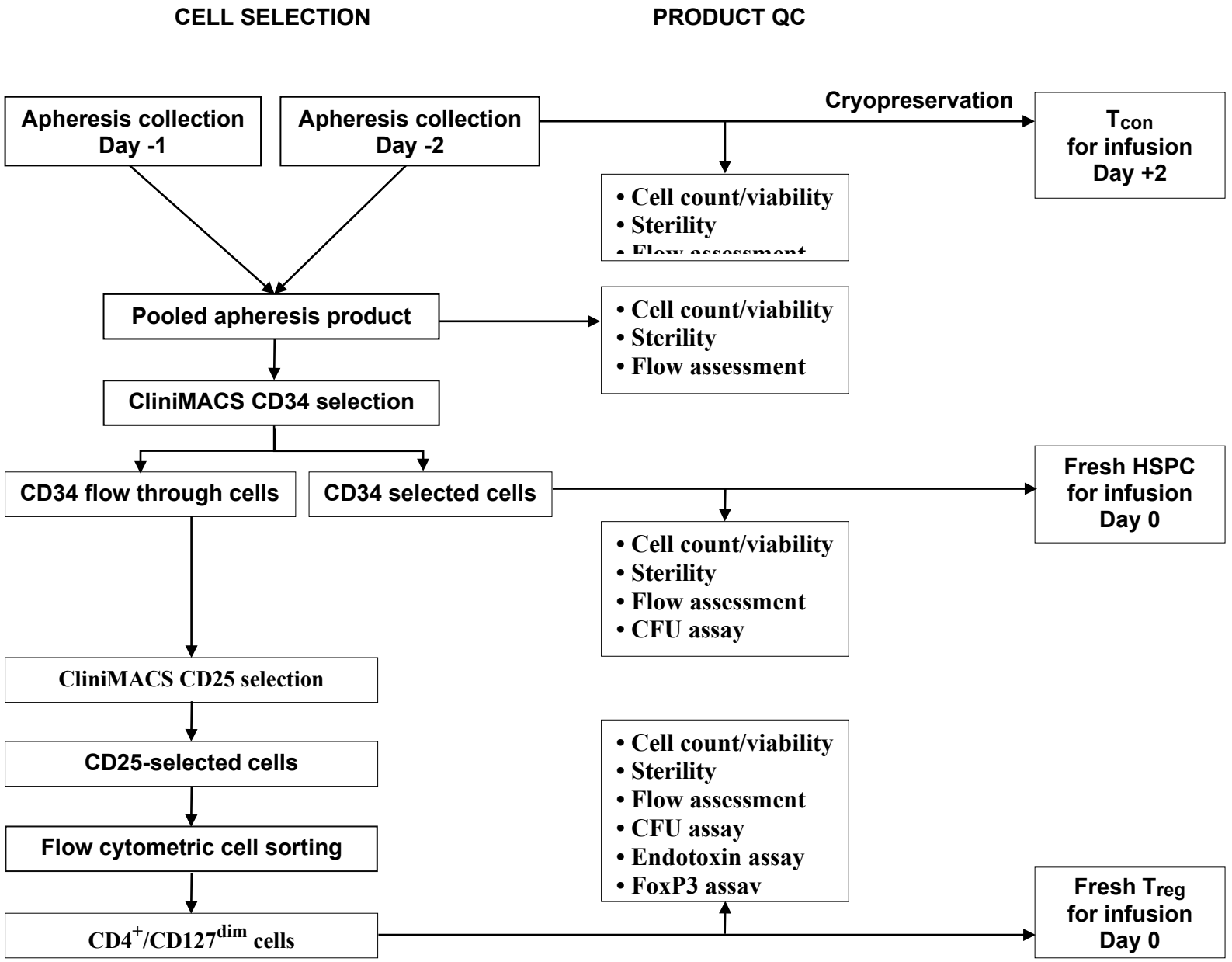
4.10.1 Venous Access

Recipients will have appropriate long-term central venous access placed, per institutional standard practice, prior to beginning the conditioning regimen.

4.10.2 Irradiation of blood products

All blood products, except the infused $CD34^+$ cells, T_{reg} and T_{con} will be irradiated in accordance with institutional standards.

Figure 2


CELL SELECTION
PRODUCT QC
Apheresis collection Day -1
Apheresis collection Day -2
Cryopreservation
T_{con} for infusion Day +2
• Cell count/viability
• Sterility
• Flow assessment
Pooled apheresis product
• Cell count/viability
• Sterility
• Flow assessment
CliniMACS CD34 selection
CD34 flow through cells
CD34 selected cells
Fresh HSPC for infusion Day 0
• Cell count/viability
• Sterility
• Flow assessment
• CFU assay
CliniMACS CD25 selection
CD25-selected cells
Flow cytometric cell sorting
$CD4^{+}/CD127^{dim}$ cells
• Cell count/viability
• Sterility
• Flow assessment
• CFU assay
• Endotoxin assay
• FoxP3 assay
Fresh T_{reg} for infusion Day 0

4.10.3 Anti-bacterial Prophylaxis

A quinolone or appropriate alternative if quinolone-allergic will be given according to institutional guidelines.

Pneumocystis pneumonia prophylaxis will be provided for adults and children according to institutional guidelines.

4.10.4 Anti-viral Prophylaxis

EBV surveillance via quantitative PCR on recipient serum will be performed every 2 weeks starting at Day +14 until Day +100, then accordance with institutional guidelines with active treatment initiated upon documented evidence of infection. Treatment with rituximab shall be in accordance with institutional guidelines and will be initiated upon documented evidence of infection.

4.10.5 Anti-fungal Prophylaxis

Fungal prophylaxis will be provided for adults and children according to institutional guidelines.

4.10.6 Antibiotic treatment for culture positive results from graft products.

If bacterial culture results from graft products become positive, recipients will be initiated on appropriate treatment-dose antibiotic unless the culture positivity is determined to be a false positive due to contamination. Subjects who have bacterial culture positivity from their donor products will not be removed from the trial or censored from analysis.

4.11 Management of Graft Failure

Primary graft failure is defined as the failure to achieve an absolute neutrophil count of > 500 cells/μL after Day +30. Secondary graft failure is defined as a sustained loss of hematopoiesis after engraftment has occurred. Subjects experiencing primary and/or secondary graft failure will be removed from the study and treated according to institutional guidelines at the investigator's discretion.

4.12 Management of Post-Transplant Relapse

Subjects who relapse with their disease after transplant will be removed from the study and will be treated according to institutional guidelines.

4.13 Criteria For Removal From Study

Subjects may be removed from study if any one of the following events occur:

- Significant protocol violation or noncompliance, either on the part of the subject or investigator.
- Graft failure
- Refusal of the subject to continue treatment and/or observations.
- Decision by the Investigator, or subject's physician that removal from the study is in the subject's best medical interest.
- Unrelated medical illness or complication.

- Lost to follow-up.
- Disease progression or a requirement for alternative therapy.

4.14 Duration of Therapy

The active duration of therapy is during the conditioning regimen and up to Day +100 after transplantation and follow up for up two years after transplantation.

4.15 Duration of Follow Up

Afterwards, the subject will be followed per treating physician until death even in the event of graft failure or disease progression.

4.16 Alternatives

After discharge from the hospital after the transplant procedure, the subject will be required to live in the local area up to at least day +100 after transplant so that the subject can be monitored closely for signs of infection, graft vs host disease or other complications. If the subject chooses not to enroll in this protocol, the subject will be offered conventional chemotherapy and/or palliative care.

4.17 Compensation

Subjects will not receive any compensation for participation in this study.

4.18 Evaluations During the Study

DONOR Evaluation/Monitoring

NOTE: The following laboratory tests/evaluations will be performed for all donors registered in the study within 40 days prior to donor leukapheresis.

SEE TABLE 1 FOR DONOR EVALUATION SCHEDULE

4.18.1 Baseline

4.18.1.1 Complete medical history and physical examination, and review of systems

4.18.1.2 Prothrombin time and partial thromboplastin time

4.18.1.3 Serum chemistries including electrolytes, glucose, ALT, AST, creatinine, bilirubin, alkaline phosphatase, albumin

4.18.1.4 Serum or urine Beta HCG for female donors

4.18.1.5 Serologies for: HIV including HIV 1 and 2 antibodies, HTLV I/II Ab; Hepatitis B including HBsAg, HBcAb; Hepatitis C ab; RPR for syphilis; Cytomegalovirus (CMV); Epstein-Barr Virus (EBV); Herpes Simplex Virus (HSV); Varicella zoster Virus

4.18.1.6 ABO/Rh typing and HLA typing: HLA-A,B,C loci (typed by serology) and HLA-DRB1, DQ, DP loci

4.18.1.7 Baseline samples for chimerism studies

4.18.1.8 For NMDP donors, the study team will utilize standard NMDP guidelines and clearance as the baseline evaluation

4.18.1.9 Donor Research Samples:

a. Phenotypic analysis of T cells subsets, particularly absolute counts and percentage of CD4+ CD25+ CD127- Foxp3+ regulatory T-cells in donor peripheral blood by immunoflow cytometry.

b. Mixed lymphocyte reaction (MLR) to show investigational drug product suppression potential *in-vitro* by mixing effector cells (donor T_{regs}) with activated responder cells (donor PBMCs). Responder cell numbers assessed after 72 hours by immunoflow cytometry.

c. Immune reconstitution assessment using T-cell and B-cell receptor gene rearrangement test.

Donor research samples are needed to characterize the T_{reg} in the donor in order to understand how this might influence the efficacy of the clinical trial results. In graft engineering, the measurement of cell subsets at the time of collection is important for understanding if a sufficient collection can be achieved.

For example, CD34+ cells are measured in order to see if there are sufficient cells for hematopoietic engraftment. It is critical to determine whether the count of T_{regs} in the donor prior to collection correlates to the total T_{reg} collected and outcome. This is accounted for through flow cytometry (a); to determine if these T_{regs} are functional, accounted for by MLR (b); and need to know if factors in the donor graft like T-cell and B-cell diversity affect immune reconstitution after transplantation (c) as T_{regs} are known to influence this.

4.19.2 Graft Evaluation

4.19.1. All donor apheresis collections will be assessed as follows.

a. Cell count by validated cell counting system

b Viability by popidium iodide (PI) or 7-aminoactinomycin D (7AAD) exclusion (flow cytometric assay)

c. Sterility in accordance with 21CFR§610.12 using Fluid Thioglycollate medium and Tryptic Soy Broth 14-day cultures.

d. Phenotypic analysis by flow cytometry for cell surface expression of CD34; CD3; CD19; CD25; CD127 and intracellular FoxP3 expression.

4.19.2. Final Products for infusion, including CD34-selected and T_{reg} fractions will be assessed prior to infusion as follows.

a. Cell count by hemocytometer.

b. Viability by PI, 7AAD (flow cytometric assay); or trypan blue exclusion as according to institutional SOPs.

c. Sterility in accordance with 21CFR§610.12 using Bactec 14-day cultures will be initiated but will not be completed prior to infusion. Gram stain will be completed on the CD34-enriched and sorted T_{reg} products prior to release for infusion.

d. Phenotypic analysis by flow cytometry for cell surface expression of CD34; CD3; CD19; CD25; CD127; and intracellular FoxP3 expression.

e. Endotoxin testing post-selection on $CD34^+$ enriched and sorted T_{reg} products.

4.19.3. T_{con} for infusion may be thawed bedside for immediate infusion or in lab for post-thaw assessment and/or dosing per institutional SOPs.

a. Cell count by hemacytometer.

b. Viability by PI, 7AAD (flow cytometric assay); or trypan blue exclusion as according to institutional SOPs.

4.20 Evaluation/Monitoring – Recipient

NOTE: The following laboratory tests/evaluations will be performed for all recipients registered in the study within 30 days prior to initiation of conditioning regimen, except as indicated. Additionally, as per our institutional practice, all subjects will be closely monitored in our out-patient Infusion Treatment Area (ITA) from discharge up to Day +100. These visits in the ITA typically occur 3x week during Day +30 to Day +60 and then are reduced to either once to twice/week depending on subject acuity. Each visit entails lab tests including a CBC; comprehensive metabolic panel; and history and physical exam by a transplant MD and physician assistant or nurse practitioner.

4.20.1 Eligibility and other pre-infusion evaluations

4.20.1.1 Medical history including bone marrow biopsy/aspirate for confirmation of histologic and/or morphologic diagnosis and disease status and staging

4.20.1.2 Physical examination including performance status (ECOG)

4.20.1.3 Informed consent per institutional standard timing

4.20.1.4 Vital signs including weight, blood pressure, pulse rate and respiratory rate

4.20.1.5 Pulmonary testing (DLCO), cardiac performance (LVEF)

4.20.1.6 Automated CBC (WBC, RBC, hematocrit, hemoglobin, platelets) with differential

4.20.1.7 Prothrombin time (PT), partial thromboplastin time (PTT)

4.20.1.8 Serum chemistries including electrolytes; glucose; ALT; creatinine; bilirubin; alkaline phosphatase; albumin

4.20.1.9 Serum or urine Beta HCG for female recipients

4.20.1.10 Urinalysis

4.20.1.11 Quantitative Immunoglobulins for IgG; IgA; IgM

4.20.1.12 Serologies for: HIV (including HIV-1 RNA PCR); HIV 1+2 Ab; HTLV I/II Ab; cytomegalovirus (CMV); hepatitis B (including HBsAg and HBcAb); hepatitis C ab; Syphilis; and if indicated, Epstein-Barr virus (EBV); herpes simplex virus (HSV); varicella zoster virus (VZV). Also HLA-typing for HLA-A,B,C loci (typed by serology) and HLA-DRB1, DQ, DP loci (typed by PCR/SSOP if available) *

* HLA typing per standard institutional timing.

4.20.1.13 Within 40 days of the cellular infusions, sample of peripheral blood to site laboratory for percentage and absolute cell count by immunoflow cytometry of CD3; CD4; CD5; CD8; CD 19/20; CD16/56; CD45RA; CD45RO; CD62L; and CD44.

4.20.2 Follow Up Evaluations (Days +30, +60, +90, +180 and +365, except as indicated)

4.20.2.1 Physical examination with vital signs

4.20.2.2 Assessment of relapse of disease using bone marrow aspirate/biopsy or other tests such as PET/CT scan as clinically indicated on Days +100; +180; 365; and then yearly for 2 years post-HCT.

4.20.2.3 Automated CBC (WBC; RBC; hematocrit; hemoglobin; platelets) with differential

4.20.2.4 Serum chemistries: glucose; electrolytes; creatinine; ALT; bilirubin; alkaline phosphatase; albumin

4.20.2.5 Sample of peripheral blood to site laboratory for absolute count and percentage immunoflow cytometry of CD3; CD4; CD5; CD8; CD19/20; CD44; CD45RA; CD45RO; CD62L; CD16/56, and lineage-specific chimerism studies. TREC analysis will also be performed. These samples will also be collected on Days 0 (prior to the infusion), +7, and +14.

4.20.2.6 Quantitative Immunoglobulin levels to include: IgG; IgA; IgM

4.20.2.7 Summary of acute GvHD (Appendix C)

4.20.2.8 Assessment for the presence of chronic GvHD (Appendix C)

4.21 Potential Benefits

Subjects who participate in this study have the potential of achieving a durable remission and long term survival from their hematologic malignancy. Additionally, because post-transplant immune suppression will not be given as part of protocol, there may be a smaller risk of serious infections. The risk of graft vs host disease also may be reduced since the subject is receiving a T-cell depleted graft. Additionally, the infusion of as regulatory T-cells may also reduce the risk of GvHD.

5. INVESTIGATIONAL AGENT/DEVICE/PROCEDURE INFORMATION

5.1 Investigational Agent

This protocol is submitted to FDA IND 14686. The specific investigational agent for which this IND was obtained was for "$CD4^+CD25^+CD127$-$foxp3^+$ regulatory T-cells" referred to as T_{reg} cells. The IND application was submitted 4 April 2011, and was approved to proceed on 3 June 2011.

6. ADVERSE EVENTS AND REPORTING PROCEDURES

6.1 Potential Adverse Events

Recipients of allogeneic hematopoietic cell transplantation face significant risks. The risk of treatment related morbidity and mortality following allogeneic transplant is related

to the underlying disease, disease status at the time of transplant, prior therapies and other coexisting health problems.

Significant risks include:

1) **Infection**. Risks include bone marrow suppression, hospitalization, invasive procedures, prior exposure to micro-organisms and immunosuppressive medications for the prevention and treatment of graft vs host disease. Infections may be caused by bacteria; viruses; protozoa; or fungus.
2) **Organ toxicity**. There is a risk of life-threatening toxicity to the heart; lungs; liver; kidney; and brain. Risks include prior therapy; concurrent health problems; and the high-dose chemotherapy and radiation used in the preparatory regimen.
3) **Graft versus host disease**. Acute GvHD can range from a mild and treatable problem to a life-threatening complication. Chronic GvHD can vary from a mild to an extensive disorder affecting almost any body tissue. Chronic GvHD can negatively affect functional status and quality of life.
4) **Secondary malignancies**. Radiation, chemotherapy and immunosuppressive medications increase the risk of secondary malignancies.
5) **Graft failure**. Although rare, graft failure can be a fatal complication.
6) **Infertility** in both genders and premature menopause in women at risk.
7) **Relapse**. All subjects face a risk of relapse. The risk is highest for those subjects with active or resistant disease at the time of transplantation.

6.2 Adverse Event Reporting

Definitions:

Unanticipated Problem: any incident, experience, or outcome that meets *all* of the following criteria: (1) Is unexpected in terms of nature, severity, or frequency in relation to (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and (b) the characteristics of the subject population being studied; **and** (2) Is related or possibly related to participation in the research; **and** (3) places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously know or recognized.

Adverse Event: Any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product, medical treatment or procedure and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), a symptom, or disease temporally associated with the use of a medicinal product, medical treatment or procedure whether or not considered related to medicinal product or treatment.

Life-threatening Adverse Event: Any adverse event that places the subject, in the view of the investigator, at immediate risk of death from the reaction.

Unexpected Adverse Event: An adverse event, the nature or severity of which is not consistent with the applicable product information (Investigator's Brochure, product insert). For studies that do not involve investigational products or devices, an unexpected adverse event is an adverse event that is not described in the transplant medical literature or consent form.

Serious Adverse Event (SAE): Any adverse event occurring that results in any of the following outcomes: death, a life-threatening adverse event, a persistent or significant disability/incapacity, a congenital anomaly, requires intervention to prevent permanent impairment or damage (21CFR§312.32). The PI is also bound by 45 CFR§46, Subpart A which is the "Common Rule" for the Protection of Human Subjects, therefore, the Stanford IRB definition applies (report "unanticipated problems" involving risks to study participants or others). All grades 3 to 5 organ-related adverse events not due to the primary malignancy or pre-existing condition and concomitant medications will be collected from Day 0 through 90 days after the T_{con} dose which is given on Day +2, ie, through Day +32 post-HCT.

Grade 4 infusion reactions will be reported expeditiously and as a separate listing in the FDA Annual Report.

Distinction between Serious and Severe: The term severe is used to describe the intensity (severity) of a specific event, for example mild, moderate or severe. The event itself however, may be of relatively minor medical significance, for example a severe headache. This is not the same as serious, which is based on the subject/event outcome and is usually associated with events that pose a threat to the subject's life or functioning. Seriousness, not severity, serves as a guide for defining regulatory obligations.

Hematopoietic cell transplantation (HCT) is an aggressive therapy for the treatment of a number of life threatening malignant and non-malignant disorders. Individuals presenting for HCT generally have exhausted other avenues of therapy that will result in any lasting benefit. The treatment related mortality (TRM) of autologous transplantation is approximately 5%. The TRM of a sibling myeloablative allogeneic transplant is approximately 20% and the TRM for an unrelated myeloablative allogeneic transplant ranges from 20 to 50%.

As an aggressive therapy HCT is associated with a large number of significant adverse and serious adverse events. The toxicities associated with HCT are related to the following:

1) Underlying disease
2) Therapy antecedent to HCT
3) Health status of the transplant recipient including co-existing conditions
4) High-dose preparative regimen employed in preparation for transplant
5) Therapies directed at reducing transplant related complications (eg, immunosuppressants for the prevention of GvHD), and
6) Treatment of complications of HCT.

The use of toxicity grading scales such as the NCI CTC is a standard in the medical community for the reporting of SAEs in the investigation of new drugs or devices. The

use of this type of scale is less helpful in the evaluation of SAEs associated with a treatment, such as HCT. In an effort to report to regulatory agencies the toxicities that are relevant and meaningful for the evaluation of risks and benefits to potential HCT recipients the following guidelines will serve to determine what is reported as SAEs in recipients of HCT.

The following SAEs require expedited reporting (as soon as possible but within at least 7 calendar days of the investigator learning of the event):

1) Deaths

Death while the subject is receiving treatment on this protocol up to 90 days post-transplant if it is felt to be treatment related

This includes deaths from the common and expected grade 3-4 toxicities noted below. Deaths that occur outside of Stanford will be reported whenever possible. It must be noted that obtaining detailed information on the cause and circumstances of a death occurring at another institution can be difficult. Excludes deaths related to relapse of underlying disease, which will be reported at the time of protocol renewal.

2) All serious and unexpected toxicities.

Defined as those toxicities not identified in the transplant literature or in the consent form.

The following will generally not be reported as SAEs:

1) Hospitalizations.

Approximately 50% of allogeneic transplant recipients will be readmitted to the hospital. The most common indications for readmission of an allogeneic HCT recipient are fever, failure to maintain nutritional status and graft versus host disease.

2) Relapse of disease.

Relapse unfortunately remains a significant problem following both autologous and allogeneic transplantation. The risk of relapse is influenced by both subject and disease variables. The risk of relapse following allogeneic transplant is extremely dependent on the disease being treated but ranges from 10% (for subjects with severe aplastic anemia) to 80% (for subjects with refractory acute leukemia).

3) Common and expected Grade 3 to 4 toxicities of HCT that are well-described in the transplant literature, the product inserts or stated in the consent form and do not result in death.

This includes but is not limited to neutropenia, thrombocytopenia, anemia, thrombotic microangiopathy, bleeding requiring transfusions, edema, hypertension, hypotension, gastritis, mucositis, nausea, vomiting, diarrhea, hematuria, central venous catheter infections, febrile episodes, sepsis, mental status changes, insomnia, mood alterations, seizures, tremor, pain, hypoxia, pleural effusion, pneumonitis, incontinence, infertility, laboratory abnormalities, veno-occlusive disease, graft failure, cardiac arrhythmias and graft versus host disease.

Secondary Malignancies.

The occurrence of secondary malignancies and associated mortality is a known risk of cancer therapies. The occurrence of secondary malignancies will be reported at the time of the protocol annual review.

6.3 Adverse Event Reporting

Protocol directors/principal investigators (PDs/PIs) are responsible for reporting any serious adverse events (SAEs) to both the IRB and regulatory authorities. Stanford's Human Subject Manual defines reportable events as "all serious adverse events, related or unrelated to the study treatment, occurring at Stanford or elsewhere, and unanticipated problems." The panel has established the reporting timeline as within five to fifteen days of first learning of the event. PDs and PIs must also report any SAEs to the biopharmaceutical sponsor or FDA (when the PD acts as sponsor-investigator) according to 21CFR§312.32. By definition, serious adverse event is any untoward or unexpected event or medical occurrence, associated with the use of a drug or device or procedure that at any dose: results in death; is life-threatening; requires in-patient hospitalization or prolongation of existing hospitalization; results in persistent or significant disability/incapacity; or is a congenital anomaly/birth defect. The term "unexpected" is used to further quantify an event/experience when it is not consistent with the current investigator brochure or with the risk information in the investigational plan. "Associated" with the investigational drug/biologic/device means that there is a reasonable possibility that the experience/event may have been caused by the drug or was contributed to, at least in part, by the drug or device or procedure. Finally, a "life -threatening" event means that in the view of the investigator, the event places the subject or subject at immediate risk of death from the reaction as it occurred. Timelines for reporting SAEs to sponsors or the FDA are more specific and depend on the outcome of the event (hospitalization, disability, death).

PDs and PIs are responsible for adhering to the timelines for reporting SAEs. When the protocol director is the principal investigator for an NIH/NCI/NHLBI sponsored study, the investigator must notify the Investigational Drug Branch (IDB) of the NCI or NHLBI by telephone of all Grade 4 and Grade 5 expected and unexpected events (see the Common Toxicity Criteria Index at www.ctep.nih.gov) within 24 hours of learning of the event. Protocol-specific adverse event case report forms must be completed and continued close follow-up is required until the event resolves.

When a protocol director is both the principal investigator and sponsor of a clinical trial (investigator-initiated), the requirements for reporting all SAEs to the FDA and other investigators rest with the PD. Specifically, the PD must notify the FDA of any study-related death or life-threatening event via telephone within 24 hours of learning of the event. A written safety report (MedWatch 3500A) must be sent to the FDA within seven days. Any other investigators who are participating in the study must be notified via an IND Safety Report within 15 days of learning of the event.

6.4 Action Plan for Positive Results on Treatment Component Safety Testing

In the unlikely event that a positive sterility test result is obtained in either cell product manufacturing facility after administration of any treatment component , (ie, T_{con};

$CD34^+$ HSPC; or T_{reg}) to a study subject (whether fresh or cryopreserved), or after administration of the product to the subject, the following steps will be initiated IMMEDIATELY:

a. Clinical Laboratory personnel will notify the BMT-CTF, via the BMT-CTF IND Manager or Medical Director, of positive test results.

b. BMT-CTF personnel will notify the IND Sponsor-Investigator (who is also the Stanford Protocol Director / Principal Investigator) at 650-906-8582. Information provided to the Sponsor-Investigator / Protocol Director will include identification of which treatment component(s) are affected/were administered to the subject. The Sponsor-Investigator will be updated with any substantive changes, including the final report on the identification and sensitivity from the positive sterility test.

c. If the BMT-CTF personnel are unable to reach Sponsor-Investigator within 15 minutes, the in-patient attending physician caring for the subject on the hospital service will be contacted via hospital page with direct communication. NOTE: If not within 15 minutes, the Sponsor-Investigator will be notified as soon as possible. The Sponsor-Investigator and/or designee will contact the attending physician, who will determine the extent of the work-up of a positive culture in consultation with appropriate infectious disease consultants, as well as determine an appropriate action treatment plan.

d. The Sponsor-Investigator/attending physician will discuss the positive results with the subject, and specify the clinical therapy, antibiotic regimen and/or monitoring plan.

e. A contaminated sample of a treatment component that has been administered to a subject will be handled in the same fashion as a Grade 4/5 toxicity. The IND Sponsor-Investigator in the role of the Stanford Protocol Director will be responsible to notify the IRB of the event via an Unanticipated Problem (UP) report within 5 working days. If the infused treatment component is the HSPC or T_{reg} drug product, the , the Sponsor-Investigator will also notify the FDA via an expedited 7-day IND Safety Report.

In addition to the above, appropriate Safety reporting will be done as per Volume 6 AS 3.1 Sterility Testing. A sample of each product is retained by Quality Systems and will be sent to the Microbiology Laboratory for repeat testing and speciation. An Out-of-Specification (OOS) Investigation will be conducted by the Quality Systems staff of the manufacturing laboratory including root cause analysis, review of viable environmental monitoring results collected at the time of manufacturing on personnel, equipment and reagents. Whether or not attribution is established, a formal Corrective and Preventive Action plan will be issued by BMT-CTF Quality staff and appropriate remediation will be performed including retraining of manufacturing personnel, elimination of any contaminated reagents and re-cleaning of the production facility followed by viable microbiological monitoring to establish effectiveness of cleaning.

STUDY CALENDAR

Assessment Schedule - RECIPIENT

Protocol IRB-21257 / BMT236

Subject Name	MRN
Last, First	nnnnnn-n
mm/dd/yyyy	D0+

BMT Date: mm/dd/yyy	Pre-conditioning	Day 0	D+7 ±1 day	D+14 ±1 day	D+30 ±7 days	D+60 ±7 days	D+90 ±10 days	D+180 ±14 days	D+365 ±14 days	2 years ±4 weeks
Study Assessments	**Baseline**									
Consent	X									
Eligibility checklist	X									
PT/PTT	X									
Beta HcG for females	X									
Pre transplant Virology Panel	X									
HLA Typing	X									
Urinalysis	X									
Pulmonary Function Tests	X									
Cardiac Function Evaluation	X									
Eligibility checklist	X									
Flow Cytometry	X				X	X	X	X	X	
(CD3,CD4,CD5,CD8,CD19/20, CD16/56, CD45RA, CD45RO, CD62L, CD44)	X	X	X	X	X	X	X	X	X	
Serum Ig levels (IgA, IgG, IgM)	X				X	X	X	X	X	
TREC	X				X	X	X	X	X	
EBV	X				X	X	X	X	X	
GvHD ASSESSMENT					X	X	X	X	X	X

BMT Date: mm/dd/yyy	Pre-conditioning	Day 0	D+7 ±1 day	D+14 ±1 day	D+30 ±7 days	D+60 ±7 days	D+90 ±10 days	D+180 ±14 days	D+365 ±14 days	2 years ±4 weeks
CBC w/ Diff	X				X	X	X	X	X	
Serum Chemistry	X				X	X	X	X	X	
Karnofsky performance score	X				X	X	X	X	X	
BMBx, Aspirate, Cytogenetics	X						X	X	X	
Lineage specific chimerism studies					X		X	X	X	
CT/PET scan as clinically indicated							X	X	X	

Table 3
DONOR ASSESSMENT SCHEDULE
ASSESSMENT SCHEDULE - DONOR

Study Assessments	**BaseLine** *
Consent	X
Eligibility checklist	X
CBC w/ Diff	X
Chem 7	X
Pre transplant Virology Panel	X
EBV	X
PT/PTT	X
PE	X
Serum or urine Beta HCG ***	X
ABO/RH typing	X
HLA typing ****	X
EKG	X
Chest Xray	X

* Within 40 days prior to leukapheresis

** Electrolytes; glucose; ALT; AST; creatinine; bilirubin; alkaline phosphatase; albumin

*** Female donors
**** HLA-A,B,C loci (**typed by serology**) and HLA-DRB1,DQ,DP loci

7. MEASUREMENT OF EFFECT

7.1 Anti-tumor Effect

Subjects will undergo disease evaluations on Day +100, +180, +365 and then annually until 5 years from HCT date. The testing should be done +/- 14 days from targeted evaluation date although the Day + 180 and yearly evaluations will be done as close to the date as possible.

7.1.1 Definitions

7.1.1.1 **Acute Leukemia** – relapsed defined as

a. Leukemic blasts > 20% are documented in the blood or bone marrow after HCT

b. Leukemic blasts > 5% and < 20% are documented in the blood or bone marrow and supported by reappearance of cytogenetic abnormality or

c. Leukemic blasts > 5% and < 20% are documented in the blood or bone marrow on at least 2 occasions

d. Leukemia detected at an extramedullary site or the appearance of new dysplastic changes within the bone marrow or % blasts

7.1.1.2 **Chronic Myelogenous Leukemia** – relapsed defined as

a. Immature hematopoietic cells are persistently documented in the peripheral blood, or

b. Myeloid hyperplasia in the bone marrow in the presence of a cytogenetic relapse as defined by:

- 50% of the metaphases exhibit the characteristic 9;22 translocation with at least 10 metaphases analyzed or
- At least 1 metaphase exhibits the 9;22 translocation on each of 2 separate consecutive examinations at least 1 month apart, regardless of number of metaphases analyzed

7.1.1.3 **Myelodysplastic syndrome**

a. Appearance of pre-transplant morphologic abnormalities detected in 2 consecutive bone marrow specimens taken at least 1 month apart

b. Satisfying above criteria for evaluation into acute leukemia

c. Reappearance of pre-transplant cytogenetic abnormalities in at least 50% of metaphases with at least ten metaphases examined or

d. Reappearance of pre-transplant cytogenetic abnormality in at least 2 metaphases on each of two separate consecutive examinations at least 1 month apart, regardless of the number of metaphases analyzed

7.1.1.4 **Hodgkin and Non-Hodgkin lymphoma** (relapse defined below)

Lymph nodes should be considered abnormal if the long axis is more than 1.5 cm regardless of the short axis. If a lymph node has a long axis of 1.1 to 1.5 cm, it

should only be considered abnormal if its short axis is more than 1.0 cm. Lymph nodes ≤ 1.0 x ≤ 1.0cm will not be considered as abnormal for relapse or progressive disease.

i. Appearance of any new lesion more than 1.5 cm in any axis during or at the end of therapy, even if other lesions are decreasing in size. Increased FDG uptake in a previously unaffected site should only be considered relapsed or progressive disease after confirmation with other modalities. In patients with no prior history of pulmonary lymphoma, new lung nodules identified by CT are mostly benign. Thus, a therapeutic decision should not be made solely on the basis of the PET without histologic confirmation.

ii. At least a 50% increase from nadir in the SPD of any previously involved nodes or in a single involved node, or the size of other lesions (eg, splenic or hepatic nodules). To be considered progressive disease, a lymph node with a diameter of the short axis of less than 1.0 cm must increase by ≥ 50% and to a size of 1.5 x 1.5 cm or more than 1.5 cm in the long axis.

iii. At least a 50% increase in the longest diameter of any single previously identified node more than 1 cm in its short axis

iv. Lesions should be PET positive if observed in a typical FDG-avid lymphoma or the lesion was PET positive before therapy unless the lesion is too small to be detected with current PET systems (< 1.5 cm in its long axis by CT). Measurable extranodal disease should be assessed in a manner similar to that for nodal disease. For these recommendations, the spleen is considered nodal disease. Disease that is only assessable (eg, pleural effusions, bone lesions) will be recorded as present or absent only, unless, while an abnormality is still noted by imaging studies or physical examination, it is found to be histologically negative. In clinical trials where PET is unavailable to the vast majority of participants, or where PET is not deemed necessary or appropriate for use, response should be assessed as above, but only using CT scans. However, residual masses should not be assigned CRu status, but should be considered partial responses.

8. DATA REPORTING / REGULATORY CONSIDERATIONS

8.1 Monitoring plan

The Monitoring Entity (ME) that will be responsible for monitoring the data collected, including data related to unanticipated problems and adverse events, and their respective roles (eg, Stanford Cancer Center, CRC, investigator, sponsor, coordinating or statistical center, independent medical monitor, DSMB/DSMC, or some other entity). If there is no ME, then the Protocol Director (PD) is responsible for this function.

Primary internal data monitoring will be performed by the Principal Investigator and the study coordinator. The PI will review data to assure the validity of data, as well as the safety of the subjects. The PI will also monitor the progress of the trial. The PI is responsible for maintaining the clinical protocol, reporting adverse events, assuring that consent is obtained and documented, reporting of unexpected outcomes, and reporting the

status of the trial in the continuing renewal report submitted annually to the IRB and SRC.

The DSMC of the Stanford University Cancer Center will provide oversight of the study. This multidisciplinary committee monitors the safety of participants in clinical trials, and the conduct, progress, validity, and integrity of the data for all investigator initiated clinical trials at the Stanford University Clinical Cancer Center. The Stanford DSMC audits all investigator-initiated studies on an annual basis and submits a DSMC report to the Principal Investigator.

The content of this DSMC report is comprised of an *overall rating* of the study audit, (Outstanding, Satisfactory, Minor Deficiencies, or Major Deficiencies) that is a composite of overall study conduct and the findings of the audit session. In rating the conduct of the study, the DSMC categorizes *deviations* as Major, Moderate or Minor. The report also contains the specific *recommendation* from the committee, which can consist either of Full Approval, Conditional Approval, Suspension of defer to the SRC for closure.

8.2 Stopping rules (for the individual subject and for the study as a whole)

As indicated in the dose escalation schema (Figure 1), any recipients within a dose cohort showing GvHD > Grade 2 will require trigger the inclusion of three additional recipients at that dose. Dose escalation will proceed if none of the additional recipients show GvHD > Grade 2; Grade 4 neutropenia lasting to 28 days after HCT; or Grade 3 to 5 cytokine release syndrome / acute infusion reaction.

8.3 Data management

i) Research records are maintained in a secure office of the data management staff. Electronic data is maintained in a secure database.

ii) Research samples are sent to the SCTT lab for coding prior to the freezing or processing of the cell. A link between the sample and the subject is maintained with access to the link limited to study personnel.

iii) Only study personnel within the Stanford BMT program have access to the electronic database, the research records or the link between research samples and identifying information. Stanford researchers outside of the BMT program do not have access to PHI.

iv) Research records are kept in a locked file cabinet within an office of the data management staff. Access to the data management office is limited to key access.

v) The BMT Database System Administrator limits access to the database to research personnel within the BMT program. Data sets can be generated to share with investigators outside the program by de-identifying the data.

8.4 Confidentiality

All subjects undergoing hematopoietic cell transplantation at Stanford are assigned a SPN (Stanford Patient Number) and data is entered into a secure database. Access to the database is limited to study personnel and password protected.

Subjects will not be directly identified in any publications, presentations or correspondence.

All staff within the BMT Program are required to complete HIPAA training. All research staff must complete the IRB human subjects research training.

The subject's data are identified by SPN number alone in the research documents with the information about the code kept in a secure location. Access to the research database is limited to approved clinical and research staff of the BMT Division. The code is maintained by the BMT Database System Administrator.

PHI is collected and recorded at the time of the subject's first consultation with the BMT program. Additional information is collected when the subject is enrolled in a clinical trial. PHI may include some or all of the following:

1. Names
2. Social Security numbers
3. Telephone numbers
4. All geographic subdivisions smaller than a State, including street address, city, county, precinct, zip code, and their equivalent geocodes, except for the initial three digits of a zip code, if, according to the current publicly available data from the Bureau of the Census: (1) The geographic unit formed by combing all zip codes with the same three initial digits contains more than 20,000 people; and (2) The initial 3 digits of a zip code for all such geographic units containing 20,000 or fewer people is changed to 000.
5. All elements of dates (except year) for dates directly related to an individual, including birth date, admission date, discharge date, date of death; and all ages over 89 and all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 or older
6. Fax numbers
7. Electronic mail addresses
8. Medical record numbers
9. Health plan beneficiary numbers
10. Account numbers
11. Certificate/license numbers
12. Vehicle identifiers and serial numbers, including license plate numbers
13. Device identifiers and serial numbers
14. Web Universal Resource Locations (URLs).
15. Internet Protocol (IP) address numbers
16. Biometric identifiers, including finger and voice prints
17. Full face photographic images and any comparable images;

Referring physicians are routinely sent letters by the BMT physicians to update them on their subject's status. Specific information regarding the study would not be routinely shared with the referring physician, however, we would share with referring physician's information regarding the expected side effects of the treatment, necessary precautions and also the appropriate follow-up.

Other outside agencies that may receive information include:

- The Office for Human Research Protections in the US Department of Health and Human Services
- The Food and Drug Administration
- Federal and regulatory agencies as required
- Center for International Blood and Marrow Transplant Research (CIBMTR)
- The National Marrow Donor Program or donor registry from which the cells were obtained
- Foundation for the Accreditation of Cellular Therapy
- The National Institutes of Health
- Pediatric Blood and Marrow Transplant Consortium
- Children's Oncology Group
- Data Safety Monitoring Board
- Other investigators with IRB-approved projects

8.5 Reporting Mechanisms

The Principal Investigator of the trial (or designee) is responsible for the coordination and development of all protocol amendments. Any changes to the protocol or consent will be made in the form of an amendment and will be approved prior to implementation and this process should be included in the monitoring plan.

Changes or amendments to the protocol or consent will be reported to the IRB, FDA and NHLBI.

All lRB actions will be reported to the DSMC, NHLBI, and FDA.

9. STATISTICAL CONSIDERATIONS

This is a phase 1 dose escalation trial followed by a phase 2 trial at the MTD. The addition of planned numbers and ratios of T_{reg} compared to T_{con} will occur at defined time points after hematopoietic cell infusion. Each cohort will have 3 subjects per group. The initial doses and ratios utilized will be 1 x 10^6/kg of T_{reg} cells to 1 x 10^6/kg of T_{con} cells at a 1:1 ratio. In order to progress to the next dose level, there must be no evidence of Grade 3 or 4 acute GvHD. If a single subject develops Grade 3 or 4 acute GvHD, an additional 3 subjects will be treated at the same dose level. If a 2nd subject develops Grade 3 or 4 GvHD, then the dose escalation will be stopped.

9.1 Endpoints

A. Endpoint for Safety

This is a phase 1 dose finding trial with two degrees of freedom in the definition of dose (numbers of T_{reg} and T_{con} , or, equivalently, the number of T_{con} and the ratio of T_{reg} to T_{con}). These 'dose pairs' act in different directions to produce the resulting rates of GvHD, and presumably, anti-tumor activity. The study will follow a traditional 3+3 model, as described in the diagram to find the maximum tolerated dose of T_{con} at the minimum necessary ratio of T_{reg} to T_{con} There will be 3 subjects in each cohort with a minimum of 3 subjects in each cohort. At all levels, progression to the next cohort will be dependent on no occurrence of > grade 2 acute GvHD or any other DLT which includes Grade 4 neutropenia lasting to 28 days after HCT and Grade 3 to 5 cytokine release syndrome / acute infusion reaction.

If at any point, a predefined DLT is observed, then the cohort is expanded to (no more than) 6 total at that dose pair. If no further grade 3 or 4 GvHD or pre-defined DLT is observed (so the total is now 1/6), the MTD is declared and in that case the cohort at that dose pair is expanded by up to 12 subjects (in 2 cohorts of 6) for a total of 18 subjects.

However, if at any point in the 12-subject expansion, the observed Grade 3 or 4 GvHD rate or pre-defined DLT rises to 50% or above, the next lower dose of T_{con} at the ratio of 1:1 T_{reg} to T_{con} is expanded as the MTD. Thus, if in either cohort of 3 subjects at any dose level there are 2 or more with Grade 3 to 4 GvHD, or pre-defined DLT we will de-escalate to the lower dose.

B. Endpoint for Feasibility

The feasibility of T_{reg} isolation is another primary endpoint of this trial. If the T_{reg} yield is below the dose for a specified cohort, the subject will be placed in the previous cohort. As this is a dose escalation trial that includes administering higher doses of T_{reg} cells, consideration has been taken in the event of failure to isolate the specified target dose of T_{reg} cells. We have determined that 40% represents the "unacceptable' failure rate to reach target T_{reg} dose. In the event that the observed failure rate in a dose cohort implies that the true failure rate exceeds 40%, with 80% confidence, then we will de-escalate T reg dose and treat subjects on a previous dose level. We will assess the failure rate after each cohort of 6 and in the expanded cohort of 12. If we see 4 failures out of 6 tries in a cohort, or 7 failures out of 12, this feasibility stopping rule will be triggered and the dose will be de-escalated. See table below.

Observed Failures / Subject cohort	80% Lower Confidence Limit for True Failure Rate
4 / 6	0.41
7 / 12	0.42

9.1.1 Primary endpoint

Phase 1 Primary Objective

The Primary Objective of the phase 1 part of this trial is to determine the **safety and feasibility of administration of conventional T-cells and regulatory T-cells** to subjects undergoing allogeneic hematopoietic cell transplantation with matched related donors, and to find the maximum tolerated dose of T_{con} at the minimum necessary ratio of T_{reg} to T_{con}. Thus, the primary endpoint in each subject is the occurrence of Grade 3 or 4 GvHD, as defined above.

Phase 2 Primary Objective

Once the MTD is determined, it is proposed to expand the cohort at that dosage into a single-arm, phase 2 admissible Simon 2-stage proof-of-concept study, continuing with HLA-matched donors (related or unrelated). The Primary Objective of the phase 2 Stage 1 portion is to determine the need for concomitant immune suppression with fresh T_{regs}, and the phase 2 Stage 2 portion is GvHD-free, relapse-free survival post-HCT. Historical control data from Stanford will be used as the comparator, documented as ~40% 1-year GRFS in a comparable patient population receiving T-cell replete grafts. GRFS is defined as the minimum time from HCT to relapse; GvHD; or death. The anticipated 1-year GRFS probability is 60% and the lower boundary for proceeding to a possible phase 3 trial is an GRFS probability of > 0.40. The primary hypothesis can be described as H_0: $p \leq 0.40$ versus H_1: $p > 0.40$. When the true GRFS percentage is 60% with a sample size of 41, there is 80% power at one-sided $\alpha = .05$ to rule out a GRFS percentage of < 40%.

An "Admissible Simon's two-stage design" (Simon, 1989) will be used. The null hypothesis that the true 1-year GRFS is 40% will be tested against a one-sided alternative. The probability of early termination under the null hypothesis is 64%. In the first stage, 17 patients will be accrued. If there are 7 or fewer successes in these 17 patients, the study will be stopped. Otherwise, 24 additional patients will be accrued for a total of 41. The null hypothesis will be rejected if 22 or more responses are observed in 41 patients. This design yields a type I error rate of 5% and power of 80% when the true 1-year GRFS is 60%.

	7	17	21	41	25.62782	0.6405077	0.04733667	0.8009427

Phase 2 Primary Outcome (for ClinicalTrials.gov)

Title: GvHD-free Relapse-free Survival (GRFS)

Description: Clinical effect was assessed as graft vs host disease (GvHD)-free relapse free survival (GRFS), as determined by per protocol analysis. GvHD-free is defined as

no GvHD symptoms, and relapse free survival is defined as survival at 12 months without relapse.

Time Frame: 12 months

Safety Issue: Yes

9.1.2 Stopping Guidelines

Stopping Rule for Grade 3 to 4 acute GvHD

Interim analyses for safety will be conducted for every 5 subjects accrued. The incidence of Grade 3 to 4 acute GvHD will be closely monitored and the following stopping rules will apply. Based on historical control data observed in a similar patient population, we anticipate an observed rate of Grade 3 to 4 acute GvHD to be 15% and we would not accept a true rate > 30%. The stopping rule is triggered when the 80% one-sided lower confidence limit for the rate exceeds 30%.

Observed Cases of Grade 3 to 4 GvHD / Subjects in Cohort	Action
3 / 5 *	Stop accrual
5 / 10 *	
7 / 15 *	
9 / 20 *	
10 / 25 *	
12 / 30 *	
14 / 35 *	
16 / 40 *	
18 / 45 *	
20 / 50 *	
22 / 55 *	
24 / 60 *	
26 / 65 *	
28 / 70 *	
30 / 75 *	

* protocol Phase 2 component / adaptive Simon 2-stage design

Stopping Rule for Primary Graft Failure

Interim analyses for safety will be conducted for every 5 subjects accrued. The incidence of primary graft failure (Grade 4 neutropenia lasting to 28 days after HCT) will be closely monitored and the following stopping rules will apply. Based on historical control data observed in a similar patient population, we anticipate an observed rate of primary graft failure to be 20%, and we would not accept a true rate > 30%. The stopping rule is triggered when the 80% one-sided lower confidence limit for the rate exceeds 30%.

Observed Cases of Primary Graft Failure / Subjects in Cohort	**Action**
3 / 5 *	Stop accrual
4 / 10 *	
5 / 15 *	
6 / 20 *	
8 / 25 *	
9 / 30 *	
10 / 35 *	
11 / 40 *	
12 / 45 *	
13 / 50 *	
14 / 55 *	
15 / 60 *	
16 / 65 *	
17 / 70 *	
18 / 75 *	

* protocol Phase 2 component / adaptive Simon 2-stage design

Stopping Rule for Acute Cytokine Release Syndrome / Infusion Reaction

Interim analyses for safety will be conducted for every 5 subjects accrued. The incidence of acute cytokine release syndrome / infusion reaction will be closely monitored and the following stopping rules will apply. Based on historical control data observed in a similar patient population, we anticipate an observed rate of acute cytokine release syndrome to be 10% and we would not accept a true rate > 20%. The stopping rule is triggered when the 80% one-sided lower confidence limit for the rate exceeds 20%.

Observed Cases of Grade 3 to 4 GvHD / Subjects in Cohort	**Action**
2 / 5 *	Stop accrual
3 / 10 *	
3 / 15 *	
4 / 20 *	
5 / 25 *	
5 / 30 *	
6 / 35 *	
7 / 40 *	
8 / 45 *	
9 / 50 *	
10 / 55 *	
11 / 60 *	
12 / 65 *	
13 / 70 *	
14 / 75 *	

* protocol Phase 2 component / adaptive Simon 2-stage design

Stopping Rules for evaluation of 9/10 HLA matched non-permissive subgroup

The same stopping rules will be applied as for HLA-matched as described above. Subjects from 9/10 HLA matched non-permissive subgroup will be monitored separately and in event that a stopping rule is triggered, accrual will be stopped for review.

9.1.3 Secondary endpoints

9.1.3.1 One-year OS

For overall survival (OS), the event analyzed is death from any cause. Alive at the time of last observation will be censored.

9.1.3.2 Incidence and severity of chronic GvHD

Chronic GvHD scored according to the first day of chronic GvHD onset will be used to calculate cumulative incidence curves. Subjects will be followed for 2 years from the Day 0 (day of $CD34^+$ peripheral blood stem cell infusion) for estimation of cGvHD incidence. See Appendix C.

9.1.3.3 Incidence of serious infections

Microbiologically and/or radiographically documented infections will be reported by source of infection (virus, bacteria, fungus), site of disease, date of onset and resolution, if any.

9.1.3.4 Immune reconstitution parameters

This will be measured in all subjects by:

1. The rate of peripheral blood repopulation by CD3; CD4; CD8; CD45RA; CD45RO; CD16/56; CD19; CD20; CD44; and CD62L
2. The rate of blood repopulation with recent thymus emigrants measured by T-cell receptor excision circle-positive T-cells (TRECs) via quantitative PCR.
3. The serum levels of IgA; IgG; and IgM.

Phase 2 Secondary Outcomes (for ClinicalTrials.gov)

Title: Dose-limiting Toxicity

Description: Dose-limiting Toxicity (DLT) was assessed as:

• Absolute neutrophil count <500/μL, to 28 day

• Cytokine release syndrome/acute infusion reactions as Common Terminology Criteria for Adverse Events (CTCAE) Grade 3 to 5

• Grade 3 to 4 acute GvHD. GvHD was staged as follows:

+ 1: Skin: rash <25%. Liver: bilirubin (BIL) 2-3mg/dL. Gut: diarrhea (DIA) 500-1000mL/day

+ 2: Skin: rash 25-50%. Liver: BIL 3-6mg/dL. Gut: DIA 1001-1500mL/day

+ 3: Skin: rash > 50%. Liver: BIL 6-15mg/dL. Gut: DIA >1501-2000mL/day

+ 4: Skin: generalized erythroderma. Liver: BIL >15mg/dL. Gut: DIA >2001mL/day

GvHD was graded as follows.

+ 1: Skin Stage 1-2; No Liver stage; No Gut stage

+ 2: Skin Stage 1-3 ; Liver Stage 1; +/- Gut Stage 1

+ 3: Skin Stage 2-3, Liver Stage 2-4; +/- Gut Stage 2-3

+ 4: Skin Stage 2-4; Liver Stage 2-4; +/- Gut Stage 2-4

The outcome is reported as the number of participants who received both T_{reg} and T_{con} cell infusions and had DLT events, per treatment level, a number without dispersion.

Time Frame: 28 days

Safety Issue: Yes

Title: Concomitant Single-agent Immunosuppression

Description: During study Phase 2, Stage 1, concomitant single-agent immunosuppression was assessed as in participants receiving fresh T_{reg} cells. The outcome is reported as number of such participants who received single-agent immunosuppression, by treatment level, a number without dispersion.

Time Frame:

Safety Issue:

Title: 1-year Overall Survival (OS)

Description: Overall Survival (OS) at 1 year was assessed as the number of participants per treatment level that received the hematopoietic cell transplant (HCT), and remained alive 12 months later, a number without dispersion.

Time Frame: 12 months

Safety Issue: No

Title: Incidence and Severity Chronic Graft vs Host Disease (cGvHD)

Description: The incidence and severity of chronic graft vs host disease (cGvHD) was assessed in participants who received the hematopoietic cell transplant (HCT).

Stage of chronic GvHD was assessed as follows.

• Stage 1: Skin: rash <25% of skin. Liver: bilirubin 2-3mg/dL. Gut: diarrhea 500-1000mL/day

• Stage 2: Skin: rash 25-50% of skin. Liver: bilirubin 3-6mg/dL. Gut: diarrhea 1001-1500mL/day

• Stage 3: Skin: rash > 50% of skin. Liver: bilirubin 6-15mg/dL. Gut: diarrhea >1501-2000mL/day

• Stage 4: Skin: generalized erythroderma. Liver: bilirubin >15mg/dL. Gut: diarrhea >2001mL/day

Grade of chronic GvHD was determined as follows.

• Grade 1: Skin Stage 1-2; No Liver stage; No Gut stage

• Grade 2: Skin Stage 1-3 ; Liver Stage 1; +/- Gut Stage 1

• Grade 3: Skin Stage 2-3, Liver Stage 2-4; +/- Gut Stage 2 to 3

• Grade 4: Skin Stage 2-4; Liver Stage 2-4; +/- Gut Stage 2 to 4

The outcome is reported as the number of participants by cGvHD grade and treatment level, a number without dispersion.

Time Frame: 24 months

Safety Issue: Yes

Title: Serious Infections

Description: Participants were assessed for infections judged serious by 21CFR§312.32. The outcome is reported as the number of serious infections per treatment level, in participants who received the hematopoietic cell transplant (HCT), a number without dispersion.

Time Frame: 24 months

Safety Issue: Yes

9.2. Plan of Analysis

9.2.1 Background and Demographic Characteristics

We will tabulate and summarize all background and demographic characteristics.

9.2.2 Evaluation of Efficacy

The OS and GRFS estimates at the MTD dose pair will be calculated according to the methods of Kaplan and Meier and will be calculated from Day 0 of infusion of $CD34^+$ selected peripheral blood stem cells. At the sample size (41) of the expanded cohort, the distance from the proportion surviving 1 year and the exact one-sided lower 90% confidence limit of that proportion is no more than 12%, illustrating the precision of the estimates of survival parameters.

9.2.3 Methods for handling missing data and non-adherence to protocol

No missing data expected; in the event of non-adherence, DLTs will be counted in any subject who receives any dose of cells.

9.2.4 Evaluation of Conduct of trial (including accrual rates, data quality)

Accrual rates will be reported to the DSMC and data quality will be reviewed by the Study Statistician.

9.2.5 Methods for Correlative Studies

Parameters of immune reconstitution and other secondary outcomes will be correlated with dose of each type of T-cell, using standard regression methods, assuming a range of doses are explored. The results will be descriptive, given the early phase of the study and small sample size, but estimates of confidence intervals for all summaries will be provided.

9.3 Sample Size

9.3.1 Accrual estimates

The number of subjects accrued during the Phase 1 component of the trial was 13. The maximum tolerated dose (MTD) was determined to be 3×10^6 T_{con} cells/kg in combination with 3×10^6 T_{reg} cells/kg.

This study's admissible Simon two-stage design is intended to evaluate the immune-suppressant (IS) combination, as well as efficacy and toxicity of the graft-engineered product.

In study phase 2, Stage 1, the trial will enroll 24 subjects to 1 of 2 arms, each received the established MTD of 3×10^6 T_{con} cells in combination 3×10^6 T_{reg} cells. In this stage, the two treatments will be graft-engineered product + IS vs graft-engineered product alone. In the 1st stage, the trial investigates if the rate of GvHD is non-inferior for the graft-engineered product with and without IS.

In study phase 2, Stage 2, if the GvHD rate is found to be non-inferior between the 2 arms, the 2 arms will be pooled for the interim analysis evaluating the primary endpoint and the remaining subjects enrolled in the 2nd stage will be administered the

graft-engineered product alone. Based on the admissible Simon two-stage design [2] the null hypothesis that the true 1-year GRFS is 40% will be tested against a one-sided alternative. The probability of early termination under the null hypothesis is 64%. In the first stage, 17 patients will be accrued. If there are 7 or fewer successes in these 17 patients, the study will be stopped. Otherwise, 24 additional patients will be accrued for a total of 41. The null hypothesis will be rejected if 22 or more responses are observed in 41 patients. This design yields a type I error rate of 5% and power of 80% when the true 1-year GRFS is 60%. See a table below.

	7	17	21	41	25.62782	0.6405077	0.04733667	0.8009427

9.3.2 Sample size justification

The phase 1 dose escalation trial will accrue 3 subjects/dose level. Such a cohort size provides a traditional level of confidence in the safety of each level before proceeding to the next. The implied target of about 33% Grade 3 or 4 GvHD rate is considered appropriate given the prognosis of the disease under standard therapy and the risk:benefit tradeoff. At the sample size of the expanded cohort, the distance from the proportion surviving 1 year and the exact one-sided lower 90% confidence limit of that proportion is no more than 18%, illustrating the precision of the phase 1 experiment. In the event that 2 of 3 subjects experience Grade 3 or 4 GvHD at any dose level, then subsequent subjects will be treated at the previous dose level following the Schema in Figure 2.

The Phase 2 part of this has a planned sample size of 41 subjects based on the true EFS being 60%. This sample size provides 80% power at $\alpha = .05$ to rule out a EFS percentage of < 40%. Given the complexity of the intervention, we consider the 60% EFS target as the smallest improvement on our historical control rate of 40% that would justify continuing to use this treatment concept as the basis for further development.

9.3.3 Definition of Dose Limiting Toxicity

a. Grade 4 neutropenia lasting to 28 days after HCT

b. Grade 3 to 4 acute GvHD

c. Grade 3 to 5 cytokine/release syndrome/acute infusion reaction

9.4 Interim analyses

The interim analyses consist of monitoring of the Grade 3 or 4 GvHD rates in successive cohorts defined by the dose pairs. The stopping rules are as described above. The confidence limits for secondary parameters will be presented at their nominal values as is traditional in phase 1. The stopping rules for the Phase 2 portion are outlined in Section 9.1.2. An “efficacy futility” interim analysis is not proposed due to the time lag between treatment and ascertainment of the primary outcome (1-year EFS rate).

10 CONSENT FORMS

10.1 Donor Consent Form

Provided separately.

10.2 Recipient Consent Form

Provided separately.

11 INVESTIGATOR AND FACILITIES INFORMATION

Provided separately.

12 APPENDICES

A. Participant Eligibility Checklist

ELIGIBILITY CRITERIA FOR RECIPIENT

In order for the subject to be eligible, all inclusion criteria must be yes and all exclusion criteria must be no.

Inclusion Criteria

Histopathologically-confirmed diagnosis of one of the following:

_____ Acute leukemia with refractory disease or beyond CR1, or minimal residual disease (MRD) positivity

_____ High-risk leukemia with any of the following features

- _____ Acute myeloid leukemia (AML) in CR1 as follows
  - _____ Complex karyotype (≥ 3 clonal chromosomal abnormalities)
  - _____ Any of the following high-risk chromosomal abnormalities:
    - _____ Monosomal karyotype (-5; 5q-; -7; 7q-)
    - _____ t(11q23); t(9;11); inv(3); t(3;3); t(6;9); t(9;22)
    - _____ Normal karyotype with FLT3-ITD mutation
  - _____ Other high-risk features as determined by molecular studies, or clinical presentation as assessed by a study investigator
- _____ Other high-risk leukemia in CR1, as determined by a study investigator

_____ Chronic myelogenous leukemia (accelerated, blast or second chronic phase or first chronic phase that and has not achieved a molecular remission after three or greater therapies)

_____ Myelodysplastic syndromes

_____ Myeloproliferative syndromes

_____ Non-Hodgkin lymphoma with poor risk features not suitable for autologous transplantation

_____ Hodgkin lymphoma with poor risk features not suitable for autologous transplantation

_____ Age ≥ 18-years old and for subjects in Cohort 1 only, ≤ 73 years of age. **At the start of Cohort 2A and beyond, eligibility will be expanded to allow pediatric subjects age ≥ 13 years old.**

_____ Left ventricular ejection fraction (LVEF) ≥ 45%

_____ Diffusing capacity of the lungs for carbon monoxide (DLCO) ≥ 50% corrected for hemoglobin

_____ Creatinine clearance calculated ≥ 50 mL/min or creatinine < 2.0 mg/dL

_____ SGPT and SGOT ≤ 7.5 x ULN;

_____ Total bilirubin ≤ 3 x ULN (patients with Gilbert's syndrome may be included at the discretion of the PI or where hemolysis has been excluded

_____ Negative serum or urine beta-HCG test in females of childbearing potential

_____ Availability of HLA-matched donor or 9/10 HLA-matched donor (related or unrelated) defined by Class I (HLA–A and -B) serologic typing (or higher resolution) and Class II (HLA-DRB1) molecular typing.

_____ Karnofsky Performance Status ≥ 70%

Exclusion Criteria (Recipient)

_____ Seropositive for any of the following:
HIV ab; hepatitis B sAg; hepatitis C ab

_____ Prior myeloablative therapy or hematopoietic cell transplant
_____ Candidate for autologous transplant
_____ HIV-positive
_____ Active uncontrolled bacterial, viral or fungal infection, defined as currently taking antimicrobial therapy and progression of clinical symptoms.
_____ Uncontrolled CNS disease involvement
_____Pregnant or lactating female
_____ Positive serum or urine beta-HCG test in females of childbearing potential
_____ Psychosocial circumstances that preclude the patient being able to go through transplant or participate responsibly in follow-up care
_____ Recipient of prior allogeneic transplants

_____ _____
Treating Physician Signature/Print Name Date

_____ _____
Research Coordinator Signature/Print Name Date

_____ _____
Other Third Signature/Print Name Date

ELIGIBILITY CRITERIA FOR DONOR

In order for the donor to be eligible, all inclusion criteria must be yes and all exclusion criteria must be no.

Inclusion Criteria

_____ ≥ 13 and ≤ 75 years of age

_____ Karnofsky performance status ≥ 70% defined by institutional standards cleared by the NMDP for NMDP donors

_____ Medical history and PE confirm good health status as defined by institutional standards or the NMDP for NMDP donors

_____ Seronegative for HIV-1 RNA PCR; HIV 1 and HIV 2 ab (antibody); HTLV-1 and HTLV-2 ab; PCR+ or sAg (surface antigen) hepatitis B ; or PCR+ or sAg for hepatitis C; negative for the *Treponema palladum* antibody *Syphillis* screen; and negative for HIV-1 and hepatitis C by nucleic acid testing (NAT) within 40 days of donor apheresis procedures

In the case that *T palladum* antibody tests are positive, donors must:

- Be evaluated and show no evidence of syphilis infection of any stage by physical exam and history
- Have completed effective antibiotic therapy to treat syphilis
- Have a documented negative non-treponemal test (such as RPR) or in the case of a positive non-treponemal test must be evaluated by an infectious disease expert to evaluate for alternative causes of test positivity and confirm no evidence of active syphilitic disease

_____ Must be a related or unrelated, HLA-matched donor or 9/10 HLA-matched donor.

_____ Female donors of child-bearing potential negative serum or urine beta-HCG test

_____ Capable of undergoing leukapheresis, have adequate venous access, and be willing to undergo insertion of a central catheter should leukapheresis via peripheral vein be inadequate

_____ Agreeable to 2nd donation of PBPC (or bone marrow harvest) in the event of graft failure

_____ Donor or donor's legal guardian ≥ 18 years of age, capable of signing an IRB approved consent form.

_____ Meet criteria for donation as specified by standard NMDP guidelines for NMDP unrelated donors.

Exclusion Criteria

_____ Evidence of active infection

_____ HIV-positive

_____ Medical, physical, or psychological reason that would place the donor at increased risk for complications from growth factor or leukapheresis

_____ Lactating female

_____	_____
Treating Physician Signature/Print Name	Date
_____	_____
Research Coordinator Signature/Print Name	Date
_____	_____
Other Third Signature/Print Name	Date

B. AE and SAE Reporting Guidelines

ADVERSE EVENT MONITORING AND REPORTING

Definitions:

Unanticipated Problem

Any incident, experience, or outcome that meets *all* of the following criteria: (1). Is expected in terms of nature, severity, or frequency in relation to (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and (b) the characteristics of the subject population being studied; **and** (2) Is related or possibly related to participation in the research; **and** (3) Places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously know or recognized.

Adverse Event (AE)

Any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product, medical treatment or procedure and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), a symptom, or disease temporally associated with the use of a medicinal product, medical treatment or procedure whether or not considered related to medicinal product or treatment.

Life-threatening Adverse Event

Any adverse event that places the subject, in the view of the investigator, at immediate risk of death from the reaction.

Unexpected Adverse Event

An adverse event, the nature or severity of which is not consistent with the applicable product information (Investigator's Brochure, product insert). For studies that do not involve investigational products or devices, an unexpected adverse event is an adverse event that is not described in the transplant medical literature or consent form.

Serious Adverse Event (SAE)

Any adverse event occurring that results in any of the following outcomes: death, a life-threatening adverse event, a persistent or significant disability/incapacity, a congenital anomaly, requires intervention to prevent permanent impairment or damage(21CFR§312.32). The PI is also bound by 45 CFR§46, Subpart A which is the "Common Rule" for the Protection of Human Subjects, therefore, the Stanford IRB definition applies (report "unanticipated problems" involving risks to study participants or others).

Distinction between Serious and Severe

The term severe is used to describe the intensity (severity) of a specific event, for example mild, moderate or severe. The event itself however, may be of relatively minor medical significance, for example a severe headache. This is not the same as serious, which is based on the subject/event outcome and is usually associated with events that pose a threat to the subject's life or functioning. Seriousness, not severity, serves as a guide for defining regulatory obligations.

Hematopoietic cell transplantation (HCT) is an aggressive therapy for the treatment of a number of life threatening malignant and non-malignant disorders. Individuals presenting for HCT generally have exhausted other avenues of therapy that will result in any lasting benefit. The TRM of a sibling myeloablative allogeneic transplant is approximately 20% and the TRM for an unrelated myeloablative allogeneic transplant ranges from 20 to 50%. In the setting of a non-myeloabative allogeneic transplant from a sibling donor the TRM is approximately 10% and ranges from 20 to 50% for unrelated donors.

As an aggressive therapy HCT is associated with a large number of AEs and SAEs. The toxicities associated with HCT are related to the following: 1) the underlying disease; 2) therapy antecedent to HCT; 3) the health status of the transplant recipient including co-existing conditions; 4) the preparative regimen employed in prior to transplant; 5) therapies directed at reducing transplant related complications (eg, immunosuppressants for the prevention of GvHD); and 6) the treatment of complications of HCT.

The use of toxicity grading scales such as the NCI CTC is a standard in the medical community for the reporting of AEs and SAEs in the investigation of new drugs or devices. The use of this type of scale is less helpful in the evaluation of AEs and SAEs associated with a treatment, such as HCT. In an effort to report to regulatory agencies the toxicities that are relevant and meaningful for the evaluation of risks and benefits to potential HCT recipients the following guidelines will serve to determine what is reported as AEs and SAEs.

The following SAEs require reporting to the CCTO. If the event is unexpected, it will also require reporting to the IRB:

1) Deaths

   All deaths while the subject is receiving treatment on a protocol up to 90 days after last dose of protocol treatment or any death that occurs more 90 days (allogeneic) after protocol treatment has ended that is felt to be treatment related.

   This includes deaths from the common and expected Grade 4 toxicities noted below. Deaths that occur outside of Stanford will be reported whenever possible. It must be noted that obtaining detailed information on the cause and circumstances of a death occurring at another institution can be difficult. Excludes deaths related to relapse of underlying disease, which will be reported at the time of protocol renewal.

2) All serious and unexpected toxicities.

   Defined as those toxicities not identified in the transplant literature, product inserts or in the consent form.

The following will generally not be reported as AEs or SAEs:

1) Hospitalizations.

   Approximately 50% of allogeneic transplant recipients will be readmitted to the hospital. The most common indications for readmission of an allogeneic HCT recipient are fever; failure to maintain nutritional status; and graft vs host disease.

2) Relapse of disease.

   Relapse unfortunately remains a significant problem following both autologous and allogeneic transplantation. The risk of relapse is influenced by both subject and disease variables.

3) Common and expected Grade 4 toxicities of HCT that are well described in the transplant literature, the product inserts or stated in the consent form and do not result in death. This includes but is not limited to neutropenia; thrombocytopenia; anemia; thrombotic microangiopathy; bleeding requiring transfusions; edema; hypertension; hypotension; gastritis; mucositis; nausea; vomiting; diarrhea; hematuria; central venous catheter infections; febrile episodes; sepsis; mental status changes; infections; insomnia; mood alterations; seizures; tremor; pain; hypoxia; pleural effusion; pneumonitis; incontinence; infertility; laboratory abnormalities; veno-occlusive disease; graft failure; cardiac arrhythmias and graft versus host disease.

4) Secondary Malignancies.

   The occurrence of secondary malignancies and associated mortality is a known risk of cancer therapies. The occurrence of secondary malignancies will be reported at the time of the protocol annual review.

C. Grading Graft vs Host Disease

Grading of Individual Organ Systems

Organ	Stage	Description
Skin	**+1**	Maculo-papular eruption over < 25% of body area
	+2	M-P eruption over > 25 to 50% of body area
	+3	Generalized erythroderma
	+4	Generalized erythroderma with bullous formation and often with desquamation
Liver	**+1**	Bilirubin 2.0 to 3.0 mg/dL; SGOT 150 to 750IU
	+2	Bilirubin 3.1 to 6.0 mg/dL
	+3	Bilirubin 6.1 to 15.0 mg/dL
	+4	Bilirubin >15.0 mg/dL
Gut	**+1**	Diarrhea > 30 mL/kg or > 500 mL/day
	+2	Diarrhea > 60 mL/kg or > 1000 mL/day
	+3	Diarrhea > 90 mL/kg or > 1500 mL/day
	+4	Diarrhea > 90 mL/kg or > 2000 mL/day; or severe abdominal pain with or without ileus

Overall Grade

Grade	Skin	Liver		Gut	ECOG Performance
I	+1 to +2	0		0	0
II	+1 to +3	+1	and/or	+1	0 to 1
III	+2 to +3	+2 to +4	and/or	+2 to +3	2 to 3
IV	+2 to +4	+2 to +4	and/or	+2 to +4	3 to 4