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# Post transplant CD34+ Selected Stem Cell Infusion to Augment Graft Function in Children with Primary Immunodeficiency Diseases and Bone Marrow Failure Syndromes.

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## ABSTRACT

The purpose of this study is to investigate the usefulness of infusing purified CD34+ cells of donor origin in order to augment graft function in response to declining chimerism after initially performing an allogeneic hematopoietic stem cell transplant (HSCT) for children with primary immunodeficiency diseases. This protocol will be utilized for patients with waning mixed donor chimerism that is inadequate for correction of clinical condition or disease for which stem cell transplant was performed, or for augmentation of immune function. An infusion of selected CD34+ stem cells will be given without any preparative regimen. As the children eligible for this protocol have reduced immune function and pre-existing donor chimerism, we hypothesize that stem cells will be able to engraft and the infusion will augment graft function. This therapy serves as an alternative to a second stem cell transplant that is known to be associated with significant morbidity and mortality. CD34+ stem cells will be collected from the donor used for initial stem cell transplant. Cells will be T-cell depleted (TCD) by performing a CD34 selection using the CliniMACS device (Miltenyi Biotec) in order to prevent development of new or exacerbation of existing graft versus host disease (GVHD), as avoidance of GVHD in nonmalignant diseases is desirable. There is sufficient data showing that mixed donor chimerism is adequate for reverting disease phenotype in certain primary immunodeficiencies. Observations from Europe and CCHMC show that donor chimerism might be boosted by CD34+ stem cell infusion alone without any specific preparative regimen. This therapy is likely to be associated with low toxicity due to the absence of a preparative regimen and lack of exposure to fresh donor cells capable of initiating GVHD, and offers potential significant benefit.

## I. PURPOSE OF STUDY

Hypothesis

Mixed or full donor chimerism that is adequate for disease control will be achieved with selected CD34+ stem cell infusion without preparative regimen with minimal or no therapy related toxicity or GVHD.

Objectives

1. Assess the toxicity of infusion of donor stem cells in augmentation of graft function in previously transplanted children with immune deficiencies, and bone marrow failures.
2. Assess the efficacy of infusion of donor stem cells in augmentation of graft function in previously transplanted children with immune deficiencies, and bone marrow failures.

Endpoints

The primary endpoint of the study is augmentation of graft function. Graft function will be measured in two ways. Firstly the percentage of donor chimerism, and secondly measured improvement in immune function; improvement in numbers of circulating white blood cells and/or platelets, and/or clearance of opportunistic viral infections.

- Improvement in immune function studies compared to baseline levels can be documented at 3, 6, and 12 months. Immune function studies will include quantitation of T and B cells and functional studies (mitogens and immunoglobulin levels). In the case of Wiskott-Aldrich syndrome, improvement of platelet count to >20,000/L on 2 separate occasions at least three months apart will be considered successful graft augmentation. Children requiring graft augmentation for a specific limited immune defect, e.g. inability to clear papilloma virus will also be assessed by clinical response of the infection; resolution or >50% improvement in infection will be considered successful graft augmentation.

The secondary endpoint is to determine the frequency and characteristics of potential infusion-related toxicity. Experience at our center, using CD34+ selected cells has shown infusion-related toxicity has occurred at an incidence of < 10% (zero toxicity in 9 patients participating in CCHMC IRB study # 2008-0777). While there is little expectation of infusion-related toxicity, the incidence of infusion-related reactions will be recorded and evaluated.

## II. SIGNIFICANCE OF STUDY IN RELATION TO HUMAN HEALTH

Primary or congenital immunodeficiency diseases result from inborn genetic defects of the immune system frequently manifesting itself as recurrent and chronic infections and

commonly lead to lethal complications. Currently, more than 100 immunodeficiency diseases have been identified and classified according to the area of immune system that is compromised. Most of these have been molecularly defined and are due to single gene defects.

Allogeneic hematopoietic stem cell transplantation is the only curative approach to date for most of the lethal immunodeficiency diseases including severe combined immunodeficiencies (SCID), T-cell immunodeficiencies, Wiskott-Aldrich syndrome, Chediak-Higashi syndrome and others, where it restores immune function and decreases risk of long term complications such as malignant transformation.

Although stem cell transplant from HLA (human leukocyte antigen) identical sibling donor is the most preferable and effective treatment in congenital immunodeficiency diseases, the majority of these patients do not have an available sibling donor and alternative approaches such as T-cell depleted haploidentical parental marrow and matched unrelated donor marrow are used as the only curative option with variable results. As reported by European study from 37 centers in 18 countries (1968-1999), in SCID patients 3 year survival with sustained engraftment was significantly better after HLA-identical than after mismatched transplantation (77% versus 54%) and improved over time [1,2]. The outcome for patients with Wiskott-Aldrich syndrome after transplantation, as reported by International Bone Marrow Registry and/or National Marrow Donor Program is significantly influenced by donor type showing the 5 year probability of survival of 87% with HLA-identical sibling donors, 52% with other related donors and 71% (p=0.0006) with unrelated donors [3].

In patients with nonmalignant diseases avoidance of graft versus host disease (GVHD) is most desirable. In some cases use of alternative donor transplant necessitates aggressive graft T-cell depletion that is needed to reduce GVHD. Severe T depletion predisposes these patients to somewhat delayed or incomplete immune recovery and unacceptably high rate of graft failure.

Unlike in malignant diseases where fully ablative conditioning is required to eliminate the tumor, most primary immunodeficiency diseases could be cured with low but sustained levels of donor chimerism [4]. Compartment specific chimerism after HSCT is commonly reported in SCID with T cells being of donor origin while B, NK and myeloid cells are either from recipient or mixed origin [5]. For patients with Chediak-Higashi Syndrome (CHS) 12-36% of donor NK cells and T lymphocytes expressing normal CHS1usually is sufficient to treat hematologic manifestations of CHS [6,7]. Early mixed chimerism post-transplant is commonly reported in Wiskott-Aldrich syndrome. Chimerism may eventually increase to full donor hematopoiesis in the peripheral compartment and persistent donor chimerism markedly decreases long-term risk of lymphoproliferative disease [8,9].

Even though low levels of mixed donor chimerism can be enough to revert disease phenotype, graft failure, Epstein-Barr virus lymphoproliferative disease (EBV-LPD), and severe viral infections remain major obstacles to successful transplantation of children with immune deficiencies. Sometimes repeated stem cell transplants are necessary to achieve adequate and stable immune recovery [10,11]. Multiple stem cell infusions are not infrequently needed in SCID patients, particularly if grafts are given without preparative regimen. Persistent post-transplant thrombocytopenia has been reported in some patients

with Wiskott-Aldrich syndrome where low platelet counts were associated with waning mixed chimerism in hematopoiesis exhibiting a high percentage of recipient cells.

The use of second allogeneic stem cell transplant in non-malignant inherited diseases, is usually associated with significant risks of morbidity and mortality [12]. Occurrence of acute and chronic GVHD, which is considerably higher in HLA-related mismatched and unrelated stem cell transplants, is another limiting factor in these circumstances.

Taking these facts into consideration, there is a need to develop an alternative method to boost waning donor mixed chimerism in this group of patients, to the level adequate for disease control, without causing excessive toxicities or GVHD.

Based on European and local (CCHMC) data (see below) waning mixed chimerism after stem cell transplant for certain primary immunodeficiency diseases may be reversible by infusion of large dose of selected CD34+ stem cells from the original donor without using any preparative regimen. The infusion toxicity is anticipated to be negligible with a CD34 selected product, and the likelihood of GVHD is very low due to the efficacy of the T-cell depletion procedure used. Due to recipients' immune status and a fact that they are already living with mixed donor chimerism, we believe that increased engraftment can be achieved without the use of a preparative regimen [13].

## III. PREVIOUS WORK DONE IN THIS AREA

Three patients with primary congenital immunodeficiency diseases have been treated using above proposed therapy with promising results.

The first patient, treated in Europe, received a single HLA antigen-mismatched (HLA-A in HvG-direction) stem cell transplant from his father as initial treatment for Wiskott-Aldrich syndrome at the age of 2 years. The patient received $2.2x10^8$ MNC/kg of unmanipulated bone marrow after a conditioning regimen with busulfan and cyclophosphamide. Acute GVHD was successfully treated with prednisone without occurrence of any chronic GVHD. The patient's immunodeficiency improved but thrombocytopenia persisted. Only 20% of cells were documented to be of donor origin. At the age of 12 years the patient was administered a boost of positively selected stem cells without any conditioning regimen for correction of persistent bleeding tendency. Stem cells from the primary donor (father) were selected with anti CD133-coated microbeads using a CliniMACS device (Milteny-Biotec). The graft consisted predominantly of CD133/CD34 double-positive cells with a total dose of $8.3x10^6$/kg stem cells with 8000 residual T cells/kg. Although immune suppression was avoided, no signs of GVHD occurred. The platelet count increased to 100,000 three weeks after stem cell boost and the recovery was sustained. The patient had no therapy related toxicities. Chimerism analysis showed decreased recipient cells (exact percentage is not reported).

The second patient with Wiskott-Aldrich syndrome received HLA-matched unrelated donor bone marrow transplant as a primary therapy at age of 10 month, after conditioning regimen with busulfan, cyclophosphamide and ATG. The child experienced graft failure with significant decline in donor chimerism (2-4%), clinically evident as persistent thrombocytopenia. The patient's immune function studies showed decreased absolute numbers of all lymphocyte subsets with normal NK and cytotoxic T-cell function

and normal proliferation to mitogens. The patient was receiving steroid therapy for colitis. At the age of 3 years and 10 month the child was referred to CCHMC for therapy. The child received a boost of Isolex selected CD34+ stem cells ($4.9x10^6$ CD34/kg) from his original donor without any preparative regimen or immunosuppression, as an outpatient procedure. There was one episode of fever 2 days after stem cell infusion due to a reaction to a platelet transfusion. The patient did not experience any other therapy related toxicities or signs of GVHD. Two and a half months after stem cell infusion, donor chimerism has increased from 2% to 10% with a sustained increase in platelet count to 20-25,000 (previously 8,000) without any episodes of bleeding.

The third patient is a boy with X-linked severe combined immunodeficiency (X-SCID) which received a haploidentical bone marrow transplant from his father at the age of 7 months with immunological recovery (80% donor chimerism) and clinical wellness lasting for 7 years. Over the past 2 years the patient has experienced debilitating skin warts due to papilloma virus unresponsive to topical medications and $\alpha$-interferon. This complication has been recognized in significant number of BMT survivors for SCID and can be associated with later skin cancer as observed in several patients in Europe. The inability to clear the papilloma virus appears to be due to a specific defect in immune repertoire. The child was referred to CCHMC for further management. The patient received an Isolex selected T-cell depleted CD34+ stem cells infusion ($13.6x10^6$ CD34/kg) from his primary donor (father) without any preparative regimen in attempt to stabilize T cell and NK cell function and to control papilloma virus infection. The patient had no infusional reactions, nor did he experience any toxicity for two month period after stem cell infusion. It is too early to evaluate efficacy of the procedure in this child.

In summary, all three patients with primary immunodeficiencies and post-transplant graft failure received highly T-cell depleted, selected CD34+stem cells that were infused without any preparative regimen or additional immunosuppression. None of these patients have had any infusion reactions, treatment related toxicities or GVHD. The first patient with Wiskott-Aldrich syndrome, as reported from Europe, had significant and stable platelet recovery at the time of reporting. The second patient with Wiskott-Aldrich syndrome, treated at CCHMC, shows steady improvement in platelet count and donor chimerism. It is anticipated that his platelet counts will improve with time, perhaps over a period as long as one year. While no adverse effects or toxicities were observed in the patient with SCID treated at our institution, it is too early to evaluate efficacy.

Most recently, the transplant group from Newcastle-upon-Tyne, UK have published a series of twenty boosts in nineteen patients with transplant for primary immunodeficiency with improvement in chimerism and/or graft function observed in 12/19.

## IV. RESEARCH PLAN

### 1a. How many subjects will be studied?

We plan to enroll 45 patients on this study (10 in strata I, II, and IV, 15 in stratum III). This number is considered sufficient to exclude major frequent toxicity, and will allow evaluation of efficacy.

### 1b. How will subjects be selected for this study?

Subjects will be selected from the population of patients who have undergone allogeneic hematopoietic stem cell transplant.

Patients will be assigned to four strata according to the underlying disease (due to different disease pathogenesis) and expected end-points after stem cell infusion. Strata are as follows:

1. Stratum I: patients with Wiskott-Aldrich syndrome (thrombocytopenia)
2. Stratum II: patients with SCID (immune defect)
3. Stratum III: other patients (rare immune diseases)
4. Stratum IV: patients with bone marrow failure and other related syndromes.

Five patients will be enrolled into each stratum for evaluation of graft augmentation. If none of the five patients per stratum show any graft augmentation after one year, accrual to that particular stratum will be closed due to lack of efficacy. Patients will continue to be enrolled into other strata until criteria for five patients per stratum are fulfilled. If one out of five patients per stratum will meet listed criteria for graft augmentation, the next five patients may be enrolled to that stratum. If graft augmentation is seen in 1 of 5 or 2 of 10 cases in any stratum the strategy will be considered worthy of further investigation.

Since initiation of the study, it has been noted that stratum III encompasses a wide variety of diseases and therefore the majority of subjects enrolled have been to this stratum. As at least one subject has met the criteria for improved graft augmentation, the target enrollment for stratum III will be increased to 15 to accommodate this large group of patients.

If the infusion of purified CD34+ donor cells does not increase graft augmentation, patient will be removed from study and treated according to standard procedures.

There are no specific plans at this time to treat the patient with an additional boost of CD34+ cells if graft augmentation does not occur on study. However, this decision will be made on a per case basis as clinically indicated.

2a. **Inclusion Criteria**

To be eligible for this protocol, patients must have the following:

1. Primary immunodeficiency (e.g. SCID, Wiskott-Aldrich and/or other more rare conditions), or a bone marrow failure syndrome with prior allogeneic stem cell transplant.
2. Waning donor chimerism or immune function that is inadequate to correct their disease or clinical condition, for which primary transplant was given, as determined by their attending physician.
3. Available primary donor.
4. Must not have other organ dysfunction deemed by the attending physician to preclude this procedure.

5. Age < 35 years at time of transplant
6. One of the following must be true:

    - Patients must have evidence of persistent or recurrent immunodeficiency or thrombocytopenia.

    **-OR-**

    - Primary immunodeficiency disease with known potential to progress to malignant condition if untreated.

    **-OR-**

    - Debilitating secondary disease known to be a consequence of inadequate immune response to known agent or pathogen, uncontrollable by other available medical therapies (e.g. third patient described on page 5).

2b. **Exclusion Criteria**

1. Absence of an available original donor
2. Failure to sign consent form, or inability to undergo informed consent process
3. Pregnant or lactating female
4. Uncontrolled GVHD

3. **Randomization**

Not applicable.

4**.** **Procedures**

Pre-infusion evaluation:

Patients enrolled on this protocol will be evaluated for disease status and organ function prior to stem cell infusion, required testing as follows:

1. Peripheral Donor Chimerism Studies
2. CBC with differential and platelets
3. Immunological studies including but not limited to absolute numbers of all lymphocyte subsets NK, T, B lymphocyte function, proliferation to mitogens, immunoglobulins
4. Urine pregnancy tests for girls of childbearing age
5. Renal and liver function tests

**Treatment Plan:**

Treatment Scheme

This treatment will involve these steps:

1. Pre-infusion evaluation
2. Collection of Granulocyte Colony Stimulating Factor (GCSF) mobilized peripheral blood stem cells from same donor used for first transplant as per standard procedures
3. Selection of CD34+ stem cells and T-cell depletion using CliniMACS
4. Infusion of stem cells into recipient
5. Continue monitoring

Schedule*:

| | |
|---|---|
| Pre-infusion | Clinic visit and evaluation of peripheral blood counts |
| Day 0 | Infusion of CD34+ selected stem cells |
| Day 7 | Clinical assessment |
| 3, 6, 12 months and then yearly for up to 3 years post infusion | Clinical assessment<br>Donor chimerism studies<br>Immunological studies |

*For details see Required Observations, page 10.

Additional testing such as disease specific markers (CD132, WASP) or more frequent testing might be needed at attending physician discretion if indicated by patient's clinical condition.

Preparative therapy:

No preparative therapy or additional immunosuppressive therapy will be given in preparation to stem cell infusion.

Stem Cell Infusion:

Stem Cells will be administered per standard institutional protocols.

Immune Suppression after stem cell infusion:

No additional immune suppression will be given after stem cell infusion. The stem cell product will be highly T-cell depleted, reducing risk of GVHD. In addition the patient is already a mixed chimera (has both donor and own cells) and likely tolerant of donor cells. Occurrence of GVHD in these settings is unlikely.

Support therapy:

- Any infusion related reactions will be treated with supportive therapy.

- Patients with fever (temperature of 38.0°C or above) will be treated in compliance with institution guidelines in regards to disease risk and immune status of the patient.
- Blood products may be administered according to standard of care and institutional guidelines for immunodeficient patients if needed for medical condition or prophylaxis.
- Patients may remain on current medication that they are taking for their medical problems or infection prophylaxis as per attending physician decision.
- If acute or chronic GVHD occurs, patients will be treated according to standard of care.

Stem Cell Processing:

The CD34+ cells will be separated by CliniMACs cell purification protocols which are not yet licensed in the US. This protocol will be submitted to the FDA under an existing IND.

Peripheral blood stem cells (PBSC) with a target of ≥$10x10^6$/kg CD34+ cells will be requested. All CD34+ stem cells will be infused if more than $10x10^6$/kg CD34+ stem cells are collected. The maximum number of CD34+ cells that will be infused will be defined by the maximum T-cell (CD3+) dose. Stem cells will be depleted of T-cells using a CliniMACS CD34+ cell selection device and infused into the patient with a maximum T-cell dose of $5 \times 10^4$/kg. The minimum number of CD34+ cells that will be infused is $1x10^6$/kg. Stem cell processing will occur at Hoxworth Blood Center per standard operating procedures. If T-cell depletion yields a product that does not meet the release parameters, the product will not be infused, although a portion of the product may be given if the T cell content of the entire product exceeds the specified maximum dose. (See the table below.) As the patient has not received a preparative regimen, there will be no negative consequences.

Product Release Parameters

| Attribute | Test Method | Specification |
|---|---|---|
| Volume | Measured | ≤ 20 mL/kg |
| Total Cell Count | Hemacytometer | Report Value |
| Viability | 7AAD | ≥ 80% |
| % CD34+ | Flow Cytometry | ≥ 70% |
| Viable CD34+/Kg | Flow Cytometry | ≥ $10^6$/kg |
| % CD3+ | Flow Cytometry | Report Value |
| Viable CD3+/Kg | Flow Cytometry | ≤$5 \times 10^4$/kg |
| Gram Stain | Gram Stain | Negative |

Safety of Product

Unrelated donor evaluation will be completed per the relevant unrelated donor registry standards (additional testing at discretion of physician and NMDP/registry donor center).

Related donors will be evaluated per donor standard operating procedures. As part of this research we will collect clinical data to ensure eligibility.

Product testing

The following tests will be performed on each product produced to assure product safety, purity, and potency:

Pre CD34 Selection (T cell Depletion):
- Total nucleated cell count
- Viability
- CD 34+ cells/kg of recipient
- CD 3+ cells/kg
- total volume collected
- sterility (bacterial and fungal culture)

Post CD34 Selection (T cell Depletion):
- Total nucleated cell count
- Viability
- Viable CD 34+ cells/kg of recipient
- Viable CD 3+ cells/kg of recipient
- total volume for infusion
- endotoxin assay (Limulus Amebocyte Lysate Test or equivalent)
- stat gram stain
- sterility (bacterial and fungal culture)

Culture of the product:
If the culture of the product (either fungal or bacterial) becomes positive following infusion of the cells, antibiotics or antifungal medications will be administered to the recipient. The patient/guardian and treating physician will be informed.

Endotoxin testing:
An endotoxin assay will be performed on the post-selection product within 24 hrs of infusion, and the patient's physician will be notified if more than 5 EU/kg was infused into the recipient.

Required Observations:

1. **Clinical Assessment**
   An assessment of the patient will be done on Day 7 (+/- one day), at three months (+/- four weeks), and at or around six and twelve months. This will include a complete physical exam and CBC.

2. **Donor chimerism studies**

Peripheral blood donor chimerism studies will be performed only for subjects for whom the CD34 product has been provided for inadequate donor chimerism. Chimerism studies will be performed at or around three, six and twelve months, then annually for up to 3 years after the stem cell infusion. Thereafter, chimerism studies will be performed as clinically indicated. Donor chimerism analysis will be done on peripheral blood using standard FISH or VNTR analysis.

3. **Immunological studies**
   Appropriate immunologic studies relevant to indications for CD34 infusion will be performed for stratum II and stratum III patients (see strata on page 6) at three months (+/- four weeks) and at or around six, twelve months and annually for up to 3 years post infusion. These tests will include, but not be limited to, lymphocyte subsets, T cell activation and NK function.

   Additional testing like disease specific markers (CD132, WASP) or more frequent testing might be needed at attending physician discretion if indicated by patient's clinical condition.

5. **Blood Specimens**

   Not applicable

6. **Other Previously Approved Research Studies In Which The Projected Patient Population May Also Be Involved** ***(include what precautions will be taken to avoid any cumulative risk, e.g., repeated blood sampling, radiation exposure, etc.)***

   Patients eligible for this study may be eligible for other open therapeutic studies at CCHMC. Study options will be discussed and decided on a case-by-case basis.

7. **Data Analysis/Statistical Considerations**

   Primary Endpoints

   The primary endpoint of the study is augmentation of graft function. Graft function will be measured in two ways: firstly the percentage of donor chimerism, and secondly immune function.

   Successful augmentation of graft function will be achieved if:

   - Donor chimerism is doubled compared to the value immediately pre-infusion at 3, 6, and 12 months, -OR-
   - Improvement in immune function studies compared to baseline levels can be documented at 3, 6, and 12 months. Example of immune function studies which would be used would include quantitation of T and B cells and functional studies (mitogens and Immunoglobulin levels). In the case of Wiskott-Aldrich syndrome, improvement of platelet count to >20,000/L without transfusions on 2 separate occasions at least three months apart will be considered successful graft augmentation. Children requiring graft augmentation for a specific limited immune defect, e.g. inability to clear papilloma virus will also be assessed by

clinical response of the infection; resolution or >50% improvement in infection will be considered successful graft augmentation.

Secondary Endpoints

Infusional Toxicity: Infusion of stem cells could result in infusion reaction presenting as shortness of breath, fever, or anaphylaxis. To reduce the risk of infusion reaction, CD34+ stem cells will be highly T-cell depleted. While we have the expectation of a low occurrence of this toxicity, we will monitor for the frequency and characteristics of infusion related toxicities.

Statistical Analysis

We expect the frequency of adverse events in this study to be low (likely less than 5% events). We will therefore use all 45 patients to determine the frequency of adverse events as our ability to correctly define the frequency of events in any one stratum of 10 cases is low.

Descriptive statistics will be compiled on responding and non-responding cases, including patient and graft characteristics that might influence response. It is recognized that this portion of the analysis is exploratory due to the small sample size, but these data will be used to inform the design of future studies.

Stopping Rules

The primary endpoint of this study is qualitative graft augmentation. Five patients will be enrolled into each stratum for evaluation of graft augmentation. If none of the five patients per stratum show any graft augmentation, accrual to that particular stratum will be closed due to lack of efficacy. Patients will continue to be enrolled into other strata until criteria for five patients per stratum are fulfilled. If one out of five patients per stratum will meet listed criteria for graft augmentation, an additional five patients may be enrolled to that stratum.

Any serious unexpected adverse events (grade 4 toxicity per Common Terminology Criteria for Adverse Events version 3.0) will lead to suspension of study enrollment for determination of attribution of the event to study procedure. The principal investigator will review and evaluate these events in relation to the study procedures and outcomes before further patients are enrolled. Any fever occurring within twelve hours of the stem cell infusion requiring hospital admission will be considered an expected serious adverse event. This event will require prompt reporting to the Institutional Review Board (IRB), but will not require suspension of the study. In the event of documented graft versus host disease, grade II or above at any site, the study will be suspended to assess and evaluate the situation and to reconsider study procedures. The IRB may determine that the study should be terminated based on their review of reported SAEs.

9. **Facilities Utilized In the Study**

The stem cell infusion will be performed at Children's Hospital of Cincinnati Medical Center.

## V. POTENTIAL BENEFITS

There may be no benefit from participating in this study. However, the information learned from this study may help make better the health care that is given to patients with primary immunodeficiency disorders in the future.

This CD34+ stem cell infusion procedure may be able to increase donor chimerism, resulting in correction of immunodeficiency, thrombocytopenia or enzyme deficiency sufficient to reverse present disease or clinical condition. If this happens, the patient will no longer require transfusions of platelets and have less risk of infection. This procedure may also be able to decrease the risk of malignant transformations that potentially occur in some of untreated primary immunodeficiencies. These outcomes would be a benefit to participants.

## VI. POTENTIAL RISKS, DISCOMFORTS, INCONVENIENCES AND PRECAUTIONS

### 1. Known or potential risks, discomforts, or inconveniences

Infusion reactions: Infusion reactions are unlikely because of the highly purified nature of the product. However there is a potential risk of reaction including fever, chills, anaphylaxis, rash, shortness of breath. Transfusions also carry the risk of hepatitis, human immunodeficiency virus and viral pneumonia due to cytomegalovirus.

Graft versus host disease (GVHD): GVHD is unlikely as a low number of T-cells will remain after cell selection and the recipients already have stable mixed chimerism with cells from their donors. Acute GVHD can cause a skin rash, liver dysfunction, and/or enteritis, and is potentially fatal. Chronic GVHD is a multi-organ autoimmune disease, which may resolve over several months or years or lead to death, commonly from infection.

### 2. Precautions that will be taken to monitor and avoid the above mentioned risks, discomforts, and inconveniences, including methods for detecting adverse events

Infusion reactions: The risk of transfusion reaction will be minimized by appropriate testing of the donor and products, and by use of pre-medication. Pre-medications will be given according to standard practice. Patients will be monitored according to CCHMC guidelines during stem cell infusion for any infusion reactions. To reduce the risk of product infusion, CD34+ stem cells will be highly T-cell depleted. Should an infusion reaction occur it will be treated with supportive therapy according to standard transplant procedure.

Graft versus host disease: The risk of developing a new case of GVHD is anticipated to be very low. No GVHD prophylaxis will be given. Any occurring

GVHD would be treated according to accepted standards.

**3. The method of monitoring study conduct.**

All grade 3 or greater adverse events occurring during this study will be recorded on the case report forms. The principal investigator will review each event and assess its relationship to study events as to whether the event is unrelated, unlikely related, possibly related, probably related, or definitely related to the study device. The CTCAE Version 3.0 will be used to evaluate these adverse events.

This population of patients, by nature of their various disease/conditions and due to effects from the original allogeneic hematopoietic stem cell transplantation (with a full preparative regimen), often continue to experience disease and therapy-related complications and are frequently hospitalized for observation and/or treatment of these conditions. As these patients do not receive any preparative therapy as participants in this study, the risk of study-related toxicity is expected to be minimal. Consequently any serious adverse events, as defined by the IRB guidelines, that are determined to be possibly, probably or definitely related to the study device will be reported according to IRB reporting regulations. However, serious adverse events determined to be related to the patient's underlying disease/condition or to the first transplant procedure, including hospitalizations or prolongation of hospitalizations resulting from such events, will not require expedited reporting to the IRB. These events will be reported annually with the progress report. Please note that for the purpose of this study we will exclude the report of hematologic toxicities, as these subjects, by virtue of their underlying disease and/or condition may present with baseline hematologic toxicity.

Although these subjects will be followed on study for up to 3 years post infusion, all adverse events (as described above) will be captured for the first 30 days post stem cell infusion. Thereafter, only related adverse events will be recorded on case report forms.

**4. Methods for maintaining data quality and confidentiality**

Data quality is monitored and audited by the Center for International Blood and Marrow Transplant Research and the National Marrow Donor Program. Confidentiality is maintained as per section VI.

**5. An assessment of accrual (timely enrollment) and the handling of dropouts.**

Accrual assessment will be evaluated by the principal investigator with the annual progress review. Subjects who do not complete a full six months of follow up will not be counted in the total stratum accrual and will be replaced.

**6. Risk assessment recommendation**

- ☐ Minimal risk but with direct benefit to participants
- ☐ Minimal risk but without direct benefit to participants
- ☒ More than minimal risk but with direct benefit to participants
- ☐ More than minimal risk but without direct benefit to participant

### 7. Data and Safety Monitoring Plan

This study does not require a DSMB or medical monitor because the patient is already a mixed chimera (has both donor and own cells) and likely tolerant of donor cells. In addition, the patient will not receive any preparative therapy prior to stem cell infuse, thus decreasing the risk of toxicities by the patient. Therefore, the principal investigator will be responsible for monitoring the study.

The data generated during this trial will be monitored by the Principal Investigator for safety and compliance with protocol-specified requirements. The Principal Investigator will review all protocol related lab results for each patient enrolled on the protocol, and each patient will be examined by the Principal Investigator or the physician following the patient during their required follow-up visits. All planned testing is medically indicated for patients undergoing treatment regardless of their participation in this research study. The study PI and research coordinator will review the study progress regularly. Patients entered on the trial and adverse events will be reviewed to ensure that the study is implemented as outlined in the protocol.

All decisions regarding study continuation, modification, or termination will be reported immediately or annually, as appropriate, to the IRB, FDA, and other appropriate agencies. Reports for events determined by the investigator to be possible or definitely related to study participation and reports of events resulting in death should be forwarded in compliance with current IRB policy and applicable federal regulations.

Any serious unexpected adverse events (grade 4 toxicity per Common Terminology Criteria for Adverse Events version 3.0) which occur during the stem cell infusion or within thirty days after the infusion will be expeditedly reported to and evaluated by the Principal Investigator, as well as the IRB (see section VI-3). Thereafter, only events related to the study procedures will be captured on case report forms for each subject on study.

## VII. CONFIDENTIALITY

Every effort will be made to maintain patient confidentiality. Absolute confidentiality cannot be guaranteed. Patient information may be disclosed if required by law. The FDA, Hoxworth Blood Center and the Cincinnati Children's Hospital Medical Center Institutional Review Board will have access to the records.

All study documents will be kept in a locked cabinet in the Cancer and Blood Disease Institute. Access to the files will be limited to the Principal Investigator and her designees only. Study records will be maintained in the secure database that has restricted access to only the Principal Investigator and her designees.

## VIII. PERIOD OF TIME ESTIMATED TO COMPLETE PROJECT AS DESCRIBED

This protocol will remain open until the number of patients planned for accrual has been reached, or until the study is closed for toxicity reasons. Our target accrual is 45 cases on this protocol. Estimated time for patient accrual is five years.

## IX. FUNDING

1. **Name of funding agency or private sponsors**

   This research is conducted through the Cancer and Blood Disease Institute.

2. **The institution to which funding will be made**

   Not applicable

## X. PAYMENT FOR STUDIES

1. **Third party payers**
   The patient's health plan/insurance company will need to pay for all of the costs of treatment in this study. FDA approval for cost recovery has been granted. The patient will be responsible for the costs of standard medical care, all hospitalizations and any infusion complications. Pre-authorization for the infusion will be cleared with the health plan/insurance company prior to admission.

2. **Reimbursement to participants**
   Patients will not be reimbursed for participation in this study.

## XI. METHOD TO BE USED IN PROCURING CONSENT OF SUBJECTS

All prospective patients will have the study explained by a member of the research team. All the potential hazards and possible adverse reactions will be explained to patient/parent.

Prior to the initiation of the study, acknowledgement of the receipt of this information and the subject's freely tendered offer to participate will be obtained in writing from each subject in the study.

Although many of the subjects on this study will have transplants utilizing matched unrelated donors provided and/or facilitated by the National Marrow Donor Program, this study also allows for human leukocyte antigen (HLA) compatible related donors. In some cases, the related donor will be local; therefore preparation for the stem cell collection will be performed at CCHMC and/or other area facilities. Prospective HLA compatible **related** donors will also need to provide signed informed consent before initiation of the collection procedures and associated clinical data. Prospective HLA compatible related donors will be no younger than 3 months of age at the time of

consent. A **physician other than the patient's attending physician** will consult with the prospective donor to explain the purpose, procedures, risks, and benefits of the study and the collection procedures at the donor's level of understanding. Opportunity will be given to consider the study and have questions answered.

For those patients and families for which English is not their primary language, the short form consent process will be utilized at CCHMC as per CCHMC Standard Operating Procedures. The consent will be administered orally by an appropriate member of the research team with translation by an interpreter, with time for questions and clarification. The interpreter will be provided with the long form version of the consent that is specific to the participant being consented.

This protocol, informed consent, and any amendments to the protocol will be reviewed and approved by the CHMCC IRB prior to initiation. The study will not be initiated without the approval of the IRB.

## XII. MAINTENANCE OF PROTOCOL RECORDS

All study documents will be kept in a locked cabinet in the Hematology/Oncology Clinical Research Office. Access to the files will be limited to the Principal Investigator and his designees only. Study data will be maintained in the secure database that has restricted access to only the Principal Investigator and his designees. Processing records for the T cell depletion procedure will be maintained at Hoxworth Blood Center.

## XIV. CRC *(protocol review required by CRC Scientific Advisory Committee) for protocols which utilize the CRC)*

The CRC will not be utilized for this research study.

## XV. PROTOCOL REVISIONS

Any changes to the protocol or informed consent document will be submitted to the CCHMC IRB for approval prior to the implementation of the changes.

## XVI. CONTINUING REVIEW

This study will undergo an annual review with all requested information provided to the CCHMC IRB.

## XVII. REFERENCES

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