Use of T-allo10 Cell Infusions Combined with Mismatched Related or Mismatched Unrelated Hematopoietic Stem Cell Transplantation (HSCT) for Hematologic Malignancies

Study Protocol and Statistical Analysis Plan

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USE OF T-allo10 CELL INFUSIONS COMBINED WITH MISMATCHED RELATED OR MISMATCHED UNRELATED HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) FOR HEMATOLOGIC MALIGNANCIES

DEPARTMENT OF PEDIATRICS

DIVISION OF STEM CELL TRANSPLANTATION AND REGENERATIVE MEDICINE

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Study Drug:	**T-allo10**
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Confidentiality Statement

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SYNOPSIS

Protocol Number	IRB 38734/ BMT 297
Protocol Title	Use of T-allo10 cell infusion combined with mismatched related or mismatched unrelated donor hematopoietic stem cell transplantation (HSCT) for hematologic malignancies.
Sponsor	Maria Grazia Roncarolo, MD
Protocol Director	Rajni Agarwal, MD
Name of Investigational Product	T-allo10
Clinical Phase	Phase 1
Background and Rationale	Hematologic malignancies are the most common childhood cancer. Although there are chemotherapy protocols to treat these patients, we have reached the zenith of tolerable dose intensity for chemotherapy, and a significant number of patients need HSCT for cure, often due to drug resistance, relapse, or other unfavorable features. Since less than 50% of these patients have a fully matched family or unrelated donor, the remaining patients will require an HSCT from a mismatched related or mismatched unrelated donor. A major complication of mismatched related or mismatched unrelated donor HSCT is Graft-versus-Host Disease (GvHD), which results in significant morbidity and increased non-relapse mortality. The objective of this clinical program is to develop a cell therapy to prevent GvHD and induce graft tolerance in patients receiving mismatched related or mismatched unrelated unmanipulated donor HSCT. The cell therapy consists of a cell preparation (T-allo10) containing T regulatory type 1 (Tr1) cells, which have been shown to suppress allogenic responses (1, 2). We plan to infuse the donor T-allo10 product one day before HSCT so that the Tr1 cells contained within the T-allo10 product will be able to prevent anti-host alloreactivity of the T cells present within the unmanipulated HSC graft. Indeed, Tr1 cells need to be present at the time of effector T cell activation to exert their most potent suppressive activity (3); therefore, we expect that the early infusion of T-allo10 cells will optimally modulate host alloreactivity of the donor T cells and prevent GvHD.
Objectives	Primary Objective: • To assess the tolerability and safety of escalating doses of T-allo10 cell infusions that can be feasibly manufactured to meet release specifications in mismatched related or mismatched unrelated unmanipulated HSCT in patients with hematologic malignancies. Secondary Objective: • To assess the incidence of grade III and IV acute GvHD. Exploratory Objectives: • To assess the incidence and severity of chronic GvHD. • To assess the time to immune reconstitution. • To assess Disease Free Survival.

Primary Endpoint(s)	The primary endpoints for this study are: -Tolerability of T-allo10 cells as evidenced by the incidence and severity of treatment-emergent adverse events (TEAE), laboratory abnormalities, changes in vital signs, and changes in physical examination following infusion of T-allo10 cells, recorded and graded according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 (see link). -Safety of T-allo10 cells as evidenced by the time to stem cell engraftment after HSCT. Stem cell engraftment is defined as: • An Absolute Neutrophil Count (ANC) above 500/mm^3 for three consecutive days by Day +42. • Presence of hematopoiesis at bone marrow examination, with cellularity >5 % and donor chimerism >90% by short tandem repeat (STR) analysis for the presence of donor cells by Day +42. • Absence of disease relapse as evidenced by minimal residual disease (MRD) assay < 0.1% by Day +42. - Feasibility defined by the rate of successful manufacture of the T-allo10 product to satisfy the targeted dose level and meet the required release specifications.
Secondary and Exploratory Endpoints	The secondary endpoint for this study is: -The incidence of grade III and IV acute GvHD at Day +100 following infusion of T-allo10 cells, assessed using the Modified Keystone scale administered by an independent evaluator on study visits through Day +100 according to schedule of events (Section 9.6). The exploratory endpoints for this study are: - The incidence and severity of chronic GvHD after Day +100 through Day +365, assessed by the National Institutes of Health (NIH) consensus guidelines administered by an independent evaluator on study visits from Day +100 through Day +365 according to schedule of events (Section 9.6). - The time to immune reconstitution, assessed by the time to reach >200/microliter CD3+ T cells. - Disease Free Survival at Day +365, assessed by bone marrow aspirate examination, morphology, MRD assay and donor chimerism by STR analysis.
Study Centers	This is a Stanford University, sponsor-investigator initiated protocol conducted at Lucile Packard Children's Hospital Stanford (LPCHS) and Stanford Health Care (SHC) only.
Sample Size	Up to 27 eligible patients will be given investigational cell product (T-allo10) sequentially at 3 escalating dose cohorts to determine the maximum tolerated dose (or the highest dose tested if no maximum tolerated dose is reached). Each cohort will begin by evaluating 3 patients. There will be a 28 day safety evaluation between the first and second subject in cohort one. The second subject in cohort one will be treated no sooner than 29 days after the first subject's infusion of T-allo10 (after safety assessment). Subsequent subjects in cohort one may be treated with T-allo10 at least 28 days after the preceding subject is treated with T-allo10 in the cohort. After infusion of T-allo10 in the last subject of each cohort, there will be a 28 day safety evaluation period. If the cohort meets safety and tolerability criteria, subsequent subjects may be enrolled in the cohort of the next higher cell dose. In cohorts two and three subjects may be treated with T-allo10 at least 28 days after the preceding subject is treated with T-allo10 in the cohort. If one out of 3 patients in a cohort has a DLT, 3 additional patients will be evaluated at the same dose level. If one

	out of 6 patients experiences a DLT, dose escalation will occur. If 2 out of ≤ 6 patients experience a DLT, dose escalation will cease and that dose will be designated the maximally administered dose. Up to three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose. The maximally tolerated dose (MTD) is: a) the highest dose level that can be feasibly generated to meet established release criteria at which no more than 1 out of 6 patients experience a DLT from the infusion of T-allo10 cells, or b) the dose below that at which at least 2 (of ≤6) patients have a DLT as a result of the infusion of T-allo10 cells. In addition to evaluating up to 6 patients at a given dose level with respect to toxicity, the number of patients for whom the targeted dose can successfully be manufactured to meet release criteria will be determined. Dose escalation will proceed as long as adequate numbers of T-allo10 cells are manufactured that meet release criteria for a targeted dose level for at least 3 patients out of ≤6 patients in a dose cohort. If cells generated for any patient do not meet the minimal T-allo10 dose for the specified cohort, the patient will receive the total number of manufactured T-allo10 cells, as the efficacious dose level is not known; that patient will be evaluable for toxicity, but will be considered a manufacturing failure in the targeted dose cohort (as outlined in Section 10). A maximum of 3 additional patients may be added to a dose cohort due to inability to achieve the target dose (Section 10). If any patient is withdrawn from study for any reason (withdrawal of consent, relapse before start of chemotherapy, etc.) before receiving the T-allo10 cells, the patient will not be counted in total evaluable patients and will be replaced. Up to a maximum of 3 inevaluable patients may be replaced. The maximum number of patients that may be enrolled will be 30 (18 in the dose escalation + 9 to replace 3 per dose cohort that fail to meet manufacturing specifications + 3 who withdraw from study for any reason).
Overall Duration of the Study	The recruitment period for this study is expected to be 2 years and 6 months. Subjects will be followed for 1-year post treatment. The total duration of this study is expected to be 3 years and 6 months.
Duration of Study per Subject	Subject's active participation in this study is expected to be 1 year.
Subject Population	Subjects recruited for this study will be patients from ≥3 years of age to ≤45 years of age. Subjects 1 and 2 (in Cohort 1) will be ≥ 12 years old. Pediatric and young adult patients with a hematologic malignancy undergoing an allogeneic mismatched related or unrelated unmanipulated HSCT are eligible to be enrolled in this study.
Eligibility criteria	Patient Inclusion Criteria: 1) Eligible diseases include: A. Acute Lymphoblastic Leukemia (B- or T-ALL) a. CR-1-ultra high risk features • Unfavorable cytogenetics • Hypodiploidy • Induction failure

- MRD positive after consolidation

b. CR-2

- Any of the high risk features listed in CR1
- B-ALL: any relapse considered eligible for transplant
- T- ALL

c. CR-3-any

d. Any feature that is considered high risk by the treating physician

B. Acute Myeloid Leukemia

a. CR1 -ultra high-risk features (eligible for stem cell transplantation)

- Unfavorable cytogenetics
- Persistent disease after induction 1 or 2

b. AML in 2nd or subsequent CR

c. Therapy related or Secondary AML

d. RAEB2

e. Any feature that is considered high risk by the treating physician

f. Patients with active disease, who in the opinion of the treating physician would benefit from an allogeneic stem cell transplant

C. Myelodysplastic syndrome

D. Mixed Phenotype Acute Leukemia MRD>1% after consolidation

E. Non-Hodgkin's lymphoma (NHL) or Hodgkin's lymphoma (HL) beyond first remission

F. Chronic Myeloid Leukemia

a. Chronic phase with incomplete response to TKI

b. Accelerated or Blast crisis

2) Age ≥3 to ≤50 years old. Subjects 1 and 2 (in Cohort 1) will be ≥ 12 years old

3) Available mismatched related donor (mMRD) or mismatched unrelated donor (mMUD), HLA matched 8/10 or 9/10

4) Lansky (age <16) or Karnofsky (age ≥16) performance status ≥60%

5) Able and willing to provide written, signed informed consent (assent as appropriate)

6) Have adequate organ function defined as the following:

- Serum Creatinine <1.5 X upper limit of normal (ULN) or 24-hour creatinine clearance >50 ml/min
- Serum bilirubin ≤ 2 x ULN
- Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) ≤10 x ULN

	• DLCO >60% predicted (in children, O2 saturation >92% on room air) • Left ventricular ejection fraction >45% (in children, shortening fraction >26%) 7) Male and female subjects of child bearing potential must agree to use an effective method of birth control to avoid pregnancy throughout the transplant procedure, while on immunosuppression, and if the subject experiences any chronic GvHD. Patient Exclusion Criteria: 1. Prior bone marrow or peripheral blood HSCT within the last 6 (six) months 2. HLA-matched related or unrelated donor available 3. Any active, uncontrolled infection at the time of enrollment 4. Pregnant or lactating females 5. Any severe concurrent disease which, in the judgement of the investigator, would place the patient at increased risk during participation in the study 6. Any subject with a history of significant renal, hepatic, pulmonary, or cardiac dysfunction or on treatment to support cardiac dysfunction 7. HIV positive 8. Non-cooperative behavior or non-compliance of the patient and/or of his/her family 9. Received another investigational agent within 30 days of enrollment 10. Patients with Down's syndrome
Investigational Product, Dose, and Mode of Administration	T-allo10 cells will be infused intravenously on Day -1 (day before transplant) in a 3+3 dose escalation fashion. The first cohort of 3 patients will receive $1x10^6$ cells/kg (+/- 10%). The subsequent two cohorts will receive $3x10^6$ cells/kg (+/- 10%), and $9x10^6$ cells/kg (+/- 10%), respectively.
Study Design and Methodology	This is a single center, non-randomized, non-controlled open-label Phase I trial to evaluate the feasibility of manufacturing T-allo10 drug product and the tolerability and safety of infusion of T-allo10 cells in children and young adults undergoing mismatched related or mismatched unrelated donor HSCT for hematologic malignancies (study schema). Before enrollment of a patient in the study, the physician must ensure the patient meets all eligibility criteria to be enrolled on the protocol. Eligibility criteria will be reviewed and confirmed by the Principal Investigator (PI)/Sub-Investigator (SubI) prior to subject being enrolled into the study. Patient is considered enrolled into the study on the day of apheresis and will be assigned a unique study number. Each study number will be assigned consecutively in increasing order starting with "297-01-01". The donor selection is made via a team meeting with attending physicians and an HLA expert. The mismatched donors in the patient's family or NMDP will be eligible for donation upon meeting all other criteria for bone marrow or peripheral blood stem cells (PBSC) donation. Unrelated donors selected from the NMDP must be ≥ 18 as per NMDP guidelines.

	Related donors will be selected from the patient's family members and relatives. Preference will be given to related donors over the age of 18 whenever possible. Related donors under the age of 18 must meet the requirements for apheresis in order to be eligible to participate in this study. Detailed apheresis criteria for pediatric patients is outlined in Section 6.1. Donors will be selected and informed about stem cell donation according to standard practice. This will be the responsibility of the transplantation center/NMDP. The donor informed consent will be collected (assent as appropriate). A detailed work up will be undertaken for the donors as per standard guidelines and institutional SOPs to establish eligibility for donation. Detailed inclusion criteria can be found in Section 6. The donors will undergo two procedures: 1. Apheresis for collection of unstimulated mononuclear cells for generation of T-allo10 to occur between D– 108 to D- 21. 2. Bone Marrow Harvest ($\geq 1x10^8$ TNC/kg minimum) or Apheresis for collection of mobilized PBSC ($\geq 3x10^8$ TNC/kg minimum) for the infusion of hematopoietic stem cells for transplant. The PI, according to applicable regulatory requirements, or a person designated by the PI, will fully inform the patient of all pertinent aspects of the clinical trial. Prior to a patient's participation in the clinical trial, the Informed Consent Form will be signed and dated by the patient, or the patient's legally authorized representative, and by the person who conducted the Informed Consent discussion. The Informed Consent Form used by the PI will be reviewed and approved by the IRB prior to study initiation. The patient will undergo a detailed pre-transplant work up as per institutional guidelines including: 1. Clinical history (previous and concomitant diseases and previous medications). 2. Physical examination: all organ systems (including a basic neurological examination). 3. Performance status (Lansky or Karnofsky) index. 4. Complete Blood count + differential. 5. Comprehensive metabolic panel. 6. Pregnancy test (if applicable): beta-HCG. 7. Organ functions- Pulmonary function test, EKG, echocardiography, creatinine clearance, nuclear renal scan, liver function test. 8. Markers of infectious disease. If a patient withdraws or fails to meet the screening guidelines, the patient will not be enrolled on the clinical trial. Apheresis will be performed according to institutional standards to collect unstimulated mononuclear cells to generate DC-10 cells between D-109 to D- 27. Pure red blood cell priming of the apheresis machine will be performed when the system extracorporeal blood volume exceeds 10-15% of the patient's blood volume. Institutional guidelines will be followed for catheter placement and apheresis procedures.

Patient Treatment:

Patient treatment will be divided into 4 phases

- Conditioning
- T-allo10 Cell infusion
- Stem Cell Infusion (Bone Marrow Infusion/Peripheral Blood Stem Cells)
- Follow-up

Conditioning chemotherapy will be administered to prepare the recipient for transplant as per the schedule below:

Day	Agent	Dose	GvHD Prophylaxis
-8	FTBI	120cGy X3 doses	
-7	FTBI	120cGy X3 doses	
-6	FTBI	120cGy X3 doses	
-5	FTBI	120cGy X2 doses	
-4	Cyclophosphamide	60 mg/kg	
-3	Cyclophosphamide	60 mg/kg	Start Sirolimus
-2	Rest		
-1	T-allo10 cell infusion		
0	Stem Cell Infusion (TNC/kg)	BM: $\geq 1x10^8$ PBSC: $\geq 3x10^8$	
+1			Start Mycophenolate Mofetil

The last dose of conditioning with cyclophosphamide will be administered on Day -3, allowing sufficient time (half-life of cyclophosphamide is 3 to 12 hours) for the clearance of the drug before the T-allo10 cell infusion.

On Day -1 (day before HSCT infusion), T-allo10 will be brought to the stem cell unit and thawed at the patient's bedside. Patients will receive premedication with acetaminophen 15 mg/kg not to exceed 650 mg and diphenhydramine 1 mg/kg not to exceed 50 mg as per institutional standards. The cells will be infused within 30 minutes (or as appropriate for

	patient weight), and patients will be observed for infusion related reactions for the next 24 hours. All infusion related toxicities will be recorded, and treatment emergent adverse events (TEAE) and treatment emergent serious adverse events (TESAE) will be reported according to Section 17. On Day 0, the bone marrow will be collected from the donors under general anesthesia; the target for collection is $\geq 1x10^8$ TNC/kg. The bone marrow will be processed in the Bone Marrow Transplant Cellular Therapy Facility (BMT-CTF) for red cell or plasma depletion as indicated by the blood group incompatibility. The marrow will be transported to the stem cell unit by a courier. Patients will receive premedication with acetaminophen and diphenhydramine. The bone marrow will be released by the BMT-CTF after release criteria are met. The cells will be infused at least 24 hours after the T-allo10 cell infusion. If the patient will receive HSCT from PBSC, granulocyte-colony-stimulating factor (G-CSF) (10 microgram/kg/day) will be administered to the donor for 5 days prior to undergoing the apheresis for collection of PBSC, according to the institutional guidelines. The target for PBSC collection is $\geq 3x10^8$ TNC/kg. Collected cells will be transported to the BMT-CTF and released for infusion. Patients will be observed for infusion related reactions to the HSCT graft for 24 hours following infusion. All HSC infusion related toxicities will be recorded, and adverse events (AE) and serious adverse events (SAE) will be reported according to Section 17. Patients will be started on Sirolimus on Day -3 at a loading dose of 3 mg/m^2, followed by 1 mg/m^2 daily. Sirolimus levels will be monitored at least twice a week to keep the levels between 8-12 ng/ml. Mycophenolate Mofetil (MMF) will be started on Day +1 after transplant at a dose of 15 mg/kg every 8 hours. Grading of GvHD and all treatments will be recorded in the Case Report Form (CRF). For the follow-up phase, patients will be evaluated weekly with blood count, blood chemistry, microbiological evaluation, clinical evaluation, and GvHD evaluation up to Day +60 after HSCT; these same parameters will be then evaluated monthly until Day +365. On Days +28, +42, +90, and +180, engraftment will be evaluated (absolute neutrophil count, bone marrow cellularity and STR) on peripheral blood and bone marrow. Immune reconstitution will be evaluated per Section 7.4. Time points outlined in schedules should be closely followed up to Day +60 (+/- 3 days), but a tolerance of +/- 10 days is allowed in the later phase (after Day 60). Concomitant and prophylactic antimicrobial therapies will be administered according to institutional clinical standards. Leukocyte-depleted, irradiated red cells and platelet preparations from individual donors or pool will be transfused as needed according to clinical standards.
Statistical Methodology	The primary analytic focus for this study is to determine and describe the tolerability and safety profile of the investigational cellular product at 3 escalating dose levels that can be feasibly generated to meet established release criteria. Descriptive statistics for tolerability and safety endpoints will be presented for each dose cohort. Examination of any potential dose-response relationship with respect to tolerability and safety outcomes will also be assessed. Since the study is not designed to provide adequate power for hypothesis testing related to clinical activity, the analytic focus for both the primary and secondary endpoints will be descriptive. Although hypothesis testing will be conducted for clinical activity, the interpretation will be considered exploratory.

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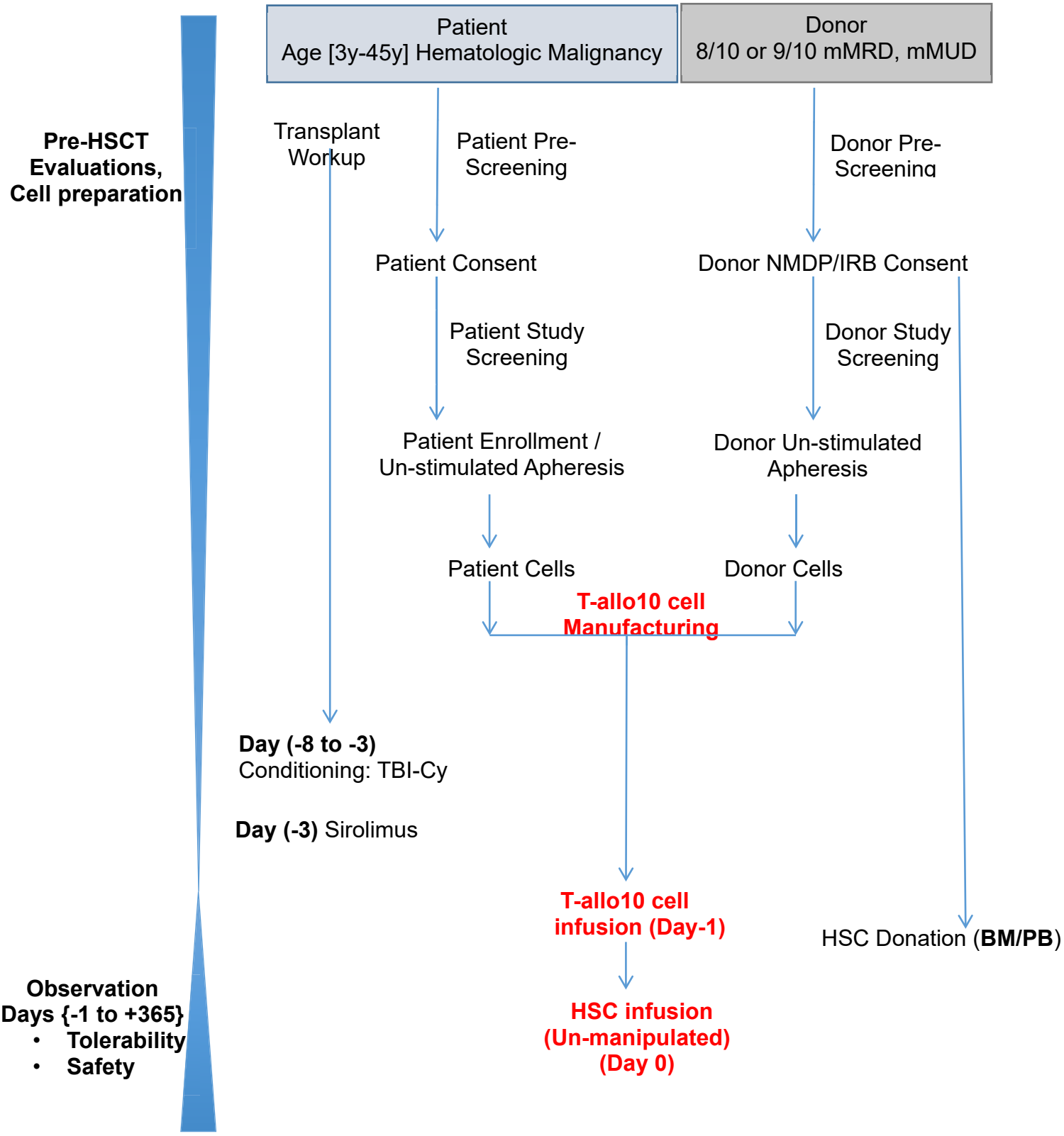
PROTOCOL SCHEMA



Recipient, Donor, and Manufacturing Timeline

Ideal Day	Window	Recipient Procedure	Donor Procedure	Manufacturing Procedure
D-27	D-109 to D-27	Recipient Apheresis (Enrollment)		
D-26	D-108 to D-26			7 day culture of recipient CD14+ cells in the presence of GM-CSF + IL-4
D-24	D-108 to D-21		Donor Apheresis (LPCHS or NMDP)	
D-19	D-101 to D-19			DC-10 & CD4+ cells co-cultured for 10 days
D-9	D-91 to D-9			Finish co-culture for T-allo10; Cells frozen at the end of co-culture; Testing of T-allo10 to meet release criteria
D-2	D-84 to D-2			CoA/Product Release (Ready for Infusion)
D -1		T-allo10 cell infusion		
D 0		Stem Cell Infusion		

Transplant Timeline

Day	Agent	Dose	GvHD Prophylaxis
-8	FTBI	120cGYx3 doses	
-7	FTBI	120cGYx3 doses	
-6	FTBI	120cGYx3 doses	
-5	FTBI	120cGYx2 doses	
-4	Cyclophosphamide	60mg/kg	
-3	Cyclophosphamide	60mg/kg	Start sirolimus
-2	Rest		
-1	T-allo10 cell infusion		
0	Stem Cell Infusion (TNC/kg)	BM: $\geq 1 \times 10^8$ PBSC: $\geq 3 \times 10^8$	
+1			Start MMF

LIST OF ABBREVATIONS

ABO	Blood grouping
Abs	Absolute
AE	Adverse event
AdV	Adenovirus
ADR	Adverse Drug Reaction
Ag	Antigen
aGvHD	Acute Graft-versus-Host Disease
ALL	Acute Lymphoblastic Leukemia
Allo	Allogeneic
ALT	Alanine transaminase
AML	Acute Myeloid Leukemia
ANC	Absolute Neutrophil Count
APC	Antigen presenting cell
AST	Aspartate transaminase
Auto	Autologous
BM	Bone marrow
BM bx	Bone marrow biopsy
BMI	Body mass index
BMT	Bone Marrow Transplant
BP	Blood pressure
BSA	Body surface area
BUN	Blood Urea Nitrogen
CBC	Complete blood count
CCTO	Cancer Clinical Trials Office

CD	Crohn's Disease
cGvHD	Chronic Graft-versus-Host Disease
CI	Confidence interval
CMAX	Maximum concentration of drug
CMV	Cytomegalovirus
CNS	Central nervous system
Co-PI(s)	Co-Investigator(s)
COA	Certificate of Analysis
CRF	Case report/record form
CR	Complete response
CSB	Cranial Spinal Boost
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTF	Cellular Therapy Facility
CXR	Chest X-ray
CY	Cyclophosphamide (Cytoxan)
DLI	Donor Leukocyte Infusion
DLT	Dose Limiting Toxicity
DSMC	Data Safety Monitoring Committee
EBV	Epstein Barr Virus
EC	Ethics Committee
ECG	Electrocardiogram
Echo/ECO	Echocardiogram
EFS	Event-free survival
F	Fahrenheit

FA	Fanconi Anemia
FACT	Foundation for the Accreditation of Cellular Therapy
FAB	French-American-British
FDA	Food and Drug Administration
FLT	FMS-like tyrosine Kinase
FTBI	Fractionated total body irradiation
GCP	Good clinical practice
G-CSF	Human granulocyte-colony stimulating factor
GI	Gastrointestinal
GFR	Glomerular filtration rate
GGT	Gamma-glutamyltransferase
GvHD	Graft-versus-Host Disease
GVL	Graft-versus-Leukemia
IB	Investigator brochure
IBW	Ideal body weight
ICF	Informed consent form
IND	Investigational new drug application
IP	Investigational product
IS	Immunosuppression
ITD	Internal tandem duplication
IV	Intravenous
HBV	Hepatitis B Virus
HCG	Human chorionic gonadotropin
HCV	Hepatitis C Virus
Hgb	Hemoglobin

HIV	Human Immunodeficiency Virus
HHV	Human Herpes Virus
HLA	Human leukocyte antigen
HR	Heart rate
HRS.	Hours
HSC	Hematopoietic Stem Cell
HSCT	Hematopoietic Stem Cell Transplantation
HSV	Herpes Simplex Virus
HTLV	Human T Cell Lymphotropic Virus
HTN	Hypertension
IRB	Institutional Review Board
IV	Intravenous
LAR	Legal authorized representative
LDH	Lactate dehydrogenase
LLN	Lower limit of normal
LPCHS	Lucile Packard Children's Hospital at Stanford
M^2	Meter squared
MAD	Maximally Administered Dose
MD	Medical Doctor
MDS	Myelodysplastic Syndrome
MFD	Maximum Feasible Dose
MMF	Mycophenolate Mofetil
mMRD	Mismatched related donor
mMUD	Mismatched unrelated donor

MRD	Minimal Residual Disease
MTD	Maximum Tolerable Dose
MUGA	Multi Gated Acquisition Scan
NCI	National Cancer Institute
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NIH	National Institute of Health
NMDP	National Marrow Donor Program
NTE	Not to exceed
OS	Overall survival
PB	Peripheral blood
PBSC	Peripheral blood stem cells
PCR	Polymerase chain reaction
PCR-SSOP	Polymerase chain reaction –sequence specific oligonucleotide probe
PD	Progressive diseased
Peds	Pediatric patients
PFS	Progression free survival
PFT	Pulmonary function test
PD	Protocol Director
pHA	Polyhydroxyalknoates
PHA SI	Phytohaemaglutinin Stimulation Index
PHI	Protected Health Information
PI	Principal investigator
PLT	Platelet
PMRD	Partially matched donor
PO	‘per Os’ –daily

PR	Partial response
Q	Every
QD	Once daily
QIg	Qualitative Immunoglobulin
QOD	Every other day
r-ATG	Polyclonal rabbit anti-thymocyte globulin
RAEB	Refractory anemia with excess blasts
RN	Registered Nurse
RNA	Ribonucleic acid
RR	Response rate
SAE	Serious adverse event
SAP	Statistical analysis plan
SCCI	Stanford Center for Clinical Informatics
SCID	Severe Combined Immune Deficiency
SCT	Stem cell transplantation
SD	Stable disease
SCT&RM	Stem Cell Transplantation and Regenerative Medicine
SDV	Source document verification
SHC	Stanford Health Care
Siro	Sirolimus
SOP	Standard Operating Procedure
STR	Short tandem repeat
SUSAR	Suspected Unexpected Serious Adverse Reactions
TBI	Total body irradiation
Td	Tetanus, (reduced) Diphtheria

TCR	T cell Receptor
TEAE	Treatment-emergent adverse events
TGFβ	Transforming Growth Factor beta
TH	Thursday
TNC	Total Nucleated Cells
TRM	Transplant-related mortality
Treg	T regulatory cells
tTreg	Thymic derived T regulatory cells
TTP	Time to progression
UCB	Umbilical Cord Blood
ULN	Upper limit of normal
UNK	Unknown
URD	Unrelated donor
VNTR	Variable number tandem repeat
VZV	Varicella Zoster Virus
WB	Whole blood
WBC	White blood cell
WHO	World Health Organization

1 INTRODUCTION

1.1 Hematopoietic Stem Cell Transplantation in Hematologic Malignancies

Hematologic malignancies comprise 34% of all childhood cancers. Approximately one third of these patients will have an urgent need for Hematopoietic Stem Cell Transplantation (HSCT) to achieve better long-term outcomes. Allogeneic HSCT is the curative option for several hematologic malignancies; however, its broad application is limited by the frequent occurrence of GvHD, a life-threatening complication mediated by alloreactive donor T cells recognizing host healthy tissues (4). The incidence and severity of GvHD directly correlates with the degree of HLA histo-incompatibility between the donor and the host, posing a significant limitation to the clinical applicability of allogeneic HSCT. Less than 50% of the patients have a HLA matched sibling or unrelated donor suitable for transplant. The rest of the patients need either to find a HLA mismatched related donor or a HLA mismatched unrelated donor for the source of HSC. Patients who receive HSCT from mismatched related or mismatched unrelated donors are at high risk for developing severe GvHD. Importantly, the benefit of the transplant resides not only in the possibility of reconstituting new blood tissue but also in providing allogeneic T cells which can kill the leukemic cells (GvL effect).

We analyzed the incidence of acute and chronic GvHD in patients (ages 3 to 30 years) undergoing mismatched related or mismatched unrelated HSCT at Lucile Packard Children's Hospital Stanford (LPCHS) and Stanford Hospital and Clinics (SHC). In this analysis, the incidence of acute GvHD was 57%, and of these patients, 26% developed grade III-IV GvHD leading to significant morbidity and mortality. In addition, 30% of patients developed chronic GvHD. The presence of acute and chronic GvHD requires treatment with systemic pharmacological immunosuppression, which is associated with serious side effects including delayed immune reconstitution, increased incidence of infections and organ damage. These results confirm the need for novel strategies to reduce GvHD and improve long-term tolerance between mismatched donor-host pairs, with the ultimate goal of extending the application and improving the outcomes of HSCT from allogeneic donors with partial HLA histocompatibility.

1.2 T-allo10 Cells

T-allo10 cells consist of CD4+ cells of donor origin that have been exposed to host alloantigens *in vitro* in the presence of IL-10. During this primary stimulation, the CD4+ cells acquire a hyporesponsive state (anergy) to subsequent stimulation with the same alloantigens (2, 5, 6). In addition, they become able to suppress primary responses of autologous CD4+ and CD8+ T cells towards the same alloantigens (7). This property is mainly due to the induction of a specific population of T regulatory cells called T regulatory type 1 (Tr1) cells during the *in vitro* culture in the presence of IL-10. IL-10 is an immunomodulatory cytokine that plays a central role in controlling inflammation, down-regulating immune responses, and inducing immunological tolerance (8, 9). IL-10 inhibits proliferation and cytokine production by T cells via down-regulating production of inflammatory cytokines as well as expression of costimulatory molecules on antigen presenting cells (APC). Importantly, IL-10 induces long lasting antigen specific T cell anergy and differentiation of Tr1 cells in both mice and humans (6, 10). Tr1 cells have been described in several preclinical and clinical models in association with induction and maintenance of immunological tolerance (1, 10, 11).

Tr1 cells are characterized by a unique cytokine production profile since they produce high levels of IL-10, TGF-β and IL-5, low levels of IFN-γ and IL-2, and no IL-4 and IL-17 (12, 13, 14). Tr1 cells

are anergic *in vitro* and actively suppress immune responses by T effector cells mainly via IL-10 and TGF-β. Until recently, Tr1 cells could be identified only based on their cytokine production profile. We have recently demonstrated that CD49b+LAG-3+ cells are good markers to define Tr1 cells (12). The vast majority of memory CD4+CD49b+LAG-3+ T cells secrete large amounts of IL-10 but not significant levels of IL-4 and IL-17, do not constitutively express FOXP3, and display regulatory activity both *in vitro* and *in vivo* (12). Both CD49b and LAG-3 are stably expressed on functional human Tr1 cell clones. CD49b is expressed on Tr1 cells irrespectively of their activation state, whereas LAG-3 is expressed on Tr1 cells upon activation when the cells produce IL-10 and display suppressor activity. CD49b and LAG-3 have been used to identify Tr1 cells in the peripheral blood of healthy donors and, at higher frequency, of tolerant patients (12). Furthermore, CD49b and LAG-3 can track Tr1 cells in cell products generated *in vitro* (12).

Tr1 cells were first identified and characterized in Severe Combined Immune Deficient (SCID) patients who were immune reconstituted after HSCT from HLA mismatched donors (15, 16). These patients developed spontaneous split chimerism, with T and natural killer (NK) cells of donor origin and B and professional APC of host origin in the absence of GvHD. Cultures of the PBMC of these chimeras led to the isolation of donor derived CD4+ and CD8+ T cell clones that were specific for the HLA of the host. These data indicated that alloreactive T cells were not deleted from the T cell repertoire of the patients. However, a high proportion of these T cell clones had the Tr1 cytokine production profile as described above and IL-10 dependent low proliferation when activated with the host alloantigens *in vitro* (16). These findings correlated with the absence of GvHD and a state of active tolerance between host and donor cells. Similar data were also obtained from β thalassemic patients when these patients developed persistent mixed chimerism following HSCT (7). The presence of Tr1 cells *in vivo* was confirmed by detection of CD49b+ and LAG-3+ T cells at higher frequency in the peripheral blood mononuclear cells (PBMC) of these tolerant patients as compared to normal donors' PBMC (12). This provides a strong rationale for the clinical application of these cells to favor immunological tolerance.

Several protocols have been developed to generate Tr1 cells *in vitro* that are suitable for *in vivo* use, and it is clear that, although IL-10 is indispensable for Tr1 cell induction, efficient *in vitro* production of Tr1 cells also requires APC.

Tr1 cell induction *in vitro* is optimal when DC-10 are used as APC. DC-10 are monocyte-derived dendritic cells generated *in vitro* in the presence of exogenous IL-10 in addition to GM-CSF and IL-4, commonly required for mature myeloid derived DC generation *in vitro* (17). Stimulation of CD4+ cells with allogeneic DC-10 and IL-10 is more efficient in generating a Tr1 cell product that can suppress primary antigen-specific proliferative responses of autologous naïve CD4+ cells (12, 18).

The T-allo10 cell culture we are planning to use in the present trial is optimized to maximize the induction of Tr1 cells, detectable as IL-10 producing CD4+CD49b+LAG-3+ T cells, starting from purified CD4+ cells cultured with DC-10 as APC in the presence of IL-10. This cell product is suitable for controlling host alloantigen specific immune responses by donor T cells in the transplant.

1.3 Adoptive Immunotherapy with T-allo10 Cells in Allogeneic HSCT

The clinical use of different types of T regulatory cells has been explored by several groups to improve the outcome of allogeneic HSCT by boosting the natural mechanisms of tolerance mediated

by the T regulatory cells. In particular, adoptive transfer of CD4+CD25+ Treg containing a high proportion of FOXP3+ T cells has been performed by several groups, and has been shown to be safe (19, 20, 21).

The main advantage of adoptive immunotherapy with Tr1 cells compared to Treg cells is the host alloantigen specificity that is established in the donor Tr1 cells during *in vitro* culture in the presence of IL-10 and host alloantigens (T-allo10 cells or IL-10 anergized T cells). The Tr1 cells present in the resulting T-allo10 cell product can exert their function without purification. Indeed, the presence of additional CD4+ cells can also mediate transfer of immune competence.

We first performed a cell therapy trial (ALT TEN trial) using IL-10 anergized T cells prepared *in vitro* and administered to adult patients transplanted with purified CD34+ HSC from haploidentical donors with the objective to favor immune reconstitution without severe GvHD, in the absence of any immunosuppression (22). These donor-derived, host alloantigen specific, IL-10 anergized T cells were infused in a total of 12 patients approximately one month after transplant and after the patients showed myeloid engraftment. Overall, feasibility and safety of infusion of the IL-10 anergized T cells was demonstrated. Four out of eight evaluable patients fully immune reconstituted, remained disease free and had evidence of a tolerance signature on long-term follow-up (22). Although assessed in a limited number of patients, this outcome suggests efficacy of the IL-10 anergized T cell therapy when compared to the outcomes obtained from haploidentical transplants with purified CD34+ HSC alone in which infections and disease-relapse occurred in the majority of the patients (23, 24). The safety data from the ALT TEN trial supports the decision to administer the T-allo10 cells in children and young adults (≥3 to ≤30) who have no other effective treatment options. For an abundance of caution, we will evaluate the safety of the T-allo10 cell product for up to 28 days in patients who are ≥ 12 years of age in the first dose cohort prior to treating children < 12 years of age. We are planning to initiate our proposed Phase I dose escalation study with a dose of 10^6 T-allo10 cells/kg. This cell dose is similar to that used in other adoptive transfer trials with Treg cells (21) although it is higher than that previously used in the ALT TEN trial. The rationale for choosing a higher initial cell dose in the proposed trial is related to the following considerations:

i. T-allo10 cells are infused with 8/10 or 9/10 HLA mismatched related and mismatched unrelated unmanipulated HSCT. The ALT TEN trial patients received IL-10 anergized T cells after purified CD34+ HSCT from haploidentical donors with minimal T cell content (≤ $2.6x10^4$ CD3+ T cells/kg). In the present trial the presence of a higher number of T cells (≥ 10^7 CD3+ T cells /kg and ≥ 10^8 CD3+ T cells /kg in BMT and PBSC transplants, respectively) (25) within the HSCT will require an incremental increase in the number of Tr1 cells in order to suppress the alloreactive donor T cells, which are responsible for the GvHD incidence. The T-allo10 cells will contain a higher frequency of Tr1 cells as compared to the IL-10 anergized T cells used in the ALT TEN trial. Nevertheless, because of the presence of higher number of T cells within the graft, the infused T-allo10 cells may be less able to undergo homeostatic expansion, as compared to the ALT TEN trial patients who were severely lymphopenic (26).

ii. T-allo10 cells are infused in the presence of immunosuppression, which is the standard of care for the study population receiving the mismatched related and mismatched unrelated unmanipulated HSCT. The ALT TEN trial patients were not treated with immunosuppressive drugs for GvHD prophylaxis at the time of the HSC graft and at the time of IL-10 anergized T cell infusion.

In the trial described herein, we propose to administer T-allo10 cells, one day before the infusion of hematopoietic stem cells for the transplant in the setting of mismatched related or mismatched unrelated unmanipulated HSCT, as opposed to the timing of cell administration in the ALT TEN trial where Tr1 cells were infused after 4 weeks of stem cell transplantation. We expect that early T-allo10 cell infusion will modulate the host alloreactivity of the donor T cells present in the unmanipulated mismatched HSCT. At the same time, the donor T-allo10 cells specific for the host alloantigen will not interfere with the ability of the other donor T cells to mount immune responses against pathogens.

We will start immunosuppression with sirolimus and mycophenolate mofetil (MMF), as described in Section 9.2. As compared to calcineurin inhibitors, sirolimus is permissive and favorable to the *in vivo* expansion of Tr1 cells (27) and MMF does not appear to interfere with T regulatory cell function (21).

2 OBJECTIVES

2.1 Primary Objectives

To assess the tolerability and safety of escalating doses of T-allo10 cell infusions that can be feasibly manufactured to meet release specifications in mismatched related or mismatched unrelated unmanipulated HSCT in patients with hematologic malignancies.

2.2 Secondary Objective

- To assess the incidence of grade III and IV acute GvHD.

2.3 Exploratory Objective

- To assess the incidence and severity of chronic GvHD.
- To assess the time to immune reconstitution.
- To assess Disease Free Survival.

3 INVESTIGATIONAL PLAN

3.1 Study Description

This is a single center, non-randomized, non-controlled open-label Phase I trial to evaluate the feasibility of generating T-allo10 cells that meet the established release criteria, and the tolerability and safety of administration of escalating doses of T-allo10 cell infusions in mismatched related or unrelated donor HSCT for hematologic malignancies.

The study design is outlined in the protocol schema. Up to 27 eligible patients will be evaluated sequentially in 3 escalating dose cohorts of T-allo10 cells. Each cohort will begin by evaluating 3 patients. There will be a 28 day safety evaluation between the first and second subject in cohort one. The second subject in cohort one will be treated no sooner than 29 days after the first subject's infusion of T-allo10 (after safety assessment). Subsequent subjects in cohort one may be treated with T-allo10 at least 28 days after the preceding subject is treated with T-allo10 in the cohort. After infusion of T-allo10 in the last subject of each cohort, there will be a 28 day safety evaluation period. If the cohort meets safety and tolerability criteria, subsequent subjects may be enrolled in the cohort of the next higher cell dose. In cohorts two and three subjects may be treated with T-allo10 at least 28 days after the preceding subject is treated with T-allo10 in the cohort. If 1 out of 3 patients in a cohort has a DLT, 3 additional patients will be evaluated at the same dose level. If 1 out of 6 patients experience a DLT, dose escalation will occur. If 2 out of ≤ 6 patients experience DLT, dose escalation will cease and that dose will become maximally administered dose. Up to three (3) additional patients will be evaluated at the next lowest dose level if only 3 patients were treated previously at that dose. The maximum tolerated dose (MTD) is the highest dose level that can be feasibly generated to meet established release criteria at which no more than 1 out of 6 patients experience a DLT from the infusion of T-allo10 cells, or the dose below that at which at least 2 (of ≤ 6) patients have a DLT as a result of the infusion of T-allo10 cells.

In addition to evaluating up to 6 patients at a given dose level with respect to toxicity, the number of patients for whom the targeted cell dose number can be successfully manufactured will be determined. Dose escalation will proceed as long as an adequate cell dose can be manufactured for at least 3 of the first ≤ 6 patients in a dose level for evaluation. If cells generated for any patient do not meet the minimal T-allo10 cell dose for the specified cohort, the patient will receive the total number of manufactured T-allo10 cells, as the efficacious dose level is not known; that patient will be evaluable for toxicity, but will be considered a manufacturing failure in the targeted dose cohort (as outlined in Section 10). A maximum of 3 additional patients may be added to a dose cohort due to inability to achieve target doses.

3.2 Study Stopping Rules

The stopping rules for this study will be as follows:

- Any Treatment Related Mortality (TRM) for any subject at any dose within 100 days of HSCT.
    - TRM – Treatment Related Mortality is defined as death possibly related to the T-allo10 cell infusion as per Section 17.2.2.
- Failure to engraft in 2 out of 3 or 3 out of 6 patients.

- If 4 out of 6 patients develop grade III and above acute GvHD.

If any of the above stopping rules are met, enrollment to the study will be suspended pending discussions with the FDA and DSMC.

3.3 Number of Patients

Each dose cohort is expected to evaluate a minimum of 3 patients and a maximum of 6 patients to determine MTD. In addition, the study will allow for up to 3 additional patients to be evaluated in each of the dose cohorts (total of 9 additional patients) due to inability to achieve target doses. If any patient is withdrawn from study for any reason (withdrawal of consent, relapse before start of chemotherapy) before receiving the T-allo10 cells, the patient will not be counted in total evaluable patients and will be replaced. Up to a maximum of 3 inevaluable patients may be replaced. The maximum number of patients that may be enrolled will be 30 (18 in the dose escalation + 9 to replace 3 per dose cohort that fail to meet manufacturing specifications + 3 who withdraw from study for any reason).

3.4 Number of Sites

This trial will be conducted at Lucile Packard Children's Hospital Stanford (LPCHS) and Stanford Health Care (SHC).

3.5 Investigational material

3.5.1 T-allo10 cells

T-allo10 cells are donor derived CD4+ cells that have been exposed *in vitro* to the host (patient) antigen presenting cells (APC) in the presence of IL-10. These CD4+ cells are hyporesponsive (anergic) to the host alloantigens and contain host alloantigen-specific Tr1 cells.

3.5.2 T-allo10 cell dose

T-allo10 cells will be administered in suspension as a single intravenous infusion at doses of 10^6/kg (+/- 10%) or 3 x10^6/kg (+/- 10%) or 9x10^6/kg (+/- 10%). If any patient does not meet the minimal T-allo10 cell dose for their specified cohort, they will receive the total manufactured T-allo10 cells. If de-escalation is needed the de-escalation dose will be defined as per Section 10. All dosing for the T-allo10 cells will be based on actual body weight.

4 STUDY POPULATION

4.1 Patient Inclusion Criteria

1. Eligible diseases include:
    A. Acute Lymphoblastic Leukemia (B- or T-ALL)
        a. CR-1-ultra high risk features
            - Unfavorable cytogenetics
            - Hypodiploidy
            - Induction failure
            - MRD positive after consolidation
        b. CR-2
            - Any of the high risk features listed in CR1
            - B-ALL: any relapse considered eligible for transplant
            - T- ALL
        c. CR-3-any
        d. Any feature that is considered high risk by the treating physician
    B. Acute Myeloid Leukemia
        a. CR1 -ultra high-risk features (eligible for stem cell transplantation)
            - Unfavorable cytogenetics
            - Persistent disease after induction 1 or 2
        b. AML in 2nd or subsequent CR
        c. Therapy related or Secondary AML
        d. RAEB2
        e. Any feature that is considered high risk by the treating physician
        f. Patients with active disease, who in the opinion of the treating physician would benefit from an allogeneic stem cell transplant
    C. Myelodysplastic syndrome
    D. Mixed Phenotype Acute Leukemia MRD>1% after consolidation
    E. Non-Hodgkin's lymphoma (NHL) or Hodgkin's lymphoma (HL) beyond first remission
    F. Chronic Myeloid Leukemia
        a. Chronic phase with incomplete response to TKI
        b. Accelerated or Blast crisis

2. Age ≥3 to ≤50 years old. Subjects 1 and 2 (in Cohort 1) will be ≥ 12 years old
3. Available mismatched related (mMRD) or mismatched unrelated (mMUD) donor, HLA matched 8/10 or 9/10
4. Performance status Lansky (age < 16) or Karnofsky score (age ≥ 16) ≥ 60%.
5. Able and willing to provide written, signed informed consent (assent as appropriate)
6. Have adequate organ function defined as the following:
   - Serum creatinine < 1.5 × ULN or 24-hour creatinine clearance > 50 ml/min
   - Serum bilirubin ≤ 2 × ULN
   - ALT and AST ≤ 10 × ULN
   - DLCO > 60% predicted (in children, O_2 saturation > 92% on room air)
   - Left ventricular ejection fraction >45% (in children, shortening fraction >26%)
7. Male and female subjects of childbearing potential must agree to use an effective means of birth control to avoid pregnancy throughout the transplant procedure, while on immunosuppression, and if the subject experiences any chronic GvHD

4.2 Patient Exclusion Criteria

1. Prior bone marrow or peripheral blood stem cell transplantation within the last 6 (six) months
2. HLA-matched related or unrelated donor available
3. Any active, uncontrolled infection at the time of enrollment
4. Pregnant or lactating females
5. Any severe concurrent disease which, in the judgment of the investigator, would place the patient at increased risk during participation in the study
6. Any subject with a history of significant renal, hepatic, pulmonary dysfunction, or cardiac dysfunction or on treatment to support cardiac dysfunction
7. HIV-positive
8. Non-cooperative behavior or non-compliance of the patient and/or of his/her family
9. Received another investigational agent within 30 days of enrollment
10. Patients with Down's syndrome

4.3 Donor Selection

The donor selection is made via a team meeting with attending physicians and an HLA expert. The mismatched donors in the patient's family or NMDP (National Marrow Donor Program) will be eligible for donation upon meeting all other criteria for bone marrow or peripheral blood stem cells (PBSC) donation.

Unrelated donors selected from the NMDP must be ≥ 18 as per NMDP guidelines.

Related donors will be selected from the patient's family members and relatives. Preference will be given to related donors over the age of 18 whenever possible. Detailed apheresis criteria for pediatric patients is outlined in Section 6.1.

Donors will be selected and informed about stem cell donation according to standard practice. This will be the responsibility of the transplantation center/NMDP. Donors screening and workup will be performed according to 21CFR1271 (see link) requirements and institutional guidelines. NMDP patients will be screened according to NMDP institutional requirements. Confirmatory testing will be performed at LPCHS or SHC as required by law. The donor informed consent will be collected. A detailed work up will be undertaken for the donors as per standard guidelines and institutional SOPs to establish the eligibility for donation.

4.4 Study Timeline

4.4.1 Primary Completion

Recruitment period for this study is expected to be 2 years and 6 months. Subjects will be followed for 1 year after treatment.

4.4.2 Study Completion

Total duration of this study is expected to be 3 years and 6 months.

5 PATIENT SCREENING ASSESSMENTS

5.1 General considerations for Patient and Donor Screening

Patients who are considered to be appropriate for the study will be initially pre-screened based upon review of the medical record, their oncologic history, and performance status. In order to confirm patient eligibility for the study, patients must first undergo an informed consent process to permit review of qualifying laboratories and to perform additional tests. All patients will undergo the screening procedures according to Section 12.1.

Donors will undergo a separate screening and qualification evaluation in accordance to Section 6.

5.2 Informed Consent

All participants will be provided a consent form describing the study with sufficient information for participants to make an informed decision regarding their participation in this study. Details of the consent process are outlined in Section 19.3. IRB approval will be obtained prior to initiating the consenting process.

5.2.1 Pediatric Assent

All participants between the ages of 7 and 17 years old will be provided with an assent describing all procedures to be performed throughout this study. See Section 19.3.1 for details regarding the assent process.

5.3 Patient Medical History

The patient's complete history through review of medical records and by interview will be collected and recorded. Concurrent medical signs and symptoms must be documented to establish baseline

severities. A disease history, including the date of initial diagnosis and list of all prior treatment, responses, and duration of response to the prior treatment also will be recorded.

5.4 Patient Physical exam

A complete physical examination will be performed. The exam will include general appearance of the patient, height (at baseline only) and weight, examination of the skin, eyes and ears, nose, throat, lungs, heart, abdomen, musculoskeletal system, and nervous system. Performance status will be scored on all subjects. Subjects under the age of 16 will be scored according to the Lansky Scale. Subjects who are ≥16 will be scored according to the Karnofsky Performance Status.

5.5 Patient Transplant workup

The patient will undergo an extensive pre-transplant work up. Work-up procedures must be completed prior to the start of conditioning. Infectious disease markers (IDMs) may be repeated to comply with Federal guidelines concerning transplantation. Recipient work up will follow the institutional guidelines.

6 DONOR STUDY PROCEDURES AND DONOR GRAFT PROCESSING

6.1 Donor Inclusion Criteria

Donors will be evaluated for participation in this study according to the following inclusion criteria:

1. Donors must meet all criteria for donation as per 21 CFR1271 Subpart C.

2. Unrelated donors must be ≥ 18 as per NMDP guidelines.

3. Related donors will be selected from the patient's family members and relatives. Preference will be given to related donors over the age of 18 whenever possible. If minor donors are to be enrolled, they will be a minimum of 12 years old. Detailed apheresis criteria for pediatric patients is outlined in section 6.3 of the protocol.

4. Related donors must meet all requirements to donate as per LPCH SOP for donors.

5. Able and willing to provide written, signed informed consent (assent as appropriate).

6.2 Donor Exclusion Criteria

Donors will be excluded from participating in this study according to the following exclusion criteria:

1. Donors who do not meet 21 CFR 1271 Subpart C requirements per the FDA to donate.

2. Donors who are unwilling or unable to sign informed consent (assent when appropriate).

3. Pregnant females will not be eligible to donate as per NMDP and LPCH guidelines.

6.3 Donor Screening

All donors will undergo HLA-typing. If original typing was not done with PCR-based typing, it must be repeated.

Donors will be evaluated with a full history and physical examination at SHC (Stanford Health Care) or through national or international bone marrow registries (i.e., National Marrow Donor Program), all under FACT guidelines.

Donors screening laboratories will include complete blood count with differential, serum chemistries including electrolytes, creatinine, liver function tests including bilirubin and alkaline phosphatase. Donor infectious disease screening laboratories will include: HIV1 and HIV2 (by antibody) and HIV PCR, Hepatitis B surface antigen and core antibody, Hepatitis C by PCR and antibody, *Treponema pallidum* (syphilis), HTLV1/2 and CMV by total antibody according to required testing set forth in 21 CFR Part 1271 Subpart C. In addition, donors will be screened for West Nile Virus, Zika Virus, and *Trypanosoma cruzi*. Viral and other infectious disease studies will be performed as per standard donor work-up guidelines. Donors will be screened for Creutzfeldt Jacob Disease through a questionnaire, which asks about previous exposure and travel/residence history.

The donor selection is made via a team meeting with attending physicians and an HLA expert. The mismatched donors in the patient's family or NMDP will be eligible for donation upon meeting all other criteria for bone marrow or peripheral blood stem cells (PBSC) donation.

Unrelated donors selected from the NMDP must be ≥ 18 as per NMDP guidelines.

Related donors will be selected from the patient's family members and relatives. Preference will be given to related donors over the age of 18 whenever possible.

Related donors under the age of 18 must meet all requirements for apheresis. Enrollment of related donors will be limited to those who can undergo unstimulated apheresis with minimal risk criteria as follows:

- Apheresis can be performed with peripheral venous access obtained without sedation and not with a central line
- The apheresis does not need a blood prime before the procedure(as per institutional apheresis SOP
- Related donors must meet all apheresis requirements as outlined in the Apheresis SOP for pediatric donors

6.3.1 Related Donor Additional Screening

All related donors will undergo an independent evaluation and examination by a licensed health care professional other than the intended recipient's primary transplant team. All related donors will be evaluated according to the institutional SOP for Donor Evaluation and Workup.

6.4 Donor Consent

Donors must be consented prior to any study related procedures.

6.4.1 Unrelated Donor Consent

All unrelated donors will be consented as per NMDP guidelines for unrelated donor consent.

6.4.2 Related Donor Consent/Assent

Related donors will be consented as per institutional guidelines. All donors between the ages of 7 and 17 years old will be provided with an assent describing all procedures to be performed throughout this study. See Section 19.31 for details regarding the assent process.

6.5 Donor Apheresis for Generation of T-allo10 Cells

All donors will undergo an apheresis procedure for collection of mononuclear cells for generation of T-allo10 cells. This collection will occur prior the scheduled recipient HSCT based on the T-allo10 manufacturing timeline.

Institutional guidelines will be followed for peripheral venous access and apheresis procedures. The cells collected will then undergo CD4 selection using CliniMACS®.

6.6 Donor Stem Cell Collection for HSCT

Donors will undergo either bone marrow collection or apheresis for collection of stimulated mononuclear cell for the purpose of HSCT. Absolute number of CD34+ stem cells and CD3+ T cells in the graft will be calculated.

6.6.1 Unrelated Donor Apheresis

Unrelated donor apheresis will be performed by the NMDP authorized transplant center. The HSCT cell dose for PBSC will be a minimum of $\geq 3x10^8$ TNC/kg.

6.6.2 Unrelated Donor Bone Marrow Collection

Unrelated donor bone marrow collection will be coordinated by the NMDP and will follow the NMDP guidelines for donor bone marrow collection. Cell dose for BM will be a minimum of $\geq 1x10^8$ TNC/kg.

6.6.3 Related Donor Apheresis

Related donors will undergo apheresis as per institutional guidelines stated above. Cell dose for PBSC will be a minimum of $\geq 3x10^8$ TNC/kg.

6.6.4 Related Donor Bone Marrow Collection

Related donor bone marrow collection will be performed according to institutional bone marrow collection guidelines. Cell dose for BM will be a minimum of $1x10^8$ TNC/kg.

6.7 Donor Timeline

Donor Study Evaluations	Screening	4 weeks before Recipient HSCT (D-108 to D-21)	Day -2 to D 0[e] of Recipient HSCT
Consent [a]	X		
Donor Workup [b]	X		
Donor Apheresis [c]		X	
Bone Marrow/ PBSC collection [d]			X
Infectious Disease Confirmation	X	X	
a	Consent to be completed per institutional and NMDP Guidelines prior to any study related procedures		
b	Workup to be completed per institutional and NMDP Guidelines		
c	Non-mobilized apheresis of single unit collection		
d	Bone marrow/ PBSC collection as per institutional and NMDP guidelines.		
e	Donors may be collected up to 2 days prior to D0 depending on HSCT source and institutional guidelines.		

7 PATIENT STUDY PROCEDURES

7.1 Patient Enrollment: Day of recipient apheresis

Before enrollment of a patient into the study, the physician must ensure the patient meets all eligibility criteria to be enrolled on the protocol. Eligibility criteria will be reviewed and confirmed by the Principal Investigator/Sub Investigator prior to subject being enrolled into the study.

Patient is considered enrolled into the study on the day of apheresis and will be assigned a unique study number. Each study number will be assigned consecutively in increasing order starting with "297-01-01".

7.2 Description of Study Procedures

Planned study procedures and the timing of these procedures are outlined in Section 9.6.

7.2.1 Medical History

A comprehensive medical history will be taken on all patients at Screening, as outlined in Section 5.3. Changes in the grading of any previously recorded medical history must be recorded as an adverse event as defined in Section 17.

7.2.2 Physical Exams

A comprehensive physical exam will be taken on all patients at Screening, as outlined in Section 5.4. Interval physical exams will be taken at all subsequent study visits according to Section 9.6.

The following assessments should be done in conjunction with the physical exam once the patient starts the conditioning regimen:

1. Mucositis Evaluation
2. Veno-occlusive disease (VOD)

7.2.3 Vital Signs

Vital signs including blood pressure, pulse, respiratory rate, and temperature will be assessed according to Section 9.6.

7.3 Standard Laboratory Assessments

The following standard laboratory tests will be collected for recording according to Section 9.6:

Hematology	WBC (with Differential if WBC > 500/mm3), RBC, hemoglobin, hematocrit, platelet count, MCV, absolute neutrophils, absolute lymphocytes, absolute monocytes, absolute eosinophils, absolute basophils.
Serum Chemistry	Sodium, potassium, chloride, carbon dioxide, BUN, glucose, creatinine, total protein, albumin, total bilirubin, direct bilirubin, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), AST (SGOT), ALT (SGPT), gamma-glutamyltransferase (GGT), calcium, magnesium, phosphorus.
Infectious Disease Surveillance	CMV, Adenovirus, BK virus, HHV6, EBV, Aspergillus galactomannan
Chimerisms	% donor whole blood (WB), % donor bone marrow (BM), CD3 WB & BM, CD15 WB & BM, CD19 WB & BM, CD34 WB& BM, CD56 WB& BM
Other	Pregnancy test for females of child-bearing potential; urinalysis

7.4 Immune Function Tests

Immune function testing will include the following tests as per standard of care and according to Section 9.6. All tests will be sent to the SHC laboratory. Table 7.4.1 describes the minimum required values that need to be reached before specific tests may be performed. Table 7.4.2 describes the frequency interval for each test once the minimum required values have been reached.

Table 7.4.1 Immune function test table describing minimum required values to initiate specific tests

	Minimum Required Values	Lymphocyte > 200/µL	CD3 > 200/µL	CD4 > 300/µL or 6 months post SCT whichever earlier	Positive PHA response	1 month post-1st immunization	Positive *in vitro* Ag-specific response
Specific Test	CD3 count abs.	X	X				
	CD4 count abs.		X				
	CD8 count abs.		X				
	CD19 count abs.		X				
	CD3- /16+ 56+ count abs.		X				
	Lymphocyte Mitogen Proliferation Panel			X			
	Immunize with Td				*X		
	Lymphocyte antigen (Tetanus) Proliferation Panel					X	
	Quantitative Immunoglobulin (QIg) and tetanus specific immunoglobulin titers						X

*For patients treated at SHC, their Immunization will occur at the one-year post HSCT mark and all activities post Td will be postponed.

Table 7.4.2 Immune function test frequency table

Test	**Frequency**
T, B, & NK Lymphocyte Subsets	Weekly until Day +60, then monthly.
Lymphocyte mitogen proliferation panel	Monthly until positive response.
Immunization with Td	1st immunization within 28 days after positive mitogen response; 2nd immunization 28 days (+/- 10 days) after the 1st immunization
Lymphocyte antigen (Tetanus) Proliferation Panel	Repeat test 1 month (+/- 10 days) after 2nd immunization if first test result is negative.

7.5 Research sample collection and handling

Refer to Section 9.6 for timing and frequency of all research lab sample collections. Detailed instructions for collection, type of tubes, volumes, handling and processing of samples are found in the T-allo10 Laboratory Manual.

7.6 Bone Marrow Aspirate

Bone marrow aspirate will be performed at specific time points as outlined in Section 9.6.

7.7 Recipient Apheresis

The recipient will undergo an apheresis procedure for the purpose of T-allo10 cell generation. The apheresis procedure will be performed according to institutional standards.

7.8 Screening and Baseline Assessments

Patient screening and baseline assessments are described in detail in Section 12.1 and Section 12.2.

7.9 Randomization Procedures

No randomization procedures will be followed.

7.10 Unblinding Procedure

This is an open label study; therefore, no unblinding procedure is required.

7.11 Graft-versus-Host Disease Assessments

7.11.1 Independent Evaluator

All patients who receive the T-allo10 investigational product will be evaluated by an independent evaluator. The GvHD evaluator will assess patients according to Section 9.6.

7.11.2 Physician Evaluations

In addition to the independent GvHD evaluator, patients will be assessed by the attending physician for GvHD according to Section 9.6 and according to standard of care and intuitional procedures.

8 Investigational Product Administration

8.1 Dose Calculation

T-allo10 cell dosage will be calculated based on the patient's weight in kilograms (at the time of apheresis) according to the cohort management plan outlined in Section 10.

8.2 Administration

On Day -1, T-allo10 cells will be brought to the stem cell unit from the BMT-CTF and thawed at the patient's bedside. Prior to cell infusion, the cell product identity will be double-checked by two authorized staff (MD or RN), and identification of the product and documentation of administration will be entered into the patient's medical record, as is done for blood product administration. Patients will receive pre-medication 30 minutes prior to the T-allo10 cell infusion with acetaminophen at a dose of 15 mg/kg not to exceed 650 mg total dose and diphenhydramine 1 mg/kg not to exceed 50 mg total dose. The cells will be infused within 30 minutes (or as appropriate for patient weight) and patients will be observed for infusion related reactions for the next 24 hours. All infusion related toxicities will be recorded, and grade 3 and 4 toxicities will be reported as per reporting guidelines in Section 17.3.

8.3 Safety Monitoring

Patients will be closely monitored for infusion related toxicities. Vital signs will be recorded as described in Section 9.3. Safety monitoring will be performed according to Section 17.

9 PATIENT TREATMENT PLAN

Patient treatment will be divided into 4 phases:

- Conditioning
- T-allo10 Cell Infusion
- Bone Marrow Infusion
- Follow-up

Study visit details are described in Section 9.6 and Section 12.

9.1 Conditioning

Patients will receive fractionated total body irradiation (FTBI) and cyclophosphamide as per the schedule below. Supportive care for FTBI and cyclophosphamide will follow institutional guidelines.

9.1.1 Cranial Spinal Boost (CSB)

Patients with central nervous system (CNS) disease at presentation or relapse will receive a CSB as per institutional guidelines.

Day	Agent	Dose	GvHD Prophylaxis
-8	FTBI	120cGy X3 doses	
-7	FTBI	120cGy X3 doses	
-6	FTBI	120cGy X3 doses	
-5	FTBI	120cGy X2 doses	
-4	Cyclophosphamide	60 mg/kg	
-3	Cyclophosphamide	60 mg/kg	Start Sirolimus
-2	Rest		
-1	T-allo10 Cell Infusion		
0	Stem Cell Infusion (TNC/kg)	BM: $\geq 1 \times 10^8$ PBSC: $\geq 3 \times 10^8$	
+1			Start MMF

9.2 GvHD Prophylaxis

Patients will be started on Sirolimus at a loading dose of 3 mg/m^2 on Day -3 and then 1 mg/m^2 daily thereafter. Drug levels will be monitored at least twice a week and dose adjusted to maintain levels between 8-12 ng/ml. See Section 13.4.1. Mycophenolate Mofetil will be started on Day +1 after transplant at a dose of 15 mg/kg every 8 hours. In case of GvHD occurrence, grading of GvHD and all treatments will be recorded in the CRF. Taper schedules can be found for both medications in Section 13.4.

9.3 T-allo10 Cell Infusion

On Day -1, T-allo10 cells will be brought to the stem cell unit and thawed at the patient's bedside. Patients will receive pre-medication 30 minutes prior to the T-allo10 cell infusion with acetaminophen at a dose of 15 mg/kg NTE 650 mg total dose and diphenhydramine 1 mg/kg NTE 50 mg total dose. The cells will be infused within 30 minutes (or as appropriate for patient weight) and patients will be observed for infusion related reactions for the next 24 hours. All infusion related toxicities will be recorded, and grade 3 and 4 toxicities will be reported as per reporting guidelines in Section 17.3.

Patients will have vital signs recorded as per table below:

1. Pre-infusion	5. 1 hour after the start of infusion
2. 15 minutes after the start of infusion	6. 2 hours after the start of infusion
3. 30 minutes after the start of infusion	7. 4 hours after the start of infusion
4. 45 minutes after the start of infusion	8. 24 hours after the start of infusion

Patient must be monitored during infusion for **ANY** adverse reactions, including the following expected infusion related reactions: (see Section 17.3 for adverse event reporting)

- Fluid overload
- Respiratory distress (shortness of breath, wheezing)
- Nausea
- Vomiting
- Chest pain
- Chills (rigors)
- Fever
- Rash/hives
- Hypertension
- Anaphylaxis
- Hypotension
- Back or flank pain
- Bradycardia
- Tachycardia

9.4 Bone Marrow or PBSC Infusion

On Day 0, HSC (bone marrow or PBSC) will be collected from the donors as per institutional standards. The minimal target for bone marrow collection is 1 x 10^8 nucleated cells per kilogram. The minimal target for PBSC collection is 3 x 10^8 nucleated cells per kilogram. The HSC will be processed in the BMT-CTF for red cell or plasma depletion as indicated by the blood group incompatibility. The HSC will be transported to the stem cell unit by a courier. Patients will receive premedication with acetaminophen 15 mg/kg NTE 650 mg total dose and diphenhydramine 1 mg/kg NTE 50 mg total dose. The HSC will be released by the BMT-CTF after release criteria are met. The cells will be infused, at least 24 hours after the T-allo10 cell infusion. The cells will be infused and patients will be observed for infusion related reactions for the next 24 hours. All infusion related toxicities will be recorded and grade 3 and 4 toxicities will be reported as per reporting guidelines in Section 17.

Patient must be monitored during infusion for **ANY** adverse reactions including the following infusion related reactions: (see Section 17 for adverse event reporting)

- Fluid overload
- Respiratory distress (shortness of breath, wheezing)
- Nausea
- Vomiting
- Chest pain
- Chills (rigors)
- Fever
- Rash/hives
- Hypertension
- Anaphylaxis
- Hypotension
- Back or flank pain
- Bradycardia
- Tachycardia

9.5 Follow up Phase

The patient will be evaluated weekly according to the Schedule of Events through Day +60 following HSCT. After Day +60, the patient will be evaluated monthly, unless indicated otherwise on the Schedule of events (Section 9.6 for details). Specific details of the requirements of the individual tests are detailed in Section 7.

Immune reconstitution will be evaluated per Section 7.4.

Time points outlined in Schedule of Events should be closely followed. Visit windows up to Day +60 will be +/- 3 days. Following Day +60 a tolerance of 10 days (+/-) is permitted.

9.6 Schedule of Events (Recipient)

Study Evaluations	Screening	Baseline	Recipient apheresis	Conditioning	Day -1 (T-allo10 infusion)	Day 0 (HSCT infusion)	Weekly until Day +60 (+/- 3 days)	Day +28 (+/- 3 days)	Day +42 (+/- 3 days)	Day +60 (+/- 3 days)	Monthly until Day +365 (+/-10 days)	Day +90 (+/- 10 days)	Day +180 (+/- 10 days)	Day +365 (+/-10 days)	Early Termination (+/-10 days)
Informed consent	X														
Medical/Clinical history	X														
Physical Exam [i]	X	X		X	X	X	X				X				X
Performance status (Lansky or Karnofsky)	X	X		X	X	X	X				X				X
Vital Signs	X	X		X	X	X	X				X				X
Echocardiogram or MUGA	X														
Pulmonary function evaluation	X														
HIV testing	X														
Standard transplant work-up [h]	X														
CBC with differential	X	X		X	X	X	X				X				X
Serum Chemistry [a]	X	X		X	X	X	X				X				X
Urinalysis	X	(X)		(X)	(X)	(X)	(X)				(X)				(X)
Infectious Disease Surveillance [b]		X		(X)	(X)	(X)	X				(X)		X	X	(X)
Pregnancy Test [g]	X	X													(X)
Sirolimus Levels [f]				X	X		2X								
Recipient apheresis (Enrollment)			X												
T-allo10 Cell Infusion [c]					X										
HSCT Cell Infusion [c]						X									

Study Evaluations	Screening	Baseline	Recipient apheresis	Conditioning	Day -1 (T-allo10 infusion)	Day 0 (HSCT infusion)	Weekly until Day +60 (+/- 3 days)	Day +28 (+/- 3 days)	Day +42 (+/- 3 days)	Day +60 (+/- 3 days)	Monthly until Day +365 (+/-10 days)	Day +90 (+/- 10 days)	Day +180 (+/- 10 days)	Day +365 (+/-10 days)	Early Termination (+/-10 days)
Infusion Toxicities					X	X									
Disease (Re)staging –BM aspirate (+/- biopsy as indicated)[i]	X							X	X	(X)		X	X	X	(X)
Chimerism-Bone Marrow[j]								X	X	(X)		X	(X)	X	(X)
Chimerism-Peripheral Blood[j]								X	X	(X)		X	X	X	(X)
Immune Function Test [d]	X						(X)				(X)				(X)
Research Lab Sample [e]	X	X	X		X	X	X				X				X
GvHD evaluation							X				X				X
Adverse Events Assessment		X	X	X	X	X	X				X				X
Concomitant Medications	X	X	X	X	X	X	X				X				X

a		See Section 7.3 for details regarding serum chemistry
b		Includes adenovirus, BK virus, CMV, EBV, HHV6 and fungal surveillance
c		See section 9 for details regarding T-allo10 and HSCT infusion
d		See Section 7.4 for details regarding Immune Function Test
e		See Section 7.5 for details regarding Research laboratory studies
f		Sirolimus levels will be performed at least twice weekly and as clinically indicated
g		Pregnancy test must be repeated prior to chemotherapy starting according to institutional standards
h		Standard transplant workup will follow institutional guidelines and must be completed prior to the start of conditioning. It does not need to be completed prior to enrollment.
i		Physical exams include review of systems and weight. Height taken at baseline.
j		If Day 28 peripheral blood and bone marrow meets all criteria of engraftment, Day 42 disease restaging and chimerism will not be performed. In replacement Day 60 disease restaging and chimerism to be done if clinically needed
(X)		As clinically indicated

10 COHORT MANAGEMENT PLAN

Dose escalation will proceed in cohorts of 3–9 patients. Each dose cohort is expected to enroll a minimum of 3 patients and a maximum of 6 evaluable patients at each dose level to determine MTD. In addition, the study will allow for up to 3 additional patients to be enrolled in each of the dose cohorts (9 additional patients for complete study) due to inability to achieve target cell doses. If any patient is withdrawn from study for any reason (withdrawal of consent, relapse before start of chemotherapy, etc.) before receiving the T-allo10 cells, the patient will not be counted in total evaluable patients and will be replaced. Up to a maximum of 3 inevaluable patients may be replaced. The maximum number of patients that may be enrolled will be 30 (18 + 9 + 3).

There will be a 28 day safety evaluation between the first and second subject in cohort one. The second subject in cohort one will be treated no sooner than 29 days after the first subject's infusion of T-allo10 (after safety assessment). Subsequent subjects in cohort one may be treated with T-allo10 at least 28 days after the preceding subject is treated with T-allo10 in the cohort.

After infusion of T-allo10 in the last subject of each cohort, there will be a 28 day safety evaluation period. If the cohort meets safety and tolerability criteria, subsequent subjects may be enrolled in the cohort of the next higher cell dose.

In cohorts two and three subjects may be treated with T-allo10 at least 28 days after the preceding subject is treated with T-allo10 in the cohort. Subjects 1 and 2 (in Cohort 1) will be ≥ 12 years' old.

Dose Escalation Schedule	
Dose Level	**Dose of (IND Agent)**
Cohort 1	1 X 10^6/kg (± 10%)
Cohort 2	3 X 10^6/kg (± 10%)
Cohort 3	9 X 10^6/kg (± 10%)

If de-escalation from the lowest dose of $1x10^6$/kg is needed, we will follow the dose de-escalation schedule indicated in the table below.

Dose De-escalation Schedule	
Dose Level	**Dose of (IND Agent)**
Cohort -1	8 X 10^5/kg (± 10%)
Cohort -2	6 X 10^5/kg (± 10%)
Cohort -3	4 X 10^5/kg (± 10%)

Dose escalation will follow the rules outlined in the table in section 10.1 below.

10.1 Safety and tolerability:

The MTD is the highest dose level that can be feasibly generated to meet established release criteria at which no more than 1 out of 6 patients experience a DLT from the infusion of T-allo10 cells, or the dose below that at which at least 2 (of ≤6) patients have a DLT as a result of the infusion of T-allo10 cells.

Number of Patients with DLT at a Given Dose Level	**Escalation Decision Rule**
0 out of 3	The cohort meets safety and tolerability criteria and we may enroll patients in next higher cohort.
1 out of 3	Enter up to 3 more patients at this dose level. • If 0 of these 3 patients experience DLT, the cohort meets safety and tolerability criteria and we may enroll patients in next higher cohort. • If 1 or more of this group suffer DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose. Up to three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
2 out of ≤ 6	Dose escalation will be stopped. This dose level will be declared the maximally administered dose

	(highest dose administered). Up to three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose. If cohort 1 is unable to meet safety and tolerability criteria, dose de-escalation will occur and cohort -1 should be tested for tolerability and safety by enrolling at least 3 patients in that cohort.
≤1 out of 6 at highest dose level below the maximally administered dose	This is the MTD and would be recommended as the phase 2 dose. At least 6 patients must be entered at the recommended phase 2 dose.

If the third dose level is completed without DLT, an MTD may not be determined. This will be considered the 'highest cell dose' studied, and will be the dose level that may be considered for further study. Alternatively, if no toxicity or clinical activity is observed after completion of the 3rd dose level, consideration may be given to adding additional dose levels in a protocol amendment.

In addition, if the targeted T-allo10 cell dose cannot be generated for at least 3 patients out of ≤ 6 patients in a dose cohort, accrual to that dose level will stop and the dose escalation phase of the study will end as manufacturing will not be feasible for that dose level. The highest dose level that can be feasibly manufactured tested for safety and tolerability will be the 'highest cell dose' studied, and the MTD may not be determined.

If the targeted T-allo10 cell dose number for a dose cohort cannot be generated for any patient, the patient will receive the total manufactured T-allo10 cell dose, as the efficacious dose level is not known. That patient will be considered a feasibility failure, and will be evaluated for safety in the dose cohort for the actual cell dose administered. For each patient who fails the feasibility criteria, another patient is enrolled in that cohort (up to a total of 9 patients). If additional patient(s) who receive cells in a previously defined safe dose cohort experience DLT(s) that meet the criteria for MTD (2 out of ≤ 6 patients experience DLT), dose escalation will pause pending discussions with the IRB and FDA regarding dose modification/escalation decisions.

11 DOSE LIMITING TOXICITIES

11.1 Definitions

A dose limiting toxicity (DLT) is defined as the following:

- Any Grade 3 or 4 related TEAE
- Any Grade 3 or 4 suspected AE

The following exceptions will be made in Section 11.2.

11.2 DLT Exceptions

The following Infusion Reactions are expected with the infusion of cellular therapy. The following adverse events will be considered DLTs only if they occur as specified below:

- Fluid overload that does not resolve within 24 hours
- Chills that cannot be reversed after 6 hours with standard care
- Hypotension that does not resolve after 3 fluid boluses of 10 ml/kg each
- Respiratory distress (shortness of breath, wheezing) that does not resolve within 30 minutes following the T-allo10 cell infusion
- Fever that cannot be reversed within 24 hours within standard care
- Back or flank pain that cannot be reversed within 12 hours with standard care
- Bradycardia that cannot be reversed within 24 hours
- Rash/Hives that cannot be reversed within 24 hours with standard care
- Hypertension that does not resolve within 2 hours with standard care
- Tachycardia that does not resolve within 24 hours and is not associated with fever
- Chest pain that does not resolve within 30 minutes of the infusion

Acute GvHD will not be considered as DLT for the study but is followed as the secondary endpoint. To ensure safety of the patients, acute grade III and above GvHD is one element in the list of safety stopping rules. The stopping rule addresses excessive acute grade III and above GvHD in Section 3.2.

11.3 DLT Assessment Window

DLTs associated with the infusion of T-allo10 cells will be evaluate through 24 hours following the T-allo10 cell infusion. Adverse events will be reported as described in Section 17.

DLTs will be assessed from the time of T-allo10 cell infusion until 28 days following the infusion.

11.4 DLT Reporting Procedures

During the DLT assessment window, any DLTs should be reported to Sponsor-Investigator and DSMC as follows:

11.4.1 Sponsor-Investigator- DLTs will be reported to the sponsor within 24 hours.

11.4.2 Data Safety Monitoring Committee (DSMC)

- ***Within 5 working days***

  Any DLT that is:

  - Suspected: associated with the use of the study drug,
  - unexpected,
  - fatal or life-threatening, or serious (not fatal or life threatening) and
  - places participants at greater risk of harm

12 PATIENT STUDY EVALUATIONS AND PROCEDURES

12.1 Screening Evaluations and Procedures

All screening assessments must be done prior to enrollment.

- Informed consent and assent (if applicable) must be obtained prior to any study-related procedures. If an assessment was done prior to informed consent as part of standard of care it may be used for screening.
- Complete medical/clinical history (including previous and concomitant diseases) using NCI CTCAEv4.
- Physical examination per Section 7.2.2.
- Performance status (Karnofsky or Lansky).
- Complete Blood Count (CBC) with differential.
- Serum Chemistry per Section 7.3.
- Urinalysis
- Vital signs per Section 7.2.3.
- Standard transplant work up per institutional standards*.
- Echocardiogram or MUGA with ejection fraction/shortening fraction as appropriate.
- Pulmonary function evaluation: Pulmonary function tests (if age appropriate and feasible) or oxygen saturation.
- HCV RNA testing and nucleic acid amplification test for HIV.
- Bone marrow aspirate (and biopsy where appropriate) to evaluate disease status.
- Immune function test per Section 7.4.
- Research lab sample per Section 7.5.
- Pregnancy test in all females of child-bearing potential.
- Adverse events assessments using NCI CTCAEv4.
- Concomitant medications.

* Standard transplant workup will continue in baseline and does not need to be completed prior to enrollment.

12.2 Baseline Evaluations and Procedures

Baseline evaluations will occur from enrollment to start of conditioning for HSCT.

- Recipient Apheresis per Section 7.7.
- Continue remaining standard transplant work up per institutional standards.
- Physical examination per Section 7.2.2.
- Vital signs per Section 7.2.3.
- Performance status (Lansky or Karnofsky).
- CBC with differential.
- Serum Chemistry per Section 7.3.
- Infectious disease surveillance per institutional standards.
- Pregnancy test for all females of child bearing potential.
- Urinalysis if clinically indicated.

- Research lab sample per Section 7.5.
- Concomitant medications.
- Adverse events assessments using NCI CTCAEv4.

12.3 Treatment Evaluations and Procedures

Treatment evaluations are divided into the following phases:

- Conditioning: Day -8 to Day -2
- T-allo10 cell infusion day: Day -1
- HSC infusion day: Day 0
- Post-transplant Days: +1 until Day +365

12.3.1 Conditioning: Day -8 to Day -2

- Physical examinations per Section 7.2.2.
- Vital signs per Section 7.2.3.
- Performance status (Lansky or Karnofsky).
- Infectious disease surveillance if clinically indicated.
- Serum Chemistry per Section 7.3.
- CBC with Differential.
- Urinalysis if clinically indicated.
- Adverse events assessments using NCI CTCAEv4.
- Concomitant medications.

12.3.2 T-allo10 Cell Infusion: Day -1

- Physical examination per Section 7.2.2.
- Performance status (Lansky or Karnofsky).
- Vital signs during infusion as per Section 9.3.
- Serum Chemistry as per Section 7.3.
- CBC with Differential.
- Urinalysis if clinically indicated.
- Sirolimus level if clinically indicated.
- Infectious disease surveillance if clinically indicated.
- T-allo10 cell infusion as per Section 9.3.
- Adverse events assessments using NCI CTCAEv4.
- Infusion toxicities assessment per Section 9.3.
- Research lab samples as per Section 7.5.
- Concomitant medications.

12.3.3 HSC Infusion: Day 0

- Physical examination per Section 7.2.2.
- Performance status (Lansky or Karnofsky).

- Vital signs during infusion as per Section 9.4.
- Serum Chemistry as per Section 7.3.
- CBC with Differential.
- Urinalysis if clinically indicated.
- Sirolimus level if clinically indicated.
- Infectious disease surveillance if clinically indicated.
- Stem cell infusion as per Section 9.4.
- Adverse events assessments using NCI CTCAEv4.
- Infusion toxicities assessment per Section 9.4.
- Research lab samples as per Section 7.5.
- Concomitant medications.

12.3.4 Post-Transplant follow up phase:

Assessments to be done weekly (+/- 3 days) through Day +60, then monthly (+/- 10 days) through Day +365

- Physical examination per Section 7.2.2.
- Vital signs as per Section 7.2.3.
- CBC w/differential.
- Urinalysis if clinically indicated.
- Serum Chemistry as per Section 7.3.
- Sirolimus level.
- Performance status (Lansky or score Karnofsky).
- Graft versus host disease (GvHD) evaluations per Section 7.11.
- Infectious disease surveillance if clinically indicated.
- Immune function test as per Section 7.4.
- Research lab samples as per Section 7.5.
- Adverse events assessments using NCI CTCAEv4.
- Bone marrow aspirate (and biopsy where appropriate) to evaluate engraftment/disease status per Section 9.6.
- Chimerism-Bone marrow per Section 9.6.
- Chimerism-Peripheral blood per Section 9.6.
- Concomitant medications.

12.4 Early Termination

In the event the subject must come off study, every effort should be made to complete the early termination assessments.

- CBC with differential.
- Serum Chemistry per Section 7.3.
- Urinalysis if clinically indicated.
- Pregnancy test if clinically indicated.

- Physical examination per Section 7.2.2.
- Vital signs per Section 7.2.3.
- Performance status per Lansky or Karnofsky
- Research lab samples per Section 7.5.
- Graft versus host disease (GvHD) evaluations per Section 7.11.
- Immune function test per Section 7.4.
- Infectious disease surveillance if clinically indicated.
- Bone marrow aspirate (and biopsy where appropriate) to evaluate engraftment/disease status if clinically indicated.
- Chimerism-Bone marrow if clinically indicated.
- Chimerism-Peripheral blood if clinically indicated.
- Adverse events assessments using NCI CTCAEv4.
- Concomitant medications.

12.5 Screen Failures

If a subject does not meet eligibility requirements or withdraws from the study prior to enrollment, they will be considered a screen failure.

13 CONCOMMITANT MEDICATIONS

In general, concomitant medications deemed necessary for the support and safety of the patients are permitted in accordance with standard practice (such as medications for emesis, diarrhea, etc.). Use of neutrophil growth factors (G-CSF) or red blood cell growth factors (erythropoietin) are permitted per institutional policy. Transfusions may be given in accordance with institutional policy. All supportive medications and interventions do not need to be recorded in the patient's CRF unless specified below.

Prohibited medications are listed in Section 13.5.

13.1 Medications used to treat AE, SAE, TEAE, and TESAE

Medications used to treat any Grade 3 or 4 adverse event must be recorded on the case report form. Any medication used to treat a TEAE will be reported on the case report form.

13.2 Immunosuppressive Medications

All medications used for immunosuppression, including prophylactic medications, must be recorded on the case report form.

13.3 Supportive Medications

13.3.1 Viral Prophylaxis

All patients will be treated as high risk and will receive viral prophylaxis according to institutional standards.

13.3.2 Fever Prophylaxis

Any temperature of > 38˚C will require initiation of the Fever work-up and treatment as per institutional standard operating procedures (SOPs).

13.3.3 Fungal Prophylaxis

All patients will be treated as high risk and will receive fungal prophylaxis according to institutional SOPs.

13.3.4 VOD Prophylaxis

Patients will receive Actigall 6 mg/kg as per institutional VOD SOP. Patients who develop VOD will be assessed and treated as per the VOD SOP.

13.4 Medications for GvHD

13.4.1 Sirolimus

a) Sirolimus dosing: Sirolimus will begin on Day -3 with a 3 mg/m^2 oral loading dose, followed by 1 mg/m^2/day rounded to the nearest full milligram. Doses may be repeated if the patient vomits within 15 minutes of an oral dose.
b) Sirolimus monitoring: Sirolimus levels will be drawn twice weekly during hospitalization, with doses adjusted to maintain a target serum trough level of 8-12 ng/ml. Thereafter, drug levels will be assessed weekly to Day +100 and as clinically indicated, such as in the setting of an acute change in renal function or a new medication with known drug-drug interactions.
c) Sirolimus tapering: Tapering of sirolimus will begin at Day +120 in the absence of GvHD and disease relapse. The goal of tapering is complete discontinuation of immunosuppression by 8 months post-HCT. Tapering by approximately 10% of the Day +120 dose per week will achieve discontinuation by approximately 8 months post-HCT. Sirolimus and MMF should be tapered in an alternate fashion every other week when clinically feasible with both drugs discontinued simultaneously. (See "MMF tapering" below).
d) Drug Interactions: Fluconazole and other azoles drugs are known to increase the sirolimus blood levels; therefore, sirolimus drug levels must be monitored closely and doses adjusted accordingly. Voriconazole has previously been contraindicated with the use of sirolimus. However, recent pharmacokinetic information supports the safe use of sirolimus at 90% of the dose if co-administered with oral voriconazole[86]. To achieve appropriate dosing of sirolimus, a liquid formulation of sirolimus, available at 1 mg/ml concentration, may be required. The liquid form can be taken directly or administered within gelatin capsules.

13.4.2 Mycophenolate Mofetil (MMF)

a) MMF Dosing: MMF will begin intravenously (IV) on Day +1 at 15 mg/kg three times a day (TID). For patients with Body Mass Index ≥ 30, MMF dose will be calculated based on the adjusted ideal body weight (Adjusted IBW = IBW + 0.25 X (Actual Body Weight – IBW). Maximum MMF dose not to exceed 3 g/24hrs. MMF may be changed to oral dosing at Day ≥ 28, upon resolution of the patient's regimen-related gastroenteritis and control of GvHD. The conversion to oral dosing is one-to-one with the IV dose. If the patient develops gastrointestinal symptoms believed to be due to MMF, such as large volume diarrhea, the dose may be reduced to 10 mg/kg three times/day.
b) MMF Monitoring: MMF will be administered IV or orally at fixed doses until the initiation of the taper schedule.
c) MMF tapering: Tapering of MMF will begin on Day +100 in the absence of GvHD and disease relapse. The goal of tapering is complete discontinuation of immunosuppression by 8 months post-HCT. Tapering by approximately 10% of the Day +100 dose per week will achieve discontinuation by approximately 8 months post-HCT. Sirolimus and MMF should be tapered in an alternate fashion every other week when clinically feasible with both drugs discontinued simultaneously.
d) Drug Interactions: Oral magnesium and antacids should be avoided as they reduce the absorption of MMF. If needed, MMF should be given 2-3 hours prior to any oral magnesium or antacids.

13.5 Prohibited Medications

- Hydrocortisone is not allowed as a pre-medication for T-allo10 cells. It may be used for HSCT as per institutional guidelines.
- Hydrocortisone may be used to treat grade 4 infusion reactions related to the T-allo10 cell administration
- Patients may not receive any other investigational agents for 30 days prior to enrollment and while they are enrolled on study.

14 CRITERIA FOR SUBJECT REMOVAL FROM STUDY AND COMPLETION

14.1 Dose Limiting Toxicity (DLT)

Subjects who experience a DLT that in the opinion of the investigator would prohibit the subject from continuing in the study will be removed. All DLTs will be followed until resolution.

14.2 Withdrawal of Consent

Subjects may withdraw consent for participation in the study at any time. However, if the patient decides not to participate once conditioning has started, the subject will not receive the T-allo10 cells, but will receive a HSCT as per standard of care and institutional guidelines and will be replaced in the accrual numbers. Subjects who withdraw consent following the HSCT infusion will be followed as per standard of care.

14.3 Completion of Study Visits and Procedures

All subjects who complete study visits and procedures will be removed from the study at the time of completion.

14.4 Disease Relapse

Subjects who experience relapse of their primary disease after T-allo10 infusion and after stem cell transplant will be removed from the study.

14.5 Unable to receive T-allo10 cells

Subjects who are not able to receive T-allo10 cells for any reason will be withdrawn from the study and will continue receive their transplant under standard of care.

15 CORRELATIVE STUDIES

15.1 Laboratory Correlative Studies

All correlative tests will be processed by/for the Roncarolo Laboratory located in the Lorry Lokey Stem Cell Institute, 265 Campus Drive West, Room 3015, Palo Alto, CA. The correlative tests include but not limited to:

1. T helper cell panel
2. T regulatory cell panel
3. Recent Thymic Emigrants
4. Cytokine production
5. Functional host donor reactivity
6. Memory / Naïve (CD45RA / CD45RO) cells

16 STUDY ENDPOINTS

16.1 Primary Endpoint

The primary endpoints are as follows:

- Tolerability of T-allo10 cells as evidenced by the incidence and severity of treatment-emergent adverse events (TEAE), laboratory abnormalities, changes in vital signs, and changes in physical examination following infusion of T-allo10 cells, recorded and graded according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 (see link).
- Safety of T-allo10 cells as evidenced by the time to stem cell engraftment after HSCT. Stem cell engraftment is defined as:
    - Absolute Neutrophil Count (ANC) above 500/mm^3 for three consecutive days by Day +42.
    - Presence of hematopoiesis, by bone marrow examination, with cellularity > 5% and donor chimerism > 90% by STR analysis for the presence of donor cells by Day +42.
    - Absence of disease relapse as evidenced by minimal residual disease (MRD) assay < 0.1% by Day +42.
- Feasibility defined by the rate of successful manufacture of the T-allo10 cells to satisfy the targeted dose level and meet the required release specifications.

16.2 Secondary Endpoint

The secondary endpoint for this study is the incidence of grade III and grade IV acute GvHD at Day +100 following the infusion of T-allo10 cells, assessed using the Modified Keystone scale administered by an independent evaluator on study visits through Day +100 according to schedule of events (Section 9.6)

16.3 Exploratory Endpoint

The exploratory endpoints for this study are as follows:

- Incidence and severity of chronic GvHD after Day +100 through Day +365, assessed by the NIH consensus guidelines administered by an independent evaluator on study visits from Day +100 through Day +365 according to schedule of events (Section 9.6).
- Time to immune reconstitution, assessed by time to reach >200/microliter CD3+ T cells.
- Disease free survival at Day +365 assessed by bone marrow aspirate examination, morphology, MRD assay, and donor chimerism by STR analysis.

16.4 Analysis of Safety

Patients will be assessed for treatment emergent adverse events (TEAE) and treatment emergent serious adverse events (TESAE). Patient assessments will include alterations in vital signs, changes in physical exam, clinical laboratory studies, and other tests like ECG, PFT, radiological studies and other studies being performed per protocol.

17 SAFETY REPORTING/DATA AND SAFETY MONITORING PLAN

17.1 Definitions

17.1.1 Adverse Event (AE)

An adverse event (AE) is an undesirable medical occurrence (sign, symptom, or diagnosis) or worsening of a pre-existing medical condition that occurs after Informed Consent whether or not it is considered to be related to the investigational product, or other protocol-imposed intervention, regardless of attribution. This includes the following:

1. AE not previously reported in the Medical History that emerge during the protocol-specified AE reporting period, including signs or symptoms that were not present prior to the AE reporting period.
2. Pre-existing medical conditions judged by the Attending Physician or designee to have worsened in severity, frequency, or increased in duration during investigational product treatment during the protocol-specified AE reporting period.

Abnormal laboratory values or test results constitute recordable AE. The highest CTCAE grade (I-IV) for the adverse event will be recorded.

An abnormal laboratory value will be considered a reportable AE if the laboratory abnormality is characterized by any of the following:

- Results in interruption or discontinuation of the transplantation process
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is judged by the Attending Physician or designee to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the Attending Physician or designee will provide details about the action taken with respect to the test drug and about the patient's outcome.

17.1.2 Treatment-Emergent Adverse Events (TEAE)

A Treatment-Emergent Adverse Event (TEAE) is any AE that occurs after initiation of the investigational product infusion (T- allo10 cell infusion) regardless of severity, or any event that was present at baseline and worsened in intensity. Adverse events that occur prior to the T-allo10 cell infusion but after the start of the conditioning regimen will be primarily attributable to the conditioning regimen, disease or extraneous causes. After T-allo10 cell infusion, toxicities will be evaluated for temporal and causal relationship to the conditioning regimen versus cell infusion. Some symptoms may overlap and attribution will not be clearly definable, in which case toxicities will be attributed as possibly related to conditioning and cell infusion.

Toxicities will be attributed to the T-allo10 cell infusion if:

1. They were NOT present before the T-allo10 cell infusion; OR
2. If they increase in Grade in temporal association with the T-allo10 cell infusion; AND
3. They are not clearly explained by other factors.

Similarly, after the stem cell infusion, symptoms that occur may overlap and attribution between the T- allo10 cell infusion and the stem cell infusion may not be clearly definable. If toxicities were not present before the stem cell infusion or are known stem cell infusion toxicities, they will be attributable to the stem cell infusion. Any symptom that occurs after the T-allo10 cell infusion and increases in grade with the stem cell infusion could be possibly related to the T-allo10 infusion and will be reported as TEAE.

17.1.3 Serious Adverse Event (SAE)

An SAE is any AE that is any of the following:

1. Fatal (i.e., the AE actually causes or leads to death).
2. Life threatening (i.e. the AE, in the view of the investigator, places the subject at immediate risk of death).
3. Requires or prolongs inpatient hospitalization.
4. Results in persistent or significant disability/incapacity (i.e., the AE results in substantial disruption of the subject's ability to conduct normal life functions).
5. A congenital anomaly/birth defect in a neonate/infant born to a mother exposed to the investigational product(s).
6. Considered a significant medical event by the investigator (e.g., may jeopardize the subject or may require medical/surgical intervention to prevent one of the outcomes listed above).

All AE that do not meet any of the criteria for serious should be regarded as **non-serious AE**. The terms "severe" and "serious" are not synonymous. Severity refers to the intensity of an AE (as in mild, moderate, or severe pain); the event itself may be of relatively minor medical significance (such as severe headache). "Serious" is a regulatory definition and is based on subject or event outcome or action criteria usually associated with events that pose a threat to a subject's life or vital functions. Seriousness (not severity) serves as the guide for defining regulatory reporting obligations.

Severity and seriousness should be independently assessed when recording AE and SAE on the CRF. The Attending Physician or designee is responsible for ensuring that all AE and SAE are recorded on the CRF and reported to the Sponsor-Investigator in accordance with protocol instructions.

17.1.4 Suspected Adverse Reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the investigational therapy caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the investigational therapy and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by an investigational therapy.

17.1.5 Unexpected adverse reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected", also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

17.1.6 Pregnancies

Pregnancies occurring after the infusion of investigational product are considered immediately reportable events. While not considered a serious adverse event unless a serious criterion is met, pregnancies occurring in subjects enrolled on the study must be reported and followed to outcome. The Attending Physician or designee should complete the pregnancy report form and provide it to the Sponsor-Investigator within one working day of knowledge of the pregnancy. Following delivery or termination of pregnancy, the follow-up pregnancy report should be completed and provide it to the Sponsor-Investigator. Spontaneous abortions should always be reported as SAE. Follow-up information regarding the outcome of the pregnancy and any postnatal sequelae in the infant will be required.

17.1.7 Protocol Deviations

Any change, divergence or departure from the IRB approved protocol will be documented and reported to the Investigator-Sponsor.

17.2 Adverse Event Characteristics

17.2.1 Severity

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

17.2.2 Relationship to Investigational Product

The Attending Physician must document their opinion of the relationship of the AE, TEAE or SAE to the investigational product as follows.

NOT RELATED: The event can be readily explained by the subject's underlying medical condition, a concomitant therapy or other cause and the Investigator believes no relationship exists between the event and the investigational product. In this case, the Investigator should document the condition,

concurrent/underlying illness, medication, study procedure, or other cause they believe to be the cause of the adverse event.

UNLIKELY: The event does not follow a reasonable temporal sequence from administration of the investigational product nor does the event follow a known or expected response pattern to the investigational product and may have another cause. In this case, the Investigator should document the condition, concurrent/underlying illness, medication, study procedure, or causality believed to have contributed to the adverse event.

POSSIBLE: The subject's condition, concurrent/underlying illness, medication, or study procedures cannot explain the event, and there is a plausible temporal relationship between the event and the investigational product administration.

PROBABLE: The temporal relationship between the administration of the investigational product and the adverse event strongly suggests a relationship, and/or the adverse event cannot be reasonably explained by another condition, concurrent/underlying illness, medication, study procedure or other cause, or the adverse event abates with discontinuation of the investigational product, and recurs with re-administration.

Adverse events that occur prior to the T-allo10 cell infusion but after the start of the conditioning regimen will be primarily attributable to the conditioning regimen, disease or extraneous causes. After T-allo10 cell infusion, toxicities will be evaluated for temporal and causal relationship to the conditioning regimen versus cell infusion. Some symptoms may overlap and attribution will not be clearly definable, in which case toxicities will be attributed as possibly related to conditioning and cell infusion.

17.3 Adverse Event and Serious Adverse Event Reporting

At each contact with the subject, the Attending Physician or designee must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate CRF.

All AE and SAE occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the investigational product is not the cause or the subject is lost to follow-up. SAE that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any SAE that occurs for up to 30 days after the study period and is considered to be at least possibly related to the study treatment or study participation should be recorded and reported immediately.

A TEAE is any AE that occurs after the investigational product infusion. After 24 hours, the patient will receive infusion of the stem cell making it more difficult to determine if the AE is related to the infusion of the T-allo10 cells or the stem cell transplantation. If it is not clear, then attribution may be attributed to both infusions. If it is an expected AE of the stem cell infusion, then it would be attributed to the stem cell infusion.

17.3.1 Type and Duration of Follow-Up of Adverse Events

The Attending Physician or designee should follow all unresolved AE and SAE until the events are

resolved or stabilized, the subject is lost to follow up, or it has been determined that the study treatment or participation is not the cause of the AE/SAE. Resolution of AE and SAE (with dates) should be documented on the Adverse Event CRF or CRF and in the subject's medical record to facilitate source data verification (SDV). For some SAE, the Sponsor-Investigator will follow up with the Attending Physician or designee by telephone, fax, email, and/or a monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE report (e.g., hospital discharge summary, consultant report, or autopsy report).

17.3.2 Post-Study Adverse Event

The Attending Physician or designee should notify the study Sponsor-Investigator of any death or other SAE occurring at any time after a subject has discontinued or terminated study participation if felt to be related to prior study treatment. The Sponsor-Investigator should also be notified if the Attending Physician or designee should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that participated in this study. The Attending Physician or designee should report these events to the Sponsor-Investigator on the study CRF. If the study CRF is no longer available, the Attending Physician or designee should report the event directly to the Sponsor-Investigator via phone or email.

17.3.3 Investigator Reporting Requirements

Attending Physician or designee will submit reports of SAE to the Sponsor-Investigator within one working day of learning of the events. For initial SAE reports, Attending Physician or designee should record all case details that can be gathered in the SAE Report Form. Corresponding information should also be entered into the CRF as soon as possible. Relevant follow-up information should be submitted to the Sponsor-Investigator as soon as it becomes available and/or upon request.

The Sponsor-Investigator must comply with the applicable regulatory requirements related to the reporting of SAE to the IRB. All IND Safety Reports will be reported to the IRB by the Sponsor-investigator.

The Sponsor-Investigator will notify the IRB of AE/TEAE by the timeline for reporting and associated event type:

Within 5 working days

Any study event that is:

- suspected: associated with the use of the study drug,
- unexpected,
- fatal or life-threatening, and
- places participants at greater risk of harm

Within 10 working days:

Any study event that is:

- suspected: associated with the use of the study drug
- unexpected,
- places participants at greater risk of harm, and
- serious, but not fatal or life-threatening.

Annually on Continuing Review:

Any study event that is:

- expected, and
- All AE/TEAE determined not to meet the criteria of serious.

17.3.4 Fatal/Life-Threatening SAE Related to Investigational Product

Any life-threatening (i.e., imminent risk of death) or fatal SAE that is attributed by the Attending Physician or designee to the investigational product will be telephoned or emailed to the Sponsor-Investigator immediately, followed by submission of case details on the SAE CRF within one working day.

17.4 Sponsor-Investigator Reporting to FDA and DSMC

17.4.1 Suspected Unexpected Serious Adverse Reactions (SUSARs)

All events qualifying as Suspected Unexpected Serious Adverse Reactions (SUSARs) will be reported to the FDA and to the DSMC by the Sponsor-Investigator. SUSARs are required to be reported to the FDA within 7 calendar days for life threatening events and those resulting in death, or 15 calendar days for all others. SUSARs will be reported to the DSMC and IRB within 5 business days for life threatening events and those resulting in death, or 10 business days for all others. These timeframes begin with the first notification of the SUSAR to the Sponsor-Investigator from the Attending Physician or designee.

The Sponsor-Investigator will notify FDA of SUSARs using FDA Form MedWatch 3500A. The following describes the safety reporting requirements by timeline for reporting and associated type of event:

Within 7 calendar days

Any study event that is:

- suspected: associated with the use of the study drug
- unexpected,

- fatal or life-threatening, and

Within 15 calendar days

Any study event that is:

- associated with the use of the study drug,
- unexpected, and
- serious, but not fatal or life-threatening.

-or-

- a previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting (reporting within 15 calendar days from when event was deemed reportable).

17.4.2 Serious Adverse Event Reporting on Cell Therapy Products to the FDA

A sample from all products that are non-conforming or do not meet release specifications will be used to conduct an out of specification investigation and the remainder either disposed of according to our facility biohazardous material disposal SOP or the FDA will be contacted by the PI and manufacturing team to determine whether the product is suitable for infusion. The manufacturing facility will report all products manufactured including those that did not meet release criteria or were otherwise not infused in the annual IND report and the annual facility report if the LCGM submits a facility master file with the FDA.

All HCT/P (Human Cells, Tissues, and Cellular and Tissue-Based Products) deviations involving 361 cell products will be reported using MedWatch From FDA3500A according to FDA publication "Guidance for Industry: MedWatch Form FDA 3500A: Mandatory Reporting of Adverse Reactions Related to Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps) available at:

http://www.fda.gov/BiologicsBloodVaccines/SafetyAvailability/ReportaProblem/ucm153082.htm

17.4.3 Reporting to the DSMC

The Sponsor-Investigator will report:

1. Any death on the study regardless of expectedness within 24 hours of knowledge
2. Suspension of study for concerns of safety or Investigational Product related toxicity within 24 hours
3. Investigational Product related SAEs and DLTs within five business days of knowledge
4. Clinical update prior to dose escalation including:
    a. Description of the cohorts
    b. Dose levels
    c. Any Dose Limiting Toxicities observed
5. All other adverse events; protocol and subject deviations; Protocol specific data and safety monitoring reports, any internal and external audit reports every six months or as requested by

the DSMC.

17.5 Data Safety Monitoring Committee (DSMC)

The purpose of the DSMC is to provide an unbiased review of the safety data generated by the clinical trial. The independent DSMC will review clinical trial data prior to dose escalation and on an as-needed basis but not less than semi-annually, if there are subjects enrolled on the study. The DSMC will review serious adverse events, adverse events, and protocol deviations associated with the research to ensure the protection of human subjects. The Sponsor-Investigator or designee will inform the Chairperson of the DSMC of the potential need for ad hoc meetings and will coordinate such meetings. A statistician, serving as a non-voting member to the DSMC, will provide data preparation support to the DSMC. The DSMC will assess the progress of the clinical trial, including the safety data and feasibility and will recommend to the sponsor whether to continue, modify, or stop the clinical trial. The DSMC recommendations will be provided to the Sponsor-Investigator for final disposition. The Sponsor-Investigator will report DSMC recommendations to the IRB as required.

17.5.1 DSMC review for grade III-IV acute GvHD

If two consecutive patients in any cohort develop grade III-IV acute GvHD, we will consider a reevaluation and the DSMC will review the data. However, patients with grade III-IV GvHD improving after MMF withdrawal or after starting specific anti infectious treatment will be exempted. This exception will allow us to rule out confounding factors that might mimic gut GvHD symptoms, like the presence of preexisting/superimposed GI infections or drug-related toxicity (e.g. MMF). In addition, patients with a complete response within 4 weeks of GvHD standard treatment (per GvHD treatment guideline) will be exempted. In case of no improvement within 4 weeks from MMF withdrawal and/or anti infectious treatment start and GvHD standard treatment, the patient will meet the criteria requiring review by the DSMC.

17.6 Graft versus Host Disease (GvHD) Evaluator

An independent qualified physician will be appointed by the Protocol Sponsor and Protocol Director to evaluate acute and/or chronic graft versus host disease.

18 INVESTIGATIONAL PRODUCT MANAGEMENT

18.1 T-allo10

The product (T-allo10) will be transported to the BMT-CTF from where it will be taken to the bedside. ISBT128 labels will be used for packaging the product. The empty bags will be disposed of as per the standard operating procedures of the hospital.

18.2 Cyclophosphamide

18.2.1 Administration

Cyclophosphamide will be given according to LPCHS high dose cyclophosphamide administration guidelines at a dosage of 60 mg/kg on Days -4 and -3. Mesna must be administered with cyclophosphamide on Days -4 and -3. Mesna is given as per institutional standards.

18.2.2 Dose adjustments

Dose adjustments for the patients will be made as per institutional standards.

18.2.3 Patient Monitoring

Patients should receive an ECG prior to the first dose of cyclophosphamide as per high-dose cyclophosphamide administration guidelines.

18.3 Mesna

18.3.1 Administration

Mesna will be administered with cyclophosphamide on Days -4 and -3 as per institutional standards.

18.4 Total Body Irradiation (TBI)

Total body irradiation (TBI) will be given by the Stanford Radiation Oncology department.

- Administration-TBI may be given outpatient prior to admission for the stem cell transplantation or inpatient as per institutional standards. TBI will be given as fractionated dosing 120 cGy three times (3) daily for three (3) days Day -8, -7,-6; and twice (2) daily on Day -5 to total 1320 cGy.
- Dose Adjustments- no dose adjustments will be made for TBI.
- Patient Monitoring- patients must be instructed not to use lotions during the days of TBI.

18.4.1 Cranial Spinal Boost (CSB)

Patients with central nervous system (CNS) disease at presentation or relapse will receive a CSB as per institutional guidelines.

19 GENERAL CONSIDERATIONS

19.1 Basic Principles

This research will be carried out in accordance with the clinical research guidelines established by the Basic Principles defined in the U.S. 21 CFR Parts 50, 56, and 312, the principles enunciated in the Declaration of Helsinki concerning medical research in humans (“Ethical Principles for Medical

Research Involving Human Subjects," Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996, Edinburgh 2000 and clarifications, Washington 2002 and Tokyo 2004), and the Good Clinical Practice (GCP) guidelines of the International Conference on Harmonization (ICH) of the Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH E6 (May 1996).

19.2 Institutional Review Board (IRB)

Prior to initiation of any study procedures, the Clinical Study Protocol, Informed Consent Form, and Product Information will be submitted to the IRB for review and approval. In addition, any amendments to the protocol or informed consent document will be reviewed and approved (if necessary) by the IRB. The Principal Investigator (PI) at the clinical site assumes responsibility for ensuring that the protocol is submitted to the IRB for any required periodic review. The PI must receive a letter documenting the IRB approval at the clinical site prior to the initiation of the study. Any subsequent IRB correspondence must also be submitted to the Investigator. The Investigator is responsible for providing the appropriate reports to the reviewing IRB during the course of the clinical study. This will include the following:

- Informing the IRB of the study progress periodically as required, but at least annually
- Reporting any unanticipated adverse event per IRB Policies & Procedures
- Reporting any deviations from the clinical protocol to protect the life or well-being of a subject in the case of an emergency within 5 working days after the emergency occurred
- Providing any other reports requested by the IRB

After the final visit of the last subject, a final report will be sent to the IRB per institutional Policies & Procedures that includes a summary of the results of the study by the PI.

The IRB must be constituted and operate in accordance with the principles and requirements described in the U.S. Code of Federal Regulations (21 CFR Part 56).

19.3 Informed Consent

Participation in this trial incurs risks to the participant as well as the possibility of benefits afforded by the investigational agent. Since the target population is pediatric, the consent process will involve both the Legally Authorized Representative (LAR) of the participant, usually the parents, as well as the participant him- or herself. The assent process will be carried out per institutional policies. The participant is not required to take part in the assent process if the parents/LAR, Principal Investigator (PI), or Medical Director (MD) does not think the child is capable of comprehension. All parents or LAR of the subject must have the ability to understand and the willingness to sign a written informed consent.

The PI, or designee will lead the consent process by describing the disease, the rationale for the proposed experimental therapy, the treatment plan, the timeline and logistics as well as the risks, and the potential benefits of the study. Alternatives to the therapy including standard-of-care treatments will be described. The Patient's Bill of Rights will be reviewed. The voluntary nature of participation will be explained, as well as the right to withdraw from the study at any point in time. Adequate time

will be taken and questions will be asked of the participants to verify comprehension. When possible, the participant and the parents or LAR will be sent home with a copy of the informed consent form (ICF) and where applicable, the Pediatric Assent Form, to read and review. The PI or designee will be available for discussion at any time during the visit. To authorize participation in the trial, the parents or LAR will need to sign the ICF. A copy of the ICF, and where applicable, a Pediatric Assent Form (PAF), will be given to the participant, parents, or LAR. If both parents are unable to sign the consent, the reasons for the omission of one parent should be documented in the patient's medical record and Research Binder. Where applicable, the PAF should also be signed and documented as for the ICF.

The consenting process for donors is outlined in Section 6.2.

19.3.1 Pediatric Assent Process

Where applicable, for children ≥ 7 and < 18 years of age, pediatric assent will be documented as described above. If an assented child turns 18 years of age while enrolled on study, they will be consented at the earliest opportunity.

19.4 Study Termination

The Investigator reserves the right to terminate the study in the interest of subject safety and welfare. The Sponsor reserves the right to terminate the study at any time for administrative reasons.

19.5 Regulatory Documentation

Documents that must be provided to the Sponsor and/or its designee prior to study initiation are as follows:

- Up-to-date curriculum vitae for each investigator and sub-investigator
- Applicable local regulatory documentation (e.g., FDA 1572 Form)
- A copy of the formal written notification to the investigator regarding approval of the protocol by an IRB that is in compliance with regulatory guidelines. The written notification is to be signed by the chairman or authorized designee and must identify the specific protocol. In cases where an IRB member has a known conflict of interest, abstention of that individual from voting should be documented; an investigator (or sub-investigator) may be a member of the IRB, but may not vote on any research in which he or she is involved.
- Name and address of the IRB with a statement that it is organized and operates according to GCP and the applicable laws and regulations, and a current list of the IRB members. If accompanied by a letter of explanation from the IRB, a general statement may be substituted for this list.
- A copy of the IRB approved informed consent form and other adjunctive materials (e.g., advertising) to be used in the study, including written documentation of IRB approval of these items.

- Name and address of any local laboratory conducting tests for the study, a dated copy of the laboratory reference values for tests to be performed during the study and a copy of the certification or other documentation establishing adequacy of the facility.

- Required financial agreement.

In addition to the documents required prior to the study, other documentation may be required during the course of the study.

19.6 Study Documentation

All documents pertaining to the study, including a copy of the approved protocol, copy of the Informed Consent Form, and case report forms, will be retained in the permanent archives of the study site. These will be available for inspection at any time by the Sponsor and/or its designee, or the U.S. Food and Drug Administration (FDA).

19.7 Data Handling and Record Keeping

As electronic trial data handling and/or remote electronic trial data systems will be used, the Sponsor and/or its designee will:

- Ensure and document that the electronic data processing system(s) conforms to the Sponsor's established requirements for completeness, accuracy, reliability, and consistent intended performance

- Maintain a security system that prevents unauthorized access to the data

- Maintain a list of the individuals who are authorized to make data changes

- Maintain adequate backup of the data

- An unambiguous subject identification code will be used that will allow identification of all the data reported for each subject

- The Sponsor and/or its designee will retain all of the Sponsor-specific essential documents pertaining to the trial in conformance with the applicable regulatory requirement(s)

- Documentation and records generated by this clinical trial will have a retention period not less than 2 years after all marketing applications in all regions where the drug is being developed and have been approved for the drug or 2 years after the last shipment / delivery of the investigational product under the IND, if a marketing application has not been approved for the drug. Subsequent to the fulfillment of the record retention requirements, Stanford may destroy all records in a confidential manner.

- The original data collection forms will be stored electronically in a secure server at Stanford University. The server is password protected and all data are encrypted. Access to the server is limited to those who have been granted permission by the PI and to whom Stanford grants access – these personnel will have a legitimate reason for accessing the data. Physical access to the server is also restricted by lock and key. When results of this study are reported in

journals, publications, or at meetings, identification of those taking part will not be disclosed. Medical records of subjects will be securely maintained in the strictest confidence, according to current legal requirements. They will be made available for review, as required by the FDA, Health and Human Services (HHS), or other authorized users under the guidelines established by the Federal Privacy Act and rules for the protection of human subjects.

19.8 Use of Information and Publication

The use of data and information from this clinical investigation is only at the discretion of the PI. All dissemination of trial data medical care and medical decisions related to the investigation and the conclusions of the investigation, by publication, public talks, or through any media will be made with the authorization of the PI.

Publications and public material pertaining to the trial will not disclose patient PHI. When referring to data from specific patients, the patient's identification and PHI will be protected by coding with non-traceable identifiers.

20 STATISTICAL ANALYSIS METHODOLOGY

A formal Statistical Analysis Plan (SAP) will be prepared and finalized before database lock for the final analysis for the study report. The SAP will provide details regarding the definition of analysis subjects (populations), analysis variables, and analysis methodology to meet all study objectives.

The principle and key elements of the SAP are provided as follows:

- In general, tolerability, safety and feasibility data will be summarized for each dose cohort with descriptive statistics, including means, standard deviations, medians, minimums and maximums for continuous variables, the number of subjects and percent in each category for categorical variables.
- Data from each individual will be tabulated as appropriate. Tolerability, safety and feasibility endpoints will be tabulated by dose cohort and time point.

20.1 Tolerability and safety endpoints

The tolerability and safety of Investigational Product (IP) will be assessed by:

- Treatment-emergent adverse events.
- Treatment-emergent serious adverse events.
- Changes in clinical laboratory tests (clinical chemistry, hematology).
- Changes in vital signs (blood pressure, pulse, respiratory rate and body temperature).
- Changes in physical exams. Signs and symptoms assessed may require additional testing as clinically indicated such as ECG, PFT, radiographic studies, etc.
- Time to stem cell engraftment after HSCT.

Tolerability data will be analyzed per standard methods and interpreted descriptively. Tolerability data will be summarized for each dose cohort separately and for all dose cohorts combined. Adverse events will be assessed using the CTCAE version 4.0 for type and severity of event. Serious Adverse Events will be summarized for each dose cohort and for all dose cohorts combined. Reasons for discontinuation will be tabulated.

Laboratory includes hematology, serology, serum chemistry, and urinalysis; laboratory collected prior to T-allo10 infusion will be the baseline laboratory. The study will utilize local lab for all clinical laboratory testing. Laboratory data will be tabulated based on the following result class.

- Normal: result is within the local lab normal range
- Abnormal: result is either higher or lower than the normal range

All abnormal values will be assessed for clinical significance; clinical significance will be captured in the case report form. Number and percent of subjects within each result class will be tabulated by time point for each lab test without formal inferential statistics. If data permits, shift in result class from baseline to post baseline may also be tabulated.

Vital signs collected immediately prior to receiving study drug will be the baseline vital signs. Observed vital sign values and change from baseline in vital signs at each visit will be summarized without formal statistical testing.

Vital sign result may also be tabulated based on the following result class.

- Normal: result is within the normal range.
- Abnormal: result is either higher or lower than the normal range.

All abnormal values will be assessed for clinical significance; clinical significance will be captured in the case report form. Number and percent of subjects within each result class will be tabulated by time point for each vital sign.

Findings of physical examinations will be tabulated by treatment groups and abnormal values will be assessed for clinical significance; clinical significance will be captured in the case report form.

Safety data will be analyzed per standard methods and interpreted descriptively. Safety data will be summarized for each dose cohort separately and for all dose cohorts combined.

For stem cell engraftment, time to engraftment and rate of engraftment will be tabulated for each dose cohort and for all dose cohorts combined.

20.2 Feasibility endpoint

In addition to tolerability and safety, establishing feasibility of manufacturing T-allo10 cells that meet release criteria is essential to evaluating the primary objective of this study. Feasibility will be defined as the rate of successful manufacture of the T-allo10 cells to satisfy the targeted dose level and meet the required release specifications. Patients will be enrolled on a given dose level until adequate number of patients are enrolled to complete the safety evaluation at that dose level. Specifically, dose escalation will proceed as long as an adequate cell dose can be manufactured that meets release specifications to reach the number of patients required for the safety evaluation at the targeted dose level. For example, this might mean that 6 to 9 patients may need to be enrolled to result in 6 patients evaluable for safety in that dose cohort level. However, if an adequate T-allo10 cell dose can only be manufactured for a small fraction (< 3) of the initial 6 patients at a given dose level, evaluation of that level and beyond will not take place. Specifically, if after the first 6 patients have been enrolled at a given dose level, and the cell dose generated for more than 3 patients is less than the targeted dose for the given dose level, accrual to that dose level will stop and the dose escalation phase of the study will also end, as manufacturing will not be feasible for that dose level. Since the upper 90% one-sided confidence interval for 3/6 is 79.9%; thus, it would be unlikely that the true feasibility rate is 80% or greater for a given, which would be desirable.

20.3 Efficacy endpoints

Although a secondary endpoint, the efficacy analysis will focus on rate of acute grade III or IV GvHD. We will report descriptive data of rate of acute grade III or IV GvHD and its comparison with historical data on rate of acute grade III or IV GvHD in similar population. This analysis will be used to determine if a phase 2 study whose primary endpoint will be efficacy is warranted.

20.4 Analysis Datasets

All patients who receive experimental treatment will be analyzed for safety and efficacy.

Subjects not treated for any reason will be included in the disposition tabulation but will be excluded from the tolerability and safety analysis.

20.5 Disposition and Study Population Characteristics

Disposition summaries will be prepared to include number and percent of subjects screened, treated, and subjects that completed the study, or if not, the reason for discontinuation. Reason for screening failure will also be tabulated.

Subject characteristics summaries will include demographics (age, gender, race, and ethnicity), baseline characteristics (weight, height, BMI), diagnosis, donor age and relationship to patient, and medical history.

Disposition summary will be based on treatment group (i.e., planned treatment) whereas the baseline characteristics summaries will be based on the actual treatment group.

Subjects with protocol deviations will be identified. The nature of the deviation and potential impact on the deviation on study outcome will also be assessed.

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22 APPENDICES

22.1 Lansky Performance Score

Lansky Performance Score

The Lansky performance scale is used to determine the functional status of the recipient and should be used for recipients < 16 years of age.

Lansky Score	Performance Scale (For use with children ages 1 through 16 years)
100%	Fully active, normal
90%	Minor restrictions in physically strenuous activity
80%	Active, but tires more quickly
70%	Both greater restriction of, and less time spent in, play activities
60%	Up and around, but minimal active play; keeps busy with quieter activities
50%	Gets dressed but lies around much of the day; no active play; able to participate in all quiet play and activities
40%	Mostly in bed; participates in quiet activities
30%	In bed; needs assistance even for quiet play
20%	Often sleeping; play entirely limited to very passive activities
10%	No play; does not get out of bed
0%	Dead

22.2 Karnofsky Performance Score

Karnofsky Performance Score

The Karnofsky performance scale is used to determine the functional status of the recipient and should be used for recipients 16 years of age and older.

KARNOFSKY PERFORMANCE STATUS SCALE DEFINITIONS		
RATING	(%)	CRITERIA
Able to carry on normal activity and to work; no special care needed.	100	Normal no complaints; no evidence of disease.
	90	Able to carry on normal activity; minor signs or symptoms of disease.
	80	Normal activity with effort; some signs or symptoms of disease.
Unable to work; able to live at home and care for most personal needs; varying amount of assistance needed.	70	Cares for self; unable to carry on normal activity or to do active work.
	60	Requires occasional assistance, but is able to care for most of his personal needs.
	50	Requires considerable assistance and frequent medical care.
Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing	40	Disabled; requires special care and assistance.
	30	Severely disabled; hospital admission is indicated although death not imminent.
	20	Very sick; hospital admission necessary; active supportive treatment necessary.
	10	Moribund; fatal processes progressing rapidly.
	0	Dead