The Children's Hospital *of* Philadelphia

**Division of Oncology, Blood and Marrow Transplant Section**

# 13BTθ51 UNRELATED AND PARTIALLY MATCHED RELATED DONOR PERIPHERAL STEM CELL TRANSPLANTATION WITH α/β T CELL AND B CELL DEPLETION FOR PATIENTS WITH HEMATOLOGIC MALIGNANCIES

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*Table of Contents*

ABSTRACT ..... 4
1. OBJECTIVES ..... 4
2. BACKGROUND ..... 4
3. PATIENT AND DONOR ELIGIBILITY ..... 8
4. METHODS ..... 9
   4.1 Pre-transplant evaluation……9
   4.2 Stem cell moblization……9
   4.3 Conditioning……10
   4.4 Graft vs host disease prophylaxis and treatment……..12
5. STEM CELL PROCESSING……12
6. STATISTICAL CONSIDERATIONS AND DATA ANALYSIS PLAN..... .15
7. DATA MONITORING..... 19
8. FINANCIAL CONSIDERATIONS ..... 19
9. RISKS OF STEM CELL TRANSPLANT ..... 19
10. DRUG INFORMATION ..... 24
11. POTENTIAL BENEFITS..... 24
12. RISK/BENEFIT ANALYSIS ……24
13. PROTECTION OF SUBJECTS/ADVERSE EVENT REPORTING ..... 24
14. CONSENT/ASSENT CONSIDERATIONS ..... 26
15. REFERENCES ..... 27
16. APPENDIX: IMMUNE RECONSTITUTION……30

*ABSTRACT*

PRIMARY HYPOTHESIS: The primary hypothesis is that hematopoietic stem cell transplantation (HSCT) with α/β T cell depletion plus CD19+ B cell depletion utilizing the CliniMACS device for unrelated and partially matched related donors will improve the efficacy of T depletion, resulting in durable engraftment and decreasing the risk of severe acute and chronic GVHD.

SECONDARY HYPOTHESIS: The method of α/β T cell depletion (TCD) plus CD19+ depletion utilizing the Clinimacs device for unrelated and partially matched related donors will promote faster immune reconstitution compared with other methods of T depletion.

PURPOSE
This is a single arm study for patients with hematologic malignancies with alternative donor sources receiving unrelated or partially matched related mobilized peripheral stem cells (PSCs) using the CliniMACS system for α/β T cell depletion plus CD19+ B cell depletion to determine efficacy as determined by engraftment and GVHD, and one year leukemia free survival.

1. OBJECTIVES
   A. Evaluate engraftment of patients receiving unrelated donor or partially matched related donor peripheral stem cells that have been α/β T cell depleted and CD19+ B cell depleted using the CliniMACS device.
      a. Evaluate primary non-engraftment.
      b. Evaluate engraftment kinetics (time to ANC >500, platelets >20 x $10^9$/l).
   B. Estimate incidence and extent of acute and chronic graft vs. host disease.
      a. Overall acute GVHD incidence.
      b. Incidence of grade IV acute GVHD.
      c. Incidence of chronic GVHD.
   C. Assess incidence treatment-related mortality (TRM).
   D. Assess probability of one year leukemia free survival (LFS).
   E. Assess tempo of immune reconstitution.
      a. Assess incidence of viral infections, including CMV, adenovirus, HHV6 and EBV.
      b. Assess tempo of lymphocyte reconstitution and recovery of TRECs.
   F. Pre-transplant evaluation will include KIR typing of the patient and donor. Data generated will be compared against the outcomes.

2. BACKGROUND

Allogeneic stem cell transplant is curative for many patients with leukemias in which chemotherapy has <20% chance of achieving long-term remission. However, only 25-30% of patients will have an HLA identical sibling donor. A well matched unrelated donor, or mismatched related donor may be considered for these patients, and leukemia free survival (LFS) is 30-60% depending upon patient age and disease status. However, acute and chronic graft vs.host disease (GVHD) are significant morbidities for patients who receive these

alternative donor transplants. Even in HLA matched unrelated donor transplants, both acute and chronic GVHD remain significant impediments to good quality of life and morbidity. Severe (grades III-IV) acute GVHD occurs in up to 50% of patients, and extensive chronic GVHD in >20%. To overcome the morbidity of GVHD, various methods of T lymphocyte depletion have been developed. In vivo methods include the use of anti-thymocyte globulin [1,2], alemtuzumab [3] and post-transplant cyclophosphamide [4]. These broadly deplete T cells. Ex vivo depletion includes mechanical methods [5,6]CD34+ selection, and narrow and broad specificity antibodies [7-10]. A study comparing various methods of ex vivo T depletion for alternative donor bone marrow transplants demonstrated superior leukemia free survival for those transplants that were T depleted with narrow specificity antibodies [11]including T10B9, which targets TcRα/β.

The technology of T cell depletion has evolved from methods that pan-deplete T cells, to more precise methods used for mobilized peripheral stem cells. Positive selection of peripheral CD34+ stem cells using cell sorting allowed the use of mismatched donors with reduced GVHD and reliable donor engraftment in the majority of patients[12]. However, this selection method also eliminated other cells that may facilitate engraftment or help maintain a graft vs leukemia (GVL) effect, such as NK cells. Newer methods have become available using magnet-activated cell sorting maintained NK and other cells. These include CD3+ depletion [13] , and most recently, depletion of TcRαβ+ T lymphocytes. In this method, γδ+ T cells are retained, with potential anti-infectious and anti-tumor properties.

*Studies with T Depletion and Alternative Donors*

T cell depletion of unrelated and partially matched related (PMR) donors has been done for over 20 years at the Children's Hospital of Wisconsin and the Children's Hospital of Philadelphia for patients who lack matched related donors [14-18]. This has enabled us to transplant patients who do not have either a matched related or unrelated donor who represent more than half of patients who may benefit from an allogeneic transplant. The technology used for TCD has changed through the years, as has the stem cell source:

| Year | Stem cell source | Technique | T depletion (approx) | Comments | |
|---|---|---|---|---|---|
| 1991-2001 | Bone marrow | T10B9 Ab + C | 1.7 log | α/β selective | |
| 2000-2009 | Bone marrow | OKT3 + C | 1.7 log | increased GVHD | |
| 2000-2009 | PSCs | CD34+ selection | 4.6 log | ?increased ES; delayed immune reconstitution | |
| 2005- | PSCs | CD3+ depletion +/- CD19+ | 3-4 log | slow immune reconstitution | |

C= complement; PSCs= mobilized peripheral stem cells; ES- engraftment syndrome

Partial T depletion was first commenced at CHOP using complement mediated lysis with monoclonal antibody T10B9 obtained through the Medical College of Wisconsin. T10B9 is a narrow specificity antibody which resulted in a more selective depletion of T cell receptor (TCR) α/β and sparing of TCR γ/δ. CD3 depletion with T10B9 reduced T cells by

approximately 1.7 log, which reduced the risk of severe GVHD, and allowed for engraftment for 95% of patients. However, the use of T10B9 was limited by its source and the use of biological reagents such as rabbit complement. Technologic advances, both in mobilization of donor peripheral stem cells and graft engineering allowed broad T depletion first by CD34+ selection, and subsequently, CD3+ depletion using magnetic bead separation approaches.
In patients receiving partially matched related donor transplants, "mega-doses" of up to 20 x $10^6$/kg CD34+ have been used to facilitate engraftment and overcome potential barriers to engraftment [19].

*2.1. CliniMACS Device: Studies with CD3+ Depletion*

The depletion of CD3+ cells, rather than CD34+ positive selection is the most recent method we have used for partial or complete T cell depletion in patients with hematologic malignancies who lack a matched related donor. Cells that may be important in maintaining a graft vs. leukemia effect, such as NK cells, CD34 negative stem cells, and other cells that may be "graft facilitating", are not eliminated with the method. Depletion of CD3+ cells by the CliniMACS system results in approximately 3.5 log T cell depletion (range 2.8-4.1) and 74% CD34+ and NK cell recovery [20-22]. CD19 (B cell) depletion, to prevent donor derived Epstein-Barr virus, was added once the appropriate reagents became available. We have transplanted over 60 patients with this method, including unrelated donors and partially matched related donors. Patients were given an addback of 1-5 x $10^5$/kg CD3+ cells at the time of infusion, unless the donor was haploidentical. Only one patient failed to engraft, which have been due to poor viability of the product upon arrival. The incidence of extensive chronic GVHD is approximately 15% in patients given CD3 addback of 1-5 x $10^5$/kg ,. Higher risk of GVHD is reported in other series [23]which may be related to the less effective T cell depletion as compared to the CD34+ enrichment approach. Chronic GVHD has been limited in most patients, but immune reconstitution is slow (Oshrine, in prep). The 2 year overall survival is 65% in patients with hematologic malignancies, and relapse remains a major cause of treatment failure. Several patients have been salvaged following relapse with DLI or second transplants.

*Studies of αβ and γδ T cells*

Two distinct T cell lineages are generated in the thymus and defined by expression of two different forms of the T cell receptor, an αβ TCR or a γδ TCR. These separate T cell lineages arise from progenitor cells that lack CD4 and CD8 co-receptors and are called double-negative thymocytes [24]. αβ T lineage cells upregulate CD4 and CD8 to generate double-positive cells that constitute the majority of thymocytes. These DP cells undergo TCRα gene rearrangement and the resulting αβ TCR hetodimer yields mature CD4 or CD8 single positive T cells[25]. Most of the γδ T lineage cells remain DN cells.

Studies in mice have shown that ex-vivo activated γδ T cells facilitate engraftment across the major histocompatibility complex, but do not contribute to GVHD, as do αβ T cells [26-28]. Studies have shown that allogeneic γδ T cells are tolerant of host antigens, but still enhance donor engraftment[29] . In addition, γδ T cells may have anti-infectious and antitumor properties [30-33]. In a previous study of partially matched related donor transplant, there was a correlation in disease free survival with the number of γδ T cells that recovered in the first 100 days after a T depleted graft[34] . Patients who recovered higher numbers of circulating γδ T cells had a significantly higher disease free survival compared to those with normal or decreased numbers

(54% vs 19%). A method that enriches for these T cells while removing T cells that cause GVHD allows for an exciting new development in unrelated donor and partially matched related donor HSCT.

*Studies with Miltenyi CliniMACS α/β Depletion*

Recently, a large scale method for selective depletion of αβ T cells from mobilized PSCs was developed that appears as effective in reducing the risk of GVHD as CD34+ cell enrichment. This may allow reduction of post transplant immunosuppression. The Miltenyi CliniMACS device is used to deplete αβ T lymphocytes, and the final product retains NK cells, dendritic cells, and γδ T cells which are removed with CD3+ depletion. In vitro, the retained CD3+gd and NK cells synergistically exert an anti-leukemic and anti-viral effector function [35].

Donor PSCs were incubated with TCRαβ+biotin reagent followed by ClinMACS anti-biotin reagent and separated by the CliniMACS device. The median T depletion was 3.9 log, and the recovery of γδ and NK cells was 92% and 80% respectively. CD34+ recovery was 66%. In mice, these grafts resulted in rapid engraftment of myeloid and lymphoid cells [36]. Pilot studies in haploidentical PSCT, including children, have been encouraging. An initial study of 5 children with advanced leukemias and haploidentical mobilized PSCs noted a 4.5 log depletion of αβ T cells, with 72% recovery of CD34+ cells and 80% recovery of γδ T cells [37]. No GVHD prophylaxis was given with chemotherapy conditioning only. All patients had rapid hematologic engraftment and rapid immune reconstitution. Median time to reach 100 CD3/μl was only 30 days. One patient had grade III skin GVHD, but there was no other acute GVHD noted. Of note, ATG was used during conditioning to achieve additional host immunosuppression.

Recent presentations at the meetings of the American Society of Hematology and European Bone Marrow Transplant meetings have expanded upon this pilot study that was performed in Tubingen Germany [38-40].Ten children with high risk leukemia underwent haploidentical PSCT using grafts that were both TcRαβ and CD19 depleted using the CliniMACS device. Conditioning included melphalan, thiotepa, fludarabine or clofarabine, and OKT-3 or Thymoglobulin. There was no GVHD prophylaxis. All patients engrafted, with median time for neutrophil recovery and platelet recovery 9 and 15 days, respectively. Immune reconstitution was rapid with 350 CD3+ cell/μl at day 30. Mild acute GVHD was seen in 7 patients, and 1 patient had skin grade III that resolved with topical therapy. There was no chronic GVHD. In Rome 13 children with very high risk (relapsed/refractory) leukemias underwent this therapy. Conditioning was myeloablative, including TBI, thiotepa, fludarabine and ATG. All patients engrafted, with 2 patients developing grade I skin GVHD[38]. At the 2012 meeting of the EBMT there were several additional abstracts regarding experience with αβT depletion. In the study by Pira et al, 22 children (15 leukemias, 7 non-malignant diseases) underwent haploidentical PSCT with αβ T depletion. Median recovery of CD34+ cells was 99% and αβ T cells were reduced by 4.5 logs (3.2-6.1)(25). All patients experienced rapid engraftment and only 2 patients developed mild skin GVHD[39].

The median log depletion of T cells is higher with the TCR αβ depletion (4.7 log) compared to CD3 depletion (3-4 log), and similar to CD34+ selection (4.6 log). The TCR αβ depletion is reportedly extremely consistent [40].

2.2 CliniMACS Device: Studies with CD19+ B cell Depletion

An increased risk of Epstein Barr Virus (EBV) induced lymphoproliferative disorder is disadvantage of methods that remove nearly all T cells from donor grafts, but leave behind B cells. Many patients will have been exposed to EBV prior to transplant and will harbor the virus endogenously. Reactivation of EBV is tightly controlled by the immune system, in particular by T lymphocytes. The simultaneous removal of donor B cells from products that are rigorously depleted of T cells greatly reduces the risk of EBV reactivation during the period before immunity recovers [41-42].In our hands, the Miltenyi CliniMACS CD19 system results in a 2.98 ±0.4 log reduction in B cells. We have had several patients with EBV reactivation (they were EBV serology positive), as demonstrated by rising PCR, but no patients who developed lymphoproliferative disorder.

2.3 Conditioning Regimens and Graft vs Host Disease Prophylaxis

For this protocol we have only two options depending upon disease and patient status. These regimens are well established and are considered standard of care. There are no other standard of care options outside the research for the targeted population. Both the TBI and busulfan myeloablative regimens have been used at CHOP and MCW for over 150 patients and have been fairly well tolerated. The risk of transplant related mortality (TRM) is <15%, and all patients have engrafted.

Additional graft vs host disease prophylaxis will not be used routinely based upon European studies (37,44). However, if it is felt that the patient would be put a greater than expected risk for GVHD, based on the cell product or other unforeseen problem, GVHD prophylaxis may be given if approved by the study PI and/or sponsor.

In addition, the pre-transplant Thymoglobulin (ATG) with its half life of several weeks, will assist in GVHD prophylaxis

*3. PATIENT AND DONOR ELIGIBILITY*

3.1. Age. Patient age ≤ 23 years. Both genders and all races eligible.

3.2. Disease eligibility

3.2.1. Leukemias/lymphomas:

a. Acute myeloid leukemia, primary or secondary
Disease status: remission or ≤10% bone marrow blasts

b. Myelodysplasia

c. Acute lymphoblastic leukemia
Disease status: in hematologic remission

d. Chronic myelogenous leukemia:
Disease status: chronic phase, accelerated phase or blast crisis now in second chronic phase.

e. Mixed lineage or biphenotypic acute leukemia

f. Lymphoblastic lymphoma
Disease status: remission

g. Burkitt's lymphoma/leukemia:
Disease status: in remission

3.3. Evaluation of organ status as per CHOP BMT SOP

3.4 Infectious disease criteria

No active untreated infection. Patients with possible fungal infections must have had at least 2 weeks of appropriate anti-fungal antibiotics and be asymptomatic.

3.5 Signed consent by parent/guardian or able to give consent if ≥18 years.
3.6 Negative pregnancy test for females of childbearing potential.
3.7 Exclusion Criteria:
3.7.1 Patients who do not meet disease, organ or infectious criteria.
3.7.2 No suitable donor
3.7.3 Recived previous allogeneic transplant
3.8 Donor eligibility
3.8.1 Donor testing
3.8.1.1 Unrelated donor meets National Marrow Donor Program criteria for donation

- Infectious disease testing
- CHOP BMT procedures apply for determining donor eligibility, including donor screening and testing for relevant communicable disease agents and diseases.

3.8.1.2 Donor matching. High resolution typing at all loci to be performed.
3.8.1.2.1 Unrelated:

- Up to one antigen mismatch A, B or DR
- One antigen mismatch at class I and one allele mismatch at another class I locus
- Donor and collection center willing to undergo mobilization and apheresis

3.8.1.2.2 Related donor:

- HLA match, other than sibling.
- One-two allele or antigen mismatch
- Haploidentical parent or other related donor
- Donor must be willing to undergo G-CSF mobilization and stem cell apheresis.

4. METHODS

4.0 This section describes the standard of care procedures that are done as part of usual CHOP BMT care.

4.1. Pre-transplant evaluation to include:

4.1.1 Organ function evaluation including ECG, Echocardiogram, comprehensive metabolic panel, pulmonary function tests if age appropriate

4.1.3 Mismatched related donor: KIR typing of patient and donor will be done whenever possible(may be done retrospectively)

4.2. Stem cell mobilization

4.2.1 Donor (or guardian) must sign the consent for donation of stem cells following explanation of risks and benefits.

4.2.2 Donor should receive a recommended G-CSF regimen of 15mcg/kg/d starting day-5, with leukopheresis on day -1, and 0 (if necessary). Collection center SOP's apply. Target CD34+ dose/kg patient weight to be collected is 10-20 x

$10^6$. Based upon prior experience, we anticipate collecting sufficient donor cells in one or two collections.

4.2.3 Unrelated donors: Peripheral stem cells collected as per standards of the National Marrow Donor Program.

4.3. Conditioning: There are only two options for conditioning regimens: a total body irradiation or non-irradiation regimen. There are no other standard of care options outside the research for the targeted population.

4.3.1. Institutions should follow their SOP's for the administration of the following chemotherapy and radiation therapy.

4.3.2. Myeloablative conditioning: TBI or Busulfan IV.

Total body irradiation 1200 cGy/6 fractions: may be given before or after chemotherapy-examples below.

| Day | |
|---|---|
| -11 | ATG |
| -10 | ATG |
| -9 | ATG |
| -8 | TBI |
| -7 | TBI |
| -6 | TBI |
| -5 | Thiotepa |
| -4 | Thiotepa |
| -3 | Cyclophosphamide |
| -2 | Cyclophosphamide |
| -1 | |
| 0 | Transplant |

| Day | |
|---|---|
| -9 | ATG* |
| -8 | ATG* |
| -7 | Thiotepa & ATG* |
| -6 | Thiotepa |
| -5 | Cyclophosphamide |
| -4 | Cyclophosphamide |
| -3 | TBI |
| -2 | TBI |
| -1 | TBI |
| 0 | Transplant** |

**Transplant may occur after completion of TBI if cell processing completed

| Day | |
|---|---|
| -9 | Busulfan & ATG* |
| -8 | Busulfan & ATG* |
| -7 | Busulfan & ATG* |
| -6 | Busulfan |
| -5 | Thiotepa |
| -4 | Thiotepa |
| -3 | Cyclophosphamide |
| -2 | Cyclophosphamide |
| -1 | |
| 0 | Transplant |

a. Thiotepa 5 mg/kg/dose
b. Cyclophosphamide 60 mg/kg/dose
c. Total body irradiation 200 cGy x 6 fractions. Shielding of heart/lungs last two fractions.
d. Busulfan: Busulfan will dosed and administered as per standard clinical care SOP.
e. The timing of conditioning drugs may be adjusted as needed by +/-2 days. Changes in conditioning timing will be decided by a physician based on clinical care.
f. If needed for clinical or donation reasons, we will allow up to two days rest from conditioning to cell infusion.

4.3.4 ATG* (Thymoglobulin) 3 mg/kg rATG .

4.3.4.1. ATG may be given to patients with MDS receiving unrelated donor product. ATG will be administered to subjects who will receive a haplo related donor product.

4.3.4.2. Timing of ATG for Busulfan arm and TBI after chemotherapy arm: Days -9, -8, -7 (+/- 2 days)

4.3.4.3. Timing of ATG for TBI before chemotherapy arm: Days -11, -10, -9 (+/- 2 days)

Clinical Care Prophylaxis:

4.3.3. CMV prophylaxis for CMV serology positive recipients with negative donors is recommended: Foscarnet daily post transplant until engrafted, and then ganciclovir or valganciclovir until day +100.

4.3.4. IVIG as per institutional guidelines. Suggest q 2-4 week therapy to maintain IgG>500.

4.3.5. Rituximab 375 mg/$m^2$ day +1 if **patient** EBV positive.

4.3.6. Graft rejection prophylaxis may be given if clinically indicated, especially for subjects with MDS.

4.4. Graft vs. host disease

4.5. Treatment- as per institutional guidelines with steroids and/or other agents.

5. Investigational Stem cell processing using the CliniMACS device will be performed in the Stem Cell Laboratory of the treating institution. This cell processing is the research aspect of this study and can only be done as part of this research study. All cell processing laboratories must be accredited by the Foundation for the Accreditation of Cellular Therapy (FACT) and maintain complete SOP's and procedure records.

   Processing of cells using the CliniMACS will occur in accordance with the Investigator Brochure and Technical Manual following the laboratory SOPs and using aseptic technique.

   5.1. Product Quality Assessment

   The laboratory performing processing shall be responsible for product quality assessment. Testing prior to product release for infusion must include:

   - Total nucleated cell counts (before, during, and after processing)
   - Viability assessment (Trypan Blue and/or 7-AAD) (before and after processing)
   - Gram stain prior to product release
   - Flow cytometry assessment of minimally: CD3, αβ TCR, γδ TCR, B cells (CD20 post processing, CD19 or CD20 prior to processing), and NK cells (CD56+/CD3-)
   - Removal of samples for sterility cultures (before and after processing)
   - Removal of samples for endotoxin assessment (after processing)

   5.1.1. Sterility testing is performed to test for bacterial and fungal cultures on all stem cell products. This testing is performed as part of standard sample testing

procedure at each institution. The culture method used is FDA approved.

5.1.2. Notification procedure for a positive test. If a culture is positive, the testing laboratories notifies the CHOP Stem Cell Laboratory Director or, in their absence, a designee who promptly informs the attending transplant physician. This notification is documented on the hard copy of the test results, and it will include the physician's name, and the date and time of the communication.

If the initial sample from a stem cell product is found to be contaminated, possibly during collection, the Stem Cell Laboratory informs the collection facility. This notification is also documented on the hard copy of the Microbiology test results and it will include the collection center staff member contacted and the date and time the communication. The collection facility is requested to undertake a review of the collection procedure. The Microbiology test results for each stem cell product are filed with the processing records and kept according to SOPs.

Laboratory personnel report a positive culture on the Event Report form. . Positive cultures will be reported to the IRB and the FDA as per the Table in Section 11.

Stem cell products will already have been infused, and the treating physician, working with the Medical Director, will respond accordingly, including monitoring of the patient, follow up cultures and treatment as clinically indicated.

Nearly identical procedures are in place at MCW, except that primary notification of a positive culture result is to the Cell Processing Laboratory (CPL) Laboratory Manager.

5.1.3. Products will be assessed for CD34+, T cell content (TCRαβ+, TCRγδ+, CD3), and B cell content (CD19 and/or CD20) by flow cytometry using validated methods. For the CD34+ determination the laboratory follows the ISHAGE gating protocol and Laboratory staining and analysis SOPs.

5.1.4. Release criteria for stem cell products

5.1.4.1 Viability of $\geq$70% . For viability <70%, the PI and the transplant physician must be notified

5.1.4.2 Negative gram stain.

5.1.4.3 Cumulative cell doses include:

- CD34+ Cells- Minimum 2.0 x $10^6$ per kg, Target 5.0 x $10^6$ per kg
- αβ TCR+ T cells-No minimum, maximum 5.0 x $10^5$/kg
- CD20+ B cells- None specified.

If the defined doses cannot be met due to processing issues or characteristics of the product, approval by the institutional PI or, in their absence, by one of the co-investigators is required for release.

5.1.4.4 Additional required test

Endotoxin testing is performed on a sample of the final infusion product. If the endotoxin values are >5 EU/kg of the recipient weight the PI must be notified.

5.1.5 α/β TCR and CD19 depletion

Processing consists of the following steps:

- Stem cell product washed to remove platelets
- Biotin-labeled αβ TCR reagent is added for 30 minutes at room temperature
- Antibody reagent removed by washing (X2)
- Anti-Biotin and CD19 bead reagents are added for 30 minutes at room temperature
- Reagents removed by washing
- Product is applied to the tubing set on the CliniMACS device and depletion program is started
- The αβ TCR/CD19 depleted fraction is collected and adjusted for infusion based on flow cytometry analysis

Cell numbers and volume of reagents and product are as per the CliniMACS manual. Wash steps all use CliniMACS buffer with HSA, and may be performed using a bag method, or using an automated cell washer per laboratory protocols.

5.1.6 Volume and formulation of final product. The goal will be to provide fresh (non-cryopreserved) product for infusion. The product is infused after concentration and resuspension in 0.9% Sodium Chloride Injection USP with human serum albumin or equivalent according to Lab SOPs.

5.1.7 In rare instances, donor timing will require cryopreservation of the cell product. In this case, the product will be cryopreserved after processing according to relevant BMT SOPs and thawed at the time of infusion. For cryopreservation, the TCRαβ/CD19 depleted products are combined with equal volumes of a cryoprotectant. containing 20% Dimethyl Sulfoxide (DMSO) in 5% Human Serum Albumin (HSA), or equivalent according to Lab SOPs. After the addition of the 2X cryoprotectant to an equal volume of the cell suspension (1:1) the final concentration of DMSO will be 10%. The products then undergo automated controlled rate freezing with recording of the freezing curves and is stored in the vapor phase of liquid nitrogen in a monitored and alarmed freezer.

5.1.8 Once release testing is completed the product label is completed and the product is transported by the laboratory to the infusion site in a validated transport container. Accompanying forms will those specified by laboratory SOPs and in accordance with FACT requirements, including a summary of records used for donor eligibility determination.

Prior to transfer to the infusion team the product is to be examined by an infusion team member and the processing laboratory staff member to confirm identity of the recipient and the product, observation of the product and product container for appearance, and confirmation of all information on the product label. This transfer must be documented.

Infusion information (date and times, identity of infusion team member, etc.) must be recorded on forms as provided by the laboratory, or in the electronic chart record and must include any adverse events associated with infusion.

5.1.9 Post transplant evaluations

5.1.10 Post transplant evaluations are done as part of usual care. These evaluations are not research procedures and are done as needed for good clinical care.

5.1.10.1 Routine laboratory studies: CBC, chemistry panel as needed for timely clinical care

5.1.10.2 Engraftment: Suggest checking Chimerism days +30 (overall only) and Chimerism and/or MRD monitoring monthly x 12 months as needed for clinical care

Sorting: overall, CD3, CD19, NK

5.1.10.3 Evaluation of infection:

a. CMV, adenovirus, EBV: Suggest weekly PCR monitoring until day +100 as needed for clinical care

5.1.10.4 Immune reconstitution: see Appendix I for suggested guidelines

## *6 STATISTICAL CONSIDERATIONS AND DATA ANALYSIS PLAN*

### 6.1 Definition of study endpoints

6.1.1 Engraftment. Defined as first day of ANC>500 for the first of 3 consecutive days

6.1.2 Grade IV aGVHD. Acute GVHD will be graded using the standard Glucksberg grading system (31)

6.1.3 Day 100 treatment-related mortality (TRM). Defined as death from non-disease related causes in the 100 days from stem cell infusion

6.1.4 One-year event free survival (EFS). Events include death, relapse and progressive disease. Relapse is defined as presence of malignant cells in the blood or other body site after initiation of conditioning in a patient previously in remission. Progressive disease is defined as objective and significant increase in disease burden in a patient not in remission, including a new site of disease and an increase in blast count >25%

### 6.2 Sample Size Considerations and Study Duration

….. The study will evaluate the rates of engraftment, grade IV aGVHD, day 100 TRM, and one-year EFS, among patients who undergo SCT with α/β depleted grafts. Since this is a single arm study, no formal hypothesis will be tested and the sample size is based on the width of a 95% confidence interval (CI) around the estimated rates. It is estimated that with 110 patients on study, the half width of a 95% CI for the rate of engraftment (or grade IV aGVHD or day 100 TRM or one-year EFS) will not be wider than 9%. This CI is narrow enough to assess whether

this approach offers enough hope to be worth pursuing in additional definitive studies.

We anticipate recruiting 10-15 patients per year, and expect the recruitment period to be approximately ten years to obtain a total of 110 patients. With an additional one year follow up, the total duration of the study is expected to be eleven years. This study provides the option of stem cell transplant to subjects that do not have a matched related donor available.

## 6.3 Stopping Rules

The early stopping rules are established based on excess rates of day 100 TRM, grade IV acute GVHD, non-engraftment, and EBV LPD. We assume the maximum acceptable rate is 15% for day 100 TRM (based on current CliniMACS studies), is 15% for grade IV aGVHD, is 10% for non-engraftment, and is 5% for EBV LPD. The stopping rules are constructed for each type of the event separately. We will evaluate these events continuously, after enroll five patients until the end of the study. The stopping rule for each type of event will be triggered if there is significant evidence that the event rate exceeds the maximum acceptable rate, that is, if the lower bound of the one-sided 95% CI exceeds the maximum acceptable rate. If the number of patients with a particular type of event equals or exceeds the number in the tables below, then the study should be suspended pending further evaluation.

### 6.3.1 Stopping Rules for day 100 TRM

The detailed stopping rule based on the incidence of day 100 TRM is summarized in the following table, for an acceptable rate of 15% and an unacceptable rate of 30%. For example, if 4 or more out of 10 subjects have TRM in the100 days from stem cell infusion, the study will be suspended. Under this stopping rule, we would stop the study early with a probability of 0.003 if the true day 100 TRM event rate is 5%, stop early with a probability of 0.19 if the true event rate is 15%, stop early with a probability of 0.90 if the true event rate is 25%, and stop early with a probability of 0.99 if the true event rate is 30%. These probabilities are calculated from a simulation study.

| Number of patients | Stop if day 100 TRM >= | Number of patients | Stop if day 100 TRM >= |
|---|---|---|---|
| 5-6 | 3 | 54-58 | 14 |
| 7-10 | 4 | 59-64 | 15 |
| 11-14 | 5 | 65-69 | 16 |
| 15-18 | 6 | 70-74 | 17 |
| 19-23 | 7 | 75-80 | 18 |
| 24-28 | 8 | 81-85 | 19 |
| 29-33 | 9 | 86-91 | 20 |
| 34-38 | 10 | 92-96 | 21 |
| 39-43 | 11 | 97-102 | 22 |
| 44-48 | 12 | 103-108 | 23 |
| 49-53 | 13 | 110 | 24 |

### 6.3.2 Stopping Rules for grade IV aGVHD

The detailed stopping rule based on the incidence of grade IV acute GVHD is summarized in the following table, for an acceptable rate of 15% and an unacceptable rate of 30%. For example, if 4 or more out of 10 subjects have grade IV acute GVHD, the study will be suspended. Under this stopping rule, we would stop the study early with a probability of 0.003 if the true grade IV aGVHD event rate is 5%, stop early with a probability of 0.19 if the true event rate is 15%, stop early with a probability of 0.90 if the true event rate is 25%, and stop early with a probability of 0.99 if the true event rate is 30%. These probabilities are calculated from a simulation study.

| Number of patients | Stop if grade IV aGVHD >= | Number of patients | Stop if grade IV aGVHD >= |
|---|---|---|---|
| 5-6 | 3 | 54-58 | 14 |
| 7-10 | 4 | 59-64 | 15 |
| 11-14 | 5 | 65-69 | 16 |
| 15-18 | 6 | 70-74 | 17 |
| 19-23 | 7 | 75-80 | 18 |
| 24-28 | 8 | 81-85 | 19 |
| 29-33 | 9 | 86-91 | 20 |
| 34-38 | 10 | 92-96 | 21 |
| 39-43 | 11 | 97-102 | 22 |
| 44-48 | 12 | 103-108 | 23 |
| 49-53 | 13 | 110 | 24 |

### 6.3.3 Stopping Rules for non-engraftment

The detailed stopping rule based on the incidence of non-engraftment is summarized in the following table, for an acceptable rate of 10% and an unacceptable rate of 25%. For example, if 4 or more out of 10 subjects have non-engraftment, the study will be suspended. Under this stopping rule, we would stop the study early with a probability of 0.009 if the true non-engraftment event rate is 5%, stop early with a probability of 0.18 if the true event rate is 10%, stop early with a probability of 0.95 if the true event rate is 20%, and stop early with a probability of 0.999 if the true event rate is 25%. These probabilities are calculated from a simulation study.

| Number of patients | Stop if non-engraftment >= | Number of patients | Stop if non-Engraftment >= |
|---|---|---|---|
| 5-8 | 3 | 57-63 | 11 |
| 9-14 | 4 | 64-71 | 12 |
| 15-20 | 5 | 72-79 | 13 |
| 21-27 | 6 | 80-86 | 14 |
| 28-34 | 7 | 87-94 | 15 |

| | | | |
|---|---|---|---|
| 35-41 | 8 | 95-102 | 16 |
| 42-48 | 9 | 103-110 | 17 |
| 49-56 | 10 | | |

### 6.3.4 Stopping Rules for EBV LPD

The detailed stopping rule based on the incidence of EBV LPD is summarized in the following table, for an acceptable rate of 5% and an unacceptable rate of 20%. For example, if 3 or more out of 10 subjects have EBV LPD, the study will be suspended. Under this stopping rule, we would stop the study early with a probability of 0.005 if the true EBV LPD event rate is 1%, stop early with a probability of 0.16 if the true event rate is 5%, stop early with a probability of 0.99 if the true event rate is 15%, and stop early with a probability of >0.99 if the true event rate is 20%. These probabilities are calculated from a simulation study.

| Number of patients | Stop if EBV LPD occurs in | Number of patients | Stop if EBV LPD occurs in |
|---|---|---|---|
| 5-7 | 2 | 54-67 | 7 |
| 8-16 | 3 | 68-81 | 8 |
| 17-28 | 4 | 82-95 | 9 |
| 29-40 | 5 | 96-110 | 10 |
| 41-53 | 6 | | |

## 6.4 Statistical Analyses

The primary analyses will estimate the rates and 95% exact binomial CIs, for the endpoints engraftment, grade IV acute GVHD, day 100 TRM, and one-year EFS. Patients who die will be considered failure for the engraftment success evaluation. Kaplan-Meier curves will be calculated and plotted for engraftment and EFS. Also, cumulative incidence analysis will be used to analyze time to relapse and time to TRM considering each other as competing risk.

In addition, descriptive statistics will be calculated on the kinetics and immune reconstitution. Some of these parameters are expected to have skewed distributions. Therefore, we will use box and whisker plots, and non-parametric summaries, (e.g., quartiles) to summarize and display the data. To assess relationships between various parameters of immune reconstitution and CD34+ and T cell, Pearson correlations will be calculated, possibly after transformation, or Spearman's correlations.

Toxicities will be tabulated for each toxicity type, per the Common Toxicity Criteria, beyond those mentioned above. For each patient, the worst grade toxicity overall will be calculated and the distribution of worst toxicities will be provided.

This study’s analyses will be descriptive to provide guidance to future studies.

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## *7 DATA MONITORING*

A data base of patient entry, data, including engraftment, acute and chronic GVHD, and outcomes, will be kept at the Children's Hospital of Philadelphia. Monitoring will be ongoing. In addition to Sponsor and PI review of each SAE a Safety Monitoring Committee (SMC) will review the SAE's to assess the need to stop the study. The SMC is comprised of a minimum of two physicians. The SMC will meet once a year and review enrollment and outcome with regard to the stopping rules. Targeted areas of safety monitoring will include the following: treatment-related mortality and engraftment. All SMC communication and meetings will be documented and maintained for this study

NCI Common Toxicity criteria will be used for adverse event reporting and toxicity grading.

## *8 FINANCIAL CONSIDERATIONS*

Patients will be billed for aspects of the transplant related to clinical care. This includes stem cell collection, processing and associated costs, conditioning and hospitalization and post-transplant follow up.

## *9 RISKS OF STEM CELL TRANSPLANT*

9.1 Risks specific to αβ depletion. These risks are a result of research participation:

9.1.1 Possible increased risk of graft rejection. Graft rejection is more common in patients who did not receive prior chemotherapy, and in T-depleted transplants compared with transplants where the T cells are not removed, but this risk may be decreased due to the presence of a low number of T cells in the infused product for patients and the high number of CD34+ cells.

9.1.2 Risks of stem cell processing: The blood cells that have been collected will be brought to the Stem Cell Laboratory, and processed under sterile conditions. The risks to this procedure include contamination with bacteria and other agents and malfunction of equipment with a loss or decrease of stem cells. These risks are extremely small.

9.2 General risks that are associated with bone marrow transplant and standard of care conditioning regimen

9.2.1 Infection: Bacterial, fungal, viral. These can be life threatening. Aggressive antibiotic use, including antifungal and antiviral therapy, will be initiated as needed.

9.2.2 Graft vs. host disease (GVHD). This can be acute or chronic, mild or severe. Severe acute GVHD (grades III and IV) are associated with an increased risk of mortality from infectious complications.

Chronic GVHD occurs most commonly in patients who have had acute GVHD, but may occur in patients who did not have any acute symptoms. It usually develops after the third month post-transplant. It has been more common in patients who have received peripheral stem cells or marrow that have not had T cells removed. Patients can have problems with skin, liver, intestine, joints,

mucous membranes, eyes, or other organs. Scarring may result. Medicines can help, but may not completely eliminate all the symptoms. Chronic GVHD may have lingering symptoms for years, or may go away completely. Infection is a major risk for patients with chronic GVHD, as the immune systems often do not return to normal.

9.2.3 Graft Rejection: This occurs when the patient's body does not accept the transfused donor stem cells.

9.2.4 Bleeding due to thrombocytopenia.

9.2.5 Veno-Occlusive Disease (VOD): This can occur as a result of chemotherapy, radiation therapy, or both. Symptoms include jaundice, with liver dysfunction, weight gain, and extra fluid in the abdominal cavity. It may often be managed successfully, and completely resolve. However, complications can arise that can be fatal.

9.2.6 Mucositis and diarrhea: The conditioning therapy causes mucositis. This can result in painful mouth sores and diarrhea. Narcotic pain medicine is generally required for mucositis, which resolves upon engraftment.

9.2.7 Capillary leak syndrome: This may occur as a result of chemotherapy and radiation therapy. The blood vessels may become "leaky" and fluid enters the abdominal cavity and tissues. Edema or anasarca may result and this may result in or worsen renal failure. Pulmonary capillary leak may cause respiratory failure or death.

9.2.8 Unexpected organ damage: This includes unpredictable life-threatening heart, lung, kidney, or liver damage may occur as a result of conditioning and other factors that occur post SCT. Multisystem organ failure usually results in death despite intensive care treatment.

9.2.9 Late effects from conditioning and/or prior therapy: Growth hormone deficiency, hypothyroidism, sterility, cataracts, decreased renal function, decreased heart and lung function. Secondary malignancies may also occur as a result of chemotherapy and/or radiation therapy. The risks of conditioning and prior therapy upon the developing brain are unknown.

10. Drug Information

**ANTILYMPHOCYTE GLOBULIN (ALG, Thymoglobulin):**

| | Common<br>Happens to 21-1000 children out of every 100 | Occasional<br>Happens to 5-20 children out of every 100 | Rare<br>Happens to <5 children out of every 100 |
|---|---|---|---|
| Immediate:<br>Within 1-2 days of receiving drug | Fever, hives | wheezing, trouble breathing, low blood pressure | |
| Prompt: | Decrease in number of lymphocytes in blood, increased risk of infections | | |

| | | | |
|---|---|---|---|
| Within 2-3 weeks, prior to the next course | | | |
| Delayed:<br>Any time later during therapy, excluding the above conditions | | | |
| Late:<br>Any time after the completion of treatment | | | Increase in viral infections, lymphoma from Epstein Barr virus |

**ALG Dosage: 3 mg/kg x 3**

**BUSULFAN (Myleran):**

| | Common<br>Happens to 21-100 children out of every 100 | Occasional<br>Happens to 5-20 children out of every 100 | Rare<br>Happens to <5 children out of every 100 |
|---|---|---|---|
| Immediate:<br>Within 1-2 days of receiving drug | nausea, vomiting, loss of appetite | | Seizures |
| Prompt:<br>Within 2-3 weeks, prior to the next course | Decrease in the number of red & white cells & platelets made in the bone marrow, mouth sores | | |
| Delayed:<br>Any time later during therapy, excluding the above conditions | | | Skin bruising, liver toxicity, veno-occlusive disease, |
| Late:<br>Any time after completion of treatment | Absence of sperm, stopped monthly periods, inability to have children | | Lung scarring, secondary leukemia |

**Busufan dosage:**

<10 kg: 3.2 mg/kg/dose IV daily x 4 days

>10kg but <4 years old- 4 mg/kg/dose IV daily x 4 days

>10 kg and >4 years old: 3.2 mg/kg/dose IV daily x 4 days

**Dose Modification based on Pharmacokinetic Assessments:**

First dose pharmacokinetics will be performed in order to target an area under the curve (AUC) .Daily dose targeting AUC 3600-6000 (micromole/liter)*minute.

*Busulfan Monitoring Instructions*

Draw 1-3cc blood into a green top tube, label with date and time, and place on ice at the following times:
**For Dose 1 Only**: Busulfex™ should be infused over 2 hours and the first sample drawn at the end of this infusion (120 minutes) and at 135 minutes, 150 minutes, 3 hours, 4 hours, 5 hours and 6 hours after the start of the infusion.
**For follow-up doses**- immediately prior to dose, at the end of the 2 hour infusion (120 minutes), 135 minutes, 150 minutes and 4 and 6 hours after the start of the infusion.

**CYCLOPHOSPHAMIDE (CTX, Cytoxan):**

| | Common<br>Happens to 21-100 children out of every 100 | Occasional<br>Happens to 5-20 children out of every 100 | Rare<br>Happens to <5 children out of every 100 |
|---|---|---|---|
| Immediate:<br>Within 1-2 days of receiving drug | Loss of appetite (L), nausea (L), vomiting (L) | Metallic taste (L), abnormal hormone function affecting levels of salt in the blood and urine, causing too much or too little urine[1] | Temporary blurred vision[1], heart damage with abnormal heart rhythms[1], decay of muscle tissue in the heart[2] |
| Prompt:<br>Within 2-3 weeks, prior to next course | Decrease in the number of red and white blood cells and platelets made in the bone marrow, hair loss | Bleeding and inflammation of the urinary bladder (L) | |
| Delayed:<br>Any time later during therapy, excluding the above conditions | Decreased ability of the body to fight infection or disease, absence of sperm or stopped monthly periods, inability to have children(L) | | Damage/scarring of lung tissue [3] (L) |
| Late:<br>Any time after completion of treatment | | | A new cancer or leukemia resulting from this treatment, damage/scarring of bladder tissue |

*[1] Less common with lower doses.*
*[2]Only with very high doses.*
*[3] Risk increased in someone who has had chest radiation.*
*(L) Toxicity may also occur later.*

**Cyclosphosphamide dosage:**

- 60 mg/kg/dose for 2 days

**RITUXIMAB (Rituxan):**

| | Common<br>Happens to 21-100 children out of 100 | Occasional<br>Happens to 5-20 children out of every 100 | Rare<br>Happens to <5 children out of every 100 |
|---|---|---|---|
| Immediate: Within 1- 2 days of receiving drug | Fever, chills, vomiting, headache, cell death causing electrolyte imbalance, Low blood pressure, high blood pressure, trouble breathing Swelling of face, hands and feet, Rash | | Heart arrhythmia, Fatal infusion reactions |
| Prompt: Within 2-3 weeks, prior to the next course | Fatigue, insomnia, generalized weakness, Pain, muscle spasm, Diarrhea, liver enzyme increases Numbness and/or tingling in hands and feet, cough, Viral, bacterial and fungal infectins, Night sweats, dizziness, High blood sugar | Nose bleeds, chills, Face swelling, Skin ulcers | Pancreatitis |
| Delayed: Any time later during therapy, excluding the above conditions | Decreased immunoglobulins | Low blood counts | Severe, possibly fatal skin reactions/rashes |
| Late: Any time after Completion of therapy | Poor growth, sterility (inability to have children) | Lung scarring, cataracts, decreased thyroid function | New cancer, Inflammation of the brain tissue caused by a virus |

**TOTAL BODY IRRADIATION:**

| | Common<br>Happens to 21-100 children out of 100 | Occasional<br>Happens to 5-20 children out of every 100 | Rare<br>Happens to <5 children out of every 100 |
|---|---|---|---|
| Immediate:<br>Within 1- 2 days of receiving drug | Slight redness of the skin, nausea | Inflammation of salivary glands | |
| Prompt:<br>Within 2-3 weeks, prior to the next course | Poor appetite | | Inflammation of the lungs with trouble breathing |
| Delayed:<br>Any time later during therapy, excluding the above conditions | Sleepiness, headache | | |
| Late:<br>Any time after completion of therapy | Poor growth, sterility (inability to have children) | Lung scarring, cataracts, decreased thyroid function | New cancer |

**Total Body Irradiation Dosage**

200 cGy bid days -3,-2,-1*

- *TBI may be administered prior to chemotherapy for ease in scheduling unrelated donors.
- Thorax shielding after 800 cGy
- Patients with leukemia or lymphoma and prior central nervous system relapse: 600 cGy in 3 fractions cranial irradiation prior to initiation of conditioning.

THIOTEPA:

| | Common<br>Happens to 21-100 children out of every 100 | Occasional<br>Happens to 5-20 children out of every 100 | Rare<br>Happens to <5 children out of every 100 |
|---|---|---|---|
| Immediate:<br>Within 1-2 days of receiving drug | Nausea, vomiting, loss of appetite | Pain at the injection site, dizziness, headache | Hives, skin rash |
| Prompt:<br>Within 2-3 weeks, prior to next course | Decrease in the number of red and white blood cells and platelets made in the bone marrow. At high doses used before marrow transplants: mouth sores, inflammation of the passage between the throat and stomach, skin redness, peeling, blisters | At high doses used before marrow transplants: inappropriate behavior, confusion, drowsiness, increased liver enzymes in the blood, increased bilirubin in the blood, darkening of the skin | Sudden high fever |
| Delayed:<br>Anytime later during therapy, excluding the above conditions | Absence of sperm or stopped monthly periods, inability to have children | | |
| Late:<br>Anytime after completion of therapy | | | |

11. POTENTIAL BENEFITS

Potential benefits of this study include decreased risk of GVHD and faster engraftment with the high number of CD34+ cells and αβ T cell + B cell depletion. This may result in decreased period of neutropenia and transfusion dependence. Faster immune system recovery has been reported with αβ T cell depletion compared with other methods.

12. RISK/BENEFIT ANALYSIS

The general risks of blood stem cell transplant include the risks of conditioning and infection. The specific risks of the study, αβ depletion, may include increased risk of graft rejection. The study therapy may potentially benefit the patient by reducing the risk of GVHD with unrelated donors or partially matched related donors, and decrease time to trilinear engraftment and immune reconstitution.

13. PROTECTION OF SUBJECTS

In the event of publication or presentation, patients will be represented by UPN only. Information regarding this study will be kept in a locked file in the SCT data office.

As per the Statistical Analysis, stopping rules are in effect for monitoring severe GVHD, graft rejection, EBV LPD, and treatment-related mortality.

**REPORTING ADVERSE EVENTS:**

All fatal or life-threatening serious adverse events occurring from initiation of transplant conditioning to 28 days after infusion of stem cells must be promptly reported (within 72 hours) to the institution's IRB and to the coordinating Center (CHOP).

The Table below details the adverse event reporting plan. Serious adverse events must be followed until resolution or deemed irreversible. The minimum information required at initial reporting is the patient identifier, description of the event, and the investigator's opinion of its attribution to one of the following: i) the study intervention (processing of PBSC according to Stratum), ii) other transplant related cause or iii) relapse.

We will follow standard regulatory SAE reporting guidelines as per 21 CFR 812.150. The treating physician and Medical Director will define attribution (in their opinions) for all expected and unexpected SAEs and will define any potential device effect as per 21 CFR 812.3(s).

Adverse events (AE's) will use the descriptions and grading scales found in the revised national Cancer Institute (NCI) Common Toxicity Criteria (CTC).

All required reports shall be submitted to the FDA at:

Food and Drug Administration
Center for Biologics Evaluation and Research
Office of Therapeutics Research and Review
HFM-99, Room 220N
1401 Rockville Pike
Rockville, MD 20852-1448
Phone: 301-827-5101
Fax: 301-827-5397

**Expedited Reporting for CliniMACS Trial**

| Unexpected Event | | Expected Event | |
|---|---|---|---|
| **Grades 2-3 Attribution of Possible, Probable or Definite** | **Grades 4 and 5 Attribution of Possible, Probable or Definite** | **Grades 1-4** | **Grades 5 Attribution of Possible, Probable or Definite** |
| Grade 3-4 – Report by phone to IRB within 72 hrs. Expedited report to follow within 10 working days.<br><br>Positive culture in stem cell product will be reported at time of annual report, unless believed to have led to infection in the recipient. If infection in recipient is felt to be related to positive culture in stem cell product then Expedited report within 10 working days to both IRB and FDA<br><br>(Grade 1-2 – Adverse Event Expedited Reporting NOT Required) | Report by phone to IRB within 72 hrs. Expedited report to follow within 10 working days.<br><br>This includes all deaths within 28 days of stem cell infusion regardless of attribution.<br><br>Any late death attributed to the infusion (possible, probable, or definite) should be reported within 10 working days. | Adverse Event Expedited Reporting NOT Required | Report by phone to IRB within 72 hrs. Expedited report to follow within 10 working days.<br><br>This includes all deaths within 28 days of stem cell infusion regardless of attribution.<br><br>Any late death attributed to the infusion (possible, probable, or definite) should be reported within 10 working days.<br><br>Routine readmission for fever, dehydration or GVHD will not be reported.<br><br>Expected toxicities such as infection (except where related to the stem cell infusion), mucositis, fever, and hematological and metabolic toxicities will not be reported. |

14. *CONSENT/ASSENT CONSIDERATIONS*

All parents/guardians meet with a transplant physician for a family meeting to discuss indications for SCT, potential benefits and risks. The consent form is given at that time, and additional discussion ensues prior to entry on study. The investigators may discuss this study with potential child participants in an age appropriate discussion but will not obtain assent. This discussion may occur with or without parents, depending upon the situation.

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APPENDIX: IMMUNE RECONSTITUTION

| | 4 months | 8months | 12 months | 18 months | 2 years | 3 years | 5 years |
|---|---|---|---|---|---|---|---|
| ALC | x | x | x | x | x | x | x |
| CD3 | x | x | x | x | x | x | x |
| CD4 | x | x | x | x | x | x | x |
| CD4/CD45RA | X | x | x | x | x | x | x |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| CD4/CD45RO | X | x | x | x | x | x | X |
| CD8 | X | x | x | x | x | x | x |
| CD16/56 | X | x | x | x | x | x | X |
| CD19 | x | x | x | x | x | x | X |
| CD19/IgD/CD27 | x | x | x | | x | x | X |
| PHA | x | x | x | x | x | x | X |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| TRECS | x | x | x | | x | x | X |
| | | | | | | | |
| IgG | x | x | x | x | x | x | X |
| IgA | x | x | x | x | x | x | X |
| IgM | x | x | x | x | x | x | X |
| IgE | x | x | x | x | x | x | X |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| Lineage chimerism (myeloid, NK, B, T) | x | x | x | | x | x | X |
| | | | | | | | |