Phase II trial of T-cell depleted hematopoietic stem cell boosts without conditioning for poor marrow graft function following allogeneic hematopoietic stem cell transplantation

PROTOCOL FACE PAGE FOR
MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL

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1.0 PROTOCOL SUMMARY AND/OR SCHEMA

This protocol includes two parallel single arm phase II trials to assess the efficacy and confirm the safety of T cell depleted (TCD) hematopoietic progenitor cell, apheresis (HPC(A) boost or bone marrow (BM) HPC (M) boost from original donor for patients who have poor graft function (PGF) after allogeneic hematopoietic stem cell transplantation.

Candidates for this trial will include patients who have undergone allogeneic hematopoietic stem cell transplantation and whose post transplant course has been complicated by poor graft function. Patients who underwent transplant at another facility and suffer from PGF will be eligible as well. Patients will be treated on one of two arms based on their condition at time of TCD boost administration. The two arms are: (A) patients with no active infection or end organ compromise or GVHD and (B) patients with active infection, active or controlled GVHD and/or organ compromise.

Poor graft function is defined as follows: cytopenia in the presence of hypocellular BM (<25%), and full donor chimerism. The severity of PGF is adapted from severity levels of aplastic anemia and include **Severe PGF:** requires 2 of the following – absolute neutrophil count (ANC)< $0.5x10^9$/L, platelets <$20x10^9$/L, reticulocyte count< $20x10^9$/L. **Very severe PGF** is defined by the above criteria with ANC< $0.2x10^9$/L

Donors of the TCD boost will be the original donors. The boost will be given without any pre-infusion conditioning therapy.

The primary endpoint of the study is recovery of neutrophil, red cell and platelet counts. Secondary endpoints include overall survival, incidence and severity of acute and chronic graft versus host disease (GvHD) post TCD boost, incidence of infections, effect on donor T cell chimerism and pace of immune reconstitution.

2.0 OBJECTIVES AND SCIENTIFIC AIMS

To study the efficacy and confirm the safety of the administration of HPC(A) or HPC (M) TCD stem cell boost in patients with poor graft function after hematopoietic stem cell transplant (HSCT) from histocompatible matched or mismatched related or unrelated donors for various hematologic or immunologic disorders.

Primary objective:

To evaluate peripheral blood counts recovery at three months following the administration of TCD HPC(A) or HPC (M) boost, without further conditioning.

Secondary objectives:

1. To explore count recovery by the severity of PGF at the time of the TCD boost
2. To evaluate overall survival after administration of the TCD boost.
3. To assess the incidence of acute and chronic GvHD after administration of the TCD boost.
4. To assess the frequency of infections after administration of the TCD boost
5. To assess the effect of the TCD boost on donor T cell chimerism.

6. To characterize the trend of immune reconstitution after administration of the TCD boosts.

Correlative studies:

To explore the interaction between the donor stem cells and the BM stroma in patients with poor graft function

3.0 BACKGROUND AND RATIONALE

3.1 Graft failure

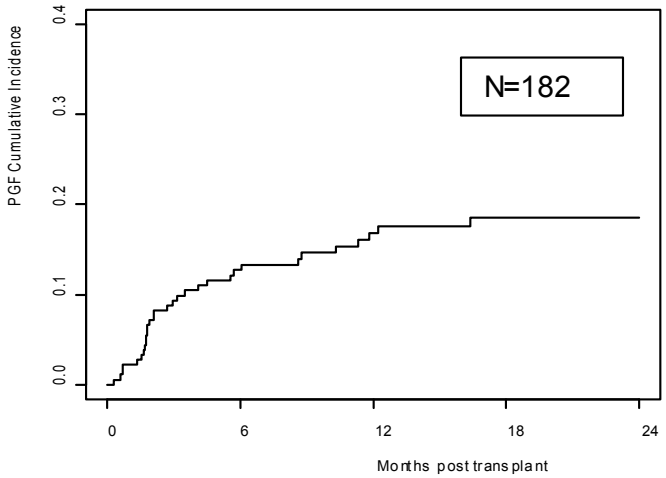
Bone marrow failure following allogeneic hematopoietic stem cell transplantation (graft failure) is a complex syndrome characterized by pancytopenia and hypocellular or acellular bone marrow. Several pathophysiological mechanisms are possible. The best characterized form of graft failure is immune rejection (graft rejection- GR). Preexisting anti-HLA antibodies[1,2] or residual host T lymphocytes can eliminate the donor stem cells and typically these patients have only host cells[3,4].

Another form of graft failure following allogeneic HSCT is due to qualitative or quantitative deficiencies of hematopoietic stem cells (poor graft function – PGF). A distinctive feature of PGF is persistence of donor cells in the recipient. PGF occurs in 5-27% of patients undergoing allogeneic HCST[5] and the severe form is associated with high morbidity and mortality due to infectious and hemorrhagic complications[6]. A number of factors are associated with PGF and this includes: (1) inadequate stem cell dose due to poor harvest/collection; (2) stem cell damage during ex-vivo manipulation or storage; (3) mismatched donor, (4) graft-versus-host disease (GvHD)[7]; (5) infection or medications used to treat an infection; or non-infectious medications (6) use of a T-cell depleted graft [8].

PGF can be primary with suboptimal recovery of blood counts after the initial HSCT, or secondary, with decreasing blood counts after successful and prompt hematopoietic engraftment. We have defined the severity categories of PGF according to the criteria used to define the severity of acquired aplastic anemia; severe PGF is defined as BM cellularity of <25% with 2 of the following – absolute neutrophil count (ANC)< $0.5x10^9$/L, platelets <$20x10^9$/L and reticulocyte count< $20x10^9$/L. Very severe PGF is defined by the above criteria with ANC< $0.2x10^9$/L. PGF can be a transient event secondary to a reversible insult, such as an acute infection/sepsis or drugs and blood counts can recover after removal of the insult. However, persistence of low blood counts require further intervention, either in the form of a second transplant or infusion of more stem cells without further pre-infusion conditioning therapy.

In a recent analysis of 182 patients who underwent HPC(A) TCD transplant at MSKCC between 9/2009-7/2012 (presented as a poster in ASH meeting 2012) the cumulative incidence of PGF was close to 20% at 1 year post transplant. Infections and anti-viral therapies were the most common etiologies for PGF (87%) followed by GVHD, medications related and unknown etiology in one case. It is important to note that while infections and anti viral therapies were the most common etiologies for PGF treating the infection did not always result in improvement in graft function. At time of analysis 36% of patients who were diagnosed with PGF died from either infectious complications or GVHD, 33% patients had recovery of blood counts either after treatment of an identified offending

agent (mostly viral infection) or discontinuation of medications, 15% had counts recovery after HPC(A) TCD stem cell boost, 10% had persistent PGF and in 6% there was evidence of relapsed disease.



3.2 Therapeutic Interventions for Poor graft function

The best course of treatment for PGF is not well defined. Hematopoietic growth factors, namely granulocyte colony-stimulator factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF) or erythropoietin have been used but often with less than optimal response[9]. The administration of additional hematopoietic stem cells following prior conditioning (second transplants) has had limited success secondary to toxicity of the conditioning regimen and high rates of grade III-IV GVHD[10]. An alternative approach is to administer additional donor- derived stem cells, un-manipulated or TCD (i.e. CD34+ selected), without additional conditioning (stem cell boost). The use of un-manipulated donor stem cell boost, however, is associated with a high incidence of acute and chronic GVHD and poor survival rates[11]. A boost of HPC(A) TCD stem cells following conventional or T-cell depleted allogeneic HSCT has been more successful mainly related to the low incidence of GVHD[12, 13], however reported in a very small cohort of patients. An important study that demonstrated the benefit of a TCD boost for PGF directly compared patients treated with either a HPC(A) TCD boost, an un-manipulated stem cell boost or supportive care alone. Trilineage recovery was more common and non-relapse mortality was lower in those receiving donor stem cells following TCD boost (CD34+ selection) compared to the other two groups. In addition, the incidence of GVHD was significantly lower in the TCD group compared to the conventional boost arm[6].

3.3 TCD stem cell boost for Poor graft function

A retrospective analysis of patients who were treated for severe PGF at MSKCC identified 35 patients who underwent allogeneic TCD boost of either peripheral blood (N=14) or bone marrow (N=21) stem cells; all boost grafts were from the original donors, without any further pre- boost conditioning therapy. The etiologies for PGF in this group of patients included infections (N=17), GVHD (N=12), low cell dose (N=2) and idiopathic (N=5). The median time between the first transplant and the TCD boost was 4.5 months (0.7-90.7 months). 7 patients died early post TCD boost (within the first 28 days post boost) and were not evaluable for blood count recovery.

Objective improvement in counts (ANC>0.5x10^9/L without G-CSF support and Plt>50x10^9/L) occurred in 20 patients with a median of 3.4 months (range 0.36-22.2 months) post TCD boost. In 17 out of the 20 patients there was further improvement of peripheral blood counts (ANC>1.0x10^9/L and Plt>100x10^9/L) with median of 8.8 months. For the 20 patients who had objective improvement in counts, the median cell dose was 1.8x10^7 SBA-E- cells/kg when bone marrow, HPC(M) was used as source of stem cells and 6.7x10^6 CD34+ cells/kg for patients who received HPC(A) TCD boost. Among the other 8 patients who were alive post day 28 after the TCD boost and had persistent poor graft function without objective improvement in blood counts, 2 had ongoing GVHD and in the other 6 patients the lack of response was of unknown reason. The stem cell boost infusion was well tolerated in all patients and no grade 2 or higher adverse events were reported. New onset GVHD, acute or chronic, was not documented in any of the patients, however, GVHD flare was documented in 2 patients with preexisting GVHD. The 2 and 5 years survival post TCD boost were 48.6% and 37.1%, respectively. The median survival for patients who achieved minimal objective count recovery was 77.58 months and for those who achieved optimal count recovery 80.34 months as compared to 6.61 months for patients who had no peripheral blood counts recovery. The 2 years OS for patients with peripheral blood counts recovery was 90% as compared to 18% for patients without counts recovery. Patient's medical condition at time of TCD boost administration had significant impact on response to treatment and survival. The best outcomes were seen in patients with no evidence of end-organ dysfunction (defined as creatinine>2, total bilirubin>2, need for mechanical ventilation and active infection) and who were still outpatients. In this group of patients 56% were alive and with stable peripheral blood counts on day 100 post administration of TCD boost as oppose to the other group of patients where only 10% were alive and with stable counts.

3.4 Study rationale

PGF is characterized by full donor chimerism and therefore we hypothesize that further stem cell infusion without further immunosuppression/conditioning will improve the overall graft function. This will be the first prospective study to assess an intervention to treat PGF in the form of TCD boost from the original donor. Based on the retrospective data of the 35 patients who received TCD boost for PGF at MSKCC, the goal will be to administer the boost as early as possible after the diagnosis of PGF is made. The large differences (while not significant due to low number of patients) noted between patients who received the TCD boost when they had active infection and/or GVHD and /or organ dysfunction and patients who received a boost without any of the above at time of TCD boost administration is the rationale for having the two cohorts of patients. This study will also provide the opportunity to study, in a prospective manner, the natural history of PGF.

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.1 Design

This protocol includes two single-arm phase II trials to assess the efficacy and confirm the safety of administration of TCD stem cell boost from the original donor for patients with poor graft function after allogeneic hematopoietic stem cell transplantation.

4.2 Intervention

Patients who are diagnosed with poor graft function will be given TCD boost without prior preparative therapy. Patients will be assigned to one of two groups based on their medical condition at time of boost administration: (A) patients who are free of active infection and GVHD and without end organ dysfunction and (B) patients with active infection, active or prior history of GVHD and/ or end organ damage.

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

5.1. a HPC(A)

Donor peripheral blood progenitor cells: stimulation, harvesting, isolation and T-cell depletion.

For related donors, beginning 5-6 days before the day of HPC(A) TCD boost infusion, the normal donor will receive GCSF per institutional guidelines. On the fifth and sixth days of this course of G-CSF, the donor will undergo daily leukapheresis designed to provide a minimum of 5x106 CD34+ cells/kg of the transplant recipient's weight. For unrelated donors, the G-CSF will be administered and the leukapheresis obtained according to the National Marrow Donor Program protocol IND, and institutional guidelines. Mononuclear cell fractions (i.e., CD34+ cells) collected on the fourth and fifth days will be pooled.

5.1. b HPC(M)

Donor peripheral blood progenitor cells: harvesting, isolation and T-cell depletion.

For related donors, beginning 1-2 days before the day of HPC(M) TCD boost infusion, the normal donor will undergo a bone marrow harvest per institutional guidelines. For unrelated donors, the bone marrow (HPC(M)) will be obtained according to the National Marrow Donor Program protocol IND, and institutional guidelines. The collected HPC(M) will be subsequently red blood cell reduced per MSKCC Cell Therapy Laboratory procedure to yield a cell product enriched for white blood cells which will undergo further processing for CD34 enrichment with the CliniMACS Cell Selection System (see 5.2).

5.2 CliniMACS Cell Selection System for Positive Selection of CD34+ Progenitor Cells and Reduction of CD3+ T-Cells

The CliniMACS Cell Selection System (Miltenyi Biotec, Auburn, CA) including the CliniMACSplus Instrument, a CliniMACS Tubing Set, the CliniMACS CD34 Reagent and the CliniMACS PBS/EDTA Buffer is intended for the selection and enrichment of human CD34 positive hematopoietic progenitor cells from a leukapheresis product. The CD34 antigen is a cell membrane glycoprotein expressed by early hematopoietic stem and progenitor cells. The CD34 positive cell separation process may be useful in several areas of clinical stem cell transplantation, including purging of tumor cells, T-cell depletion, *ex vivo* cell expansion and gene therapy. When re-infused after myeloablative chemotherapy, CD34 positive HPC(A) cells have been shown to reconstitute all

hematologic lineages and exhibit both short and long term repopulating capacities. The CliniMACS System uses selective CD34 monoclonal antibodies conjugated to superparamagnetic particles. The CD34 positive target cells are selected in an automated continuous flow separation system. The CD34 positive cells are specifically labeled by incubation with the CliniMACS CD34 Reagent. After unbound CD34 Reagent is washed from the suspension, the cells are ready for the automated cell separation process. The CliniMACS System passes the antibody-labeled suspension through a separation column in which strong magnetic gradients are generated. The Selection Column retains the magnetically labeled CD34 positive cells, while unwanted cells flow through the Selection Column and are collected in the Negative Fraction Bag. The system performs several washing steps, disposing most of the wash liquid into the Buffer Waste Bag. The Separated CD34 positive cells are then released from the column by removing the magnetic field and collecting the CD34+ cells into the Cell Collection Bag. While the CliniMACS System/CD34 Reagent has been FDA approved for HPC(A), the MSKCC Cell Therapy Laboratory has successfully validated performing the selection procedure on HPC(M).

5.3 The components of the CliniMACS System include:

5.3.1 The CliniMACS Instrument

The CliniMACS Instrument is a bench-top instrument consisting of a supporting structure to hold the column/tubing assembly and various bags, a series of check valves through which the tubing set is fitted, a high strength magnet between the poles of which the separation column is placed, a peristaltic pump through which a section of tubing is placed, software to control the instrument and user interface and a computer touchpad with a display window. The instrument is operated at ambient temperature and it is intended to be a multi-use platform instrument.

The software for the CliniMACS Instrument controls the function of the electromechanical components of the instrument and the user interface. Two separate computers, one a microcontroller located on a control board of the CliniMACS Instrument and the second a PC compatible computer which operates the user interface are incorporated with the instrument. Software Version 2.31, the current version of software which is directly traceable to the version of software utilized in pre-clinical testing and European Safety trials, and has been inspected and approved by TÜV product services with the CE Mark.

5.3.2. CliniMACS Tubing Set

The CliniMACS Tubing Set consists of a tubing element combined with a pair of proprietary cell selection columns. These form a functionally closed, sterile system for processing the cells. The separation column is a proprietary component of the CliniMACS System consisting of a plastic column housing with polypropylene frits in each end. The interior of the column housing is filled with a matrix of sub-millimeter iron beads coated with a heat-cured biocompatible resin. The columns are placed at appropriate locations in the CliniMACS Tubing Set to facilitate the cell selection process. The first column serves as a device to remove components that bind non-specifically to the column. The second column which is placed within a magnetic field performs the actual cell selection. The columns are incorporated sterile as part of the tubing set and are intended for single use only.

The tubing element consists of a series of tubes, connectors, spikes, Luer locks, and collection bags. The tubing of the tubing element is comprised of materials that have been qualified for use in this application by testing to ISO 10993. The principal constituents are polyvinyl chloride (PVC) and silicone. The connectors are made of various polymers (e.g., ABS and PVC) suitable for use in a blood contact environment. They are solvent bonded to the PVC tubing. The silicone pump tubing is softened with petroleum ether for manufacturing and mechanically fixed to connectors. The cell wash bags are composed of PVC.

The CliniMACS Tubing Set is packaged in a thermoformed tray and heat sealed with a Tyvek® lid. The CliniMACS Tubing Set is sterilized by ethylene oxide gas in a validated sterilization cycle and supplied as a single-use component for the CliniMACS Instrument.

5.3.3 CliniMACS CD34 Reagent

The CliniMACS CD34 Reagent is a dark (amber), non-viscous colloidal solution containing the antibody conjugate in buffer solution. The conjugate consists of a monoclonal antibody towards the human CD34 antigen. The murine monoclonal IgG1 antibody is covalently linked to dextran beads having an iron oxide/hydroxide core. The concentration of the conjugate is equivalent to 20 micrograms (µg) per mL of antibody protein, 800 µg/mL of dextran and 800 µg/mL of iron. The colloid is buffered in a phosphate-buffered saline (PBS) containing ethylenediaminetetraacetic acid (EDTA) and Poloxamer 188. The nominal concentrations of its components are 0.0095 M phosphate, 0.004 M potassium, 0.163 M sodium, 0.139 M chloride, 0.005 M EDTA and 0.03 % (w/v) Poloxamer 188. The pH is 7.4 - 7.7. Poloxamer 188 is added to the CliniMACS CD34 Reagent to stabilize it during shipping, handling and storage. The CliniMACS CD34 Reagent is supplied sterile and pyrogen-free in glass vials containing 7.5 mL and is intended for single use and for in vitro use only.

5.3.4. The CliniMACS PBS/EDTA Buffer

The CliniMACS PBS/EDTA Buffer is an isotonic and isohydric buffer solution with a pH-value of 7.2 and osmolarity of 290 mosmol/L. Its formulation is shown in the following table.

Table 1 Formulations of the CliniMACS PBS/EDTA Buffer

Ingredient	Compendial	Amount
NaCl	Ph. Eur.	8.0 g/L
KCl	Ph. Eur.	0.19 g/L
Na2HPO4 anhy.	Ph. Eur.	1.15 g/L
KH2PO4	Ph. Eur.	0.19 g/L
Na2EDTA	Ph. Eur.	0.37 g/L
Water for Injection	Ph. Eur.	ad 1L

The CliniMACS PBS/EDTA Buffer is used as external wash and transport fluid for the in vitro preparation of human heterogeneous cell populations intended to be separated with the CliniMACS Cell Selection System

5.5 Transplantation of the T-cell depleted stem cells

The HPC(A) or HPC (M) TCD boost will be infused intravenously over 5-30 minutes with monitoring of vital signs. The patient is premedicated as for blood product transfusions.

6.0 CRITERIA FOR SUBJECT ELIGIBILITY

Patients who underwent allogeneic stem cell transplantation and are diagnosed with either of the following are candidates for this trial.

1. Patients who are diagnosed with PGF are candidates for this trial.
2. Patients who underwent transplant at another facility and suffer from PGF will be eligible as well as long as a donor is available. PGF can be primary (no counts recovery after the preparative regimen) or secondary (cytopenia after engraftment has occurred).
3. Patients with auto-immune cytopenia with auto antibodies to neutrophils or platelets or positive Coombs test that did not respond to immunosuppressive agents within 3 months from initiation of therapy are eligible as well.

6.1 Subject Inclusion Criteria

1. Persistent cytopenia requiring growth factors and/or blood products AND evidence of hypocellular BM (<25%). Persistent cytopenia (at least 4 week period) is defined by presence of TWO of the following:
    1. ANC $<1.0x10^9$/L without filgrastim support or any ANC value that requires recurrent support by filgrastim (administered at least once a week).
    2. Plt$<50x10^9$/L
    3. Hb<8 or PRBC transfusion dependent (once every 2 weeks or more) with reticulocyte count of $< 40x10^9$/L.

   This criteria for persistent cytopenia and hypocellular bone marrow does not apply to patients with auto-immune cytopenia (category #3 in Section 6.0), ONLY PGF patients.

2. Full donor myeloid chimerism. Patients after T cell depletion transplant can have a significant mixed T cell chimerism and this can affect the testing of marrow chimerism. In this case, the neutrophil chimerism will be used to determine eligibility for this trial. Patients will be excluded if neutrophils are less than 90% donor cells; a higher percentage of host cells could be due to relapse or impending relapse.

3. Age: pediatrics and adults patients. No age exclusion.

4. Each patient must be willing to participate as a research subject and must sign an informed consent form.

5. For infections and end organs related criteria (at time of TCD boost administration) see table below:

Criteria	Arm A	Arm B
Infections	Without active uncontrolled bacterial, fungal or viral infection. **CMV**- If CMV viremia is <137 IU/ml, but patients are on therapy for CMV. **HHV-6** < 40,000 copies/ML and without active trend up.	No limitation
Karnofsky/Lansky performance scale	>60%	No limitation
GVHD	Without evidence of active or prior history of GVHD.	Patients with active GVHD, or with GVHD controlled by steroids or other immunosuppressive medications
Renal function	Creatinine Clearance >60ml/min	No limitation
Hepatic function	Liver enzymes (<x3 ULN), Bilirubin (<x2 ULN) and stable in the 30 days prior to TCD boost	No limitation
Cardiac function	No evidence of uncontrolled heart failure or active angina	No limitation
Pulmonary function	Spontaneous breathing, not requiring ventilatory support.	No limitation

6.2 Subject Exclusion Criteria

Patients will be excluded from the trial if at time of enrollment:

1. Evidence of relapsed disease by morphologic, cytogenetic or molecular diagnostic tools.
2. Hypersplenism documented by imaging study (US or CT)
3. Pregnant women
4. Patient who underwent TCD boost without counts recovery and are considered for another TCD boost will be treated off protocol.

7.0 RECRUITMENT PLAN

Patients who fulfill the eligibility criteria as listed in section 6.0 will be recruited for this study by an attending physician of the Adult and Pediatric BMT service. This protocol will take due notice of NIH/ADAMHA policies concerning inclusion of women and minorities in clinical research population.

8.0 PRETREATMENT EVALUATION

The patient will undergo comprehensive medical evaluation which will include:

1. Complete physical exam and medical history.
2. GVHD assessment
3. Infectious status assessment, including PCR studies for CMV, HHV6, EBV and adenovirus. Imaging studies if indicated.
4. Complete medications review
5. Complete blood counts (CBC) and reticulocyte count
6. Complete metabolic panel
7. Chimerism studies – bone marrow and peripheral blood lineage (T cells, B cells and Myeloid)
8. Splenic size evaluation by US or CT scan only for patients for whom it is clinically relevant
9. Anti-neutrophil antibodies, anti-platelet antibodies and Coombs test.
10. Bone marrow aspiration and biopsy including (1) complete disease evaluation for patients with hematologic malignancies and (2) cellularity evaluation for all patients

9.0 TREATMENT/INTERVENTION PLAN

1. CBC will be checked weekly for the first two months or until independent of G-CSF and transfusion support, then every two weeks (or more frequently if clinically indicated) until day 100 post TCD stem cell boost. In patients who by day 100 have partial recovery, monitoring blood counts will continue every 2-4 weeks.
2. Bone marrow aspirate and biopsy for assessment of engraftment will be done approximately on day 28 (+/- 7 days) and day 100 post TCD boost (+/- 10 days) and afterwards as required per BMT guidelines.
3. Peripheral blood lymphocyte phenotyping and in-vitro response to mitogen will be assessed on day 28 and day 100 post TCD boost and afterwards as part of routine post transplant assessment until CD4 reaches 200 and PHA>100th percentile.

10.0 EVALUATION DURING TREATMENT/INTERVENTION

All patients will be closely monitored and evaluated as per MSKCC BMT standard of care guidelines. Study specific assessment schedule listed in table below.

Procedures	Pre-treat	Days Post-Boost												
		7	14	21	28	35	42	49	56	63	70	77	84	100
Window	45	(+/-) 3	(+/-) 3	(+/-) 3	(+/-) 7	(+/-) 3	(+/-) 3	(+/-) 3	(+/-) 3	(+/-) 7	(+/-) 7	(+/-) 7	(+/-) 7	(+/-) 7
Eligibility	X													
Informed consent	X													
History/Physical	X													
CBC	X	X	X	X	X		X		X		X		X	X
Reticulocyte Count	X	X	X	X	X		X		X		X		X	X
Blood Chemistry/CMP (1)	X	X		X			X		X		X		X	X
Infection Testing (2)	X													
ECG and/or ECHO (only for new cardiac symptoms)	X													
US or CT (only if clinically indicated)	X													
Pregnancy Test (3)	X													
Creatinine Clearance	X													
Anti-Neutrophil Antibodies	X													
Coombs Test	X													
Bone marrow aspirate and biopsy (standard of care and research samples)	X				X									X
Correlative Studies – PB Research Sample (4)					X									X
Immune evaluation	X				X									X
Chimerism (Blood and BM)	X				X									X
GvHD evaluation (5)			X	X	X	X	X	X	X	X	X	X	X	X
Toxicity assessment		X	X	X	X	X	X	X	X	X	X	X	X	X

(1) BUN, creatinine, electrolytes, glucose, total protein, albumin, AST, ALT, bilirubin, alkaline phosphatase
(2) CMV, EBV, HHV-6, adeno virus by PCR
(3) Required for FCBP only
(4) Peripheral blood samples will be collected for correlative research studies
(5) GvHD assessment to begin at engraftment

10.1 Correlative Studies

BM aspirate will be obtained prior to administration of the TCD boost as well as on day 30 and day 100 post TCD boost. These studies will give an insight to the events at the level of the stem cells and its interaction with the bone marrow microenvironment.

These correlative studies will be performed in Dr. Malcolm Moore's lab, where the results will be analyzed as well. The studies will include the following:

1. To study early hematopoietic progenitors colony forming cells assays will be used. These will include: erythroid burst forming units (BFU-E), Megakaryocytes colony forming units (CFU-Meg) and granulocyte -macrophage colony forming unit (GM-CFU).

2. To study a more primitive hematopoietic progenitors the cobblestone area-forming cell (CAFC) assay will be used.

3. To study the interaction between the patients BM stroma and the donor stem cells a cobblestone area-forming cell (CAFC) assay will be set from BM aspirate pre boost and an aliquot of donor stem cells will be added to the established culture.

Peripheral blood sample will be obtained on days +28, +100 post TCD boost and will be sent to the clinical immunology laboratory to assess immune reconstitution with response to administration of the TCD boost. Peripheral blood samples will also be collected on those time-points and processed at HOTB for correlative studies.

11.0 TOXICITIES/SIDE EFFECTS

Toxicities will be graded according to NCI CTAE version 4.0.

Toxicities associated with stem cell infusion: The T-cell depleted peripheral blood stem cells are infused in a small volume (approximately 10-20 ml). Possible side effects include side effects associated with blood product infusions such as: changes in blood pressure, fever, headache, shortness of breath, chills, sweats, nausea/vomiting, bad taste in the mouth. Pre-medications are given to reduce these side effects.

Graft-versus-host-disease (GvHD): acute and chronic GVHD may develop after allogeneic transplantation and can be disabling and fatal. CD34+ selection and CD3+ depletion reduces the number of T cells in the HPC(A) or HPC(M) graft but GVHD can occur after TCD transplant. Acute and/or chronic GVHD will be treated with immunosuppressive drugs as per transplant service guidelines.

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

Definitions of events in the study period that are important for analysis and treatment

1. **Peripheral blood counts recovery**
   Peripheral blood counts recovery will be documented by CBC checks every 2 weeks. Response will be defined by stable blood counts (ANC>1000, Hb>8 with absolute reticulocyte count>$20x10^9$/L ,and Plt>50,000) without support of blood product transfusions and/or growth factors.

2. **Chimerism**
   Chimerism will be performed by analysis of bone marrow cells as well peripheral blood cells subpopulations (T cells, B cells, neutrophils) by standard karyotype or FISH analysis for sex mismatched host-donor or short tandem repeat analysis at 1 month and at 100 days post TCD boost and afterwards at regular time points as per routine BMT guidelines.

3. **Immunologic reconstitution**

   Our previous studies have identified time points at which the various immunologic functions can be expected to return. Immunophenotyping including CD4/CD8 will be performed on circulating lymphocytes of all patients as well as T cell proliferation in response to PHA at 1 month and 100 days post-TCD boost. These markers will be used to

define immune reconstitution.

4. Graft versus Host Disease

Standard BMT-CTN and IBMTR systems clinical criteria as defined by Rowlings, et al.[14] will be used to establish and grade acute GvHD. Data will be collected weekly and upto day 100 after the TCD boost to characterize the severity of symptoms and signs caused by acute GvHD and to evaluate possible confounding factors.

Real time data collection will include descriptive characteristics of rash and estimated body surface area involved, extent of dermal/epidermal separation, identification of concomitant causes of increased bilirubin other than GvHD, presence or absence of nausea, vomiting or anorexia persistent after engraftment, peak diarrhea volume with annotations concerning the presence after engraftment, peak diarrhea volume with annotations concerning the presence or absence of urinary mixing and estimates of true diarrhea volume, presence or absence of abdominal cramps, presence or absence of frank stool blood or melena, concomitant causes of GI symptoms other than GvHD, biopsy results, identification of any agents used for treatment and autopsy results.

Chronic GvHD will be diagnosed and graded according to the NIH consensus criteria[15].

13.0 CRITERIA FOR REMOVAL FROM STUDY

If at any time the patient is found to be ineligible for the protocol as designated in the section on Criteria for patient/subject eligibility, the patient will be removed from the study. Also patients may be removed from the study if requested by the patient. Management will continue as per standard allogeneic HSCT guidelines.

14.0 BIOSTATISTICS

This protocol investigates the efficacy of T-cell depleted boosts for poor graft function (PGF) in two cohorts of patients. The first cohort is patients who have PGF without any active infection, GVHD, and end organ compromise at the time of TCD boost administration (Cohort A). The second cohort is patients who have PGF with active infection, GVHD, or end organ compromise at the time of TCD administration (Cohort B). The protocol will have two single-arm phase II trials to investigate the efficacy separately in the two cohorts. The primary endpoint in both cohorts is the achievement of objective count recovery as defined in section 12.0 at 100 days following the intervention without the aid of growth factors. Patients who die prior to 100 days or those who achieve count recovery but lose their counts prior to 100 days will be treated as treatment failures.

Cohort A:

A Simon's 2-stage optimal design will be used to investigate the primary endpoint in patients without active infection or end organ disease. Based on historical MSKCC data, the boost treatment strategy will be considered promising for further investigation if 65% achieve objective recovery at 100 days. However, if 40% or fewer achieve sustained recovery, the strategy will not be considered

promising. Based on these rates, the trial will accrue a maximum of 28 patients. In the first stage of the trial, response will be evaluated in the first 13 patients. The trial will stop due to a lack of efficacy if 5 or fewer achieve objective recovery; otherwise, an additional 15 will accrue. At the end of the trial, if at least 15 out of the 28 patients achieve an objective recovery at 100 days, the boost intervention will be considered promising for further investigation. The type I and type II errors are both set at 0.10.

The estimated accrual is 7-10 patients/year and the overall study duration is 3-4 years.

The Cohort A trial includes early termination in the event that patients have high rates of grade III-IV acute GVHD. The stopping rules below are based on a maximum of 28 patients receiving the boost intervention.

Failure Type	**# of failures needed to stop the study**	**Failure rate in the population**	**Probability boundary is crossed**
Acute GVHD (Grade III-IV)	3 in the first 8 patients 4 in the first 14 patients	0.10	0.09
	5 in the first 20 patients 6 in the first 27 patients 7 at any point	0.35	0.96

Cohort B:

A Simon's 2-stage optimal design will be used to investigate the primary endpoint in patients with active infection and/or end organ disease. The boost treatment strategy will be considered promising for further investigation if 35% achieve objective recovery at 100 days. However, if 10% or fewer achieve sustained recovery, the strategy will not be considered promising. Based on these rates, the trial will accrue a maximum of 19 patients. In the first stage of the trial, response will be evaluated in the first 11 patients. The trial will stop due to a lack of efficacy if one or no patient achieves objective recovery; otherwise, an additional 8 will accrue. At the end of the trial, if at least 4 out of the 19 patients achieve an objective recovery at 100 days, the boost intervention will be considered promising for further investigation. The type I and type II errors are both set at 0.10.

The estimated accrual is 4-5 patients/year and the overall study duration is 4-5 years.

The Cohort B trial includes early termination in the event that patients have high rates of grade III-IV acute GVHD. The stopping rules below are based on a maximum of 19 patients receiving the boost intervention.

Failure Type	**# of failures needed to stop the study**	**Failure rate in the population**	**Probability boundary is crossed**
Acute GVHD (Grade III-IV)	2 in the first 4 patients 3 in the first 8 patients 4 in the first 14 patients 5 at any point	0.10	0.10
		0.35	0.89

This trial includes a number of secondary objectives that will be explored separately for the two cohorts.

1. The objective count recovery will be tabulated by the severity of PGF at the time of the boost.

2. Overall survival following the TCD boost will be estimated using Kaplan-Meier methodology.

3. The incidence of grade II-IV and III-IV acute GVHD and chronic GVHD will be estimated using cumulative incidence functions. Competing risks for these events include relapse and non-relapse mortality.

4. The frequency of serious infections as defined by CIBMTR will be tabulated for the two patient cohorts.

5. The levels of T-cell chimerism will be compared for patients who do and who do not achieve the 100-day objective count recovery endpoint.

6. Immune reconstitution will be explored using graphical and descriptive measures.

As outline in section 10.1, this trial includes a number of correlative studies. These studies will be performed and analyzed in Dr. Malcolm Moore's lab.

15.0 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.1 Research Participant Registration

Confirm eligibility as defined in the section entitled Criteria for Patient/Subject Eligibility.

Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures.

During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist.

All participants must be registered through the Protocol Participant Registration (PPR) Office at Memorial Sloan-Kettering Cancer Center. PPR is available Monday through Friday from 8:30am – 5:30pm at 646-735-8000. Registrations must be submitted via the PPR Electronic Registration System (http://ppr/). The completed signature page of the written consent/RA or verbal script/RA, a completed Eligibility Checklist and other relevant documents must be uploaded via the PPR Electronic Registration System.

15.2 Randomization

This is not a randomized trial.

16.0 DATA MANAGEMENT ISSUES

A Research Study Assistant (RSA) will be assigned to the study and will be responsible for both pediatric and adult accruals. The responsibilities of the RSA and principal investigator include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordinate the activities of the protocol study team. The data collected for this study will be entered into The Clinical Research Data Base (CRDB), a secure database. Source documentation will be available to support the computerized patient record.

16.1 Quality Assurance

Weekly registration reports will be generated to monitor patient accruals and completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period and potential problems will be brought to the attention of the study team for discussion and action. Random-sample data quality and protocol compliance audits will be conducted by the study team, at a minimum of two times per year, more frequently if indicated.

16.2 Data and Safety Monitoring

The Data and Safety Monitoring (DSM) Plans at Memorial Sloan-Kettering Cancer Center were approved by the National Cancer Institute in September 2001. The plans address the new policies set forth by the NCI in the document entitled "Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials" which can be found at: http://cancertrials.nci.nih.gov/researchers/dsm/index.html. The DSM Plans at MSKCC were established and are monitored by the Office of Clinical Research. The MSKCC Data and Safety Monitoring Plans can be found on the MSKCC Intranet at: http://mskweb2.mskcc.org/irb/index.htm

There are several different mechanisms by which clinical trials are monitored for data, safety and quality. There are institutional processes in place for quality assurance (e.g., protocol monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research QA) and departmental procedures for quality control, plus there are two institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees: Data and Safety Monitoring Committee (DSMC) for Phase I and II clinical trials, and the Data and Safety Monitoring Board (DSMB) for Phase III clinical trials, report to the Center's Research Council and Institutional Review Board. During the protocol development and review process, each protocol will be assessed for its level of risk and degree of monitoring required. Every type of protocol (e.g., NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group, etc.) Will be addressed and the monitoring procedures will be established at the time of protocol activation.

17.0 PROTECTION OF HUMAN SUBJECTS

Risks: The TCD boost will be given to patients with PGF with evidence of full donor chimerism and the source of the TCD boost is the same as the original donor. In a retrospective analysis of 35 patients who received TCD for the same indication, there were no new cases of GVHD, however in 2 patients there was a flare of GVHD. While the risk of GVHD is small due to depletion of the T cells, it still exists since T cells are not completely eliminated from the product.

Benefits: improvement of blood counts will lower the risk of bleeding and infectious complications.

Possible toxicities/side effects: Toxicities and side effects of the TCD boost are listed in section 11 and reporting of serious adverse events is found in section 17.2.

Consent Process: Participation in this study is voluntary. All patients will be required to sign a statement of informed consent which must conform to MSKCC IRB guidelines.

Alternatives: Alternative treatment options will be presented to the patient prior to taking part in this study. Alternative treatment options may include not participating in this study and follow up as per regular standard of care post transplant with continuation of supportive therapy.

Costs: The patient's health plan/insurance company will need to pay for all of the costs of standard medical care. Patients will not be paid for taking part in this study. Research tests will be done at no cost to the patient.

Confidentiality: Every effort will be made to maintain patient confidentiality. Research and hospital records are confidential.

17.1 Privacy

MSKCC's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board (IRB/PB).

17.2 Serious Adverse Event (SAE) Reporting

Any SAE must be reported to the IRB/PB as soon as possible but no later than 5 calendar days. The IRB/PB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office at sae@mskcc.org. The report should contain the following information:

Fields populated from CRDB:

- Subject's name (generate the report with only initials if it will be sent outside of MSKCC)
- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following
    - A explanation of how the AE was handled
    - A description of the subject's condition
    - Indication if the subject remains on the study
    - If an amendment will need to be made to the protocol and/or consent form.

The PI's signature and the date it was signed are required on the completed report.

For IND/IDE protocols:
The CRDB AE report should be completed as above and the FDA assigned IND/IDE number written at the top of the report. If appropriate, the report will be forwarded to the FDA by the SAE staff through the IND Office.

17.2.1

This protocol is being conducted under an IDE and the safety reporting to the FDA will be IDE compliant as per 21 CRF 812.150, 21 CFR 812.3 and 21 CFR 312.32.

17.2.2 Definition of SAE

A Serious Adverse Event (SAE) is any undesirable experience that meets any of the following criteria: fatal, life-threatening, disabling, results in hospitalization or prolongation of hospitalization, results in a congenital anomaly or occurrence of malignancy.

All SAEs will be reported according to the approved Adult and Pediatric BMT Adverse Event Reporting Guide, version 2.0 dated 29 December 2014.

18.0 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

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