CD34-Selected Allogeneic Hematopoietic Cell Transplantation with Myeloablative Conditioning and $CD8^+$ Memory T-cell Infusion For Patients with Myelodysplastic Syndrome, Acute Leukemia, and Chronic Myelogenous Leukemia

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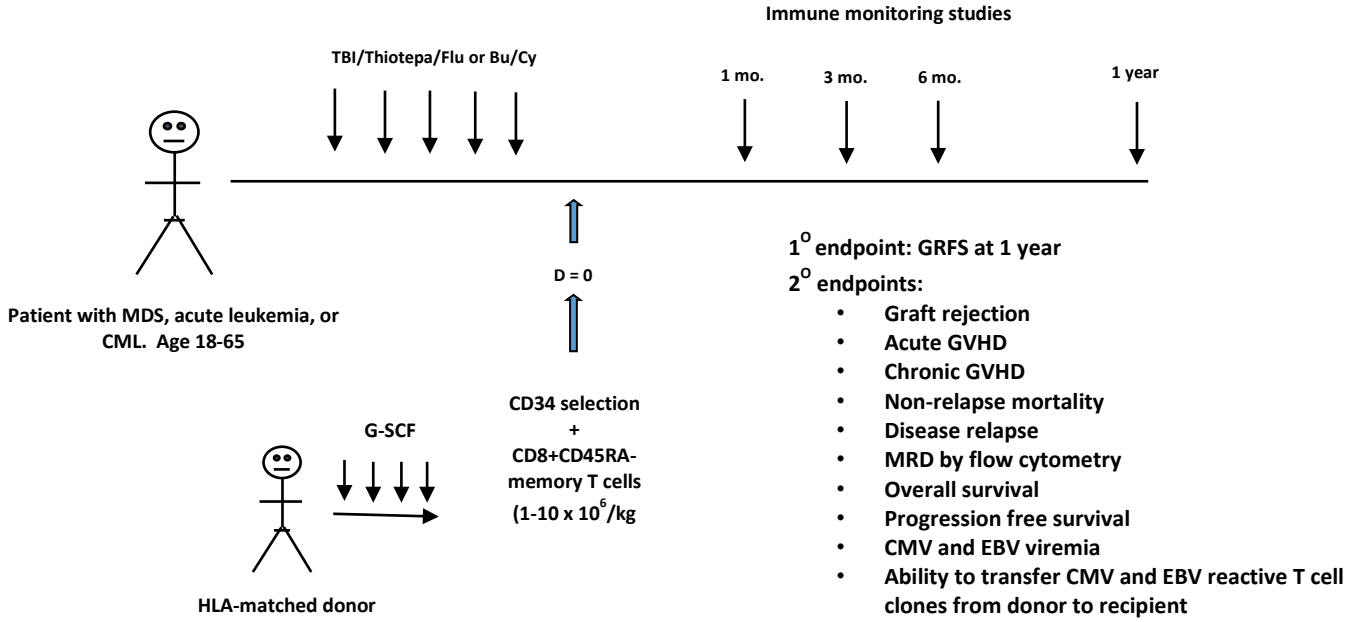
Protocol Synopsis

TITLE	CD34-selected allogeneic hematopoietic cell transplantation with myeloablative conditioning and $CD8^+$ memory T-cell infusion for patients with myelodysplastic syndrome, acute leukemia, and chronic myelogenous leukemia (CML)
STUDY PHASE	Phase 2
STUDY DESIGN	Single-arm, non-randomized safety and efficacy study using Simon stage 2 design
STUDY SITE	Stanford University Medical Center, CA 94305
INDICATION	Subjects with myelodysplastic syndrome (MDS), acute myeloid leukemia (AML), or acute lymphoblastic leukemia (ALL) eligible for full-dose myeloablative conditioning, or chronic myelogenous leukemia (CML)
INVESTIGATIONAL PRODUCT	Allogeneic phenotypic $CD8^+$ memory T-cells from HLA-matched donors infused at the time of hematopoietic cell transplantation
PRIMARY ENDPOINT and OBJECTIVE(S)	To determine the rate of graft-versus-host-disease (GvHD)-free, relapse-free survival (GRFS) at one year post-transplant
SECONDARY ENDPOINTS AND OBJECTIVE(S)	To determine the rate of graft rejection, acute and chronic GvHD, non-relapse mortality, relapse, overall survival, disease-free survival, EBV and CMV reactivation, and the expansion and migration of EBV and CMV-reactive $CD8^+$ memory T-cells
STUDY OBSERVATION PERIOD	The total duration of subject study participation is 1 year. Subjects will be followed indefinitely for all outcomes including overall survival, disease-free survival, and late complications including chronic GvHD and infections.
TREATMENT SUMMARY	CD34 selection with immunomagnetic beads is an effective method of depleting alloreactive donor T-cells from a peripheral blood graft and results in a significant reduction in acute and chronic GvHD. However, depletion of donor T-cells also leads to an increased rate of disease relapse and infection. We will test the hypothesis that combining phenotypic $CD8^+$ memory T-cells to a purified $CD34^+$ graft will retain the low incidence of GvHD, yet also provide enhanced antitumor activity, and transfer of EBV and CMV immunity.
SAMPLE SIZE	32 subjects and HLA-matched donors.

SUMMARY OF SUBJECT ELIGIBILTY	Subjects with histologically-confirmed MDS, acute leukemia, or CML. Subjects age 18 to 65 years who are eligible for full-dose myeloablative conditioning. Subjects with HLA-matched donors.
CONTROL GROUP	Historically-matched control subjects with MDS, AML, ALL, or CML receiving CD34-selected transplants with myeloablative conditioning without $CD8^+$ memory T-cell infusion

Study Schema



Donors will undergo apheresis for processing of $CD34^+$ selected cells and $CD8^+CD45RA^-$ T_M cells. These cells will be infused on Day 0 following a standard non-ATG containing regimen used for CD34-selected transplants consisting of fTBI (1375 cGy); thiotepa (10 mg/kg); and fludarabine (125 mg/m^2) or non-TBI conditioning consisting of Busulfan (14.4 mg/kg) and Cyclophosphamide (120 mg/kg).

1. OBJECTIVES

1.1 Objectives

1.1.1 Primary Objective

To determine the rate of graft-versus-host-disease (GvHD)-free, relapse-free survival (GRFS) at one year following CD34-selected allogeneic hematopoietic cell transplantation using myeloablative conditioning combined with an infusion of phenotypic $CD8^+$ memory T-cells from HLA-matched donors for subjects with myelodysplastic syndrome (MDS), acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), or chronic myelogenous leukemia (CML).

1.1.2 Secondary Objectives

To determine the rate of graft rejection, acute and chronic GvHD, non-relapse mortality, relapse, overall survival, and disease-free survival.

1.1.3 Exploratory Objective

To determine in some donor recipient pairs the expansion and migration of EBV and CMV-reactive $CD8^+$ memory T-cells and correlation with EBV and CMV reactivation.

1.2 ClinicalTrials.gov Outcomes

1.2.1 Primary Outcome

Title: Graft-versus-Host Disease (GvHD)-free and relapse-free survival (GRFS)

Description: The rate of participants who do not experience GvHD and also do not experience relapse are collectively considered to be GRFS. Relapse will be assessed according to the myelodysplastic syndrome or leukemia response criteria. The participants will be assessed for GRFS though 1 year post-transplant. The outcome will be reported as the number of participants, a number without dispersion.

Timeframe: 1 year

Safety Outcome?: No

1.2.2 Secondary Outcomes

Title: Graft Rejection

Description: Graft rejection will be determined on the basis of reaction against the donor hematopoietic cells. The outcome will be reported as the number of participants who experience graft rejection though 1 year post-transplant, a number without dispersion.

Timeframe: 1 year

Safety Outcome?: No

Title: Acute Graft-versus-Host Disease (GvHD)

Description: The participants will be assessed for acute graft-versus-host disease (GvHD) though 1 year post-transplant. The outcome will be reported as the number of participants who experience acute GvHD, a number without dispersion.

Timeframe: 1 year

Safety Outcome?: Yes

Title: Chronic Graft-versus-Host Disease (GvHD)

Description: The participants will be assessed for chronic, steroid-requiring graft-versus-host disease (GvHD) though 1 year post-transplant. The outcome will be reported as the number of participants who experience chronic GvHD, a number without dispersion.

Timeframe: 1 year

Safety Outcome?: No

Title: Non-relapse Mortality

Description: Non-relapse mortality will be assessed as the number of participants who have died though 1 year post-transplant, without a relapse or recurrence of their myelodysplastic syndrome or leukemia. Relapse will be assessed according to the myelodysplastic syndrome or leukemia response criteria. The outcome will be reported as the number of affected participants, a number without dispersion.

Timeframe: 1 year

Safety Outcome?: No

Title: Relapse

Description: Relapse will be assessed according to the myelodysplastic syndrome or leukemia response criteria. The outcome will be reported as the number of participants who experience relapse though 1 year post-transplant, a number without dispersion.

Timeframe: 1 year

Safety Outcome?: No

Title: Overall Survival (OS)

Description: Overall Survival (OS) will be assessed as the number of participants who remain alive at 1 year post-transplant. The outcome will be reported as a number without dispersion.

Timeframe: 1 year

Safety Outcome?: No

2. BACKGROUND

2.1 Allogeneic Hematopoietic Cell Transplantation for MDS, Acute Leukemia, and CML

Allogeneic hematopoietic cell transplantation (HCT) is a potentially curative therapy for subjects with high-risk MDS and acute leukemia. Following allogeneic HCT, a significant percentage of subjects with otherwise incurable blood cancers using best of care non-transplantation therapies may achieve long-term disease control and are cured. These patients are characterized by developing donor type hematopoiesis and have complete resolution of all disease-related signs, symptoms, and markers. Generally, patients who undergo allogeneic HCT are doing so as a "last ditch" effort for long-term disease control and possibly cure as best of care non-transplant therapies are unlikely to provide this.

The antitumor activity of allogeneic HCT is from the direct cytotoxic effect of the conditioning regimen combined with the immunologic graft-versus-tumor (GVT) effect mediated by alloreactive donor T-cells. A myeloablative conditioning regimen is the current standard of care for patients with MDS and acute leukemia, as shown in a recent randomized controlled trial demonstrating significantly lower relapse rates and superior overall survival with myeloablative conditioning compared to reduced intensity conditioning (RIC) for fit patients age 18 to 65 with MDS or acute leukemia. [1] The main causes for treatment failure following allogeneic HCT are disease relapse, acute and chronic GvHD, and infections. A strategy to augment donor-mediated GVT activity and improve infection control without increasing the risk of acute and chronic GvHD may improve transplant outcomes including overall survival.

2.2 CD34-Selected Allogeneic HCT for MDS, Acute Leukemia, and CML

CD34 selection with immunomagnetic beads is an effective method of depleting alloreactive donor T-cells from a peripheral blood graft and results in a significant reduction in acute and chronic GvHD. [2-9] Studies of CD34-selected HCT for various hematologic malignancies performed at Memorial Sloan Kettering Cancer Center (MSKCC) reported incidences of acute GvHD (limited to Grade II) of 8%, and chronic GvHD of 9% in recipients of matched related grafts and incidences of acute and chronic GvHD of 9% and 29%, respectively, in recipients of matched unrelated donors. [3,4] None of the subjects received GvHD prophylaxis beyond T-cell depletion of the graft. Results from single-center studies have been validated in the National Blood and Marrow Clinical Trials Network (BMT CTN) study 0303 in which 44 subjects with AML in CR1 or CR2 were conditioned with total body irradiation, thiotepa, and cyclophosphamide with rabbit antithymocyte globulin (ATG) followed by CD34-selected allogeneic HCT from an HLA-matched sibling donor. The incidence of acute GvHD Grade II-IV was 22.7% and the incidence of extensive chronic GvHD was 6.8% at 24 months. [5] Similar results have been reported in subjects with high-risk MDS. In a recent study of 102 subjects at MSKCC with advanced MDS who received CD34-selected HCT, the cumulative incidence of acute GHVD (Grade II to IV) at 180 days was 16% and the cumulative incidence of chronic GvHD at 1 year was 4%. [10] A recent analysis of the BMT CTN 0303 subjects compared to a subset of subjects on BMT CTN 0101 who received a conventional transplant for AML in CR1/CR2 showed a significantly lower rate of chronic GvHD at 2 years with a CD34-selected graft than a conventional graft (19% vs 50%, respectively, $p < 0.001$, **Figure 1**) with no difference in overall or disease-free survival. [11]

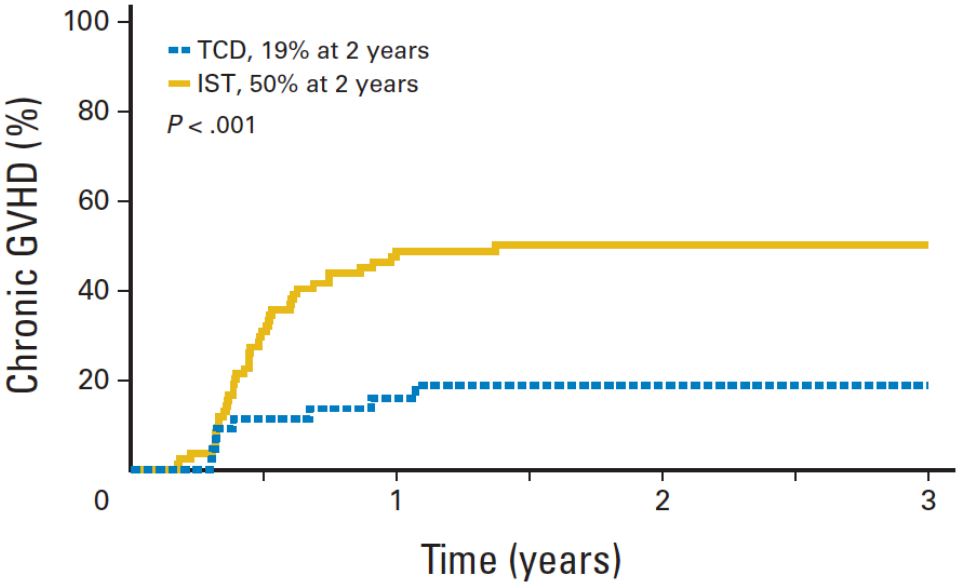


Figure 1. Cumulative incidence of chronic GvHD in recipients of CD34-selected T-cell depleted (TCD) grafts compared to recipients of unmanipulated grafts with post-transplant immunosuppressive therapy (IST), Pasquini, *et al*, *J Clin Oncol* 2012.

Another advantage of CD34-selected HCT is that no post-transplant GvHD prophylaxis is required, thus eliminating the risks of side effects like nephrotoxicity associated with calcineurin inhibitors (CNIs) and the combined toxicity of an ablative regimen and methotrexate, which may exacerbate mucositis and cytopenias. The ability to avoid post-transplant immunosuppression also makes CD34-selected HCT an ideal platform for post-transplant adoptive cellular therapies targeting minimal residual disease or infections. [12]

Subject accrual to a multicenter phase 3 trial (BMT CTN 1301, NCT02345850) was recently completed; this trial randomized subjects with MDS or acute leukemia to one of three arms following myeloablative conditioning and transplantation from an HLA-matched donor. Subjects were randomized to either: a CD34-selected graft, an unmanipulated graft followed by post-transplant cyclophosphamide (PTCy), or an unmanipulated graft with conventional post-transplant immunosuppressive therapy consisting of a CNI combined with methotrexate; all three arms are currently accepted standard-of-care approaches for GHVD prophylaxis. The results from the trial may still be more than 18 months from being publically disclosed, albeit interim DSMB analyses throughout has found no evidence of superiority or inferiority in any of the three arm that was sufficient to warrant early closure for safety or efficacy concerns.

2.3 $CD34^+$ Selection Impact on Disease Relapse, Infection, and Graft Rejection

A major concern limiting the widespread adoption of CD34-selected, T-cell depleted (TCD) donor grafts is loss or attenuation of the GVT effect mediated by alloreactive donor T-cells, which appears to lead to a higher risk of disease relapse. This was first demonstrated in patients with chronic myelogenous leukemia (CML), where a retrospective study of 46 patients who underwent TCD transplants were compared to 40 patients who received conventional grafts. [13]

The 3-year probability of disease relapse was significantly higher in the TCD group than in the non-TCD group (62% vs 24%, p = .0003). The infusion of supplemental donor T-cells was beneficial to the TCD group as after a donor lymphocyte infusion (DLI), 17 of 20 patients in the TCD group and 2 of 3 patients in the non-TCD group achieved a complete remission.

While the CML experience clearly showed a higher risk for disease relapse with a TCD graft, this relationship has not been clearly demonstrated in patients with acute leukemia or MDS. For example, in the BMT CTN 0303 study, the relapse rate for patients with acute leukemia in CR1 receiving a CD34-selected graft was 17.4% at 36 months, similar to historical controls receiving conventional grafts. [5] Another retrospective study compared 115 patients who received TCD grafts after myeloablative conditioning at MSKCC to a cohort of 181 patients who received unmodified grafts after conditioning with busulfan/fludarabine and GvHD prophylaxis with tacrolimus and mini-methotrexate at MD Anderson Cancer Center (MDACC). There were no significant differences in the rate of disease relapse at 3 years between the TCD and unmodified graft recipients (18% vs 25%, p = 0.3). [8] Similarly, in a study of 102 patients with advanced MDS who received CD34-selected grafts at MSKCC, the cumulative incidence of relapse was 11.8% at 1 year and 15.7% at 2 years, [11] similar to historical controls receiving unmodified grafts. The prospective randomized phase 3 BMT CTN 1301 study will be pivotal in comparing CD34-selected HCT to an unmodified graft with either PTCy or standard CNI-based immunosuppression in patients with advanced MDS and acute leukemia.

Another important limitation of CD34-selected TCD grafts is the increased risk for infection, particularly viral reactivation with CMV and EBV, with a higher incidence of EBV-associated lymphoproliferative disorders. [14-18] Prior studies of CD34-selected HCT have demonstrated delayed reconstitution of CD4+ T-cells with a large proportion of patients having absolute CD4+ counts < 200 cells/uL for more than seven months, which may contribute to the increased risk for severe, recurrent, or opportunistic infections. [3]

Early studies of TCD grafts were limited by higher rates of graft rejection than with conventional grafts, with reported graft failure rates as high as 27%. [19-21] These clinical results confirmed pre-clinical data that donor-derived T-cells facilitate engraftment. However, following modification of the conditioning regimen, and in particular the use of ATG to promote engraftment, several centers have reported consistent engraftment with CD34-selected TCD grafts. [2,5,22,23]

2.4. $CD8^+$ Memory T-cells Have GVT Activity Without Causing Severe GvHD in Murine Models of Transplantation

Murine studies showed that in many strain combinations memory T-cells, including memory $CD4^+$ and memory $CD8^+$ T-cells induce significantly less GvHD than naïve T-cells ($CD62L^{hi}CD44^{lo}$) or combinations of naïve and memory T-cells. [24-30]

Accordingly, our group at Stanford compared isolated naïve CD4+, $CD8^+$, or total T-cells, and/or memory $CD4+CD44^{hi}$; $CD8^+CD44^{hi}$; and total memory $CD44^{hi}$ T-cells from unprimed donors for their capacity to induce GvHD, promote chimerism and mediate antitumor activity against a naturally occurring B-cell lymphoma (BCL1) in an MHC-mismatched model. [31] Only the $CD8^+CD44^{hi}$ memory T-cell subset containing both central and effector memory cells was

capable of eradicating the lymphoma cells without inducing GvHD. In contrast, CD4+ and $CD8^+$ naïve T-cells, memory $CD44^{hi}$ CD4+ T-cells, naive total T-cells, and memory $CD44^{hi}$ total T-cells either induced lethal GvHD or lacked potent antitumor activity. The tumor-bearing recipients of $CD8^+CD44^{hi}$ T-cells had a clear survival advantage over those given $CD8^+$ naive T-cells because of the lethal GvHD induced by the latter. The $CD8^+CD44^{hi}$ T-cells were also used in a model of treatment of progressive lymphoma growth after BMT, and were able to promote complete chimerism and eradicate the tumor without causing GvHD.

In other preclinical murine models of bone marrow transplantation using various MCH-matched strain combinations conducted by our group, the only cell subset that we could identify that provided GVT reactions against FBL3 leukemia tumor cells without inducing lethal GvHD was $CD44^{hi}CD8^+$ memory T-cells. [32]

Taken together, our studies and those by other groups suggest that $CD8^+$ memory T-cells promote donor cell engraftment and retain GVT reactions without precipitating severe GvHD.

2.5 Methods for the Enrichment of Human $CD8^+$ Memory T-cells

Since we successfully treated progressive tumor growth after BMT without inducing GvHD in mice with purified donor $CD8^+$ memory T-cells, we developed methods to translate this work to human clinical trials. In studies using healthy donor PBMC apheresis collections and immunomagnetic bead separation on Miltenyi columns, we enriched for $CD8^+CD45RO^+CD45RA^-$ phenotypic memory T-cells to > 95% purity. [33]

Briefly, phenotypic $CD8^+$ memory T-cells ($CD8^+CD45RA^-$) were isolated from healthy donors following one or two 12-liter volume apheresis collections. The $CD45RA^-$ cells were obtained by labeling peripheral blood collections with the research grade CliniMACS CD45RA Microbeads (Catalogue number 130-020-003) and Reagent (Miltenyi Biotec) followed by selection on the CliniMACS Plus instrument using program Depletion 3.2 with CliniMACS Depletion tubing sets. The flow through $CD45RA^-$ cells were labeled with the research grade CliniMACS CD8 Microbeads (Catalogue number 130-030-810) and Reagent and selected using program Enrichment 3.1 with standard CliniMACS tubing sets. Post-selection products were washed and re-suspended in 100 mL of Normosol-R with 1% human serum albumin (HSA) and evaluated immediately and after 24 to 48 hours of storage at 4°C. Products were infused fresh (ie, not cryopreserved) and there was no change in cell viability or phenotype during the 48-hour storage time compared to immediate analysis. Cell viability (consistently > 90%), recovery and purity were determined by flow cytometry, and a representative example is shown in **Figure 2** with > 95% of cells consistent with the $CD8^+$ memory T-cell subset.

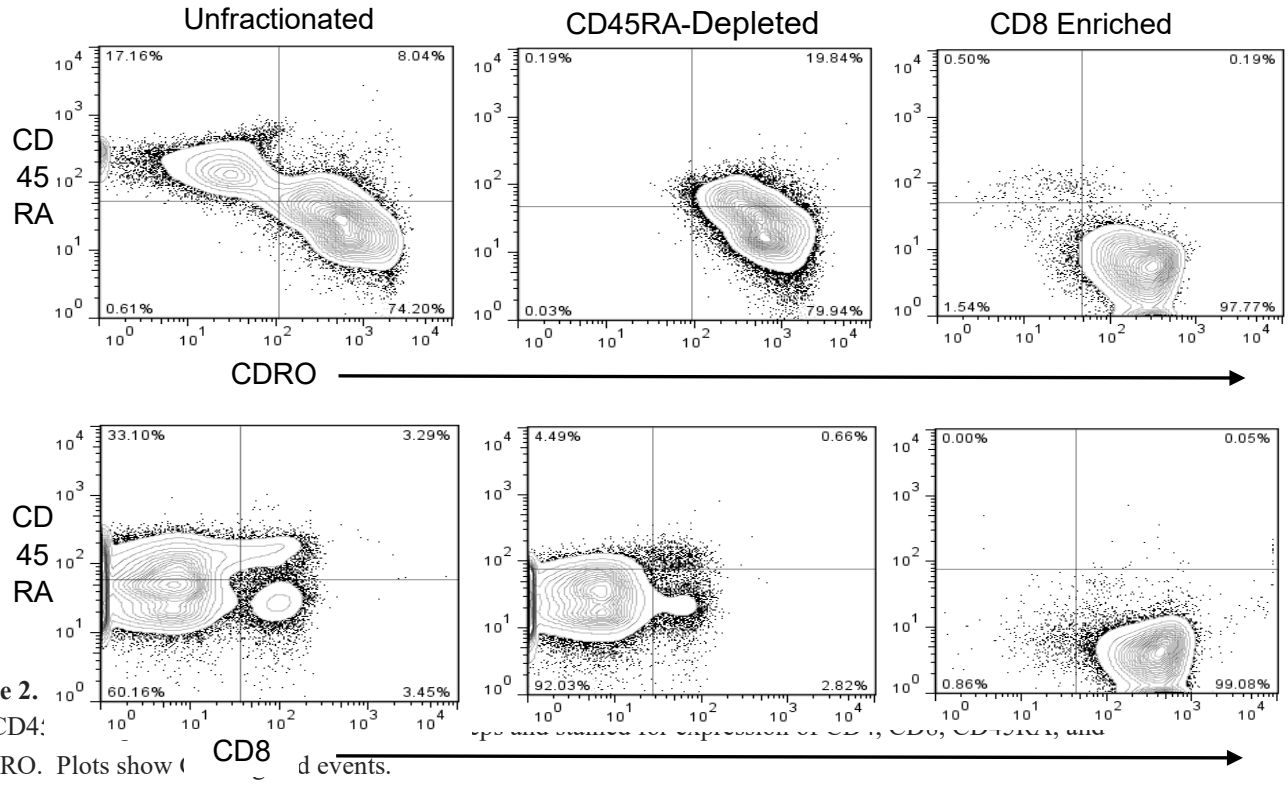


Figure 2. post-CD4[illegible] CD45RO. Plots show [illegible] events.

Cell yields were determined using apheresis products from normal HCT donors and the mean $CD8^+$ memory T-cell post-enrichment cell count obtained from a single 12-liter apheresis product was 8 x 10^8 with a range of 5 to 8 x 10^8 $CD45RA^-CD8^+$ cells. Therefore, following 2 standard volume aphereses or one large volume (18 to 22 liters) apheresis per donor, we estimated that the highest feasible dose of memory $CD8^+$ T-cells available for infusion would be 10 x 10^8 cells. Assuming that most subjects weigh less than 100 kg, attainable numbers of $CD8^+$ memory T-cells for a phase 1 dose escalation trial would be 1 x 10^6/kg; 5 x 10^6/kg; and 10 x 10^6/kg.

In further studies, the purified phenotypic $CD8^+$ memory T-cells were tested for immune reactivity *in vitro* by stimulation with irradiated allogeneic PBMCs from normal donors. [33] The responder $CD8^+$ memory T-cells showed a modest increase in the mean 3H-thymidine incorporation in cultures with allogeneic stimulator cells compared to control cultures without allogeneic stimulators (**Figure 3A**). The $CD8^+$ memory T-cell supernatants showed a marked increase in the concentration of IFN-γ after culture with allogeneic stimulator cells and a minimal increase in the concentration of IL-2 (**Figure 3B**). The results of the human mixed leukocyte response (MLR) experiments were consistent with the responses observed with $CD8^+$ memory T-cells from mice where the murine responder cells also showed a modest increase in 3H-thymidine incorporation after stimulation with allogeneic cells, and the production of IFN-γ was considerably greater than that of IL-2.

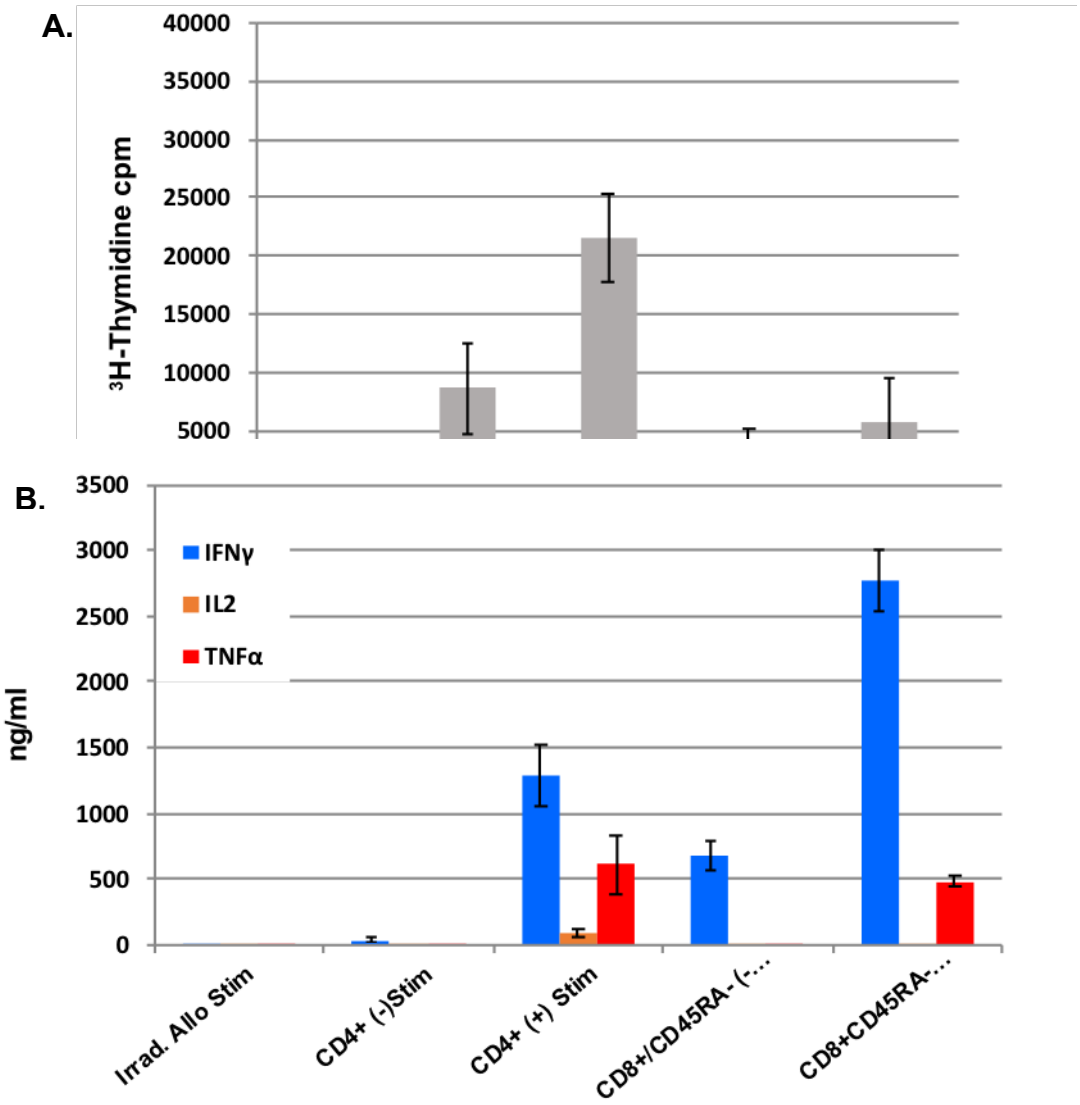


Figure 3A. Proliferation of CI
stimulators (Stim) and assessed
shown (n = 4). **B**. Cytokine se
irradiated allogeneic stimulator
Bead Arrays for INFγ, IL-2, an

2.6 Phase 1 Trial of CD8+ Memory T-cell Infusion for Relapsed Hematologic Malignancies after Allogeneic HCT

To test the DLI treatment concept of human donor-derived $CD8^{+}CD45RO^{+}CD45RA^{-}$ memory T-cells in patients, a 15-subject phase 1 safety and feasibility study was conducted under an approved IND (BB-IND-14844) entitled "A Phase I Study of $CD8^{+}CD45RO^{+}CD45RA^{-}$ Memory T-cell Donor Lymphocyte Infusion for Relapse of Hematolymphoid Malignancies Following Matched Related Donor Allogeneic Hematopoietic Cell Transplantation" (NCT01523223). The study followed a standard 3+3 dose escalation format using a low $CD8^{+}$ memory T-cell dose of 1×10^{6} cells/kg, an intermediate dose of 5×10^{6} cells/kg and a final dose of 10×10^{6} cells/kg. The objectives of the protocol were:

1. To determine the feasibility of purifying allogeneic $CD8^{+}$ memory T-cells suitable for clinical application and to determine the safety and maximum tolerated dose (MTD) of these cells in subjects with recurrent or refractory hematolymphoid malignancies following allogeneic HCT.

2. To determine disease response, time to disease progression, event-free survival, and overall survival following treatment with allogeneic $CD8^+$ memory T-cells.
3. To assess donor specific chimerism before and at designated time points after treatment with allogeneic $CD8^+$ memory T-cells.

Eligible subjects were 18 to 75 years of age. Phenotypic $CD8^+$ memory T-cells were isolated following 1 or 2 days of unmobilized donor apheresis using a tandem immunomagnetic selection strategy including CD45RA depletion followed by $CD8^+$ enrichment on the CliniMACS Plus instrument. Fifteen subjects received $CD8^+$ memory T-cells at 3 dose levels. The subject characteristics and outcomes are summarized in Table 1 and have been previously reported. [34]

Table 1. Subject Outcomes Following $CD8^+$ Memory T-cell DLI for Disease Relapse after Allogeneic HCT

Subject	Diagnosis	Disease Status at $CD8^+$ Memory Infusion	$CD8^+$ Memory Dose (x 10^6/kg)	Response following DLI	Time from DLI to Relapse/Progression (Days)	Time from DLI to Death or Last Follow-up (Days)	Status at Last Follow Up
1	AML	CR2	1.0	CR2	973	1057	Expired
2	AML	Active Disease	1.0	CR2	480	591	Expired
3	AML	CR3	1.0	CR3	579	1328	Alive in CR
4	AML	CR2	5.0	CR2	120	430	Expired
5	CML	CR3	5.0	CR3	-	1069	Alive in CR
6	CLL	Active Disease	5.0	PD	NR	168	Expired
7	AML	Active Disease	7.8	PD	NR	128	Expired
8	AML	Active Disease	8.0	PD	NR	151	Expired
9	NHL	Active Disease	10.0	PD	NR	495	Alive with disease
10	NHL	Active Disease	5.2	SD	90	412	Alive with disease
11	AML	CR2	10.0	CR2	-	328	Alive in CR
12	AML	CR3	7.7	CR3	147	286	Alive with disease
13	NHL	Active Disease	10.0	SD	158	194	Expired
14	MM	Active Disease	10.0	PD	NR	221	Alive with disease
15	ALL	Active Disease	10.0	PR	-	118	Alive with disease

AML, acute myeloid leukemia; CML, chronic myeloid leukemia; CLL, chronic lymphocytic leukemia; NHL, non-Hodgkin lymphoma; MM, multiple myeloma; ALL, acute lymphoblastic leukemia; CR, complete remission; PD, progressive disease; SD, stable disease; PR, partial response; NR, no response

All subjects in dose levels 1 and 2 received the intended $CD8^+$ memory T-cell dose of 1 to 10^6/kg and 5 to 10^6/kg, respectively. Five of the subjects treated at dose level 3 received the 10 to 10^6/kg $CD8^+$ memory T-cell dose; however, the intended dose could not be obtained in

4 subjects, who received doses of 5.2 to $10x10^6$/kg. The maximum tolerated dose (MTD) was not reached.

No adverse events occurred during $CD8^+$ memory T-cell infusion, and no dose-limiting toxicities were observed. GvHD developed in only 1 subject, was limited to Grade II involvement of the liver, and resolved following a course of corticosteroids.

Thirteen subjects (87%) received cytoreductive therapy prior to $CD8^+$ memory T-cell infusion, and 6 subjects (40%) had returned to complete remission (CR) at the time of infusion. The median follow-up from the time of $CD8^+$ memory T-cell infusion was 328 days (range, 118 to 1328 days). Ten subjects (67%) maintained or achieved an objective response (7 CR; 1 partial response; 2 stable disease) for at least 3 months following $CD8^+$ memory T-cell infusion, and four of these responders had active disease at time of infusion. Among the 10 responders; 7 have relapsed at the time of this report after a median 165 days (range, 90 to 973 days) to relapse. Eight subjects are currently alive; 3 are alive in CR and 5 are alive with active disease. For the entire study cohort, the median event-free survival and overall survival following $CD8^+$ memory T-cell infusion were 4.9 months (95% CI, 1 month to 19.3 months) and 19.6 months (95% CI; 5.6 months to not reached), respectively.

In summary, this phase 1 trial showed that $CD8^+$ memory T-cell DLI is well tolerated and associated with a low incidence of GvHD. The MTD was not reached even at the higher dose level of 10 x 10^6/kg; however, this yield was unable to be achieved using the current-cell selection strategy. Some subjects achieved a prolonged CR in this small heterogeneous study population with heavily pretreated hematologic malignancies. There did not appear to be a dose response to CD8 memory T-cell infusions as responses were observed at all doses used, and likewise there was not a dose that was associated with increase in toxicity or side effects. Importantly, this clinical trial defined that the dose of phenotypic $CD8^+CD45RO^+CD45RA^-$ T_M cells reliably obtained for use in a clinical trial using our enrichment strategy is 1 to10 x 10^6/kg.

2.7 Phase 2 Trial of Consolidative $CD8^+$ Memory T-cell Infusion for Subjects with Mixed Chimerism after Allogeneic HCT

Based on the results of the Phase 1 study that confirmed safety and suggested some level of efficacy, we initiated a second clinical trial entitled "Post-Transplant Infusion of Allogeneic CD8 Memory T-cells as Consolidative Therapy After Non-Myeloablative Allogeneic Hematopoietic Cell Transplantation in Patients with Leukemia and Lymphoma", (NCT02424968) under IND BB-14844. The study rationale is based on multiple publications, including our own, that showed that persistence of mixed hematopoietic cell chimerism after allogeneic HCT was associated with a significantly higher risk of disease relapse. [35-44] Once a patient's disease has relapsed after allogeneic HCT, the ability to return the patient to a durable remission is poor. Therefore, a strategy that can help promote conversion from mixed chimerism (MC) to full donor chimerism (FC) and that does not promote significant GvHD has the potential to improve the outcomes of transplant recipients by reducing the risk of disease relapse.

The primary objective of this study will be to determine the rate of conversion from MC to FC following a post-transplant infusion (day 30 to 70) of enriched donor phenotypic $CD8^+$ memory T-cells in subjects with hematologic malignancies who received non-myeloablative HCT with

TLI-ATG conditioning. It is hypothesized that subjects who convert from MC to FC will have a reduced risk of relapse similar to subjects who are achieve FC without DLI. The secondary objectives are to determine the risk of disease relapse, overall and event-free survival, non-relapse mortality, and the incidence of acute and chronic GvHD following the infusion of allogeneic $CD8^+$ memory T-cells.

Eligible subjects are 18 to 75 years of age with a high-risk or refractory hematologic malignancy and who receive nonmyeloablative allogeneic HCT using TLI-ATG conditioning and cells from an HLA-matched donor and who have MC (< 95% donor $CD3^+$ cells) at day +30. Phenotypic $CD8^+$ memory T-cells are isolated from HLA-matched donors following 1 or 2 days of apheresis using the tandem immunomagnetic selection strategy including CD45RA depletion followed by $CD8^+$ enrichment on the CliniMACS Plus instrument. Cell purity is determined by flow cytometry analysis. $CD8^+$ memory T-cells are planned for infusion between day +30 and day +70 at a dose of 1 to 10 x 10^6 cells/kg, the dose determined from the phase 1 trial (Section 2.6, above) to be the maximum dose that can be consistently collected from 1 or 2 apheresis products.

This study will follow a single-arm, Simon 2-stage trial principle. In Simon stage 1, 9 subjects are planned for enrollment, and if three or fewer subjects responded, defined as the conversion from mixed to complete chimerism within 90 days of the $CD8^+$ T_M cell infusion, the study will be stopped for futility. If 4 or more subjects respond in Simon stage 1, then in stage 2 enrollment will be planned to continue to a full sample of 18 subjects. Success will be declared if a total of 13 or more subjects respond.

To date, 9 subjects have been enrolled on the trial and received prophylactic donor $CD8^+CD45RO^+CD45RA^-$ memory T-cells between day 30 to 70 post-transplant. [45] Following the $CD8^+$ memory DLI, subjects were assessed for the development of acute and chronic GvHD, conversion from MC to FC (≥ 95% donor $CD3^+$ chimerism) within 3 months, and for disease relapse. Secondary outcomes included the incidence of adverse events, invasive infections, non-relapse mortality, overall survival (OS), and disease-free survival (DFS). The subjects and their outcomes are shown in Table 2. [45]

Table 2. Subject characteristics and outcomes following consolidative $CD8^+$ memory T-cell DLI

Age/sex	Diagnosis and disease status		Day of DLI	$CD3^+$ chimerism pre- and 3 months post-DLI		Acute GvHD	Chronic GvHD	Remission status	Surviving	Follow up days post-HCT
71F	AML	CR1	+40	88%	97%	None	None	CR, MRD negative	Alive	747
67F	AML	CR2	+33	23%	44%	None	None	Relapsed (day +698)	Alive	730
67M	MDS	PR	+40	40%	19%	None	None	Relapsed (day +88)	Deceased	128
38F	CHL	PR	+42	84%	99%	Grade II (skin)	Mild (joints)	Relapsed (day +231)	Alive	474
47M	DLBCL	PR	+41	88%	95%	None	None	CR	Alive	390

Table 2. Subject characteristics and outcomes following consolidative $CD8^+$ memory T-cell DLI

Age/sex	Diagnosis and disease status		Day of DLI	$CD3^+$ chimerism pre- and 3 months post-DLI		Acute GvHD	Chronic GvHD	Remission status	Surviving	Follow up days post-HCT
60F	DLBCL	CR	+47	93%	100%	Grade II (skin)	None	Relapsed (day +141)	Deceased	187
30F	PMBCL	PD	+42	88%	95%	None	None	CR	Alive	306
47F	ENKTL	PR	+62	75%	72%	None	None	CR	Alive	152
65M	DLBCL	CR	+49	92%	96%	None	None	CR	Alive	131

AML = acute myeloid leukemia; CHL = classical Hodgkin lymphoma; CR = complete remission; DLBCL = diffuse large B-cell lymphoma; DLI = donor lymphocyte infusion; ENKTL = extranodal NK/T-cell lymphoma; GvHD = graft-versus-host disease; HCT = hematopoietic cell transplantation; MDS = myelodysplastic syndrome; MRD = minimal residual disease; PMBCL = primary mediastinal B-cell lymphoma; PD = progressive disease; PR = partial remission

Median age of the subjects was 60 years (range 30 to 71 years) with a median follow up of 306 days post-HCT (range 131 to 747 days). Diagnoses included non-Hodgkin lymphoma (n = 5), AML (n = 2), MDS (n = 1), and Hodgkin lymphoma (n = 1). Median donor $CD3^+$ chimerism at Day +30 was 88% (range 23 to 93%).

The cell product was highly enriched for $CD8^+/CD45RA^-/CD45RO^+$ cells, and flow analysis confirmed the selected cells were phenotypically consistent with the $CD8^+$ T-effector memory subset with a post-enrichment purity of ≥ 96% (range 96.4 to 98.8%) in all subjects.

$CD8^+$ memory T-cell DLI was well-tolerated with no adverse events. 6 of the 9 subjects (67%) converted from MC to FC within 3 months post-DLI with a median donor $CD3^+$ chimerism of 97% (range 95 to 100%). Two subjects developed Grade II acute GvHD (skin only) that was treated to resolution. One subject developed mild chronic GvHD. No subjects developed severe acute GvHD (Grade III to IV) or extensive chronic GvHD. No subjects developed invasive infections, and non-relapse mortality was 0%. Of the 3 subjects who failed to convert from MC to FC, 2 had residual disease at the time of transplant, 1 of whom had early disease relapse at Day +88. 4 of the 6 subjects who converted to FC were in complete remission (CR) at the last follow up. 1 of the 3 subjects with persistent MC was in CR at the last follow up. For the entire study cohort, the median disease-free survival is 1.1 years and the median overall survival is not yet reached.

As with the phase 1 study, the early results from our phase 2 study demonstrate (1) the feasibility of consistently obtaining a cellular product highly enriched for phenotypic $CD8^+$ memory T-cells at a yield of 1 to 10 x 10^6 cells/kg from donor apheresis products; (2) the safety of this cellular therapy with a very low incidence of mild GvHD and no cases of severe acute GvHD (Grade III-IV) or extensive chronic GvHD observed to date in any of the 24 subjects treated thus far on the phase 1 and phase 2 trials; and (3) the efficacy of $CD8^+$ memory T-cells in converting 6 of 9 subjects (67%) with MC to FC, recapitulating the results seen in our murine model. Subjects continue to accrue to trial, and further accrual and longer follow up are needed to determine if this strategy will reduce relapse rates for high-risk subjects with MC.

2.8 Proposed Study Rationale and Hypothesis

CD34 selection with immunomagnetic beads is an effective method of depleting alloreactive donor T-cells from a peripheral blood graft, and results in a significant reduction in acute and chronic GvHD. [3,46.47] Yet in these trials, overall subject survival did not appear improved perhaps due to an increase in tumor relapse; graft rejection; and infection. Our **hypothesis** is that combining $CD8^+CD45RA^-$ T_M phenotype cells to a purified $CD34^+$ graft will retain the low incidence of GvHD, yet also provide protection from graft rejection; protection from infection; and enhance anti-tumor activity compared with $CD34^+$ selection alone. The proposed trial has particular significance especially in context of the current BMT CTN Phase 3 randomized, multicenter trial (BMT CTN 1301) in which a T-cell depleted (TCD) CD34-selected graft was a SOC arm in subjects with hematologic malignancies undergoing myeloablative conditioning for HCT.

2.9 Study Overview

This single institution, single arm, Simon 2-Stage trial will test the hypothesis that combining donor $CD8^+CD45RA^-$ T_M cells with donor $CD34^+$ selected grafts will be safe, and that the adoptively transferred $CD8^+$ T_M cells into the recipient will enhance anti-tumor, and anti-infection reactions without aggravating GvHD compared with $CD34^+$ selection alone. **The primary study objective** is the one-year rate of GvHD-free, Relapse-Free Survival (GRFS) as previously defined. [48,49] As with other CD34-selected graft trials, post-transplant immunosuppression will not be used in this trial.

3. PARTICIPANT SELECTION AND ENROLLMENT PROCEDURES

3.1 Eligibility Criteria and Participant Eligibility Checklist

Inclusion and Exclusion Criteria are provided on the Eligibility Checklist, following, and which may be extracted from this document for use in screening potential subjects. otherwise indicated: Pre-transplant screening: all studies to be completed within 70 days of beginning conditioning therapy, unless otherwise indicated:

The Inclusion and Exclusion Criteria are provided on the Participant Eligibility Checklist following, which must be completed in its entirety for each subject prior to registration. The checklist may be extracted from this document for use in screening potential subjects. The completed, signed, and dated checklist must be retained in the subject's study file, and the study's Regulatory Binder.

Pursuant to Stanford Medicine SOP "Confirmation of Participant Eligibility in Clinical Trials," the treating Physician (investigator); the Study Coordinator; and an Independent Reviewer will verify that the subject's eligibility is accurate; complete; and legible in source records. A description of the eligibility verification process should be included in the EPIC or other Electronic Medical Record progress note.

Participant Eligibility Checklist

I. Protocol Information

Protocol Title:	**CD34-Selected Allogeneic Hematopoietic Cell Transplantation with Myeloablative Conditioning and CD8$^+$ Memory T-cell Infusion For Patients with Myelodysplastic Syndrome, Acute Leukemia, and Chronic Myelogenous Leukemia (CML)**
eProtocol number: **OnCore number:**	**IRB-49023** **BMT339**
Principal Investigator:	Robert Lowsky, MD

II. Subject Information

Subject name / Unique ID:	/	
Gender	☐ Male	☐ Female

III. Eligibility Criteria

RECIPIENT Inclusion Criteria

Pre-transplant screening: all studies to be completed within 70 days of beginning conditioning therapy, unless otherwise indicated.

Prospective RECIPIENTS Must MATCH ALL these Inclusion Criteria to be Eligible	**Yes**	**No**	**Supporting Documentation ***
Acute leukemia, in morphologic complete remission; **OR** myelodysplasia with < 10% blasts in the marrow, and no circulating blasts that contain auer rods; **OR** chronic myelogenous leukemia (CML) with any one of the following high-risk features: 1. Chronic Phase CML yet with additional karyotypic features at diagnosis that are beyond the reciprocal translocation (9;22)(q34;q11) known as Philadelphia (Ph) chromosome. 2. Resistant CML, defined as: • A failure of therapy to achieve a complete cytogenetic response within 12 months of diagnosis, • A failure of therapy to maintain a > 3 log-fold reduction in molecular response once beyond 12 months of treatment, • Any new leukemia-associated chromosomal/sequence abnormality detected by conventional cytogenetics; FISH; molecular or next generation sequencing. • Enlarging spleen size despite ongoing treatment. 3. Accelerated Phase CML, defined as: • 10% to 19% blasts in the blood and/or bone marrow. • > 20% basophils in the peripheral blood. • Any new leukemia-associated chromosomal/sequence abnormality detected by conventional cytogenetics; FISH; molecular or next generation sequencing. 4. Blast Crises CML, defined as: • > 20% marrow or blood blasts. Subjects with chronic myelomonocytic leukemia (CMML) must have a WBC count ≤ 10,000 cells/μL and < 10% blasts in the marrow.	☐	☐	
1. Planned myeloablative conditioning regimen at Stanford University Medical Center.	☐	☐	

Prospective RECIPIENTS Must MATCH ALL these Inclusion Criteria to be Eligible	**Yes**	**No**	**Supporting Documentation ***
2. ≥ 18 years to < 66.0 years	☐	☐	
3. Males or females	☐	☐	
4. Karnofsky or Lansky Performance Score ≥ 70%.	☐	☐	
5. Must have an HLA-matched donor as follows: donor must be an 8/8 match for HLA-A, -B and -C at intermediate (or higher) resolution, and -DRB1 at high resolution using DNA-based typing. The donors must be willing to receive G-CSF followed by collection of cells by apheresis, and must meet the Program's criteria for donation.	☐	☐	
6. Cardiac function: Ejection fraction at rest ≥ 40%	☐	☐	
7. Serum creatinine value of < 1.5 mg/dL, or an estimated creatinine clearance greater than 50 mL/minute (using the Stanford calculator for eGFR available in EPIC)	☐	☐	
8. Diffusing capacity of the lungs for carbon monoxide (DLCO) ≥ 50% (adjusted for Hgb)	☐	☐	
9. Forced vital capacity (FVC) ≥ 50%	☐	☐	
10. Forced expiratory volume (FEV1) ≥ 50%	☐	☐	
11. Total bilirubin < 2 times the upper limit of normal (ULN) (unless the elevated bilirubin is attributed to Gilbert's Syndrome)	☐	☐	
12. Alanine aminotransferase (ALT) < 2.5 x ULN	☐	☐	
13. Aspartate aminotransferase (AST) < 2.5 x ULN	☐	☐	
14. Signed informed consent	☐	☐	

RECIPIENT Exclusion Criteria

Prospective RECIPIENTS Must NOT Match ANY of These Exclusion Criteria	**Yes**	**No**	**Supporting Documentation ***
1. Prior autologous or allogeneic hematopoietic stem cell transplant	☐	☐	
2. Prior malignancies, except resected non-melanoma or treated cervical carcinoma *in situ*. Cancer treated with curative intent ≥ 5 years previously is allowed. Cancer treated with curative intent < 5 years previously will not be allowed unless approved by the Protocol Officer or one of the Protocol Chairs	☐	☐	
3. Active CNS involvement by malignant cells	☐	☐	
4. Presence of fluid collection (ascites, pleural or pericardial effusion) that interferes with methotrexate clearance or makes methotrexate use contraindicated	☐	☐	
5. Requirement for supplemental oxygen	☐	☐	
6. Uncontrolled bacterial, viral or fungal infections (currently taking medication and with progression or no clinical improvement) at time of enrollment	☐	☐	
7. History of uncontrolled autoimmune disease or on active treatment (defined as > 5 mg prednisone daily)	☐	☐	
8. Seropositive for HIV-1 or -2	☐	☐	
9. Seropositive for HTLV-I or –II	☐	☐	
10. Active Hepatitis B or C viral replication by PCR	☐	☐	
11. Documented allergy to iron dextran or murine proteins	☐	☐	

Prospective RECIPIENTS Must NOT Match ANY of These Exclusion Criteria	Yes	No	Supporting Documentation *
12. Pregnant (positive serum or urine βHCG) or breastfeeding)	☐	☐	
13. Females of childbearing potential (FCBP) or men who have sexual contact with FCBP unwilling to use an effective form of birth control or abstinence for one year after transplantation	☐	☐	
14. Unable to comply with the treatment protocol, including appropriate supportive care, follow-up and research tests	☐	☐	
15. Planned to receive post-transplant maintenance therapy except for FLT3 inhibitors or BCR-ABL tyrosine kinase inhibitors (TKIs)	☐	☐	

* All subject files must include supporting documentation to confirm subject eligibility. The method of confirmation can include, but is not limited to, laboratory test results, radiology test results, subject self-report, and medical record review.

V. Statement of Eligibility (RECIPIENT)

By signing this form of this trial I verify that this subject is: ☐ eligible / ☐ ineligible for participation in the study. This study is approved by the Stanford Cancer Institute Scientific Review Committee, the IRB of record, and has finalized financial and contractual agreements as required by Stanford School of Medicine's Research Management Group.

Study Coordinator printed name:	Date:
Signature:	

Investigator printed name:	Date:
Signature:	

Triple-check reviewer printed name:	Date:
Signature:	

Protocol Title:	**CD34-Selected Allogeneic Hematopoietic Cell Transplantation with Myeloablative Conditioning and CD8$^+$ Memory T-cell Infusion For Patients with Myelodysplastic Syndrome, Acute Leukemia, and Chronic myelogenous leukemia (CML)**
eProtocol number: **OnCore number:**	**IRB-49023** **BMT339**
Principal Investigator:	Robert Lowsky, MD

DONOR name / Unique ID: /

Gender ☐ Male ☐ Female

DONOR Eligibility Criteria - Donors for this trial will follow the general standard of care that is used to select G-CSF-mobilized hematopoietic cell donors at Stanford University

Prospective DONOR Must MATCH ALL these Inclusion Criteria to be Eligible	**Yes**	**No**	**Supporting Documentation ***
1. HLA-matched donor (matching at 8/8 antigens or alleles including HLA-A, -B, -C, and –DRB1).	☐	☐	
2. ≥ 18 years to < 66.0 years	☐	☐	
3. State of general good health	☐	☐	
4. Completed a donor evaluation with history, medical examination and standard blood tests within 60 days of starting the hematopoietic cell collection procedure. In order to fairly represent the interests of the donor, the donor evaluation and consent will be performed by a study team member other than the recipient's attending physician.	☐	☐	
5. Hepatitis A, B and C, HIV-1 and -2, HTLV, VZV, EBV, HSV, West Nile virus, Syphilis Treponema, T cruzi (Chagas), CMV, and the MPX NAT IDT (HIV/HCV/HBV) will be tested as per national standard of care guidelines for transplant donors. Donors who are HIV-positive will be excluded. Donors who are positive by serology for Hepatitis B or C are eligible as long as PCR for RNA/DNA is negative	☐	☐	
6. White blood cell count > 3.5 x 10^9/L	☐	☐	

Prospective DONOR Must MATCH ALL these Inclusion Criteria to be Eligible	Yes	No	Supporting Documentation *
7. Platelets > 150 x 10^9/L	☐	☐	
8. Hematocrit > 35%	☐	☐	
9. Capable of undergoing leukapheresis	☐	☐	
10. Able to understand and sign informed consent	☐	☐	

DONOR Exclusion Criteria

Prospective DONOR Must NOT Match ANY of These Exclusion Criteria	Yes	No	Supporting Documentation *
1. Psychological traits or psychological or medical conditions which make them unlikely to tolerate the procedure	☐	☐	
2. Pregnant or lactating female	☐	☐	

* All subject files must include supporting documentation to confirm subject eligibility. The method of confirmation can include, but is not limited to, laboratory test results, radiology test results, subject self-report, and medical record review.

V. Statement of Eligibility (DONOR)

By signing this form of this trial I verify that this subject is: ☐ eligible / ☐ ineligible for participation in the study. This study is approved by the Stanford Cancer Institute Scientific Review Committee, the IRB of record, and has finalized financial and contractual agreements as required by Stanford School of Medicine's Research Management Group.

Study Coordinator printed name:	Date:
Signature:	

Investigator printed name:	Date:
Signature:	

Triple-check reviewer printed name:	Date:
Signature:	

3.2 Recruitment and Informed Consent

Subjects will be selected based upon the eligibility requirements highlighted above. Informed consent forms will be approved by the Stanford University IRB, SRC and FDA. No payment will be provided to participate in this protocol. Costs for routine care will be borne by the subject's insurance carrier. Research costs including cell enrichment procedures of the $CD8^+$ T_M cells as well as all research studies will be borne by an NCI-sponsored Program Project Grant (PPG 4P01CA049605-27).

3.3 Potential Risk to the Recipient

Subjects who undergo allogeneic HCT have risks for a variety of complications that include transplant-related deaths. An Attending Physician will explain the risks associated with HCT in detail to the subject and their caregivers once it is deemed that treatment with this protocol is a feasible consideration and appropriate. The physician involved in the consenting process will not be the primary physician who is involved in managing the care of the subject or donor. A detailed discussion regarding the current alternative treatment which is a CD34-selected graft without a $CD8^+$ memory T-cell infusion, an unmanipulated donor graft with SOC post-transplant immune suppression or post-transplant Cyclophosphamide will be discussed with the subject and their caregiver(s). All inclusion criteria eligibility must be fulfilled. The plan will also be discussed with the subject's referring physician.

Potential Sensitization to Murine Proteins: Mouse protein antibodies are used in the CliniMACS processing procedures. If the recipient has a pre-existing allergy, he or she may be at risk for allergic reactions during infusion of the processed cells; although the residual amount of murine protein in the final product is very low (estimated maximum dose for a 50 kg subject would be 30 μg). To date, no allergic reactions are reported in subjects receiving cells processed with the CliniMACS System. Epinephrine and antihistamines will be available at the recipient's bedside during the PBSC infusion.

3.4 Protection Against Risks

The patient-subject will receive a consent form with appropriate information concerning the cell infusion. Risks include the toxicities of the acute and chronic GvHD, infection, marrow aplasia and cytopenia, disease progression, and the possible other unforeseen risks given the novel nature of the clinical trial. All questions will be answered by the Attending Physician as well as Nurse Coordinator. The physician involved in this process will not be the primary physician who is involved in managing the care of the patient-subject or donor. Once a patient-subject has agreed to proceed with the trial a formal 1 to 1.5 hour consenting process will be performed by the Research Nurse and all questions will be answered. Signed informed consent must be obtained prior to initiation of the protocol. Risks to the donors are expected to be very low and are the same as for other donors who undergo collection for traditional HCT.

To maintain confidentiality, study subject identification will not be revealed in any reports or publications resulting from the study. Information will be provided to the study subjects' physicians as necessary, to the IRB, DSMB, and FDA as necessary. Case report forms and other study records will not identify the subjects by name. To monitor data and safety of the research, the procedures as outlined in the "Data and Safety Monitoring Plan" (see below) will be followed.

3.5 Potential Benefits of the Proposed Research to the Subjects

This study represents a logical extension of our work on the use of $CD8^+$ T_M cells that have the potential to improve transplant outcomes by providing anti-tumor activity and enhancing post-transplant immune reconstitution with prevention from infection yet without increasing the risk of GvHD. The potential benefits could be realized by patient-subjects who participate in this trial, yet it is understood that the trial concept and design is investigational.

3.6 Importance of the Knowledge to be Gained

The goal of the trial is to test the treatment concept that adding HLA matched donor $CD8^+CD45RA^-$ T_M phenotype cells to purified $CD34^+$ grafts given to recipients undergoing full dose conditioning and HCT for acute leukemia and MDS is safe, and will reduce the risk of disease relapse and infections without increasing the risk of GvHD. If we are successful, it is likely that this strategy will improve overall subject survival. As well, the strategy developed in the proposed trial can be effectively adapted to other clinical transplant settings, and therefore has applicability to treat diseases beyond acute leukemia and MDS. The overall significance is a potential improvement in transplant outcomes.

3.8 Inclusion of Women and Minorities

Women and minorities and are eligible for this protocol.

3.9 Subject Withdrawal

Participation in the trial is voluntary. Subjects will be advised that they may voluntarily withdraw from the study at any time and will be instructed to notify the investigator. Subjects may choose to withdraw for any reason(s). Subjects are not obligated to reveal their reason(s) for withdrawal to the sponsor.

3.10 Subject Withdrawal from Data Collection

If a subject explicitly states they do not wish to contribute further data to the trial their decision must be respected and notified to the sponsor in writing. In this event details should be recorded in the subject's hospital records, no further CRFs must be completed and no further data sent to the sponsor.

4 TREATMENT PLAN

Study Transplant Conditioning Regimen

OPTION 1:

Fractionated TBI will be delivered in the standardized format consisting of a total dose of 1375 cGy given in fractions on days -10 -9,-8 and -7. Sequential doses are administered in an anterior/posterior or lateral orientation. The orientation of TBI chosen will be left to the discretion of the radiation oncologist but will remain consistent at our institution throughout the duration of the trial. Full value lung blocks are not used yet compensators and lung blocks yielding a minimum of 800 cGy lung dose are allowed based on our institutional practice. Depending on the method of lung shielding employed, the blocked areas should be boosted with high-energy electrons or be otherwise radiated so that the cumulative chest wall dose is approximately 1300 cGy, so as to ensure that marrow sites in the ribs are adequately treated. In

addition, male subjects receiving transplants for ALL or AML, use of boost to testes is allowed according to institutional practices.

Thiotepa will be administered at 5 mg/kg/day intravenously over 4 hours for 2 consecutive days (Day -6 and -5).

Fludarabine will be administered intravenously over 30 minutes at a total dose of 125 mg/m^2 divided into 5 daily doses of 25 mg/m^2/day (Day -6 to -2).

Treatment Schedule

Days -10 to -7	Fractionated TBI (1375 cGy), administered over 4 days
Days -6 to -5	Thiotepa 5 mg/kg/day: IV for 2 consecutive days
Days -6 to -2	Fludarabine 25 mg/m^2/day: IV for 5 consecutive days

OPTION 2: : Non TBI Conditioning – Busulfan/Cyclophosphamide

Days -7 to -4:	Busulfan: 3.6 mg/kg/dose Q24h IV. Infused over 3 hours. 1 dose per day x 4 consecutive days x 3.6 mg/kg/dose = 14.4 mg/kg
Days -3 to -2	Cyclophosphamide: 60 mg/kg/dose Q24h IV. Infused over 2 hours. 1 dose per day x 2 consecutive days x 60 mg/kg/dose = 120 mg/kg

Immune Suppression Medication

Tacrolimus .01 mg/kg IV BID or .03 mg/kg PO BID
1. Begin day -3
2. Target serum level is 5-10 ng/ml
3. If > 15 kg over IBW, dose based on adjusted IBW

Dose adjusted based on creatinine, t. bilirubin and serum drug levels.
Tacrolimus taper will begin around day +90 in the absence of GVHD and based on clinical decisions. The aim will be to discontinue Tacrolimus altogether if clinically applicable (no history of GVHD) by around day +180. In some patients who are considered higher risk for disease relapse, as determined by the medical team, the tacrolimus taper may begin in the absence of active GVHD by day +60.

4.2 Hematopoietic Stem Cell Graft Collection

Transplant donors are considered research subjects by the FDA. For HLA-matched donors, the consent process will be done at the Stanford University Medical Center along with routine donor consent procedures according to institutional guidelines. The donor informed consent document for donors will be reviewed and approved by the Stanford University IRB.

4.3 Mobilization of Donor

Following screening and enrollment, donors will undergo 1 to 2 days of apheresis collections for the processing and enrichment for $CD34^+$ cells and for $CD8^+CD45RA^-$ T_M cells as detailed in Section 5. As per standard of care, donor mobilization will be using G-CSF (filgrastim or its biosimilar equivalent) and cell collection will be done using standard procedures for apheresis. A sample donor schedule is described below, and as per standard of care the schedule can shift one day up or down depending on apheresis and CTF (cell therapy facility) availability. In general, the donor will begin GCSF on Day -6 or Day -7 of the conditioning regimen at a dose of 10 μg/kg/day actual body weight subcutaneously (rounded to a multiple of the nearest vial size of either 300 or 480 μg). Based on the volume of GCSF, the dose may be split into multiple injection sites. The mobilization phase will start on the 1st day of administration of G-CSF (Day -6 or Day -7) and continue until the final day of leukapheresis (Day -1 or Day -2). The donor will undergo their 1st apheresis on Day -2 or Day -3, and if it is determined that an additional apheresis is required to achieve adequate cell doses, then a 2nd apheresis will be on Day -1 or Day -2, respectively. Therefore, the cell collection days for the donor will be Days -3 and -2, or it can be Days -2 and -1.

4.3 Donor Leukapheresis

Leukapheresis will be performed on a continuous flow cell separator according to institutional standards and will commence on the morning of the fifth day of G-CSF treatment. The anti-coagulant used for the procedure will be acid citrate dextrose (ACD). The volume of blood processed per leukapheresis session should be approximately three to four times total blood volume as tolerated by the donor. It is expected that many donors will undergo 2 consecutive days of apheresis. Concurrent plasma (about 200 mLs) will be collected for products to be stored overnight. A unique identification and labeling system will be used to track the leukapheresis product from collection to infusion according to FACT/JACIE guidelines.

5 INVESTIGATIONAL AGENT INFORMATION

5.1 Investigational Agent: $CD8^+$ Memory T-Cell Selection

The apheresis product will be enriched for $CD8^+CD45RA^-$ memory T-cells. In a procedure we successfully used in 2 prior clinical trials, we will enrich for donor $CD8^+$ memory T-cells using a tandem immunomagnetic selection procedure that involves the CliniMACS Cell Selection System (Miltenyi Biotec). Depletion of CD45RA expressing cells uses CliniMACS CD45RA Reagent (BB-MF-11872). The CD45RA depleted fraction is then enriched for CD8-expressing cells by positive cell selection with CliniMACS CD8 Reagent (BB-MF-11704). The cells will be infused in the usual manner on Day 0 and within 6 hours of the $CD34^+$ selected stem cell infusion.

In our two prior clinical trials we determined that 2 consecutive days of aphereses per donor will yield a target cell dose of 5 to 10 x 10^6 $CD8^+$ memory T-cells/kg for all subjects, assuming most subjects weigh less than 120 kg. We will use a dose range of 5 to 10 x 10^6 $CD8^+$ memory T-cells/kg for the current study, as both dose levels (5 x 10^6/kg and 10 x 10^6/kg) were well tolerated in our prior phase 1 study. We will not exceed a dose of 10 x 10^6 $CD8^+$ memory T-cells/kg. If the number of $CD8^+$ memory T-cells falls below the desired threshold of

5 x 10^6/kg, the infusion will proceed as planned because we believe it is in the subject's best interest to receive the cell infusion. The $CD8^+$ memory T-cell infusion has the potential to help achieve donor cell engraftment, provide anti-tumor reactions and protect from infections yet without increasing the GvHD risk. It is expected that a cell yield of < 5 x 10^6/kg will be obtained less than 20% of the time.

5.2 Stability

As part of our original IND, we submitted stability data to the FDA from validation runs that extended to 72 hours from the time of completion of first apheresis, and showed negligible loss in cell viability as all validation products continued with > 90% viability. In addition, the results from our prior $CD8^+$ memory T-cells trials confirmed stability data extending to 72 hours after completion of first apheresis from 8 additional donors, and all cases maintained > 94% viability.

5.3 Reagents for cells for immediate release and conditions for storage

The CD8 and CD45 reagent systems are pharmaceutical grade and both have Master Files that are BB-MF-11704 and BB-MF-11872, respectively. Donors will undergo two consecutive apheresis days with the first day of apheresis being in the afternoon expected to begin around 2 PM and be completed by 6 PM (the collection will be stored overnight in a 4-degree Celsius bio-fridge), followed by a second apheresis the next morning starting at around 7:30 AM. The two days of collections will be batched for routine SOC $CD34^+$ cell selection that will be performed on the same day as the second collection. The $CD34^+$ flow through will be diluted for overnight storage at 4 degrees Celsius with 1 volume of Normosol-R with 2% HSA. Thereafter, the following morning the tandem immunomagnetic selection procedure for enrichment of $CD45RA^-CD8^+$ phenotype T-cells will be performed. For subject comfort, the final volume of the infusate will be reduced to < or equal to 200 mL and the $CD8^+$ memory T-cell concentration will range between 1 x 10^6 to 1 x 10^8 cells/mL. The final infusate will be diluted in the Miltenyi Biotech Selection Buffers that contain PBS with 1mmolar EDTA and 0.5% HSA. It is estimated that all CD45RA-$CD8^+$ phenotype T-cell products will be infused within 72 hours from completion of the first apheresis.

5.4 Release Requirements of the Product

Release of the selected cell products for infusion includes:

- Negative Gram staining (no organisms detected).
- Endotoxin levels ≤ 0.5 EU/milliliter of infused product volume
- 80% cell viability as assessed by trypan blue or 7-AAD dye exclusion.
- ≥ 80% of the CD3+ cells express the CD8 memory T-cell phenotype, here defined as:
  - $CD8^+/CD45RA^-/CD45RO^+$
  - ≤ 5% of cells with the $CD3^+/CD45RA^+/CD45RO^-$ phenotype
  - Sufficient cells with the CD8 memory T-cell phenotype to meet the desired dose, or at least the minimum dose of > 2 x 10^6 $CD8^+$ memory T-cells/kg

In addition, post-selection sterility cultures in accordance with sterility testing guidelines (21CFR§610.12) will be conducted to confirm the sterility of the selected cells. Any positive growth results obtained will be documented in the processing record of the selection and immediately communicated to the subject's attending physician in accordance with the plan of action for the infusion of cellular products with microbial contamination. Ancillary studies may include phenotypic analysis of the selected cells and cytokine secretion profiles from MLR assays may be used to further our understanding of the $CD8^+$ memory T-cell population characteristics but do not constitute release criteria at this time. **If the cell product does not meet release criteria after manipulation, the product will not be infused.**

In the case(s) where the manipulated cell product does not meet release criteria, the subject will be removed from the study due to a failure to receive the investigational product. If 2 of the first 5 enrolled subjects fail to receive the $CD8^+$ memory T-cell product due to a failure to satisfy the release criteria, a formal DSMC review will be initiated. An enrolled subject who does not receive the $CD8^+$ memory T-cell product will be removed from the study and replaced with another subject as the accrual target is 30 subjects who will be infused with $CD8^+$ memory T-cells.

6 DOSE MODIFICATION

As a single treatment transplant procedure, dose modification for any particular subject is not anticipated.

7 ADVERSE EVENTS AND REPORTING PROCEDURES

7.1 Adverse Events

Hematopoietic cell transplantation (HCT) is an aggressive therapy for the treatment of a number of life-threatening disorders, including cancer. In this setting, a very large number of Grade 1, 2 and 3 adverse events (AEs) are expected to occur, regardless of whether or not a subject is participating in a research study. In order to minimize the "background noise" and to focus on clinically significant, meaningful adverse events useful in the assessment of proposed trial the following AE reporting schema is proposed. This schema is intended to capture all AEs that are clinically significant and/or impactful, but minimize "not informative" AE collection.

Laboratory values without a clinical consequence or outcome will not be recorded as adverse events, unless deemed serious (see below).

Grade 1 to 3 AEs not meeting these criteria will not be collected, except as follows. Grade 1 to 3 AEs resulting in subject termination or withdrawal from the study will be collected.

An adverse event is considered a serious adverse event (SAE) if it fulfills one of the following SAE criteria per 21CFR§312.32(a):

- Results in death.
- Life-threatening (subject at risk of death at the time of the event).
- Requiring new inpatient hospitalization or prolongation of existing hospitalization.
- Resulting in persistent or significant disability.
- Other medical events that may not be immediately life-threatening or result in death or hospitalization by may jeopardize the subject or require intervention to prevent one of the outcomes listed above

Grade 4 and higher AEs will be collected and documented with causality attribution on an adverse event log (AE log), and all AEs meeting the criteria of "serious" as defined at 21CFR§312.32(a), including any that are otherwise Grade 1, 2 or 3 will be collected; identified as serious; and documented on an AE log, including causality attribution.

All SAEs will be collected as outlined in Section 7.1 from date of $CD8^+$ memory T-cell infusion through 365 days. All SAEs will be tracked until resolution or at least 60 days after the study treatment.

Vital signs for all subjects enrolled on the trial will be monitored for infusion-related toxicities (AEs) during the CD8 memory T-cell infusion, and every 30 minutes for 2 hours afterwards. Observed events will be recorded on the AE log. In particular, subjects will be observed for hemodynamic instability, allergic reactions (eg, hives, rashes, angioedema), and fevers (> 38.3º).

The development of acute GvHD, its date of onset and overall grade, is a key secondary outcome measure for subjects on this trial. Acute GvHD will be recorded on an AE log.

7.2 Adverse Event Reporting

All SAEs will be reported to the IRB; to the IND; and to the DSMC, either in an annual review or as an expedited report, in accordance with the established practices of the Stanford Division of Blood and Marrow Transplantation, as well as applicable guidelines and regulations. AEs meeting the criteria specified for an IND Safety Report as defined at 21CFR§312.32(c)(1) will be collected; documented in the adverse event log; and reported to the IND (on a MedWatch 3500A form) in the timeframe specified. The IND annual report will include summaries of the collected AEs, as specified by 21CFR§312.33.

8 CORRELATIVE/SPECIAL STUDIES

None planned.

9 STUDY CALENDAR

	Pre-Enrollment requirements [A]	**On-study enrollment [A] = $CD8^+$ Memory T-cell Infusion (Day 0)**	**Time (days) from on-study enrollment ± 20 days**		
			Day +90	**Day 180**	**Day 365**
$CD34^+$ cell Infusion		**X**			
Infusion of $CD8^+$ memory T-cells		**X**			
Informed consent	**X**				
Demographics	**X**				
Medical history	**X**		**X**	**X**	**X**
Donor evaluation	**X**				
Physical exam	**X**		**X**	**X**	**X**
Fulfills inclusion criteria	**X**				**X**
Has no exclusion criteria	**X**				

Performance status	**X**		**X**	**X**	**X**
Disease Status Assessment	**X**		**X**	**X**	**X**
CBC with differential			**X**	**X**	**X**
Serum chemistry			**X**	**X**	**X**
STR analysis [B]			**X**	**X**	**X**
Assess for ≥ Grade 2 acute GvHD [C]			**X**	**X**	**X**
Assess for moderate or severe chronic GvHD [C]			**X**	**X**	**X**

[A] All pre-transplant screening and procedures must be completed within 70 days of beginning conditioning therapy (unless otherwise indicated).

[B] STR analysis will only be performed beyond Day +90 if there is concern about mixed chimerism or relapse.

[C] Refers to if the event was recorded during the reporting periods of Day 0 to +90; Day 91 to +180; and Day 181 to +365.

10. MEASUREMENTS

10.1 Primary and Secondary Objectives and Outcome measures

Objectives and outcome measures are described at Section 1.1 and 1.2.

11. REGULATORY CONSIDERATIONS

11.1 Institutional Review of Protocol

The protocol, the proposed informed consent and all forms of participant information related to the study (eg, advertisements used to recruit participants) will be reviewed and approved by the Stanford IRB and Stanford Cancer Institute Scientific Review Committee (SRC). Any changes made to the protocol will be submitted as a modification and will be approved by the IRB prior to implementation. The Protocol Director will disseminate the protocol amendment information to all participating investigators.

11.2 Data and Safety Monitoring Plan

The Stanford Cancer Institute Data and Safety Monitoring Committee (DSMC) will be the monitoring entity for this study. The DSMC will audit study-related activities to determine whether the study has been conducted in accordance with the protocol, local standard operating procedures, FDA regulations, and Good Clinical Practice (GCP). This may include review of the following types of documents participating in the study: regulatory binders, case report forms, eligibility checklists, and source documents. In addition, the DSMC will regularly review serious adverse events and protocol deviations associated with the research to ensure the protection of human subjects. Results of the DSMC audit will be communicated to the IRB and the appropriate regulatory authorities at the time of continuing review, or in an expedited fashion, as needed.

11.3 Data Management Plan

The Protocol Director, or his/her designee, will prepare and maintain adequate and accurate participant case histories with observations and data pertinent to the study, in accordance with the established practices of the Stanford Division of Blood and Marrow Transplantation. Accrual rates will be reported to the Data Safety Monitoring Committee (DSMC) and data quality will be reviewed by the Study Statistician. The DSMC of the Stanford Cancer Center will oversee this study and will receive regular reporting of SAEs and monitoring. This Committee involves knowledgeable physicians, scientists and statisticians who oversee all phase I/II trials conducted under the auspices of the Stanford Cancer Center. Individuals who serve on this Committee who are associated with this trial will recuse themselves from discussions of study results, and decisions about revision or closure. Audits will be conducted as requested. Data will also be submitted to the FDA as required by our IND and follow the appropriate reporting rules and regulations including an annual report.

11.4 Good Clinical Practice Guidelines

The clinical investigators assure that the clinical study is performed in accordance with the protocol, the ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996, the Declaration of Helsinki (Recommendations guiding physicians in Biomedical Research involving Human Subjects; Helsinki 1964; and as amended Tokyo 1975; Venice 1983;

Hong Kong 1989; Somerset West 1996; Edinburgh 2000; Seoul 2008; and Fortaleza 2013) and applicable regulatory requirements.

12. STATISTICAL CONSIDERATIONS

12.1 Statistical Design

The study is a single arm, Simon 2-stage trial with a modification to avoid protracted temporary closure of accrual at the end of the first stage. In single and multi-center studies consisting of over 1500 adult patients treated with SOC allogeneic HCT, the 1-year probability of GRFS was 30%. [48,49] For Type 1 and 2 error rates of 0.10 and 0.2 respectively (power of 0.80), a control success rate of 0.30 and experimental rate of 0.50, the Simon optimal design calls for enrolling 15 subjects in stage 1 and if 6 or more subjects achieve success, then in stage 2 continuing to enroll to a full sample of 32 subjects and declare success if a total of 13 subjects experience success. In order to avoid a protracted temporary closure between the first and second stage, Day 100 GRFS will be evaluated at the 1st stage. If ≥ 6 subjects in Simon stage 1 are alive without relapse or Grade III-IV GvHD at Day +100, then the full cohort of 32 subjects will be enrolled for stage 2. Success will be declared if ≥ 13 subjects of 32 meet the primary endpoint of GRFS at 1 year, *and if at least 6 of the stage 1 subjects ultimately meet that endpoint as well*. With that proviso, this modified design has the same type 1 and 2 errors of a hypothetical standard Simon 2-stage design which would pause at the end of stage 1 until at least 6 subjects reach the 1-year GRFS outcome. However, the modification modestly increases the expected number of subjects treated (under the null, as discussed below).

Note that the end of stage 1 is defined as 100 days, while at the final analysis is for 1 year. Subjects who fail at 100 days will be regarded as failing in the time frame of the final analysis. No increase in type I or type II error is anticipated.

12.1.1 Randomization

This is a single arm, non-randomized study.

12.2 Interim analyses

There is no formal plan for an interim analysis because the intrinsic nature of the Simon two-stage design is such that the study will not proceed to stage 2, unless the null hypothesis is rejected, because activation of the second stage depends on an adequate number of success observed in the first stage. Therefore, the trial design incorporates stopping early for futility and safety. It is possible that $CD8^{+}CD45RA^{-}$ T_M cells will induce acute GvHD. If 2 of the first 4 subjects develop Grade 2 or higher acute GvHD, or, if after 8 subjects, > 30% develop Grade 2 or higher acute GvHD, then we will include a post-transplant GvHD prophylactic regimen. Toxicity, adverse events, and other safety endpoints will be monitored regularly and reported to the DSMB at each meeting or earlier. The expected 100-day mortality rate for a myeloablative transplant is 15 to 20% based on our data as well as CIBMTR data. The rate of mortality will be monitored up to 100 days after transplant, and if 2 of the first 4 enrolled subjects, or if 3 subjects in Simon stage one die within 100 days of transplant, this will trigger a DSMB safety review. If 2 subjects experience graft rejection in Simon stage one, this too will trigger a DSMB review.

12.3 Primary endpoint

The primary endpoint is GvHD-free, relapse-free survival (GRFS) at 1 year. Development of acute GvHD Grade 3 or higher, extensive chronic GvHD requiring immunosuppression, relapse, or death will be considered failures for this endpoint.

12.4 Sample Size

12.4.1 Accrual estimates

32 subjects is the full target accrual for the study.

12.4.2 Sample size justification

It is estimated that 1 subject per month will be accrued. The total subject accrual is expected to take approximately 32 months. Given that accrual rate, the 15^{th} (last) subject recruited for stage 1 will reach the +100 day milestone at about 18 months of study time, and the first 6 subjects recruited will have reached the 1-year point. The entire first stage group of 15 subjects will reach the 1-year milestone at month 27. Thus, sometime between the end of months 18 and 27, it will be known whether the unmodified Simon trial would be stopped. During that interim period, up to 9 new subjects would be recruited. This is an upper bound on the excess exposure under the null. It is approached only if the +100 day GRFS rate is considerably larger than the 1-year GRFS rate null, so that few of the first-stage +100 day successes continue to 1 year. We have no direct estimate of the failure rate over 1 year given GRFS at +100 days (under the experimental treatment), so we do not attempt to refine the calculation further.

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APPENDICES

APPENDIX A GvHD SCORING CRITERIA

Glucksberg clinical stage and grade of acute GvHD

Stage	Skin	Liver	Intestinal tract
1	Maculopapular rash < 25% of body surface	Bilirubin 34–50 μmol/l	> 500 ml diarrhoea/d
2	Maculopapular rash 25–50% body surface	Bilirubin 51–102 μmol/l	> 1000 ml diarrhoea/d
3	Generalized erythroderma	Bilirubin 103–225 μmol/l	> 1500 ml diarrhoea/d
4	Generalized erythroderma with bullous formation and desquamation	Bilirubin > 255 μmol/l	Severe abdominal pain, with or without ileus

Grade	Degree of organ involvement
I	Stage 1–2 skin rash; no gut involvement; no liver involvement; no decrease in clinical performance
II	Stage 1–3 skin rash; stage 1 gut involvement or stage 1 liver involvement (or both); mild decrease in clinical performance
III	Stage 2–3 skin rash; stage 2–3 gut involvement or 2–4 liver involvement (or both); marked decrease in clinical performance
IV	Similar to Grade III with stage 2–4 organ involvement and extreme decrease in clinical performance

NIH consensus criteria for chronic GvHD severity with examples

(comprehensive organ scoring criteria is reviewed in Jagasia, *et al*, BBMT 2014)

Mild	• 1 or 2 organs or sites (except lung) with score 1 • Mild oral symptoms, no decrease in oral intake • Mild dry eyes, lubricant eyedrops ≤ 3x/day
Moderate	• 3 or more organs with score 1 • At least 1 organ or site with score 2 • 19-50% body surface area involved or superficial sclerosis • Moderate dry eyes, eyedrops > 3x/day or punctal plugs • Lung score 1 (FEV1 60-79% or dyspnea with stairs)
Severe	• At least 1 organ or site with score 3 • > 50% body surface area involved • Deep sclerosis, impaired mobility or ulceration • Severe oral symptoms with major limitation in oral intake • Severe dry eyes affecting ADL • Lung score 2 (FEV1 40-59% or dyspnea walking on flat ground)

APPENDIX B. RESPONSE CRITERIA by DISEASE HISTOLOGY

AML Response Criteria (Dohner, *et al*, Blood 2017)

Table 6. Response criteria in AML

Category	Definition	Comment
Response		
CR without minimal residual disease (CR_{MRD-})	If studied pretreatment, CR with negativity for a genetic marker by RT-qPCR, or CR with negativity by MFC	Sensitivities vary by marker tested, and by method used; therefore, test used and sensitivity of the assay should be reported; analyses should be done in experienced laboratories (centralized diagnostics)
Complete remission (CR)	Bone marrow blasts <5%; absence of circulating blasts and blasts with Auer rods; absence of extramedullary disease; ANC ≥1.0 × 10^9/L (1000/μL); platelet count ≥100 × 10^9/L (100 000/μL)	MRD^+ or unknown
CR with incomplete hematologic recovery (CR_i)	All CR criteria except for residual neutropenia (<1.0 × 10^9/L [1000/μL]) or thrombocytopenia (<100 × 10^9/L [100 000/μL])	
Morphologic leukemia-free state (MLFS)	Bone marrow blasts <5%; absence of blasts with Auer rods; absence of extramedullary disease; no hematologic recovery required	Marrow should not merely be "aplastic"; at least 200 cells should be enumerated or cellularity should be at least 10%
Partial remission (PR)	All hematologic criteria of CR; decrease of bone marrow blast percentage to 5% to 25%; and decrease of pretreatment bone marrow blast percentage by at least 50%	Especially important in the context of phase 1-2 clinical trials
Treatment failure		
Primary refractory disease	No CR or CR_i after 2 courses of intensive induction treatment; excluding patients with death in aplasia or death due to indeterminate cause	Regimens containing higher doses of cytarabine (see Table 8) are generally considered as the best option for patients not responding to a first cycle of 7+3; the likelihood of responding to such regimens is lower after failure of a first
Death in aplasia	Deaths occurring ≥7 d following completion of initial treatment while cytopenic; with an aplastic or hypoplastic bone marrow obtained within 7 d of death, without evidence of persistent leukemia	
Death from indeterminate cause	Deaths occurring before completion of therapy, or <7 d following its completion; or deaths occurring ≥7 d following completion of initial therapy with no blasts in the blood, but no bone marrow examination available	
Response criteria for clinical trials only		
Stable disease	Absence of CR_{MRD-}, CR, CR_i, PR, MLFS; and criteria for PD not met	Period of stable disease should last at least 3 mo
Progressive disease (PD)*,†	Evidence for an increase in bone marrow blast percentage and/or increase of absolute blast counts in the blood: • >50% increase in marrow blasts over baseline (a minimum 15% point increase is required in cases with <30% blasts at baseline; or persistent marrow blast percentage of >70% over at least 3 mo; without at least a 100% improvement in ANC to an absolute level (>0.5 × 10^9/L [500/μL], and/or platelet count to >50 × 10^9/L [50 000/μL] nontransfused); or • >50% increase in peripheral blasts (WBC × % blasts) to >25 × 10^9/L (>25 000/μL) (in the absence of differentiation syndrome)†; or • New extramedullary disease	Category mainly applies for older patient given low-intensity or single-agent "targeted therapies" in clinical trials In general, at least 2 cycles of a novel agent should be administered Some protocols may require blast increase in 2 consecutive marrow assessments at least 4 wk apart; the date of progression should then be defined as of the first observation date Some protocols may allow transient addition of hydroxyurea to lower blast counts "Progressive disease" is usually accompanied by a decline in ANC and platelets and increased transfusion requirement and decline in performance status or increase in symptoms
Relapse		
Hematologic relapse (after CR_{MRD-}, CR, CR_i)	Bone marrow blasts ≥5%; or reappearance of blasts in the blood; or development of extramedullary disease	
Molecular relapse (after CR_{MRD-})	If studied pretreatment, reoccurrence of MRD as assessed by RT-qPCR or by MFC	Test applied, sensitivity of the assay, and cutoff values used must be reported; analyses should be done in experienced laboratories (centralized diagnostics)

ALL Response Criteria (Brown, *et al*, JNCCN 2017)

Response Criteria for Blood and Bone Marrow:

- **CR**
  - **No circulating blasts or extramedullary disease**
    - **No lymphadenopathy, splenomegaly, skin/gum infiltration/testicular mass/CNS involvement**
  - **Trilineage hematopoiesis (TLH) and <5% blasts**
  - **Absolute neutrophil count (ANC) >1000/microL**
  - **Platelets >100,000/microL**
  - **No recurrence for 4 weeks**
- **CR with incomplete blood count recovery (CRi)**
  - **Meets all criteria for CR except platelet count and/or ANC**
- **Overall response rate (ORR = CR + CRi)**
- **Refractory disease**
  - **Failure to achieve CR at the end of induction**
- **Progressive disease (PD)**
  - **Increase of at least 25% in the absolute number of circulating or bone marrow blasts or development of extramedullary disease**
- **Relapsed disease**
  - **Reappearance of blasts in the blood or bone marrow (>5%) or in any extramedullary site after a CR**

MDS Response Criteria (Cheson, *et al*, Blood 2006)

Table 3. Proposed modified International Working Group response criteria for altering natural history of MDS[7]

Category	Response criteria (responses must last at least 4 wk)
Complete remission	Bone marrow: ≤ 5% myeloblasts with normal maturation of all cell lines*
	Persistent dysplasia will be noted*†
	Peripheral blood‡
	Hgb ≥ 11 g/dL
	Platelets ≥ 100 × 10^9/L
	Neutrophils ≥ 1.0 × 10^9/L†
	Blasts 0%
Partial remission	All CR criteria if abnormal before treatment except:
	Bone marrow blasts decreased by ≥ 50% over pretreatment but still > 5%
	Cellularity and morphology not relevant
Marrow CR†	Bone marrow: ≤ 5% myeloblasts and decrease by ≥ 50% over pretreatment†
	Peripheral blood: if HI responses, they will be noted in addition to marrow CR†
Stable disease	Failure to achieve at least PR, but no evidence of progression for > 8 wks
Failure	Death during treatment or disease progression characterized by worsening of cytopenias, increase in percentage of bone marrow blasts, or progression to a more advanced MDS FAB subtype than pretreatment
Relapse after CR or PR	At least 1 of the following:
	Return to pretreatment bone marrow blast percentage
	Decrement of ≥ 50% from maximum remission/response levels in granulocytes or platelets
	Reduction in Hgb concentration by ≥ 1.5 g/dL or transfusion dependence
Cytogenetic response	Complete
	Disappearance of the chromosomal abnormality without appearance of new ones
	Partial
	At least 50% reduction of the chromosomal abnormality
Disease progression	For patients with:
	Less than 5% blasts: ≥ 50% increase in blasts to > 5% blasts
	5%-10% blasts: ≥ 50% increase to > 10% blasts
	10%-20% blasts: ≥ 50% increase to > 20% blasts
	20%-30% blasts: ≥ 50% increase to > 30% blasts
	Any of the following:
	At least 50% decrement from maximum remission/response in granulocytes or platelets
	Reduction in Hgb by ≥ 2 g/dL
	Transfusion dependence
Survival	Endpoints:
	Overall: death from any cause
	Event free: failure or death from any cause
	PFS: disease progression or death from MDS
	DFS: time to relapse
	Cause-specific death: death related to MDS