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

Coordinating Center
Stanford Cancer Center
875 Blake Wilbur Dr
Stanford, CA 94305

Protocol Director
Robert Lowsky, MD
Division of Blood and Marrow Transplantation
Stanford University Medical Center
300 Pasteur Dr, Stanford, CA 94305
rlowsky@stanford.edu

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Co-Investigators	
Wen-Kai-Weng, MD, PhD Division of Blood and Marrow Transplantation Stanford University Medical Center 300 Pasteur Dr, Stanford, CA 94305 wkweng@stanford.edu	Robert Negrin, MD Division of Blood and Marrow Transplantation Stanford University Medical Center 300 Pasteur Dr MC5623, Stanford, CA 94305 negrs@stanford.edu
Sally Arai, MD Division of Blood and Marrow Transplantation Stanford University Medical Center 300 Pasteur Dr, Stanford, CA 94305 sarai1@stanford.edu	Laura Johnston, MD Division of Blood and Marrow Transplantation Stanford University Medical Center 300 Pasteur Dr, Stanford, CA 94305 korb@stanford.edu
David Miklos, MD, PhD Division of Blood and Marrow Transplantation Stanford University Medical Center 300 Pasteur Dr, Stanford, CA 94305 dmiklos@stanford.edu	Judith Shizuru, MD, PhD Division of Blood and Marrow Transplantation Stanford University Medical Center 300 Pasteur Dr, Stanford, CA 94305 Jshizuru@stanford.edu
Everett Meyer, MD Division of Blood and Marrow Transplantation and Cellular Therapeutics Facility Laboratory Stanford University Medical Center 300 Pasteur Dr, Stanford, CA 94305 evmeyer@stanford.edu	Andrew Rezvani, MD Division of Blood and Marrow Transplantation Stanford University Medical Center 300 Pasteur Dr , Stanford, CA 94305 arezvani@stanford.edu
Lori Muffly, MD Division of Blood and Marrow Transplantation Stanford University Medical Center 300 Pasteur Dr, Stanford, CA 94305 lmuffly@stanford.edu	Samuel Strober, MD Divisions of Immunology & Rheumatology CCSR Building, Stanford, California 94305 sstrober@stanford.edu

Michael Spinner, MD Division of Blood and Marrow Transplantation Stanford University Medical Center 300 Pasteur Drive, Stanford, CA 94305 mspinner@stanford.edu	Philip Lavori, PhD (Biostatistician) Department of Health Research and Policy Biostatistics Shared Resource, Redwood Building, Stanford, CA 94305 lavori@stanford.edu

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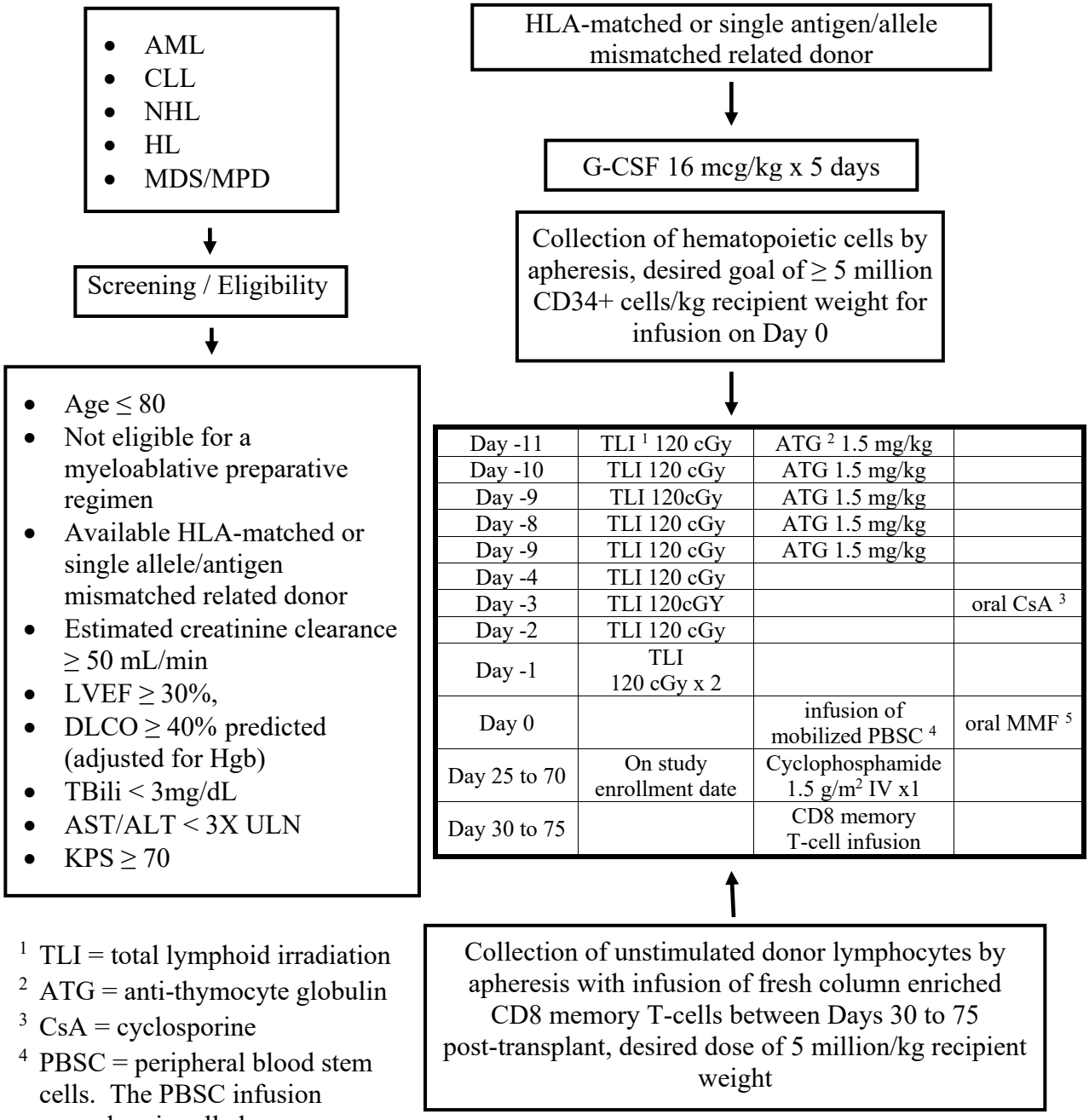
Protocol Synopsis

TITLE	A post-transplant infusion of CD8 memory donor T-cells as consolidative therapy after allogeneic non-myeloablative hematopoietic cell transplantation in patients with leukemia and lymphoma.
STUDY PHASE	Phase 2 efficacy study
STUDY SITE	Stanford University Medical Center, CA 94305
INDICATION	Patients with leukemia and lymphoma who are not eligible for full dose transplantation
INVESTIGATIONAL PRODUCT	Allogeneic CD8 memory T-cells infused 30 to 75 days after hematopoietic cell transplant
PRIMARY ENDPOINT and OBJECTIVE(S)	To determine the proportion of patients with full dose donor T-cell chimerism within 3 months after the CD8+ memory T-cell infusion.
SECONDARY ENDPOINTS AND OBJECTIVE(S)	1. To determine the risk of disease progression, overall and event free survival, and non-relapse mortality following treatment with allogeneic CD8+ memory T-cells. 2. To determine the incidence of acute and chronic GVHD following infusion of allogeneic CD8 memory T-cells.
STUDY OBSERVATION PERIOD	Patients will be followed for 6 months from the time of transplant (about 4 month after the CD8+ memory T-cell infusion)
TREATMENT SUMMARY	The efficacy of non-myeloablative allogeneic transplantation is mediated in part by the establishment of an alloreactive donor T-cell compartment following transplant with minimal toxicity. A post-transplant infusion of allogeneic CD8+ memory T-cells may facilitate the conversion to full donor chimerism without an increase in graft versus host disease. This is a single institution open-labeled single treatment study evaluating the efficacy of allogeneic CD8+ memory T-cell infusion derived from HLA-matched sibling donors.
SAMPLE SIZE	20 patients plus 20 sibling donors
SUMMARY OF SUBJECT ELIGIBILTY	Patients with the following histologically-confirmed disease: 1. Acute myeloid leukemia (AML) 2. Chronic lymphocytic leukemia (CLL) 3. Non-Hodgkin lymphoma (NHL) 4. Hodgkin lymphoma (HL) 5. Myelodysplastic syndrome (MDS) / Myeloproliferative disease syndrome (MPD) Patients not undergoing a full dose transplant conditioning Patients with HLA-matched or single antigen/allele mismatched related donors.
CONTROL GROUP	Historically-matched control patients

SCHEMA



[1] TLI = total lymphoid irradiation
[2] ATG = anti-thymocyte globulin
[3] CsA = cyclosporine
[4] PBSC = peripheral blood stem cells. The PBSC infusion procedure is called a hematopoietic cell transplant (HCT) or donor leukocyte infusion (DLI)
[5] MMF = mycophynelate mofitel

1. OBJECTIVES

1.1 Primary Objective

To determine the rate of conversion to full donor chimerism (FDC) following a post-transplant infusion (Day 30 to 75) of freshly enriched allogeneic CD8+ memory T-cells in patients with AML, NHL CLL, or HL, who received non-myeloablative TLI ATG transplant conditioning.

1.2 Secondary Objectives

1. To determine the risk of disease progression, overall and event free survival, and non-relapse mortality.
2. To determine the incidence of acute and chronic GVHD following the infusion of allogeneic CD8+ memory T-cells.

2. BACKGROUND

2.1 Allogeneic Hematopoietic Cell Transplantation

Allogeneic hematopoietic cell transplantation (HCT) is proven effective therapy for patients with a variety of hematolymphoid malignancies. Following allogeneic HCT, a significant percentage of patients who were otherwise considered to have incurable cancers using best of care non-transplantation therapies have long-term disease free control and appear 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 after exhausting all chemotherapy, and radiation therapy based treatment strategies. Often many of the patients who undergo allogeneic HCT for treatment of their lymphoma have had disease relapse even after high dose chemotherapy and autologous stem cell rescue (autologous HCT).

2.2 Relapse of Disease following Allogeneic HCT

The main cause for treatment failure following allogeneic HCT is disease relapse. Depending, in part, on the disease, disease status at the time of transplantation, and the intensity of the transplantation regimen, disease relapse occurs in roughly 25 to 80% of patients. For example, following a full dose transplant in younger patients with acute leukemia in 1st or 2nd complete remission (CR) and using a graft from an HLA-matched sibling, the 2-year risk of disease relapse was < 30% [1-8]. In contrast, the 2-year risk of disease relapse for younger patients with refractory acute leukemia who received a full dose transplant was > 70% [9-11]. Older (> 55 years of age) patients or younger patients with medical co-morbidities that preclude full dose conditioning undergo reduced intensity conditioning (RIC) allogeneic HCT. The 2-year risk of disease relapse in patients with acute leukemia in CR who received RIC allogeneic HCT was > 50% [12, 13]. Similarly, disease relapse remains the major cause of treatment failure for patients with T-cell, large B-cell and Hodgkin lymphomas and chronic lymphocytic leukemia (CLL) and occurs in > 40% who undergo RIC allogeneic HCT with chemotherapy, sub-lethal total body irradiation (TBI), or total lymphoid irradiation combined with anti-thymocyte globulin (TLI/ATG) [13-18].

2.3 Treatment for Disease Relapse Following Allogeneic HCT

Disease relapse following allogeneic HCT remains an ominous clinical event, which usually results in progressive disease and death. Treatment strategies of patients with relapse following

allogeneic HCT can often be further complicated by the overall general poor clinical status of the patient. Strategies for the treatment of disease relapse in these patients include the cessation of post-transplantation immune suppression medication, salvage chemotherapy with or without irradiation, a second allogeneic HCT, or the infusion of donor leukocytes (DLI). Despite these interventions few patients beyond those with chronic myelogenous leukemia (CML) can be returned to durable remissions and the overall survival (OS) at 2 years following disease relapse was < 20% [19, 20]. Given that disease relapse is relatively common after transplant, and that few if any patients can be returned to durable remission once relapse has occurred, a desired goal would be to improve the transplant strategy and avoid the serious problem of post-transplant disease relapse.

2.4 Graft Versus Tumor Reactions

A major mechanism of cancer eradication following allogeneic HCT is the immunological-based recognition of residual host tumor cells by donor-derived immune cells contained in the donor graft. This phenomenon, termed graft-versus-tumor (GVT) reactions, is supported by various lines of evidence. The initial observations in support of GVT reactions stemmed from studies that used T-cell depleted (TCD) donor grafts whereby graft-versus-host disease (GVHD) was eliminated; however, without donor T-cells in the donor inoculum patients suffered a high incidence of disease recurrence [21]. The importance of GVT reactions was further demonstrated by improved disease control and fewer relapses following HLA identical sibling (allogeneic) transplantation compared to syngeneic (identical twin) transplantation for patients with hematologic malignancies [22]. The three year probability for relapse of leukemia was substantially higher following syngeneic compared to allogeneic HCT in acute myelogenous leukemia (AML) (52% vs 16%) and CML (40% vs 7%) following treatment using full dose transplant conditioning regimens [22]. The increased risk of relapse in syngeneic transplants counterbalanced the beneficial effect of the lack of GVHD. Perhaps the most compelling evidence comes from the observation that the infusion of donor leukocytes obtained from the original donor in patients who have disease relapse following allogeneic HCT resulted in a GVT effect (23).

2.5 Reduced Intensity Transplant Conditioning

Following the recognition that a main mechanism of disease control after allogeneic HCT is by donor derived immune mediated GVT reactions, multiple groups, including our own at Stanford, developed reduced intensity transplant conditioning (RIC) regimens that shifted the burden of disease control from high doses of chemoradiotherapy to the donor immune system (24-28). The low intensity transplant conditioning has allowed older patients and those with medical co-morbidities to proceed with transplant whereas, before these patients were excluded from allogeneic transplantation as the full dose conditioning regimens were associated with prohibitive toxicities. As a consequence of the low dose conditioning, host hematopoietic and immune cells persist and result in a state of mixed hematopoietic and lymphoid chimerism (MC) that may convert to full donor chimerism (FDC) over a variable time course (13, 28-30). Importantly, the achievement of early (before Day 60) FDC appears to be a necessary precondition for disease control (13, 28-30). We and others have reported that irrespective of the specific type of RIC regimen used, the relapse risk is significantly higher in patients with persistent MC compared to those with FDC, and likely reflects tolerance of donor cells to recipient tissues (13, 28-30). In a report detailing the outcomes of 111 allogeneic HCT recipients from HLA-matched and single antigen mismatch donors following TLI-ATG conditioning for leukemia and lymphoma there was a very low incidence of severe (Grade 2 to4) acute GVHD (< 5%), and a low incidence of

non-relapse related mortality at 1 year (< 5%) (13). Whereas almost all patients who failed to achieve FDC experienced disease relapse, the actuarial event free survival was more than 55% in the patients who achieved > 95% donor type chimerism, with observations extending up to 6 years (13).

2.6 Strategies to Convert MC to FDC

Attaining FDC relatively early post-transplant is desirable because of its significant association with a reduced risk of disease relapse (13, 28-36). A commonly used strategy to convert MC to FDC has involved the use of low dose, or escalating doses, of donor lymphocyte infusions (DLI) at designated early time points post-transplant [31-36]. These studies confirmed that prophylactic DLI (i.e. before disease progression) induced conversion to FDC and was associated with a reduced risk of disease relapse, yet overall survival was not improved in these trials due to the increased risk of significant acute GVHD (31-36).

In summary, disease relapse is the main cause of treatment failure following allogeneic HCT and is an ominous event as most patients cannot be returned to durable remission. A significant percentage of patients who receive reduced intensity conditioning remain as MC for months and there is a significant association of MC with increased risk of disease relapse. Conversion of MC to FDC following low dose or escalating doses of DLI was associated with a reduced risk of disease relapse yet OS was not improved due to the limiting toxicity of severe acute GVHD. A pre-emptive strategy that would promote conversion of MC to FDC and retain immune mediated GVT reactions yet without promoting GVHD has the potential to benefit patients and may improve the cure rate in patients following allogeneic HCT.

2.7 Preclinical Strategies to Overcome GVHD with DLI in a Murine Model Memory T-cells Induce Significantly Less GVHD than Naïve T-cells

Recent murine studies showed that in many strain combinations memory T-cells, including memory CD4+, and memory CD8+ T-cells induce significantly less GVHD than naive T-cells ($CD62L^{hi}CD44^{lo}$) or combinations of naive and memory T-cells [37-43].

Accordingly, our group at Stanford compared freshly isolated naive CD4+, CD8+, or total T-cells, and/or memory $CD4+CD44^{hi}$, $CD8+CD44^{hi}$, and total memory T $CD44^{hi}$ cells from unprimed donors for their capacity to induce GVHD, and mediate antitumor activity against a naturally occurring B-cell lymphoma (BCL1) in an MHC-mismatched model where CD4 is predominant yet CD8 also can induce lethal GVHD (44). 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 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 [45].

Taken together, our studies and those by others suggest that CD8+ memory T-cells will have retained promote donor cell engraftment and retain GVT reactions yet have markedly reduced GVHD capacity. These studies support the hypothesis that a clinical trial of infusing CD8+ memory T-cells may be superior to DLI for the conversion of MC to FDC following allogeneic HCT.

2.8 Purification of human CD8$^+$ memory T-cells for use as DLI therapy

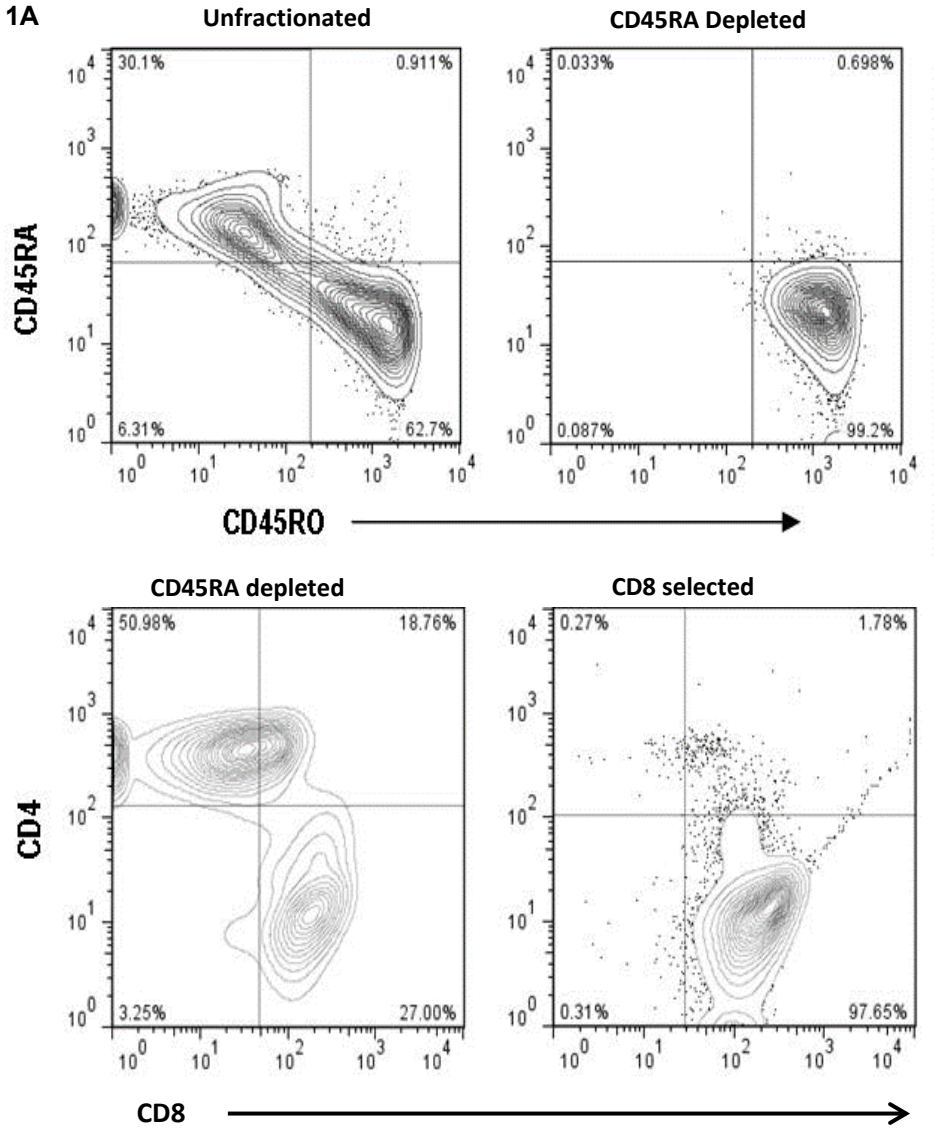


Figure 1A, Representative flow analysis of peripheral blood apheresis collections from the pre-selection, post-CD45RA depletion, and CD8 enrichment steps and stained for expression of CD4, CD8, CD45RA, and CD45RO after gating on CD3+ cells.

Since we were successful in treating progressive tumor growth after BMT without inducing GVHD in mice with enriched donor CD8$^+$ memory T-cells, we attempted to purify a similar subset of freshly isolated T-cells from normal humans for use in the currently planned study. Accordingly, we used immunomagnetic bead separation on Miltenyi columns to enrich CD8$^+$CD45RO$^+$CD45RA$^-$ T-cells from PBMCs (46). First, cells were incubated with Miltenyi

GMP grade anti-CD45RA mAb conjugated beads for negative selection. Thereafter, the CD45RA- cells were incubated with Miltenyi GMP grade anti-CD8 mAb conjugated beads to positively select the CD8+ cells. The resultant $CD8^+CD45RA^-$ T-cells had over 95% purity as shown in the FACS pattern in Figure 1A that is representative of 6 independent separations. Almost all the $CD45RA^-CD8^+$ cells were $CD45RO^+CD8^+$. Yields of the cells were also determined using apheresis products from normal HCT donors and the mean post-enrichment cell count obtained from a single apheresis product was 5×10^8 with a range of 3 to 8×10^8 $CD45RA^-CD8^+$ cells. Therefore, following 2 high volume aphereses per donor, the highest feasible dose of memory $CD8^+$ T-cells available for infusion will be 10×10^8 cells. Assuming most patients weigh less than 100 kg, attainable numbers of memory $CD8^+$ T-cells for a phase 1 dose escalation trial are 1×10^6/kg; 5×10^6/kg; and 10×10^6/kg. IND 014844 has been submitted to the FDA describing the use of manipulated donor lymphocyte infusion (DLI) cells in clinical trials.

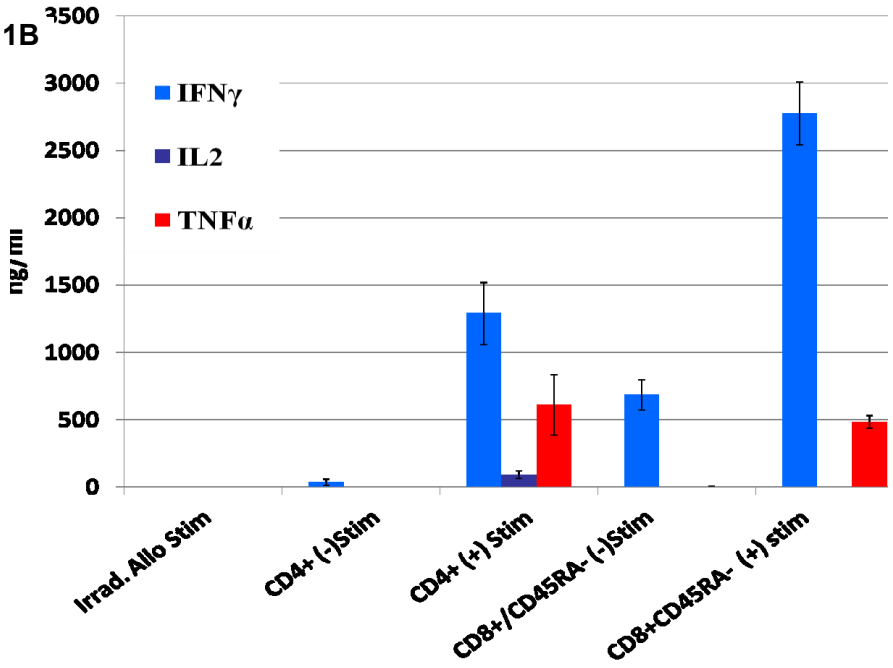


Figure 1B, Cytokine secretion assessment in $CD8^+/CD45RA^-$ and $CD4^+$ cells activated by co-culture with or without irradiated allogeneic stimulators (Stim). Supernatants from 7-day cultures were analyzed by flow cytometry using Cytokine Bead Arrays for INFγ; IL-2; and TNFα (n = 4). Results are shown with +/- standard deviation (SD)

In further studies the enriched $CD45RA^-CD8^+$ human memory T-cells were tested for immune reactivity *in vitro* by stimulation with irradiated allogeneic PBMCs from normal donors. The cultures were monitored for 3H-thymidine incorporation at 7 days and for IFN-γ and IL-2 concentrations in the supernatants. The responder T-cells increased the mean 3H-thymidine incorporation in cultures with allogeneic stimulator cells as compared to control cultures without allogeneic stimulators (data not shown). In addition, the memory $CD8^+$ T-cell supernatants had a marked increase in the concentration of IFN-γ after culture with allogeneic stimulator cells, and had a minimal increase in the concentration of IL-2 (Figure 1B). The results of the human mixed

leukocyte response (MLR) experiments were consistent with the responses observed with $CD8^+$ memory T-cells from mice. The murine responder cells also showed a marked increase in 3H-thymidine incorporation after stimulation with allogeneic cells, and the production of IFN-γ was considerably greater than that of IL-2 [46].

In summary, these studies demonstrated that an enriched population of CD8+ memory T-cells can be obtained from healthy human donors that in MLR experiments were consistent with the responses observed with CD8+ memory T-cells from mice and provided evidence for clinical safety (limited GVHD) and efficacy (retained GVT reactions).

2.9 Phase 1 Clinical Trial of CD8+ Memory T-cell Infusion for Disease Relapse after Allogeneic HCT

We conducted a single institution open-labeled single treatment study evaluating the safety and potential efficacy of allogeneic CD8+ memory T-cell infusion derived from HLA-matched sibling donors. The phase I study followed a standard 3+3 dose escalation format using a low CD8+ memory T-cell dose of 1 x 10^6 cells/kg, an intermediate dose of 5 x 10^6 cells/kg and a final dose of 10 x 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 patients 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.

To date, 8 patients with disease relapse after allogeneic HCT have been enrolled on the study and were followed for safety and potential efficacy. The Table below summarizes the patients.

Pt	Dose level	Dx	DOT	Date of Relapse	Status at CD8+ memory T-cell infusion	Date of CD8+ memory T-cell infusion	AE or GVHD	Follow-up (months)	Status at last F/U
1.1	1	AML	2-27-09	8-25-11	CR2	4-17-12	none	27	Alive in CR
1.2	1	AML	4-6-12	8-1-12	Not in CR	8-10-12	none	17	Expired
1.3	1	AML	08-14-03	4-17-12	CR3	10-11-12	none	20	Alive, Relapse
2.1	2	AML	07-17-12	1-4-13	CR2	6-6-13	none	14	Expired
2.2	2	BC- CML	08-29-07	2-1-13	CR	6-27-13	none	13	Alive in CR
2.3	2	CLL	09-03-10	3-15-12	Not in CR	7-17-13	none	3	Expired
3.1	3	AML	05-31-13	7-10-13	Not in CR	10-9-13	AE*, no GVHD	1	Expired
3.2	3	AML	03-15-13	11-18-13	Not in CR	4-2-14	AE*, no GVHD	6	Alive, Relapse

* The AE was unrelated to CD8+ memory T-cell infusion.

Detailed patient summaries are provided below and are divided by cell dose cohort,

Cohort 1: A dose of 1 x 10^6 CD8+ memory T-cells/kg

Patient 1.1: A 66-year-old female with AML relapse 910 days after TLI-ATG conditioned allogeneic transplant from her matched sibling donor. For disease relapse she received 4 cycles of myelotarg combined with azacytadine and achieved a complete marrow remission with < 5% marrow blasts yet had incomplete count recovery. She received the donor CD8+ memory T-cell infusion on 17 April 2012, and as per visit on 21 October 2014, she was 917 days post CD8+ memory T-cell infusion and continues in complete remission with normal blood counts and a marrow with no evidence of AML.

Patient 1.2: A 71-year-old female with AML who had disease progression 117 days after TLI-ATG conditioned allogeneic transplant from her matched sibling donor. The patient declined chemotherapy her post-transplant disease relapse. Therefore, CD8 memory T-cells were infused at a time of the patient had active disease. Post CD8+ memory T-cell infusion, the patient developed stable disease and was transfusion independent for 455 days. Fifteen months after the cell infusion the patient presented with leukemic cutis and declined therapy and she succumbed from progressive disease on Day 517 after the cell infusion.

Patient 1.3: A 40-year-old female with extra medullary leukemia relapse 3165 days after initial allotransplant. She received involved field radiation therapy to the extra-medullary site of disease and 4 cycles of Azacytadine and was returned to remission. On evaluation on 727 days after the CD8 memory T-cell infusion, she was alive yet had disease relapse 650 days after the cell infusion.

Cohort 2: A cell dose of 5 x 10^6 CD8+ memory T-cells/kg

Patient 2.1: A 37-year-old with AML who had disease relapse 169 days after allogeneic transplant from a matched sibling donor. The patient received 7+3 re-induction with cytarabine and Daunorubicin and failed to achieve a remission. Thereafter the patient received 2 cycles of G-CLAC (G-CSF, clofarabine and cytarabine) and achieved a marrow remission with incomplete blood count recovery. Following the CD8+ memory T-cell infusion the patient continued in remission with normal blood counts for 9 months and thereafter had chemorefractory resistant recurrence and expired from AML 418 days after the cell infusion.

Patient 2.2: A 60-year-old who underwent allogeneic HCT from a matched sibling donor for blast crises CML and developed a return of chronic phase CML 1829 days after transplant. The patient was returned to a complete remission with dasatinib and at last follow up continues in complete remission, 435 days after the CD8+ memory T-cell infusion.

Patient 2.3: A 53-year-old with multiply recurrent high risk CLL who had disease progression 545 days post allotransplant that progressed on salvage bendamustine-rituximab, and was treated to a stable disease using 3 cycles of FCR (fludarabine, cyclophosphamide, and rituximab). Shortly (32 days) after receiving the CD8+ memory T-cell infusion the patient had progressive disease and was transitioned to best supportive care and died 89 days after the cell infusion.

Cohort3: A cell dose of 10 x 10^6 CD8+ memory T-cells/kg

Patient 3.1: A 61-year-old woman with refractory AML at start of allotransplant conditioning despite several attempts to achieve remission using 3 different induction regimens. The patient had progressive AML 65 days post allotransplant and received salvage FLAG (fludarabine, high-dose cytarabine, G-CSF) chemotherapy and continued with refractory disease. For her

refractory disease the patient was infused with 7.8 x 10^6 CD8 memory T-cells/kg, instead of the desired cell dose of 10 x 10^6cells/kg. The patient continued with progressive disease and was transitioned to best supportive care and died 42 days after the cell infusion.

Patient 3.2: A 67-year-old man with overlap Myeloproliferative Disease and Myelodysplastic syndrome who on azacitidine progressed to AML. He failed to receive remission despite 2 courses of induction cytarabine and daunorubicin and underwent allogeneic HCT using his HLA-matched sibling donor. He had progressive AML 236 days after transplantation and received several cycles of re-induction chemotherapy using vorinostat with temozolomide yet his disease failed to respond. He received a CD8 memory T-cell donor lymphocyte infusion for his refractory disease; the dose of cells was 8 x 10^6 CD8 memory T-cells/kg, instead of the desired 10 x 10^6 CD8 memory T-cells/kg. At last follow-up 185 days after the cell infusion, the patient continues with a stable low level of AML and has not received additional chemotherapy.

Two adverse events, considered unrelated to the infused cell product were noted.

Patient 3.1 had neutropenia prior to the cell infusion and continued with neutropenia after the cell infusion. The neutropenia was related to active AML. Eighteen days after the CD8 memory T-cell infusion, the patient developed a fever that was treated to resolution with short course broad spectrum intravenous antibiotics and anti-fungal therapy. All cultures were negative. The reported adverse event was most likely UNRELATED to the investigational product. The serious adverse event (SAE) was reported to MedWatch on 6 November 2013.

Patient 3.2 had neutropenia prior to the cell infusion and continued with neutropenia after the cell infusion. The neutropenia was related to active AML. The patient developed fevers 33 days after the cell infusion. Imaging studies at the time revealed probable new onset pneumonia. The pneumonia was treated to resolution with broad spectrum antibiotics. A bronchoscopy revealed light growth of MSSA. The patient completed a full course of rocephin, azithromycin, and fluconazole. The PCP stain was negative. The patient continues with active AML. The adverse event was most likely UNRELATED to the investigational product.

2.10 Lymphodepletion with cyclophosphamide to promote CD8+ memory T-cell expansion and persistence

Lymphodepletion with cyclophosphamide administered several days prior to DLI has been shown to promote in vivo expansion and persistence of the infused donor lymphocytes by providing lymphoid space, eliminating anti-donor immune reactivity, and reducing competition for growth factors that promote expansion of the infused T-cells [47-49]. Lymphodepletion with cyclophosphamide is thus routinely utilized prior to the infusion of various autologous or allogeneic cellular therapies where it has been shown to augment the antitumor activity of unmanipulated DLI [50, 51], chimeric antigen receptor-modified T cells [52, 53], and adoptively transferred tumor-specific T cells [54, 55]. We therefore propose administering a single dose of cyclophosphamide 1.5 g/m2 intravenously 3-5 days prior to the CD8+ memory T-cell infusion in order to promote further expansion and persistence of the cells, augment GVT activity, and reduce relapse rates.

The Cell Processing and Quality Assurance data are highlighted below.

Cell Processing Summary

UPI	Pt.	CD8+CD45RA- cells			% Recovery	% CD8+CD45RA-
		Starting	Post CD45RA reduction	Post CD8 enrichment		
TC00206	1.1	$7.52x10^8$	2.97E+08	$5.15x10^7$	6.8%	63.0%
TC00210	1.1	$5.33x10^8$	2.48E+08	$9.68x10^7$	18.2%	82.6%
TC00229	1.2	$9.14x10^8$	7.71E+08	$2.34x10^8$	25.6%	81.4%
TC00233	1.3	$1.62x10^9$	$8.88x10^8$	$8.76x10^8$	54.1%	96.8%
TC00252	2.1	$3.20x10^9$	$2.68x10^9$	$1.20x10^9$	37.5%	96.3%
TC00253	2.2	$2.38x10^9$	$1.96x10^9$	$4.63x10^8$	34.6%	96.8%
TC00259	2.3	$4.27x10^9$	$4.31x10^9$	$1.89x10^9$	44.3%	99.4%
TC00265	3.1	$9.05x10^8$	$9.94x10^8$	$5.57x10^8$	61.5%	97.6%
TC00285	3.2	$1.17x10^9$	$1.19x10^9$	$6.54x10^8$	55.9%	98.6%

CD8+ memory T-cell Quality Assurance Assessment

UPI	Sterility	Endotoxin	Viability	Recovered Dose	Distribution
TC00206	no growth detected	<0.5EU/kg	99%	$0.8x10^6$/kg	No
TC00210	no growth detected	<0.5EU/kg	99%	$1.2x10^6$/kg	Yes
TC00229	no growth detected	<0.5EU/kg	98%	$2.5x10^6$/kg	Yes
TC00252	no growth detected	<0.5EU/kg	94%	$2.0x10^7$/kg	Yes
TC00253	no growth detected	<0.5EU/kg	96%	$8.0x10^6$/kg	Yes
TC00259	no growth detected	<0.5EU/kg	96%	$2.6x10^7$/kg	Yes
TC00265	no growth detected	<0.9EU/kg	98%	$7.8x10^6$/kg	Yes
TC00285	no growth detected	<0.5EU/kg	98%	$8.0x10^6$/kg	Yes

From the clinical trial the following observations are made

1. The CD8 memory T-cell infusion was not associated with any infusion related toxicities, or related adverse events such as the development of GVHD, or other.
2. The CD8 memory T-cell infusion appeared to have some efficacy albeit patients who have relapse of disease after allogeneic transplant have a poor overall survival at < 20% at 2 years [19, 20]. Among the 8 patients treated, one patient continues in CR more than 2 years after the CD8 memory T-cell infusion, one patient with untreated relapse continued with disease that did not progress and transfusion independence for > 1 year, and 2 additional patients remain alive but with disease relapse with observations extending to beyond a year.
3. Although no toxicities were observed at any dose, the maximum cell dose of 10×10^6 CD8+ memory T-cells/kg is **not** a feasible dose for use in a clinical trial due to our inability to obtain this high number of CD8 memory T-cells. Rather, a dose of 5×10^6 cells/kg is readily obtainable from all donors.

2.11 Study Rationale

The administration of CD8+ memory T-cells at doses of $\leq 10 \times 10^6$ cells/kg did not result in GVHD, or other related adverse events. The persistence of mixed hematopoietic cell chimerism is associated with a significantly high risk of disease relapse after allogeneic HCT [13, 28-36]. Once the patient's disease has relapsed after allogeneic HCT, the likelihood for long-term survival is poor. Therefore, a strategy that can help promote conversion of mixed to complete hematopoietic cell chimerism prior to disease relapse, and does not promote significant GVHD has the potential to help improve the outcomes of allotransplant recipients by reducing the risk of disease relapse. The goal of the current proposed trial will be to determine if $CD8^+$ memory T-cells administered prophylactically and prior to disease relapse can convert patients with mixed chimerism at 30 to 75 days after transplant to complete chimerism. We **hypothesize** that patients who convert from mixed to complete chimerism will have a reduced rate of relapse similar to patients who are complete chimeras. This study will follow a single arm Simon 2-stage trial principle. In Simon stage 1, we will enroll 8 patients, and if 3 or fewer patients respond, defined as the conversion from mixed to complete chimerism within 90 days of the $CD8^+$ memory T-cell infusion, we will stop for futility. If 4 or more respond in Simon stage 1, then in stage 2 we will continue to enroll a full sample of 20 patients and declare success if a total of 11 or more patients respond. The time frame for the $CD8^+$ memory T-cell infusion will be between 30 to 75 days after transplant, and all patients will continue with the planned immune suppression drug taper to discontinuation at 6 months after transplant providing no GVHD is observed. The primary endpoints will be safety and determination of the rate of conversion from mixed to complete donor chimerism. If > 2 patients among the first 6 enrolled experience any new Grade 3 to 4 toxicities or > 2 patients develop Grade 3 to 4 GVHD during the 60 days following the $CD8^+$ memory T-cell infusion, we will de-escalate the dose to 1×10^6 CD8+ memory T-cells/kg. Based on our preclinical data that CD8+ memory T-cells do not aggravate GVHD, and the data from the clinical phase 1 trial we do not expect toxicities at a dose of 5×10^6/kg. Secondary endpoints will include the risk of acute and chronic GVHD, freedom from disease progression, event-free and overall survival, and the relapse rate. The goal is to improve the rate of attaining complete chimerism without increasing the risk of GVHD. If following the Simon 2-stage trial the results are

lncouraging, we will expand our experience to a broader trial to determine if the infusion of $CD8^+$ memory T-cells is associated with significantly lower rates of disease relapse.

Study Design

This is a single institution open-labeled single treatment study evaluating the safety and efficacy of allogeneic CD8+ memory T-cell infusion derived from HLA-matched sibling donors. Enrollment of 18 patients is expected to be completed in less than 3 years. The study will involve a single non-randomized intervention arm of administering 5 x 10^6 cells/kg CD8+ memory T-cells 30 to 75 days after allogeneic HCT using TLI ATG conditioning. The total duration of patient study participation is 6 months. Patients will be followed indefinitely for all outcomes including survival, event-free survival and late complications such as but not limited to late chronic GVHD and infections.

3. PARTICIPANT SELECTION AND PRE-ENROLLMENT REQUIREMENTS

Refer to the Participant Eligibility Checklist in Appendix A.

3.1 Patient Inclusion Criteria for Transplant (patients must have all of the following**).**

3.1.1 Between 18 and 80 years of age, inclusive.

3.1.2 Has a HLA-matched or single allele-mismatched adult sibling serving as donor.

3.1.3 Has a myeloid or lymphoid malignant disease that is treated with TLI and ATG reduced-intensity conditioning for allogeneic transplant [any of the following: acute myeloid leukemia (AML); chronic lymphocytic leukemia (CLL); B or T-cell non-Hodgkin lymphoma (NHL); Hodgkin lymphoma (HL); Myelodysplastic syndrome (MDS); or Myeloproliferative disease syndrome (MPD)]

3.1.4 Patients who due to age, pre-existing medical conditions, or, prior therapy are considered to be at high-risk for regimen-related toxicity associated with fully ablative transplant conditioning, and therefore reduced intensity conditioning is recommended.

3.1.5 Ability to understand and the willingness to sign a written informed consent document. Patients must have signed informed consent to participate in the trial.

3.2 Patient Exclusion Criteria for Transplant (excluded if any of the following)

3.2.1. Uncontrolled bacterial, viral or fungal infection defined as currently taking medication and progression of clinical symptoms.

3.2.2. Progressive hemato-lymphoid malignancy despite conventional therapy

3.2.3. Acute leukemia not in remission

3.2.4. Chronic myelogenous leukemia (CML)

3.2.5. Active CNS involvement of the underlying malignancy

3.2.6. HIV-positive

3.2.7. Pregnant or lactating

3.2.8. Prior malignancy (EXCEPTION: diagnosed > 5 years ago without evidence of disease, OR treated ≤ 5 years ago but have a greater than 50% chance of life expectancy of ≥ 5 years for that malignancy).

E.2.9. Have a psychiatric disorder(s) or psychosocial circumstance(s) which in the opinion of the primary physician would place the patient at an unacceptable risk from transplant.

3.2.10. Organ dysfunction defined as follows:

i. Ejection fraction < 30%, or uncontrolled cardiac failure

ii. DLCO < 40% predicted

iii. Total bilirubin > 3 mg/dL

iv. SGOT or SGPT > 4 x ULN

v. Creatinine > 2 mg/dL and an estimated creatinine clearance < 40 mL/min

j. Poorly controlled hypertension despite multiple antihypertensive medication

k. KPS < 60%

l. Note: Patients positive for hepatitis B and C will be evaluated on a case-by-case basis

3.3 Donor Inclusion/Exclusion Criteria (To be eligible to participate in this trial, donors must meet the following criteria):

3.3.1 HLA-matched or single allele mismatched sibling of enrolled transplant patient

3.3.2 18 to 80 years of age, inclusive

3.3.3 In a state of general good health and have 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.

3.3.4 White blood cell count >3.5 x 10^9/liter, platelets >150 x 10^9/liter and hematocrit > 35%

3.3.5 Capable of undergoing leukapheresis

3.3.6 Able to understand and sign informed consent

3.3.7 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.

3.3.8 Females must not be pregnant or lactating

3.3.9 No psychological traits or psychological or medical conditions which make them unlikely to tolerate the procedure

3.3.10 Has not developed a new malignancy requiring chemotherapy or radiation in the interval since apheresis for initial HCT

3.4 Patient Criteria for Proceeding with CD8+ Memory T-cell Infusion: On Study enrollment date.

3.4.1 Patients must be beyond Day 30 and before Day 75 after transplant.

3.4.2 Patients must have evidence of mixed CD3 T-cell chimerism based on the Day +28 (+/- 7 days) blood sample showing ≥ 30% and <90% donor type cells.

3.4.3 Patients must have no evidence of active graft-versus-host disease at the time of the CD8+ memory T-cell infusion. Patients with a history of acute GVHD overall Grade 2 based on skin only involvement or upper GI tract involvement only will be eligible. Patients with a history of Liver or lower GI tract GVHD will not be eligible.

3.4.4 Patient must not have clinical evidence of disease progression prior to the CD8+ memory T-cell infusion.

3.4.5 Patients must be on single immune suppression therapy with either Tacrolimus or Cyclosporine at the time of CD8+ memory T-cell infusion. Prednisone at a physiologic dose of 5mg per day or less is allowed.

3.4.6 Patients must have a Karnofsky performance status of ≥ 60% at the time of the CD8+ memory T-cell infusion.

3.4.7 Patients must not have an uncontrolled bacterial, fungal or viral infection, defined as progressive symptoms despite therapy, at the time of the CD8+ memory T-cell infusion. Asymptomatic viremia is allowed.

3.4.8 Patients must have adequate organ function and performance status at the time of the CD8+ memory T-cell infusion, defined by the following:

i. Total bilirubin ≤ 4 mg/dL

ii. SGOT or SGPT ≤ 4 x ULN

iii. Creatinine ≤ 3 mg/dL or estimated creatinine clearance ≥ 40 mL/min

3.5 Donor Evaluation for CD8+ Memory T-Cell Collection

Donors must be the HLA-matched sibling who served as the donor for the allogeneic transplant. Within 7 days of cell collection, donors will be screened to confirm eligibility. Screening will consist of the following evaluations and procedures.

- A transplant physician together with the study coordinator will review the prior medical record that was performed for the initial transplant.
- Under the direction of the study coordinator, donors will fill out a standard Stanford BMT self-assessment form that highlights all new medications prescribed from the time of initial donation, as well as any new medical diagnosis or surgeries.
- Any newly prescribed medication, new medical diagnosis, or surgery since the time of the initial collection for transplant will initiate a formal medical re-evaluation.
- If no new medications or new diagnosis have occurred, and no surgery, then just as what is done for the NMDP and collection of a DLI, the prior medical evaluation will suffice providing it is ≤ 120 days from date of initial medical evaluation to the date of starting the CD8+ memory T-cell collection.

Documentation of vital signs (temperature, blood pressure, pulse rate, weight).

Donors will be evaluated with the following laboratory tests within 14 days of starting apheresis for the collection of CD8+ memory T-cells:

- CBC with complete blood count.
- Clinical chemistry assessments including sodium, potassium, chloride, creatinine, random glucose, total calcium, total protein, albumin, total bilirubin, alkaline phosphatase, SGOT, SGPT.
- A coagulation profile including PT and PTT
- Female donors of child-bearing potential will undergo serum HCG testing which must be negative to proceed.

Donors will be screened within 7 days of the CD8 memory T-cell collection to confirm eligibility by undergoing repeat testing for:

- 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.
- ABO/Rh will be performed.

Once donors have been appropriately screened, they will proceed to cell harvest.

3.6 Informed Consent Process

A conference will be held with the patient and family to discuss this study and alternative treatments available for treatment of the underlying disease. The attending physician will conduct the conference. All potential risks associated with the use of TLI, ATG, immunosuppressive drugs and allogeneic hematopoietic cell infusions including and CD8+ memory T-cell cells will be discussed as objectively as possible. It will be explained that patients offered this protocol have an underlying malignancy that render them either at high risk of relapse or that will result in life expectancies of several months to no more than one to two years with conventional treatments. These patients would be unlikely to benefit from, or tolerate an autologous transplant, and are at high risk of early transplant mortality from conventional allogeneic transplant. Informed consent from the patient will be obtained using a form approved by the Administrative Panel on Human Subjects in Medical Research of the Stanford University Medical Center. The participant will receive a copy of the signed and dated consent document. The original signed copy of the research consent document must be retained in the subject's research file.

3.7 Study Timeline

It is expected the study will accrue 7 patients per year and therefore the target goal of 20 patients will be completed within 2.5 years of the study opening.

Patients will be followed for 6 months after the mobilized PBSC infusion on Day 0. Patients' End-of-Study Visit will be scheduled for approximately Day 180 post-PBSC infusion, +/- 3 weeks. Therefore, it is expected the study will be complete within 36 months of opening. All patients will be followed for outcomes beyond the 6 months study observation period for determination of secondary outcome measures and as per institutional standards for transplant patients.

4. TREATMENT PLAN

4.1 Study Treatment Schedule

Transplant Schedule

Week 1

Monday Day -11	Tuesday Day -10	Wednesday Day -9	Thursday Day -8	Friday Day -7	Saturday Day -6	Sunday Day -5
TLI 120 cGy	TLI 120 cGy	TLI 120 cGy	TLI 120 cGy	TLI 120 cGy	Rest	Rest
ATG 1.5 mg/kg + solumedrol 1.0 mg/kg	ATG 1.5 mg/kg + solumedrol 1.0 mg/kg	ATG 1.5 mg/kg + solumedrol 1.0 mg/kg	ATG 1.5 mg/kg + solumedrol 1.0 mg/kg	ATG 1.5 mg/kg + solumedrol 1.0 mg/kg		

Week 2

Monday Day -4	Tuesday Day -3	Wednesday Day -2	Thursday Day -1	Friday Day 0	Saturday Day +1	Wednesday Day 25-70	Friday Day 30-75
TLI 120cGy	TLI 120 cGy	TLI 120 cGy	TLI 120cGy x 2 doses	Mobilized PBMC		Cytoxan 1.5 g/m^2 IV x1	CD8+ memory T-cell infusion
	Start Oral CSP →	CSP	CSP	CSP	CSP	CSP	CSP →
				Start Oral MMF	MMF	MMF stopped Day +28	

4.2 Apheresis

HLA-matched donors will undergo unmobilized apheresis for collection of CD8+ memory T-cells. Sufficient numbers of cells will be obtained following 1 to 2 apheresis procedures on consecutive days such that the target cell dose at the end of the selection will be achieved. Plasmapheresis, including volumes and frequency of collections, will be in accordance with 21CFR§640.65.

4.3 Cyclophosphamide lymphodepletion

Patients will receive a single dose of cyclophosphamide 1.5 g/m^2 intravenously over 2 hours as lymphodepletion 3-5 days prior to the CD8+ memory T-cell infusion in order to promote further expansion and persistence of the infused T cells (see section 2.10).

4.4 Administration of Study Drug

Selected CD8+ memory T-cells meeting the release criteria (described below) will be infused no more than 48 hours after the harvest. CD8+ memory T-cells will be taken to the recipient and infused fresh without cryopreservation. The cells will be infused through a central venous catheter or peripheral IV of at least 19 gauge. CD8+ memory T-cell infusion will take place over 10 to 20 minutes, just like unmodified DLI.

IN Patients will be monitored for vital signs (temperature, blood pressure, pulse rate) every 30 minutes following infusion and for at least two hours afterwards with evaluation every 30 minutes. WHO toxicity will be performed as described. Recipients will be monitored for infusion related toxicities and treated as needed. Premedication is not required and, if possible, should be avoided. If clinically indicated, Benadryl and Tylenol can be given as needed. Benadryl and will be utilized should a reaction occur and epinephrine and solumedrol will be available at the bedside.

4.5 Criteria for Removal from Study and not proceeding with the Planned CD8+ memory T-cell Infusion

All adverse events will be reviewed by Stanford Cancer Institute Data and Safety Monitoring Committee (DSMC) and patients will be removed from the study for the following reasons:

- Excess toxicities are observed such as acute GVHD beyond Grade 2 skin or upper GI tract only GVHD, uncontrolled infection or organ performance status not meeting criteria for cell infusion.
- Any other cconcurrent illness that prevents further administration of CD8 + memory T-cell Infusion
- Non-compliance with the protocol (defined as inability to receive all scheduled treatments, follow-up appointments, and tests)
- Patient request to withdraw from the study

The number of patients who do not receive the planned CD8 memory T-cell infusion will be followed. If for any reason more than 1 of the first 3 patients, or more than 2 of the first 6 patients, or more than 3 of the first 10 patients do not receive the planned infusion, the trial will temporarily close pending a DSMC audit to determine the cause. A patient enrolled on the trial but who does not proceed with the planned infusion will be replaced with another enrolled patient as the goal is to evaluate 20 patients who receive the CD8+ memory T-cell product.

4.6 Alternatives

Alternative therapies will be discussed with each patient and would potentially include allogeneic transplantation using TLI and ATG conditioning without a planned CD8+ memory T-cell infusion or allogeneic transplantation using other transplant conditioning or other investigational therapies, or best of care non-transplantation therapy.

4.7 Compensation

There will be no compensation for participation on study.

5. INVESTIGATIONAL AGENT INFORMATION

5.1 Investigational Agent

CD8+ Memory T-Cell Selection

The patient's original HLA-matched donor will undergo apheresis with collection of blood mononuclear cells as for standard DLI. No mobilization agents will be administered. Donor CD8+/CD45RA- T-cells will be isolated by tandem immunomagnetic selection using the CliniMACS Cell Selection System (Miltenyi Biotec). Depletion of CD45RA expressing cells uses CliniMACS CD45RA Reagent (BB-MF-11872). The CD45RA depleted fraction is enriched for

CD8 expressing cells by positive cell selection with CliniMACS CD8 Reagent (BB-MF-11704). In our phase 1 study, we evaluated the $CD45RA^{-}CD8^{+}$ cell yield from 8 normal donors that underwent either 1 or 2 aphereses. The mean starting TNC count was 6.8×10^{10}, the mean post-CD45RA depletion cell count was 4.5×10^{10}, and the mean post-CD8 enrichment cell count was 6.6×10^{8} with a range of 1.5 to 18.0 x $CD45RA^{-}CD8^{+}$ cells. The lower end range cell yields were in donors who underwent 1 apheresis and the higher end range cell yields were following 2 consecutive days of apheresis. In combination, we completed 4 validation runs, combined with 8 donor runs for the phase 1 protocol, our efficiency of percent CD8+ memory T-cell recovery has dramatically increased from < 20% to > 50%. Therefore, following 1 or 2 consecutive days of aphereses per donor, we have data to validate that we will achieve the target cell dose of 5×10^{6} CD8+ memory T-cells/kg for all patients, assuming most patients weigh less than 120 kg. If the number of CD8+ memory T-cells be below the desired threshold of 5×10^{6}/kg, the infusion will proceed as planned as the aggregate data support the importance of conversion from mixed to complete chimerism to decrease the risk of post-transplant disease relapse (13, 28-36). Therefore we believe it is in the patient's best interest to receive the additional cell infusion as this has the potential to help achieve complete chimerism. It is expected that a cell yield of less than the desired cell dose will be obtained less than 10% of the time.

Stability

Prior to initiating the phase 1 study we completed four validation runs and found > 90% viability based on the immediate post selection viability. We also have stability data on the 4 validation runs that extend to 48 hours of storage and found minimal loss in cell viability and all validation products continued with > 90% viability. The phase 1 study added stability data from an additional 8 donors that extended to 48 hours of storage and confirmed > 94% viability in all cases.

Reagents for cells for immediate release and conditions for storage

For post selection products infused the same day the Miltenyi Biotech Selection Buffers that contain PBS with 1mmolar EDTA and 0.5% HSA will be used. The CD8 and CD45 reagent systems are pharmaceutical grade and both have Master Files which are BB-MF-11704 and BB-MF-11872, respectively. For post selection products that will be stored for 24 or 48 hours prior to infusion, the product will be diluted for storage with 1 volume of Normosol-R with 2% HSA. For patient comfort the volume will be reduced to < or equal to 200 mL and the CD8+memory T-cell concentration will range between 1×10^{6} to 1×10^{8} cells/mL. The overnight storage temperature will be 4 degrees Celsius.

Release Requirements of the Product

Release of the selected cell products for fresh infusion includes:

- negative Gram staining (no organisms detected)
- endotoxin levels ≤ 0.5 EU/milliliter of infused product volume
- > 90% cell viability
- ≥ 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 > 2×10^{6} CD8+ memory T-cells/kg

Release Criteria for products stored 24 or 48 hours: due to the logistics of donor cell collection, cell processing and patient schedule, it is possible that some products may be stored for 24 or 48 hours before their infusion. Cell viability >90% is required for infusion of products not being stored. Release criteria for products stored 24 or 48 hours require >80% viability as assessed by trypan blue or 7-AAD dye exclusion.

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 patient'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 donor may undergo subsequent apheresis procedure(s) to collect a new product in an attempt to achieve a releasable fresh product for infusion. If at any dose level the minimum cell dose is not achieved the patient and donor may be taken off study. A patient removed from the study due to a failure to achieve the minimum CD8+ memory T-cell dose will be replaced with another patient.

5.2 Availability

CD8+ memory T-cell products will be prepared in the Stanford Blood and Marrow Transplantation Cellular Therapy Facility under the direction of laboratory director, Kevin Sheehan, PhD.
Dr Sheehan oversaw the manufacturing of the CD8+ memory T-cells for the phase 1 trial.

5.3 Agent Accountability

CD8+ memory T-cells meeting the release criteria will be administered according to the instructions specified in the approved study protocol. Infusions will be prepared and administered by a trained person designated by the investigator in response to orders written by the investigator.

6. DOSE MODIFICATIONS

The primary endpoints will be safety and determination of the rate of conversion from mixed to complete donor chimerism. In the first 6 enrolled patients (i.e., infused with CD8+ memory T-cells), if > 2 patients experience any new Grade 3 to 4 toxicities, or > 2 develop Grade 3 to 4 GVHD during the 60 days following the $CD8^+$ memory T-cell infusion, we will de-escalate the dose to 1 x 10^6 CD8+ memory T-cells/kg. In the first 12 enrolled patients, if > 3 patients develop Grade 3 to 4 GVHD during the 60 days following the $CD8^+$ memory T-cell infusion, or after the first 12 enrolled patients if > 4 patients develop Grade 3 to 4 GVHD during the 60 days following the $CD8^+$ memory T-cell infusion, the dose will be de-escalated as per above.

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 and 2 adverse events (AEs) are expected to occur, regardless of whether or not a patient is participating in a research study. In order to minimize the "background noise" and to focus on

clinically significant, impactful adverse events useful in the assessment of treatment effect, 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.

- Grade 3 and higher AEs will be collected and documented with causality attribution (see Section 7.2 Adverse Event Attribution) on an adverse event log (AE log).
- In addition, all AEs meeting the criteria of "serious" as defined at 21CFR§312.32(a), *including* any that are otherwise Grade 1 or 2, will be collected; identified as serious; and documented on an AE log, including causality attribution.
    - An adverse event is considered serious if it fulfills one of the following criteria per 21CFR§312.32(a):
        - Results in death
        - Life-threatening (patient at risk of death at the time of the event)
        - Requires inpatient hospitalization or prolongation of existing hospitalization
        - Results 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 patient or require intervention to prevent one of the outcomes listed above
- Laboratory values without a clinical consequence or outcome will not be tracked as adverse events, unless deemed serious.
- Grade 1 and 2 AEs not meeting these criteria will not be collected, except as follows. Grade 1 and 2 AEs resulting in subject dose modification (including dose termination) or withdrawal from the study will be collected.

All Serious Adverse Events (SAEs) will be tracked until resolution or at least 60 days after the last dose of 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 po).

The development of acute GVHD, its date of onset and overall grade, is a key secondary outcome measure for patients on this trial. Please refer to Section 6 "Dose Modifications" that highlight the safety rules with respect to GVHD development. Acute GVHD will be recorded on an AE log.

7.2 Adverse Event Attribution

As noted, HCT is an aggressive therapy for the treatment of cancer, and a large number of AEs are expected in this setting, including a number of specific AEs that are associated with the underlying disease; therapies administered prior to HCT; health status of the transplant recipient including co-existing conditions; the preparative regimen for transplant; concomitant therapies intended to reduce transplant-related complications (eg, immunosuppressants for the prevention of GVHD); and treatment for complications of HCT.

The Protocol Director (PD) or designee will assess each Adverse Event (AE) to determine whether it is unexpected according to the Informed Consent, Protocol Document, or Investigator's Brochure, and related to the investigation. Unless specifically determined otherwise by the investigator, the following events are considered anticipated in this setting.

- Alopecia
- Anemia
- Anorexia
- Bleeding, including requiring transfusions
- Cardiac arrhythmias
- Central venous catheter infections
- Constipation
- Diarrhea
- Edema
- Fatigue
- Febrile episodes
- Gastritis
- Graft failure
- Graft versus host disease
- Hematuria
- Hypertension
- Hypotension
- Hypoxia
- Incontinence
- Infections, including sepsis
- Insomnia
- Laboratory abnormalities
- Mental status changes and mood alterations
- Mucositis
- Nausea
- Neutropenia
- Pain
- Pleural effusion
- Pneumonitis
- Rash
- Seizures
- Sinusoidal obstructive syndrome
- Tachycardia
- Thrombocytopenia
- Thrombotic microangiopathy
- Tremor
- Vomiting

7.3 Adverse Event Reporting

All serious adverse events (SAEs) related to study procedures will be collected from signing of informed consent through CD8+ Memory T-cell infusion. Adverse events, both non-serious and serious, will be collected as outlined in Section 7.1 from date of CD8+ Memory T-cell infusion through 60 days. Beyond 60 days post-cell infusion, SAEs related to study treatment will be collected through the Week 20 follow-up visit.

All serious adverse events (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 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

N/A

9. STUDY CALENDAR

	Pre enrollment requirements	HCT Day = 0	On study enrollment = CD8+ Memory T-cell Infusion (Day 30-75)	Weeks from on study enrollment ±5 days			Weeks from on study enrollment ±2 weeks		
				Wk 1	Wk 2	Wk 4	Wk 8	Wk 12[g]	Wk 20[g]
HCT Infusion		X							
Infusion of CD8+ memory T-cells			X						
Informed consent	X								
Demographics	X								
Medical history	X			X	X	X	X	X	X
Concurrent meds	X		X	X	X	X	X	X	X
Donor Evaluation	X								
Physical exam	X		X	X	X	X	X	X	X
Vital signs [a]	X		X	X	X	X	X	X	X
Height	X								
Weight	X		X	X	X	X	X	X	X
Performance status	X		X	X	X	X	X	X	X
CBC w/diff	X		X	X	X	X	X	X	X
Serum chemistry [b]	X		X	X	X	X	X	X	X
Serious Adverse Event evaluation			X						
Disease Status Assessment [c]	X		X						
B-HCG [d]	X								
Cyclophosphamide			X[f]						
STR analysis [e]	X		X						

a: Temperature, blood pressure, and pulse. On day of infusion, vital signs to be taken any time prior to infusion, then immediately after infusion and every 30 minutes thereafter until 2 hours post-infusion.
b: Albumin, alkaline phosphatase, total bilirubin, calcium, chloride, creatinine, glucose, potassium, total protein, AST, ALT, sodium
c Response assessment as per disease histology, and may include CT, PET, and/or MRI imaging, and bone marrow biopsy or aspiration (refer to Appendix C), and is typically performed around Day 90 post transplant (Day 0)
d: Women of childbearing potential only
e: Short tandem repeat (STR) analysis relative to transplant date Day 0. Therefore, performed on Day 28 +/- 7 days, Week 8 +/- 2 weeks, Week 12 +/- 2 weeks, and Week 20 +/- 2 weeks, and thereafter as per standard of care
f: A single dose as lymphodepletion given 3-5 days prior to the CD8+ memory T-cell infusion
g: As necessary, data for the Week 12 or Week 20 visit, which is considered part of routine medical care for post-transplant patients, may be collected by phone contact to the subject and/or their regular physician.

10. MEASUREMENT and STATISTICAL METHODS

Our extensive experience with the TLI ATG conditioning for allogeneic HCT in cancer patients showed a 30% incidence of FDC at Day 90, with a 95% confidence interval of 10 to 49%. The non-relapse mortality (NRM) was 3% (95% CI: 0 to 14%) at 100 days and 6% (95% CI: 1 to 28%) at 1 year. The incidence of acute GVHD by Day 100 was 6% (0.7 to 22%).

10.1 Primary and Secondary Outcome measures

Following the infusion of CD8+ memory T-cells to promote the conversion of mixed chimerism to full donor chimerism and ultimately reduce the risk of disease relapse a variety of outcome measures will be followed.

The primary outcome is to determine the proportion of patients with full dose donor chimerism within 3 months of receiving the CD8+ memory T-cell infusion. Successful conversion from mixed to complete chimerism is defined as achieving ≥ 95% donor type in the CD3+ lineage or whole blood within 90 days of cell infusion.

The secondary outcome measures include the 1 year time-to-disease progression; overall survival (OS); and event-free survival (EFS); and non-relapse mortality. The incidence of acute and chronic GVHD following the infusion of allogeneic CD8+ memory T-cells will be determined.

Recipients will be monitored for infusion related toxicities and the rates of GVHD or marrow aplasia. Typically following an unmanipulated DLI the timing for acute GVHD or marrow hypoplasia onset is about 30 days post-infusion [32-38]. GVHD, if it occurs, will be treated promptly. First line therapy will include corticosteroids at a dose of 1-2 mg/kg depending upon severity. Additional immunosuppressive medications will be added as clinically indicated.

Evaluations will be performed to assess infusion related toxicity according to the following:

- Post-infusion toxicity evaluations (including physical exam, vitals, and weight) will be performed on days +7; +14; +28; and weeks 8; 12; 20; and 26 (+/- 5 days for the first 4 weeks post-infusion; +/- 14 days for dates thereafter) using CTCAE v4.0.
- STR analysis will be performed prior to the CD8+ memory T-cell infusion (within 35 days prior) and then will follow the scheduled events calendar assessment visits until 6 months post-transplant.
- GVHD will be according to Appendix B for GVHD Grading.
- CBC performed within 7 days prior to the CD8+ memory T-cell infusion and then will follow the scheduled events calendar until 6 months.
- Chemistries including Albumin, alkaline phosphatase, total bilirubin, calcium, chloride, creatinine, glucose, potassium, total protein, AST, ALT, and sodium will be performed within 7 days prior to the cell infusion and thereafter follow the scheduled events calendar until 6 months.
- The level of tumor burden will be evaluated at 3 and 6 months after transplant by standard imaging and blood evaluation procedures specific for the patient disease (refer to Appendix C).

Beyond 6 months all patients will be followed as per standard as per our institutional practice for HCT recipients (on average about every 6 weeks until 12 months post-transplant and regularly

thereafter) and data and laboratory tests including disease burden assessment will be recorded to determine the 1 year overall survival and event-free survival, NRM and for the development of infections and chronic GVD.

11. REGULATORY CONSIDERATIONS

11.1 Institutional Review of Protocol

The protocol, the 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 Center 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 Center 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). 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 designee, will prepare and maintain adequate and accurate participant case histories with observations and data pertinent to the study. The Stanford BMT data monitoring team along with the study coordinator will collect the information required in the Study calendar of events and maintain records for data analysis. The data will be reviewed by the study PI for accuracy against source document data.

11.4 Confidentiality

Patient records will be kept in a secure location at Stanford University Medical Center accessible only to research authorized personnel. Patient identity will be kept as confidential as possible as required by law. The patient will not be identified by name, social security number, address, telephone number, or any other personal direct identifier. Study patients will be assigned an identification code. Information about the code will be kept in a secure location and access limited to research study personnel. The results of this study may be presented at scientific or medical meetings or published in scientific journals, however patient identity will not be disclosed. Personal data included in the investigators' database will be maintained in compliance with all applicable laws and regulations.

12. STATISTICAL CONSIDERATIONS

Statistical Analysis

Analysis of 50 consecutive previous patients with persistent mixed chimerism after HCT using TLI-ATG conditioning confirmed the observed rate of conversion to complete chimerism without

an additional cell infusion was 25% (n = 12), and assume the upper limit of conversion is likely 35%. The sample size determination for the clinical trial will follow a single arm Simon 2-stage trial principle, with a null hypothesis of 35% (p0), and an alternative hypothesis of 70% (p1). We will use a significance level of 0.05 (α) and power of 0.9 (1-β). In Simon stage 1, we plan to enroll 8 patients and if 4 or more patients respond by converting to complete chimerism we will proceed to stage 2 where we will continue to enroll a full sample of 20 patients and declare success if a total of 11 or more patients respond. We will not start enrollment in Simon stage 2 until at least 3 successes are observed.

Patients will receive a CD8+ memory T-cell infusion 30 to 75 days after their initial HCT. The desired dose of CD8+ memory T-cells will be 5 x 10^6 cells/kg. It is fully expected this dose will be safe and not provoke or increase the risk of GVHD or other complications. In the phase 1 trial we infused this dose, or exceeded it, in 5 patients and observed no infusion related, early or late toxicities.

Patients will be evaluated for toxicities, defined as new Grade 3 to 4 toxicities, according to the Common Toxicity Criteria v.4 and Grade 3 to 4 GVHD (Appendix B) during the 60 days following the $CD8^+$ memory T-cell infusion. If 2 of the first 6 patients develop DLTs, the dose will be lowered to 1 x 10^6 CD8+ memory T-cells/kg. If 2 of the first 6 patients infused with this lower dose develop DLTs, then the trial will be stopped for safety reasons.

For the first 3 enrolled patients, the CD8+ memory T-cell infusion will be staggered by at least a 30-day interval between patients to allow for an assessment of DLTs for each patient. Thereafter, if no DLTs are observed, the CD8+ memory T-cell infusion will be staggered by 10-day intervals between patients. For added safety, if there is any incidence of a DLT observed the wait period will be extended to 60 days between the patients.

If a Grade 5 toxicity or death occurs and is at least likely related to the cell infusion, the trial will stop. Consideration for trial modifications will depend on institutional IRB and DSMC reviews. The appropriate regulatory offices including FDA will be notified within 5 business days of such an event.

Statistical considerations for the revised protocol

We have infused phenotypic CD8+ memory T cells in a total of 29 patients (as a treatment in 15 patients who had disease relapse after transplant, and as a prophylactic intervention in 14 patients with mixed chimerism to convert to complete chimerism to reduce the risk of subsequent disease relapse). We have confirmed the safety; there have been no infusion reactions, and no grade 3 or higher adverse events attributable to the infusion, and no aggravation or precipitation of acute GVHD.

We now propose to amend the protocol by administering a single dose of cyclophosphamide (1500mg/m2 BSA) 2-5 days prior to the CD8+ memory T cell infusion. The primary reason to amend the protocol to include cyclophosphamide is the desire to take advantage of the new clinical and pre-clinical information available that strongly suggests an increase in efficacy (success rate) with the addition (see section 2.10 in the revised Protocol). In addition to the obvious ethical impetus to provide trial participants with the optimal version of an experimental treatment, we note a scientific rationale: since the use of cyclophosphamide in this context will very likely become the new standard prior to infusing a cellular therapy, it is advisable to adapt our protocol to that reality.

The compelling ethical and scientific rationale notwithstanding, we must remain attentive to the extra assumptions required for statistical integration of the data from the patients treated without Cyclophosphamide early in the study and those treated with Cyclophosphamide after the change. Here is a review of the new design, the main issues it raises, and the analysis that led us to our new strategy:

1. The original Simon optimal, 2-stage design was predicated on a historical control success rate of 35% and an alternative success rate of 70%. There is no reason to alter the historical assumption. We propose a new embedded but independent 2-stage design (see below for details) with a single arm treated with the addition of Cyclophosphamide to the current regimen, predicated on an 80% alternative. The 10% increase in the alternative is based on the pre-clinical and clinical results cited above. The original design entertained a Type 1 error rate of 5% and a Type 2 error rate of 10%; in the new embedded trial we allow a 10% Type 1 error rate (which is more usual in phase 2) and retain the 10% Type 2 error rate.

2. We considered simply finishing the trial with the remaining 6 patients in the original design now being treated with cyclophosphamide, which would address the ethical dimension. However, the interpretation of a positive result would rest on an assumption that the alternative under Cyclophosphamide cannot be less than that without Cyclophosphamide. This is a reasonable assumption, but at the end of the trial we would likely not have strong evidence to support it, so the interpretation would still rely on a largely untested assumption. In addition, we would have little new evidence for safety, always an important consideration. By adding only 2 patients to the entire study, we are able to treat up to 8 patients with the Cyclophosphamide-containing regimen, which leads to the 2-stage design (details below) predicated on the 80% alternative assumption.

Why is this better? We have converted an assumption necessary for *interpretation* of a positive overall result into an assumption that drives the *design* of the embedded trial *whose results will test the assumption at high power* (90%). A positive result in the embedded trial is statistically definite, once it is completed, since the design assumption has been subject to test. A positive independent result in the embedded trial will buttress the overall result obtained by pooling the initial 14 and new 8 patients and calculating the one-sided 95% lower confidence limit for the success rate. It should be noted that because the embedded trial "stands alone" and does not include the initial cohort of patients treated without Cyclophosphamide, the fact that the interim results in the initial patients are known does not affect the type 1 error of the embedded trial.

3. The details of the embedded trial: Assuming a 35% historical control rate, and an 80% alternative, for 10% Type 1 and 2 error rates, the Simon optimal 2-stage design calls for a first stage with 2 patients, going on if there is at least 1 success, to the second stage with a cumulative total of 8 patients, rejecting the historical control rate (declaring a positive result) if there are at least 5 successes out of 8.

The output for the file is:

```
clinfun::ph2simon(pu=0.35,pa=0.80,ep1=0.1,ep2=0.1)
 Simon 2-stage Phase II design
```

Unacceptable response rate: 0.35
Desirable response rate: 0.8
Error rates: alpha = 0.1 ; beta = 0.1

	r1	n1	r	n	EN(p0)	PET(p0)
Optimal	0	2	4	8	5.465	0.4225

12.1 Sample Size and On-Study Enrollment Date

At least 20 patients will be required to complete the study (plus two additional patients as per above) The on-study enrollment date for this trial will be the day the recipient is infused with CD8+ memory T-cells. Prior to this date transplant recipients will be in a screening phase.

12.2 Accrual estimates

Approximately 6 to 7 patients will be enrolled annually. Time to complete enrollment will be 3 years. Time to complete the trial will be about 3.5 years.

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APPENDICES

APPENDIX A**: Participant Eligibility Checklist**

Protocol Title:	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.
Protocol Number:	**IRB-33058 / BMT288**
Principal Investigator:	Robert Lowsky, MD

II. Subject Information:

Subject Name/ID:
Gender: ☐ Male ☐ Female

III. Inclusion/Exclusion Criteria

Transplant Patient Inclusion Criteria (From IRB-approved protocol) Must have all of the following	**Yes**	**No**	**Supporting Documentation***
1. Between 18 and 80 years of age, inclusive	☐	☐	
2. Has a HLA-matched or single allele-mismatched adult sibling serving as donor	☐	☐	
3. Has a myeloid or lymphoid malignant disease that is treated with TLI and ATG reduced-intensity conditioning for allogeneic transplant [any of the following: acute myeloid leukemia (AML); chronic lymphocytic leukemia (CLL); B or T-cell non-Hodgkin lymphoma (NHL); Hodgkin lymphoma (HL); Myelodysplastic syndrome (MDS); or Myeloproliferative disease syndrome (MPD)]	☐	☐	
4. Patients who due to age, pre-existing medical conditions, or, prior therapy are considered to be at high-risk for regimen-related toxicity associated with fully ablative transplant conditioning, and therefore reduced intensity conditioning is recommended.	☐	☐	
5. Ability to understand and the willingness to sign a written informed consent document. Patients must have signed informed consent to participate in the trial.	☐	☐	

Transplant Patient Exclusion Criteria Excluded if any of the following are yes			
1. Uncontrolled bacterial, viral or fungal infection defined as currently taking medication and progression of clinical symptoms.	☐	☐	
2. Progressive hemato-lymphoid malignancy despite conventional therapy.	☐	☐	
3. Chronic myelogenous leukemia (CML)	☐	☐	
4. Acute leukemia not in remission	☐	☐	
5. Active CNS involvement of the underlying malignancy	☐	☐	
6. HIV-positive	☐	☐	
7. Pregnant or lactating	☐	☐	
8. Patients with a prior malignancy (EXCEPTION: diagnosed > 5 years ago without evidence of disease, OR treated ≤ 5 years ago but have a greater than 50% chance of life expectancy of ≥ 5 years for that malignancy)	☐	☐	
9. Has a psychiatric disorder(s) or psychosocial circumstance(s) which in the opinion of the primary physician would place the patient at an unacceptable risk from transplant.	☐	☐	
10. Organ dysfunction defined as follows:			
i. Ejection fraction < 30%, or uncontrolled cardiac failure	☐	☐	
ii. DLCO < 40% predicted	☐	☐	
iii. Total bilirubin > 3 mg/dL	☐	☐	
iv. SGOT or SGPT > 4 x ULN	☐	☐	
v. Creatinine > 2 mg/dL and an estimated creatinine clearance ≤ 40 mL/min	☐	☐	
vi. Poorly controlled hypertension despite multiple antihypertensive medication	☐	☐	
vii. KPS < 60%	☐	☐	

Donor Eligibility Criteria (From IRB-approved protocol)			
1. HLA-matched or single allele-mismatched sibling of enrolled transplant patient	☐	☐	
2. 18 to 80 years of age, inclusive	☐	☐	
3. State of general good health with completed donor evaluation with history, medical examination and standard blood tests within 60 days of starting the hematopoietic cell collection procedure	☐	☐	
4. White blood cell count $> 3.5 \times 10^9$/liter, platelets $> 150 \times 10^9$/liter and hematocrit > 35%	☐ ☐ ☐	☐ ☐ ☐	
5. Capable of undergoing leukapheresis	☐	☐	
6. Able to understand and sign informed consent	☐	☐	
7. 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.	☐	☐	
8. Females must not be pregnant or lactating	☐	☐	
9. No psychological traits or psychological or medical conditions which make them unlikely to tolerate the procedure	☐	☐	
10. Has not developed a new malignancy requiring chemotherapy or radiation in the interval since apheresis for initial HCT	☐	☐	

*****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.

IV. Statement of Eligibility

This subject is [☐ **eligible** / ☐ **ineligible**] for participation in the study.

Signature:	Date:
Printed Name:	

Signature:	Date:
Printed Name:	

Signature:	Date:
Printed Name:	

APPENDIX B: **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

APPENDIX C. RESPONSE CRITERIA by DISEASE HISTOLOGY

AML Response Criteria (Cheson, *et al.* 2003)

Response Criterion	Time of Assessment	Neutrophils (μL)	Platelets (μL)	Bone Marrow Blasts (%)	Other
Early treatment assessment	7-10 days after therapy	NA	NA	< 5	
Morphologic leukemia-free state	Varies by protocol	NA	NA	< 5	Flow cytometry EMD
Morphologic CR	Varies by protocol	> 1,000	> 100,000	< 5	Transfusion EMD
Cytogenetic CR	Varies by protocol	> 1,000	> 100,000	< 5	Cytogenics – normal, EMD
Molecular CR	Varies by protocol	> 1,000	> 100,000	< 5	Molecular – negative, EMD
Partial remission	Varies by protocol	> 1,000	> 100,000	> 50 or decrease to 5-25	Blasts < 5% if Auer rod positive

In this study day of assessment is 12 weeks and 24 weeks post stem cell infusion. For patients who are in a pathologically confirmed remission prior to infusion, a bone marrow biopsy and/or aspirate are not required; disease can be followed by confirmatory CBCs

NHL Response Criteria (Cheson, *et al.* 2007)

Response	Definition	Nodal Masses	Spleen, Liver	Bone Marrow
CR	Disappearance of all evidence of disease	(a) FDG-avid or PET positive prior to therapy; mass of any size permitted if PET negative (b) Variably FDG-avid or PET negative; regression to normal size on CT	Not palpable, nodules disappeared	Infiltrate cleared on repeat biopsy; if indeterminate by morphology, immunohistochemistry should be negative
PR	Regression of measurable disease and no new sites	≥ 50% decrease in SPD of up to 6 largest dominant masses; no increase in size of other nodes (a) FDG-avid or PET positive prior to therapy; one or more PET positive at previously involved site (b) Variably FDG-avid or PET negative; regression on CT	≥ 50% decrease in SPD of nodules (for single nodule in greatest transverse diameter); no increase in size of liver or spleen	Irrelevant if positive prior to therapy; cell type should be specified
SD	Failure to attain CR/PR or PD	(a) FDG-avid or PDT positive prior to therapy; PET positive at prior sites of disease and no new sites on CT or PET (b) Variably FDG-avid or PET negative; no change in size of previous lesions on CT		
Relapsed disease or PD	Any new lesion or increase by ≥ 50% of previously involved sites from nadir	Appearance of a new lesion(s) > 1.5 cm in any axis, ≥ 50% increase in SPD of more than one node, or ≥ 50% increase in longest diameter of a previously identified node > 1 cm in short axis. Lesions PET positive if FDG-avid lymphoma or PET positive prior to therapy.	> 50% increase from nadir in the SPD of any previous lesions	New or recurrent involvement

In this study day of assessment is 12 weeks and 24 weeks post stem cell infusion.

CLL Response Criteria (Hallek, *et al.* 2008)

Parameter	CR	PR	PD	SD
Lymphadenopathy[1]	None above 1.5 cm	Decrease ≥ 50%	Increase ≥ 50%	Change of -49% to +49%
Liver and/or spleen size	Normal size	Decrease ≥ 50%	Increase ≥ 50%	Change of -49% to +49%
Constitutional symptoms	None	Any	Any	Any
Polymorphonuclear leukocytes	> 1500/μL	> 1500/μL or > 50% improvement over baseline	Any	Any
Circulating clonal B-lymphocytes	Nil	Decrease ≥ 50% from baseline	Increase ≥ 50% from baseline	Change of -49% to +49%
Platelet count	> 100,000/μL	> 100,000/μL or increase ≥ 50% over baseline	Decrease of ≥ 50% from baseline secondary to CLL	Change of -49% to +49%
Hemoglobin	> 11.0 g/dL (untransfused and without erythropoietin)	> 11 g/dL or increase ≥ 50% over baseline	Decrease of > 2 g/dL from baseline secondary to CLL	Increase < 11.0 g/dL or < 50% over baseline, or decrease < 2 g/dL
Marrow	Normocellular, < 30% lymphocytes, no B-lymphoid nodules. Hypocellular marrow defines CRi.	≥ 30% lymphocytes, or B-lymphoid nodules, or not done	Increase of lymphocytes to more than 30% from normal	No change in marrow infiltrate

[1] sum of the products of multiple lymph nodes (as evaluated by CT scans, physical exam or ultrasound).
CR: complete remission, all of the criteria have to be met, with a marrow aspirate and biopsy performed at least 3 months after last treatment; PR: partial remission, at least one of the criteria has to be met; PD: progressive disease, at least one of the above criteria has to be met; SD: stable disease, all of the above criteria have to be met. Note that it is not necessary to assess bone marrow to confirm SD and determination of circulating clonal B lymphocytes is only necessary if other criteria are insufficient to define a response.

In this study day of assessment is 12 weeks and 24 weeks post stem cell infusion.

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

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

Deletions to IWG response criteria are not shown.

To convert hemoglobin from grams per deciliter to grams per liter, multiply grams per deciliter by 10.

MDS indicates myelodysplastic syndromes; Hgb, hemoglobin; CR, complete remission; HI, hematologic improvement; PR, partial remission; FAB, French-American-British; AML, acute myeloid leukemia; PFS, progression-free survival; DFS, disease-free survival.

*Dysplastic changes should consider the normal range of dysplastic changes (modification).[41]

†Modification to IWG response criteria.

‡In some circumstances, protocol therapy may require the initiation of further treatment (eg, consolidation, maintenance) before the 4-week period. Such patients can be included in the response category into which they fit at the time the therapy is started. Transient cytopenias during repeated chemotherapy courses should not be considered as interrupting durability of response, as long as they recover to the improved counts of the previous course.