BLOOD AND MARROW
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CLINICAL TRIALS NETWORK

REDUCED-INTENSITY CONDITIONING FOR CHILDREN AND ADULTS WITH HEMOPHAGOCYTIC SYNDROMES OR SELECTED PRIMARY IMMUNE DEFICIENCIES (RICHI)

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PROTOCOL SYNOPSIS - BMT CTN PROTOCOL #1204

Reduced-Intensity Conditioning for Children and Adults with Hemophagocytic Syndromes or Selected Primary Immune Deficiencies (RICHI)

Study Chairpersons: Carl Allen, MD, PhD and Michael Pulsipher, MD

Primary Objective: To prospectively determine the 1-year overall survival in subjects treated for hemophagocytic syndromes or primary immune deficiencies (CGD, HIGM1, IPEX, and severe LAD-I) using a standardized, reduced-intensity conditioning protocol consisting of fludarabine, melphalan and intermediate timing of alemtuzumab (Day -14).

Secondary Objectives: Secondary objectives for the study include measurement of sustained engraftment, incidence of HLH and CAEBV reactivation and death from disease, immune reconstitution and functional immune recovery at 1-year, cumulative incidence (CI) of grade II-IV and III-IV acute GVHD and chronic GVHD, transplant-related complications (VOD, CNS toxicity), infectious complications including reactivation of CMV, adenovirus, EBV, invasive fungal infection or bacterial sepsis, and overall survival and rate of sustained engraftment of specific disease subsets.

Study Design: This study is designed as a Phase II multi-center trial. The study population includes patients with HLH, HLH-related disorders, and selected primary immune deficiencies: CGD, HyperIgM Syndrome (HIGM1), IPEX Syndrome, or severe LAD-I with indications for HCT receiving a bone marrow transplant from a related or unrelated donor (see HLA typing requirements in eligibility criteria below).

Accrual Objective: The trial will accrue a minimum of 35 HLH patients.

Accrual Period: The estimated accrual period is 3 years.

Eligibility Criteria: Eligible patients are > 3 months and ≤ 45 years of age with Lansky/Karnofsky performance status ≥ 50% who have HLH or related disorders or selected immune deficiencies with an indication for HCT. Patients must have adequate organ function (cardiac, renal, hepatic, pulmonary). Only bone marrow donors are allowed on this study. The donor must be:

- An unaffected sibling donor who is a 6/6 match at HLA–A and –B (intermediate or higher resolution) and –DRB1 (at high resolution using DNA-based typing) *OR*

- An unaffected related donor (other than sibling) who is a 7/8 or 8/8 match for HLA–A, –B, –C (at intermediate or higher resolution), and –DRB1 (at high resolution using DNA-based typing) *OR*
- An unrelated donor who is a 7/8 or 8/8 match at HLA–A, –B, –C, and –DRB1 (at high resolution using DNA-based typing).

Treatment Description: All eligible patients undergoing bone marrow HCT will receive reduced-intensity conditioning (RIC) with fludarabine, melphalan and alemtuzumab beginning on Day -14.

Study Duration: Patients will be followed for 1 year post-HCT.

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CHAPTER 1

1. BACKGROUND AND RATIONALE

1.1. Background

Hematopoietic cell transplant (HCT) is required for long-term survival in many patients with primary defects of the immune system. The major obstacles to long-term survival with high quality of life in these patients, many of whom have significant morbidities at the time of transplant, are treatment-related mortality (TRM) and durable immune reconstitution. Reduced-intensity conditioning protocols have significantly improved outcomes in patients with immune deficiencies such as hemophagocytic lymphohistiocytosis (HLH) (Marsh et al., 2011). However, the decrease in treatment-related morbidity and mortality comes with increased risk of inadequate durable engraftment, though a portion of patients with unstable chimerism or graft loss may be rescued with subsequent lymphocyte infusions or repeat HCT (Marsh et al., 2010). In an institutional case series of patients with HLH and related disorders, "intermediate" (Day -14) timing of alemtuzumab administration maintained excellent survival rates with improved stable engraftment (Marsh et al., 2013b). In this study, we will test the efficacy of Intermediate-Timed Alemtuzumab Reduced Intensity Conditioning (Intermediate RIC) in a multi-center setting in patients with HLH and other primary immunodeficiencies.

1.1.1. An Opportunity to Improve Survival of Patients with HLH and Selected Primary Immune Deficiencies (PID)

Although outcomes after related and unrelated donor HCT have improved dramatically in the past decade, survival in patients with diseases of immune dysfunction has lagged behind. Myeloablative (MA) approaches have been plagued with high rates of early treatment related-mortality (section 1.2.4), but even patients transplanted with RIC approaches have not had ideal outcomes. A review of CIBMTR data between 2008 and 2010 illustrates this challenge. A total of 77 HLH patients received unrelated BM/PBSC, 22 related BM/PBSC, and 57 unrelated cord blood (CB) transplants during this three-year period. One third received various RIC approaches and 2/3 MA approaches. Overall survival (OS) at 1-year was 70% in those who received either related or unrelated BM/PBSC and 67% in recipients of unrelated CB. One-year OS of patients with immune deficiencies included in this protocol (XLP, Chediak-Higashi, CGD, HIGM1, IPEX, and LAD-I) receiving URD RIC approaches was similarly poor at 74% (personal communication, Mary Eapen, CIBMTR). Single-center experiences with fludarabine, melphalan, and alemtuzumab describe overall survival as high as 90% in HLH patients, demonstrating a clear opportunity to improve survival in this patient population (Cooper et al., 2008; Marsh et al., 2010).

1.1.2. Challenges with Rejection of Cord Blood in Patients with HLH/PID

Very little data on outcomes of cord blood transplantation in this population has been published. Early experience with use of cord blood following the Flu/Mel/Alem approach led to a high number of rejections. This anecdotal experience is borne out by CIBMTR data. Between 2008-

2010, of the 56 who underwent cord blood transplantation for HLH (2/3 MA, 1/3 RIC), 13 had primary and 7 had secondary graft failure for an overall graft failure rate of 36%. One-year survival was poor, at 67%. Patients with immune deficiencies undergoing cord blood procedures during these three years rejected 35% and 34% of the time with RIC (n= 31) and MA (n=68) approaches, respectively. Over the same time period, rejection occurred in 5% of MA procedures for immune deficiencies who received unrelated BM/PBSC (personal communication, Mary Eapen, CIBMTR). There is clearly a major problem with cord blood engraftment in these non-malignant disorders that will need to be addressed by a different approach. Relative delay in immune reconstitution with cord grafts and difficulty generating cytotoxic T cell therapy from donor cells are also challenges in this population with frequent infectious complications. With these data in mind, most investigators will choose a 7/8 or 8/8 HLA matched unrelated donor over a cord blood for these disorders. For patients who do not have a 7-8/8 matched unrelated donor available, enrollment on pilot studies aimed at improving engraftment using UCB or haploidentical donors is encouraged.

1.2. Hemophagocytic Lymphohistiocytosis

Hemophagocytic lymphohistiocytosis (HLH) is a syndrome of pathologic immune activation characterized by clinical signs and symptoms of extreme inflammation caused by dysregulation of immune effector function. Without prompt initiation of immunochemotherapy, active familial HLH is almost universally fatal (Janka, 1983). "Primary" inherited and presumed "secondary" acquired HLH are impossible to differentiate in the acute clinical setting, and distinctions between these groups are becoming increasingly blurred. In the last Histiocyte Society trial, HLH-94, there was no significant difference in outcomes between patients with presumed familial or presumed secondary HLH with overall 3-year survival of 55% (Henter et al., 2002).

1.2.1. Diagnostic Criteria for HLH

The current resource most clinicians use to define HLH is the Histiocyte Society HLH-2004 research protocol that requires either identification of recognized mutations in HLH-associated genes (including *PRF1, MUNC13-4, STX11, STXBP2 (Munc18-2), SH2D1A* (XLP1)*, BIRC4* (XLP2)*, LYST* (Chediak-Higashi Syndrome)*,* or *RAB27a* (Griscelli Syndrome)) or five out of eight clinical criteria: fever; splenomegaly; cytopenias in 2/3 lineages; hypertriglyceridemia and/or hypofibrinogenemia; hemophagocytosis in bone marrow/spleen/lymph node/liver; decreased NK-cell activity; elevated ferritin; and elevated soluble CD25 (also called soluble IL-2 receptor alpha) (Henter et al., 2007). (See Table 1.2.)

TABLE 1.2: DIAGNOSTIC CRITERIA FOR HLH USED IN THE HLH-2004 TRIAL*

The diagnosis of HLH may be established by: **

A. A molecular diagnosis consistent with HLH: Pathologic mutations of *PRF1*, *UNC13D (MUNC13-2)*, *STXBP2 (Munc18-2)*, *Rab27a*, *STX11*, *SH2D1A*, or BIRC4

-OR-

B. **Five** out of the **eight** criteria listed below are fulfilled:

1. Fever ≥ 38.3°C
2. Splenomegaly
3. Cytopenias (affecting at least 2 of 3 lineages in the peripheral blood):
   Hemoglobin < 9 g/dL (in infants < 4 weeks: hemoglobin < 10 g/dL)
   Platelets < $100X10^3$/mL
   Neutrophils < $1X10^3$/mL
4. Hypertriglyceridemia (fasting, ≥ 265 mg/dL) and/or
   hypofibrinogenemia (≤ 150 mg/dL)
5. Hemophagocytosis in bone marrow or spleen or lymph nodes or liver
6. Low or absent NK-cell activity
7. Ferritin > 500 ng/mL
8. Elevated Soluble CD25 (soluble IL-2 receptor alpha)***

Notes:

* Adapted from Henter et al. 2007

** Additionally, in the case of familial HLH, no evidence of malignancy should be apparent.

*** Elevations above age-adjusted, laboratory-specific normal levels (defined as > 2 SD from the mean) appear more meaningful than the original designation of '> 2,400 U/mL', because of variations between laboratories and age groups.

1.2.2. Initial Therapy for HLH

Without therapy, survival of patients with active familial HLH is approximately 2 months (Henter et al., 1991). The first international treatment protocol for HLH was organized by the Histiocyte Society in 1994 and led to reported survival of 55%, with a median follow-up of 3.1 years (Henter et al., 2002). The HLH-94 protocol included an 8 week induction therapy with dexamethasone, etoposide, and intrathecal methotrexate in patients with CNS inflammation. The principal goal of induction therapy is to suppress the life-threatening inflammatory process that underlies HLH. At the end of 8 weeks, patients who can be weaned off of dexamethasone and etoposide without recurrence, recover normal immune function, and have no identified HLH-associated gene defects may stop therapy. HCT is generally recommended in patients with CNS involvement, recurrent/refractory disease, persistent NK cell dysfunction or proven familial/genetic disease (Jordan et al., 2011).

1.2.3. Salvage Strategies for Recurrent/Refractory HLH

A significant number of patients with HLH will either fail to respond adequately to current therapies or have disease recurrence prior to HCT. Approximately 50% of patients treated on the HLH-94 study experienced a complete resolution of HLH, while 30% experienced a partial resolution, and approximately 20% died prior to HCT (Henter et al., 2002). Notably, most

deaths occurred during the first few weeks of treatment and may reflect either pre-existing morbidities or primary refractory disease. While it is hoped that some patients will fare better with more prompt diagnosis of HLH, others remain unresponsive to standard therapy. Initial treatment with anti-thymocyte globulin (Thymoglobulin, rabbit ATG) has been reported to give higher complete response rates, but due in part to higher recurrence rates, long term outcomes do not appear superior (Ouachee-Chardin et al., 2006).

At present there is little data regarding potential second line therapies. Case reports exist describing the use of infliximab, daclizumab, alemtuzumab, anakinra, vincristine, and other agents as salvage therapies for HLH (Bruck et al., 2011; Henzan et al., 2006; Imashuku et al., 1999; Kobayashi et al., 2007; Olin et al., 2008; Strout et al., 2010; Tomaske et al., 2002). Due to the increasing recognition of the critical role of T cells in driving HLH pathogenesis, alemtuzumab has been increasingly used as a salvage therapy. A recent retrospective analysis of 25 patients found that alemtuzumab has significant activity against refractory HLH (Marsh et al., 2013a). Though refractory HLH typically has a dismal prognosis, approximately 70% of patients in this series survived. In contrast to refractory patients, those patients who initially respond well to standard therapy but then recur as treatment is tapered or withdrawn often respond to reintensification of therapy with standard agents.

1.2.4. Fully Ablative Conditioning with Hematopoietic Cell Transplant and HLH

For patients with refractory or inherited HLH, HCT is the only curative therapy currently available. In the HLH-94 protocol, consisting primarily of matched related and matched unrelated donors, 3-year survival was 64% (Horne et al., 2005). A retrospective evaluation of HLH patients undergoing HCT with unrelated matched donors through the National Marrow Donor Program (Baker et al., 2008) and a single-institution study from Necker Hospital which included many haploidentical donors (Ouachee-Chardin et al., 2006) both reported similar outcomes with long-term survival for patients receiving ablative conditioning for HLH at ~55%. Most of the patients in these reports treated with ablative conditioning received busulfan, cytoxan and etoposide with or without ATG. A significant finding in all of these studies is a very high rate of TRM > 30% (Baker et al., 2008; Horne et al., 2005; Ouachee-Chardin et al., 2006). Causes of death were multifactoral, including infection, veno-occlusive disease, hemorrhage, and multi-system organ failure. Primary graft failure was reported in 9-22%. Ouachee-Chardin et al. attributed 50% of the TRM to reactivation of HLH, and Horne et al. correlated active disease at the time of transplant with primary graft failure. These poor Day +100 outcomes with standard myeloablative conditioning regimens highlight the need for an alternative approach to transplantation for these patients, many of whom come to transplant with pre-existing organ dysfunction, infection and persistent pathologic inflammation.

1.2.5. Reduced Intensity Conditioning Hematopoietic Cell Transplantation for HLH

Several recent series have demonstrated decreased TRM and improved overall survival with reduced-intensity conditioning strategies that include alemtuzumab (Cooper et al., 2006; Cooper et al., 2008; Marsh et al., 2010). In the long-term, protection from HLH reactivation requires stable donor chimerism greater than 10-20% (Ouachee-Chardin et al., 2006), and donor T cells alone may be sufficient to protect against HLH reactivation (Cooper et al., 2008). The immune

deficiencies described below require similar levels of chimerism for disease correction. In a single-institution study, survival for HLH patients receiving RIC was significantly superior to survival for patients receiving myeloablative conditioning (92% vs. 43%) (Marsh et al., 2010). There were no deaths before Day +100 in the RIC group. However, while survival was significantly improved, patients undergoing RIC HCT in this series had extremely high rates of post transplantation mixed chimerism (65%). Other series also report high rates of mixed chimerism in patients treated with alemtuzumab-based RIC for HLH (Cooper et al., 2008).

1.2.6. Optimizing RIC HCT for HLH Syndromes

The improved survival reported for RIC strategies for HCT for HLH may be due to decreased toxicity of fludarabine/melphalan compared to myeloablative busulfan/cyclophosphamide. Additionally, it is possible that alemtuzumab-mediated depletion of T cells and antigen-presenting cells ameliorates inflammation prior to transplant. Investigators from Cincinnati Children's Hospital reported that the timing of alemtuzumab administration appeared to influence sustained engraftment with 65% of patients having mixed chimerism: 29% of patients who received "distal" alemtuzumab (starting Day -22) and 79% of patients who received "proximal" alemtuzumab (start day range -12 to -8). Most of the patients with mixed chimerism in this study ultimately received lymphocyte infusion or second stem cell infusion. Alemtuzumab persists at cytotoxic levels in patients following RIC for up to 60 days (Morris et al., 2003), with an estimated half-life of 15-21 days (Rebello et al., 2001). While a RIC approach for HLH appears to be effective, the optimal dose and timing of alemtuzumab and other therapeutic agents used in conditioning to achieve maximum survival with improved engraftment remains to be defined.

Recent data from Cincinnati Children's Hospital suggests that intermediate timing (starting Day -14) of alemtuzumab significantly improved engraftment rates (87% Day +100 stable engraftment without donor lymphocyte infusion (DLI)) while maintaining improved overall survival (90% at one year). The cumulative incidence of mixed chimerism was also significantly better in the Intermediate RIC (31%) compared to patients who received "proximal" alemtuzumab (72%) (Marsh et al., 2013b).

1.2.7. HLH-Related Syndrome: Chronic Active EBV Disease

While over 90% of the adult population is infected with EBV, rare individuals develop a life-threatening condition of chronic active EBV disease (CAEBV), characterized by highly elevated EBV levels in blood and tissue and often associated with hemophagocytic syndrome. EBV typically infects B cells and CAEBV may arise as a B-cell lymphoproliferation. However, in most cases of CAEBV in Asians or Native Americans, very high levels of EBV are detected in T or NK cells (Reviewed in (Cohen et al., 2011)).

1.2.8. Diagnosis of CAEBV

CAEBV is defined as prolonged systemic symptoms of inflammation associated with uncontrolled EBV infection of B, T or NK cells. Unlike post-transplant lymphoproliferative

disorder, CAEBV arises in people without known immune dysfunction or iatrogenic immune suppression. Clinical Definition of CAEBV requires all of the following (Cohen et al., 2011):

1. Severe progressive illness of > 6 months duration, usually with fever, lymphadenopathy and splenomegaly that either began as primary EBV infection or was associated with markedly elevated antibody titers to EBV viral capsid antibody (≥ 1:5120) or early antigen (≥ 1:640), or markedly elevated EBV DNA in the blood;
2. Infiltration of tissues (e.g., lymph nodes, liver, lungs, CNS, bone marrow, eye, skin) with lymphocytes;
3. Elevated EBV DNA, RNA or proteins in affected tissues; and,
4. The absence of any other immunosuppressive condition.

1.2.9. CAEBV and HCT

CAEBV is infrequently reported in North America. In a 28-year series at the NIH and Baylor College of Medicine, 19 patients with EBV were evaluated. In this North American cohort, immunosuppressive agents, rituximab, autologous cytotoxic T cells, cytotoxic chemotherapy or autologous HCT achieved only short-term responses. The only cures were achieved with allogeneic HCT, with 5/8 alive 2-11 years after transplantation, including 2 of 3 who underwent subablative HCT (Cohen et al., 2011). From 57 other cases of CAEBV who underwent HCT reported in the literature, overall survival was 72%. Factors associated with survival from these cases include younger age (14.4 years vs. 21.2 years), shorter time to transplant from onset of disease (3.5 years vs. 6.5 years), and non-myeloablative conditioning (69% vs. 38%) (Cohen et al., 2011).

1.3. Selected Primary Immune Deficiencies

HLH is caused by defects in effector immune function resulting in uncontrolled immune activation and pathologic inflammation. Defects in other regulatory pathways also cause diseases due to dysfunctional immune mechanisms, including chronic granulomatous disease, hyper IgM syndrome, IPEX syndrome, and leukocyte adhesion deficiency. HCT corrects defects in all of these primary immune deficiencies and sustained partial chimerism may be sufficient for cure. HCT with myeloablative conditioning is associated with high rates of TRM in these patients due to pre-existing infections and organ dysfunction in many patients at the time of transplant (Filipovich, 2008).

1.3.1. Chronic Granulomatous Disease

Chronic granulomatous disease (CGD) is a primary immunodeficiency caused by ineffective function of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, resulting in impaired microbicidal activity of reactive oxygen in neutrophils, macrophages and eosinophils. Patients with NADPH oxidase deficiency are susceptible to recurrent, life-threatening infections by bacteria and fungi (reviewed in (Kang et al., 2011; Seger, 2010b)). CGD is a heterogeneous disease with significant phenotypic variability. The production of residual oxygen intermediates (ROIs) is determined by the specific NADPH mutation, and is a predictor of survival in patients

with CGD (Kuhns et al., 2010). However, even among patients with similar NADPH mutations, there is variability in clinical outcomes. Therefore, the specific genetic defect, the patient's infection history, the presence of active inflammation or infection and other co-morbidities are important clinical variables in risk/benefit considerations for different therapeutic options.

1.3.1.1. Diagnosis of CGD

CGD is diagnosed by clinical history of recurrent infections, classically including *Staphylococcus aureus* (lymphadenitis, liver abscess), *Burkhoderia* (necrotizing pneumonia, sepsis), *Serratia marcescens* (sepsis, skin ulcers, osteomyelitis), *Nocardia* and *Aspergillus* spp. (pneumonia, brain, bone infections), *Actinomyces* spp. (cervicofacial, pulmonary or abdominal infections), *Granulibacter bethesdensis* (lymphadenitis), *Mycobacterium tuberculosis* (pulmonary and extrapulmonary infections), and *Leishmania infantum* (intracellular protozoal infection) (Reviewed in (Seger, 2010a)). Front-line diagnostic tests are based on measurement of phagocyte NADPH oxidase (Phox) activity. The flow-cytometry-based dihydrorhodamine-1,2,3 (DHR) test measures the ability of stimulated phagocytes to oxidize DHR interacellularly, and is a sensitive screening study (Vowells et al., 1995). Definitive diagnosis is made by protein- or DNA-based study of the five Phox components: $gp91^{phox}$ (X-linked), $p47^{phox}$, $p67^{phox}$, $p22^{phox}$ and $p40^{phox}$ (Rada and Leto, 2008).

1.3.1.2. HCT for CGD

While survival has improved with the use of prophylactic antimicrobials and earlier diagnosis, the only cure for CGD currently available is allogeneic hematopoietic cell transplantation. To date, fewer than 200 transplants have been reported, with most data as case reports. A survey of European centers (n=27 patients) reported 85% survival with myeloablative conditioning and primarily matched-sibling donors (Seger et al., 2002). Two series including matched unrelated donor (MUD) transplants for CGD (n=19) with ablative conditioning reported cumulative 84% survival (Schuetz et al., 2009; Soncini et al., 2009). Reduced intensity conditioning strategies for HCT in CGD are feasible. One study from the NIH reported 70% survival (n=10 patients) with allogeneic matched related donor (MRD) transplants with non-myeloablative conditioning (cyclophosphamide/fludarabine/ATG) (Horwitz et al., 2001). Similarly, a European consortium reported successful MRD/MUD transplants with non-myeloablative busulfan- and fludarabine-based conditioning regimen with ATG, with durable engraftment, survival to 1 year, and normalized superoxide production in all three patients (Gungor et al., 2005). A CIBMTR survey (through 2009) found 59 patients who had undergone allogeneic transplant for CGD with 71% overall survival (Seger, 2010b). In CGD, patients with stable mixed chimerism recover neutrophil Phox function (Martinez et al., 2012; Horwitz et al., 2001). To date, the limited data available make it difficult to determine what the most optimal conditioning regimen is for this disease, however, a reduced-intensity conditioning transplant strategy is particularly attractive in CGD, where many patients have significant co-morbidities including severe infections at the time of transplant.

1.3.2. Hyperimmunoglobulin M Syndrome

Hyper-IgM syndrome includes a heterogeneous group of conditions characterized by defects in class-switch recombination (CSR). As a result, serum IgM accumulates with reduced or absent levels of IgG, IgA and IgE. The most common genetic form of hyper-IgM syndrome, HIGM1, is caused by defects in the gene encoding CD40 ligand (CD40L) on the X chromosome. In addition to defective humoral immunity and lack of germinal centers in peripheral lymphoid tissue, patients with hyper-IgM may also have neutropenia, anemia, impaired delayed-type hypersensitivity reactions to recall antigens, and decreased T cell proliferation *in vitro* (Reviewed in (Davies and Thrasher, 2010)). More than 50% of boys with HIGM1 develop symptoms by one year, and more than 90% are symptomatic by four years (Winkelstein et al., 2003). Symptoms include recurrent respiratory tract bacterial infections, opportunistic infections, chronic diarrhea, neutropenia, thrombocytopenia and anemia. Opportunistic infections from *Pneumocystis jeroveci* and *Cryptosporidium parvum* are also common (Lee et al., 2005). The median survival of patients with HIGM1 without HCT is less than 25 years (Levy et al., 1997; Winkelstein et al., 2003).

1.3.2.1. Diagnosis of HIGM1

HIGM1 should be considered in boys with recurrent bacterial and opportunistic infections. Lab criteria include decreased serum IgG (2 or more SD below normal for age) with a mutation in *CD40LG* or a family history of maternally related males with HIGM1 [ESID Working Party 2005: http://www.esid.org/workingparty.php?party=3&sub=2&id=73#Q16].

1.3.2.2. HCT for HIGM1

Allogeneic HCT is the only curative treatment currently available for HIGM1. The largest published case series is a European survey which reported HCT for 38 patients, with 68% long-term survival and immune reconstitution with cure in 58%. Pre-existing lung disease correlated with poor outcome and all deaths in this series were associated with infection (Gennery et al., 2004). Outcomes for MRD and MUD were not statistically different. There were some patients conditioned with non-ablative strategies but too few to analyze separately (Gennery et al., 2004). However, the cases included in the survey by Gennery et al. as well as other case series support feasibility of reduced-intensity conditioning strategies (Kikuta et al., 2006; Jasinska et al., 2013; Gennery et al., 2004). The co-morbidities of this population prior to transplant and associated risks of TRM also support investigation of a RIC approach in patients with HIGM1.

1.3.3. IPEX Syndrome

IPEX refers to immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance. The syndrome is a rare X-linked disorder that typically presents with a triad of enteropathy, autoimmune polyendocrinopathy and dermatitis (Powell et al., 1982). Most patients present with the characteristic constellation of symptoms in early infancy. IPEX is caused by defects in the inhibitory regulatory helper T cells (Tregs) due to dysfunctional or absent FOXP3 transcription factor (Wildin et al., 2001; Chatila et al., 2000). Disease manifestations are due to immune dysregulation from absent or dysfunctional Tregs. Disease-causing mutations in *FoxP3*

have been described in each of the main coding regions of the gene, as well as in some non-coding regions (Campbell and Ziegler, 2007).

1.3.3.1. Diagnosis of IPEX Syndrome

IPEX should be considered in male infants with chronic diarrhea, failure to thrive and/or onset of type 1 diabetes in infancy. The other clinical manifestations discussed above are also supportive. Definitive diagnosis is established by absent or functionally defective FOXP3+ CD4+ T cells or by mutational analysis of *FoxP3* (Hannibal and Torgerson, 1993).

1.3.3.2. HCT for IPEX Syndrome

HCT is the only curative therapy for IPEX currently available. Infections and co-morbidities are common in patients at the start of transplant. Literature is limited to case reports, including examples demonstrating feasibility of reduced intensity conditioning strategies and resolution of disease symptoms even with mixed chimerism (Burroughs et al., 2010; Dorsey et al., 2009; Rao et al., 2007; Seidel et al., 2009; Zhan et al., 2008).

1.3.4. Leukocyte Adhesion Deficiency

Leukocyte adhesion deficiency is a primary immune disorder caused by defects in migration of immune cells. It typically presents in infancy with delayed umbilical cord separation with omphalitis, deep tissue infections, impaired pus formation, and poor wound healing. LAD-I is an autosomal recessive disorder caused by mutations in the common chain (CD18) of the β 2-integrin family, resulting in deficiency of 3 integrins with functions in leukocyte function: lymphocyte function associated antigen-1 (LFA-1, CD11a/CD18), Mac-1 (CD11b/CD18), and gp 150/95 (CD11c/CD18) (Reviewed in (Etzioni, 2010)). The severity of LAD-I is related to the degree of CD18 deficiency: severe deficiency is observed in patients with less than 2% of normal surface expression of CD18; mild to moderate deficiency is observed in patients with greater than 2-30% of normal surface expression of CD18 (Fischer et al., 1988). Clinical management of LAD-I depends on clinical severity. Mild to moderate disease can usually be managed with antibiotic therapy while this is generally inadequate for patients with severe disease (Etzioni, 2010).

1.3.4.1. Diagnosis of LAD-I

Definitive diagnosis of severe LAD-I can be made in male or female patients with decreased intensity of expression of CD18 on neutrophils (< 5% of normal for age) with mutation of the β2 integrin gene and/or absence of β2 integrin mRNA in leukocytes (Conley et al., 1999).

1.3.4.2. HCT for LAD-I

HCT is the only curative therapy for patients with severe LAD-I currently available. The largest published series reported 36 children who underwent HSC transplant with 75% overall survival (Qasim et al., 2009). Reduced-intensity conditioning was used in 8 patients, with no deaths in this sub-group, and mixed-donor chimerism was sufficient to correct the symptoms of LAD.

1.4. Summary

HLH, HLH-related disorders, CGD, HIGM1, IPEX and severe LAD-I represent primary immune disorders that are typically fatal without HCT. However, transplant is often complicated by inflammation, infection and other co-morbidities. In addition, these disorders have been shown to be cured with partial chimerism, making them an ideal target for the use of reduced intensity approaches, where a portion of patients may not achieve full donor chimerism, but instead achieve stable mixed chimerism. Reduced-intensity conditioning strategies have demonstrated improved survival with decreased TRM in institutional series for patients with HLH (Cooper et al., 2006; Marsh et al., 2010; Marsh et al., 2011). However, graft loss and unstable chimerism remain challenges. An institutional case series from Cincinnati Children's Hospital demonstrated full or high-level chimerism and improved durable engraftment using intermediate (Day -14) timing alemtuzumab (Marsh et al., 2013b). This study aims to test the efficacy of the Intermediate RIC strategy in a prospective multi-center study including HLH as well as other primary immunodeficiencies where allogeneic transplant with RIC has been shown to be feasible and stable chimerism is curative.

CHAPTER 2

2. STUDY DESIGN

2.1. Study Overview

The primary goal of this Phase II clinical trial is to determine the one-year overall survival of patients treated for immune deficiencies including HLH, HLH-like disorders, CGD, HIGM1, IPEX syndrome, and severe LAD-I with MRD/MUD bone marrow transplant using a reduced-intensity conditioning strategy including intermediate-timing of alemtuzumab. The patient's donor must be willing and able to give bone marrow stem cells and be:

- An unaffected sibling donor who is a 6/6 match at HLA-A and –B (intermediate or higher resolution) and –DRB1 (at high resolution using DNA-based typing) *OR*
- An unaffected related donor (other than a sibling) who is a 7/8 or 8/8 match for HLA-A, -B, -C (at intermediate or higher resolution) and –DRB1 (at high resolution using DNA-based typing) *OR*
- An unrelated donor who is a 7/8 or 8/8 match at HLA-A, -B, -C, and –DRB1 (at high resolution using DNA-based typing).

The transplant conditioning regimen will include fludarabine, melphalan, and alemtuzumab starting at Day -14 (Flu/Mel/Alem). GVHD prophylaxis will consist of cyclosporine and corticosteroids through engraftment. Post-transplant supportive care will include infection surveillance and prophylaxis, and disease-specific supportive care.

2.2. Hypothesis and Study Objectives

2.2.1. Primary Hypothesis

Allogeneic HCT with MRD/MUD using RIC conditioning (fludarabine, melphalan) and intermediate timing of alemtuzumab (Day -14) in patients with hemophagocytic syndromes or selected primary immune deficiencies including CGD, HIGM1, IPEX syndrome, and severe LAD-I will result in a one year overall survival rate of 90%.

2.2.2. Primary Objective

To prospectively determine the one year overall survival in subjects treated for hemophagocytic syndromes or selected primary immune deficiencies (CGD, HIGM1, IPEX, and severe LAD-I) using a standardized, reduced-intensity conditioning protocol consisting of fludarabine, melphalan and intermediate timing of alemtuzumab (Day -14).

2.2.3. Secondary Objectives

Secondary objectives are to determine the impact of HCT on clinical and laboratory manifestations of hemophagocytic syndromes and selected primary immune deficiencies and the incidence of HCT complications. Secondary clinical outcomes to be collected include:

1. Rates of sustained engraftment: Donor engraftment > 5% (unfractionated blood and T cells) at one year without infusion of stem cell products after the original transplant. Incidence and timing of primary graft failure (< 5% donor cells by Day +42), infusion of second stem cell product, or graft loss (< 5% donor cells at any time from initial engraftment through Day +365), all of which are considered failures of sustained engraftment, will be analyzed.
2. Incidence of HLH and CAEBV reactivation and death from disease.
3. Immune reconstitution and functional immune recovery.
4. The cumulative incidence of neutrophil and platelet engraftment.
5. The cumulative incidence of grade II-IV and III-IV acute GVHD and chronic GVHD.
6. Transplant-related complications including hepatic veno-occlusive disease (VOD) and CNS toxicity.
7. Rates of reactivation of CMV and EBV and infection with adenovirus, invasive fungal infection, or bacterial sepsis. Prospective collection of peripheral blood at specific time-points, in order to study pharmacokinetics of alemtuzumab, engraftment, and immune reconstitution with clinical correlation in a companion biology study.
8. Overall survival and rate of sustained engraftment at one year of specific disease subsets (HLH-related diseases and selected immune deficiencies).

2.3. Patient Eligibility

2.3.1. Patient Inclusion Criteria

1. Patient is ≥ 3 months and ≤ 45 years of age at time of enrollment.
2. Meets criteria for **one** of the following immune disorders (2A-2F) requiring HCT:

2A. HLH or related disorder with indication for HCT:

a. Inherited gene mutation associated with HLH: *PRF1*, *UNC13D (MUNC13-2)*, *STXBP2 (MUNC18-2)*, *STX11*, *RAB27A* (Griscelli syndrome, type 2), *SH2D1A* (XLP1), *XIAP* (XLP2), *LYST* (Chediak-Higashi syndrome).
– OR –
b. Meets clinical criteria for HLH (Table 1.2), refractory to therapy according to HLH-94 or HLH-2004 (dexamethasone/etoposide), or recurrent episodes of hyper-inflammation.
– OR –
c. Meets clinical criteria for HLH (Table 1.2), without identified gene defects, with affected sibling – OR – decreased or absent NK cell function at the last evaluation,

– OR – a history of CNS inflammation as evidenced by pleocytosis in CSF or MRI evidence of hyper-inflammation in the CNS.

2B. CAEBV: Patients with chronic EBV infection (CAEBV) with or without associated lymphoma (in complete remission) or active HLH. Note that this diagnosis is distinct from post-transplant lymphoproliferative disorder/ EBV-associated lymphoproliferative disease (PTLD/LPD). Patients must meet all of the following:

a. Severe progressive illness, usually with fever, lymphadenopathy and splenomegaly that either began as primary EBV infection or was associated with markedly elevated antibody titers to EBV viral capsid antibody (≥ 1:5120) or early antigen (≥ 1:640), or markedly elevated EBV DNA in the blood;
– AND –
b. Infiltration of tissues (e.g., lymph nodes, liver, lungs, CNS, bone marrow, eye, skin) with lymphocytes;
– AND –
c. Elevated EBV DNA, RNA or proteins in affected tissues;
– AND –
d. The absence of HIV or post-transplant lymphoproliferative disorder.

2C. Chronic granulomatous disease with indication for HCT:

a. Oxidative burst < 10% normal with dihydrorhodamine (DHR) assay
– AND –
b. Documented CGD mutation(s) in gp91phox, p47phox, p67phox, p22phox or p40phox
– AND –
c. Severe disease as evidenced by one or more of the following:
    i. history of one or more potentially life-threatening infections
    ii. inflammatory bowel disease
    iii. failure to thrive with height <10% for age (unless parent(s) height <10%)
    iv. autoimmune complication felt to be linked to CGD

2D. X-linked Hyper IgM Syndrome (HIGM1):

a. Decreased serum IgG (more than 2 standard deviations below normal for age)
– AND –
b. Mutation in *CD40LG* – OR – family history of maternally related males with HIGM1.

2E. IPEX Syndrome:

a. Absent FOXP3+ CD4+ T cells – OR – abnormal function of FOXP3+CD4+ T cells
– AND –
b. Disease-associated mutation in *FoxP3* (bi-allelic in females) – OR – family history of maternally related males with clinical diagnosis of IPEX.

2F. Severe Leukocyte Adhesion Deficiency, type I (LAD-I):

a. Decreased CD18 expression on neutrophils (<5% normal for age)
– AND –
b. Mutation of *ITGB2* – OR – absence of *ITGB2* mRNA in leukocytes

3. Lansky or Karnofsky performance status ≥ 50%.

4. The patient's donor must be willing and able to give bone marrow stem cells and be:

- An unaffected sibling donor who is a 6/6 match at HLA-A and –B (intermediate or higher resolution) and –DRB1 (at high resolution using DNA-based typing) *OR*
- An unaffected related donor (other than sibling) who is a 7/8 or 8/8 match for HLA-A, -B, -C (at intermediate or higher resolution) and –DRB1 (at high resolution using DNA-based typing) *OR*
- An unrelated donor who is a 7/8 or 8/8 match at HLA-A, -B, -C, and –DRB1 (at high resolution using DNA-based typing).

5. Patient must have adequate organ function as measured by:
   a. Cardiac: Left ventricular ejection fraction (LVEF) > 40%; or LV shortening fraction (LVSF) > 26% by echocardiogram.
   b. Renal: Calculated or radioisotope GFR > 50 mL/min/1.73m^2
   c. Hepatic: Adequate liver function: serum conjugated (direct) bilirubin < 2x upper limit of normal for age as per local laboratory (with the exceptions of isolated hyperbilirubinemia due to Gilbert's syndrome, or hyperbilirubinemia as the result of liver inflammation in the setting of persistent, active HLH); ALT and AST < 10x upper limit of normal as per local laboratory (with the exception of elevated transaminase levels as the result of liver inflammation in the setting of persistent, active HLH).
   d. Pulmonary: Patient may not be on mechanical ventilation support or have progressive pulmonary infection at the time of transplant; Pulmonary Function Testing (PFT) with FEV1 $\geq$ 50% of normal and DLCO corrected for Hgb > 50% of normal. Patients unable to undergo PFTs should have stable respiratory status with SaO2 > 90% on a maximum of 2L/min supplemental oxygen.
6. Signed informed consent.

2.3.2. Patient Exclusion Criteria

1. Hematopoietic stem cell transplant within 6 months of enrollment.
2. Uncontrolled bacterial, viral or fungal infection (currently receiving appropriate antimicrobials and experiencing progression or no clinical improvement) at time of enrollment. We recognize that patients with CAEBV may have ongoing EBV viremia at the time of initiating transplant therapy, but other patients should have no uncontrolled bacterial, viral or fungal infections at the time of enrollment (or prior to initiating the preparative regimen).
3. Pregnant or breastfeeding.
4. Seropositive for human immunodeficiency virus (HIV).
5. Alemtuzumab within 2 weeks of enrollment.
6. History of prior or current malignancy, especially malignancies with a likelihood of relapse and progression, with the exception of (1) EBV-associated lymphomas related to immune deficiency or lymphomas associated with X-linked LPD in a good remission, as they are unlikely to relapse after treatment; (2) Resected basal cell carcinoma or treated

cervical carcinoma in situ. Cancer treated with curative intent will not be allowed unless approved by the Protocol Officer or one of the Protocol Chairs.

2.3.3. Donor Selection Criteria

1. Related donors will be identified according to institutional guidelines.
2. HLA typing of a sibling donor must be a 6/6 match for HLA–A and –B (intermediate or higher resolution) and –DRB1 (at high resolution using DNA-based typing).
3. HLA typing of a related donor other than a sibling must be a 7/8 or 8/8 match for HLA-A, -B, -C (at intermediate or higher resolution) and –DRB1 (at high resolution using DNA-based typing).
4. Unrelated donors will be identified through the National Marrow Donor Program (NMDP) or equivalent donor search organization.
5. HLA typing of an unrelated donor must be a 7/8 or 8/8 match for HLA–A, –B, –C and DRB–1 (at high resolution using DNA-based typing).
6. Donors must be willing and able to donate bone marrow stem cells.
7. For HLH or related disorders: If donor is a relative of a recipient with known HLH-associated gene mutations, the donor must not have the same HLH-causing gene mutations as the recipient (a sibling with a heterozygous mutation of an autosomal recessive HLH-associated gene would be acceptable, as would a female sibling or other female relative who is a carrier of an X-linked HLH-associated gene mutation). If donor is a relative of a recipient with unknown genetic cause of HLH, the donor must not have medical history concerning for HLH or laboratory values suggestive of significant immune dysfunction (for example, highly elevated ferritin or absent NK cell function).
8. For CGD, HIGM1, IPEX Syndrome, and LAD-I: If donor is a sibling, the donor must not meet diagnostic criteria for the immune deficiency for which the patient is receiving HCT.
9. A donor who is EBV IgG positive is recommended for recipients with history of EBV exposure, and strongly recommended for patients with CAEBV. Similarly, a donor who is CMV IgG positive is recommended for recipients with history of CMV exposure.

2.3.4. Donor Exclusion Criteria

1. Donors will be excluded if they are an identical twin of the recipient.
2. Females who are pregnant (positive serum β-HCG) or uninterruptedly breastfeeding will be excluded.
3. HIV seropositive donors will be excluded.
4. Donors receiving experimental therapy or investigational agents will be excluded unless approved by the protocol chairs and protocol officer.

2.4. Treatment Plan

If an infection occurs or a pre-existing infection worsens between the day of enrollment and the day the preparative regimen is scheduled to begin, the infection must be controlled and the patient clinically improved prior to initiating the preparative regimen. We recognize that patients with CAEBV may have ongoing EBV viremia at the time of initiating pre-transplant therapy, but other patients should have no uncontrolled bacterial, viral or fungal infections at the time of initiation of the transplant preparative regimen.

Patients will receive Intermediate RIC with Flu/Mel/Alem (Table 2.4). The experimental design is outlined in Figure 2.4.

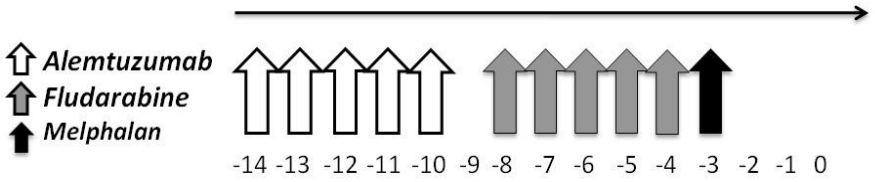

Figure 2.4: Trial Design

TABLE 2.4: Flu/Mel/Alem(-14)

Day	Treatment (adults and children > 15kg)	Treatment (children 10 to 15 kg)	Treatment (children < 10 kg)
-14	Alemtuzumab* 3mg test dose	Alemtuzumab 0.2 mg/kg	Alemtuzumab 0.2 mg/kg
-13	Alemtuzumab* (max 21.75 mg)	Alemtuzumab 0.2 mg/kg	Alemtuzumab 0.2 mg/kg
-12	Alemtuzumab* (max 21.75 mg)	Alemtuzumab 0.2 mg/kg	Alemtuzumab 0.2 mg/kg
-11	Alemtuzumab* (max 21.75 mg)	Alemtuzumab 0.2 mg/kg	Alemtuzumab 0.2 mg/kg
-10	Alemtuzumab* (max 21.75 mg)	Alemtuzumab 0.2 mg/kg	Alemtuzumab 0.2 mg/kg
-9	Rest	Rest	Rest
-8	Fludarabine 30 mg/m^2	Fludarabine 30 mg/m^2	Fludarabine 1 mg/kg
-7	Fludarabine 30 mg/m^2	Fludarabine 30 mg/m^2	Fludarabine 1 mg/kg
-6	Fludarabine 30 mg/m^2	Fludarabine 30 mg/m^2	Fludarabine 1 mg/kg
-5	Fludarabine 30 mg/m^2	Fludarabine 30 mg/m^2	Fludarabine 1 mg/kg
-4	Fludarabine 30 mg/m^2	Fludarabine 30 mg/m^2	Fludarabine 1 mg/kg
-3	Melphalan 140 mg/m^2	Melphalan 140 mg/m^2	Melphalan 4.7 mg/kg
-2	Rest	Rest	Rest
-1	Rest	Rest	Rest
0	Stem cell infusion	Stem cell infusion	Stem cell infusion

* Alemtuzumab dose is 1 mg/kg for the entire treatment course (including the test dose on Day -14) in adults and children > 15 kg.
* Calculation for Alemtuzumab daily dose for Day-13, Day-12, Day-11, Day-10 for a patient 15kg-90kg: ((1mg/kg x (patient weight in kg)) – 3mg)/4
* The maximum cumulative Alemtuzumab dose for any patient is 90 mg, making the maximum daily dose of Alemtuzumab from Day-13 to Day -10, 21.75 mg = (90mg – 3mg)/4

2.4.1. Alemtuzumab (Campath-1H)

If a patient cannot receive the complete dose due to intolerance, the choice of conditioning regimen modifications (if any) will be left to the institution; however, the patient will be followed per protocol.

For information about the Campath Distribution Program and how to procure Campath, please visit the following website: http://www.campath.com/index.html

2.4.1.1. Premedication

Premedication should be commenced 30 minutes prior to each infusion of alemtuzumab, including the first dose, and should be continued as needed for at least 48 hours after the last dose of alemtuzumab.

Recommended pre-medication includes the following combination of medications:

Acetaminophen: 15 mg/kg PO pre-therapy, then as needed every 6 hours for signs of allergic reaction (maximum 4 grams per day)

Diphenhydramine: 1 mg/kg IV or PO pre-medication, then every 6 hours as needed for signs of allergic reaction (maximum 50 mg/dose)

Meperidine: 0.5 mg/kg IV every 6 hours as needed for rigors

Hydrocortisone: 1 mg/kg IV pre-medication, then 1-2 mg/kg IV as needed for signs of allergic reaction. For patients on scheduled corticosteroids for control of HLH, dexamethasone or methylprednisolone may substitute for hydrocortisone.

2.4.1.2. Alemtuzumab administration

First day: test dose:
Day -14: The first day children ≤ 15kg will receive a dose of 0.2 mg/kg subcutaneously. Children or adults > 15 kg will receive a maximum of 3 mg subcutaneously. Subcutaneous route of administration for alemtuzumab is required on this protocol.

If the first dose is not tolerated:
If the patient has a severe, life-threatening or fatal adverse reaction to alemtuzumab (e.g., severe hypotension, severe bronchospasm), the adverse event meets the expedited reporting requirements (within 24 hours for life-threatening or fatal events or within 3 days for severe events) through the expedited AE reporting system via AdvantageEDC^{SM} (see Section 4.2.2).

If the first dose is tolerated:
Alemtuzumab will be given subcutaneously each day for the next four days (-13 to -10). The total dose will be 1 mg/kg with a maximum cumulative dose of 90 mg. For children ≤ 15 kg, the dose will be 0.2 mg/kg each day. For children or adults > 15kg the dose will be the total dose (1mg/kg) minus the 3 mg test dose divided evenly over the four days. **For example**, if the

patient weighs 40 kg, they will receive 3 mg on Day -14 and 40 mg (total dose) – 3 mg (test dose) = 37 mg/4 daily doses or 9.25 mg daily Day -13 to Day -10. (Table2.4)

Day -13 to Day -10: (total dose (1 mg/kg) – test dose (3 mg))/4 mg given daily subcutaneously (max cumulative dose: 90 mg, max daily dose (90 mg – 3 mg)/4 or 21.75 mg for any patient ≥ 90kg). (Table 2.4)

Alemtuzumab dilution guidelines: For ≤ 3 mg, drug is diluted to 3 mg/mL concentration. For doses > 3 mg undiluted 30 mg/mL concentration is used.

2.4.2. Fludarabine

Fludarabine will be administered IV, on Day -8 to Day -4 (for a total of 5 days) given over a minimum of 30 minutes daily. (Table 2.4) The infusion can take longer per institutional guidelines.

For patients ≥ 10kg, Dose: 30 mg/m^2/day IV (cumulative dose 150 mg/m^2)
For children < 10kg, Dose: 1 mg/kg/day IV (cumulative dose 5 mg/kg)

Preparation, administration and monitoring will be according to institutional standard practice.

Dose Adjustment of Fludarabine for Patients Whose Weight Exceeds > 125% IBW
Fludarabine will be dosed based on actual weight for patients ≤ 125% IBW. Those > 125% IBW will be dosed based upon adjusted ideal body weight as follows:

Adjusted ideal body weight = IBW + 0.25(Actual weight – IBW)

The following formulas for pediatric and adult IBW calculations are recommended, but IBW may be calculated according to institutional SOPs.

Recommended Ideal Body Weight Calculation for Children Age 1-17 years
IBW = (Height (cm)2 x 1.65)/1000

Recommended Ideal Body Weight Calculation for Adults
IBW (females) = (cm ÷ 2.54 – 60) x 2.3 kg + 45.5 kg
IBW (males) = (cm ÷ 2.54 – 60) x 2.3 kg + 50 kg

2.4.3. Melphalan

Melphalan will be given IV on Day -3 (Table 2.4). It is recommended to be given over a minimum of 30 minutes; however, the infusion can be shorter or longer per institutional guidelines.

For patients ≥ 10kg, Dose: 140 mg/m^2 IV
For children < 10 kg, Dose: 4.7 mg/kg IV

2.4.4. Infusion of Hematopoietic Stem Cells

Under no circumstances is the stem cell product to be irradiated. Vital signs should be monitored before beginning the infusion and periodically during administration. Pre-medications and hydration prior to stem cell infusion will be administered per institutional procedures.

2.5. GVHD Prophylaxis

Patients will receive the regimen as described in Table 2.5

TABLE 2.5: GVHD PROPHYLAXIS REGIMEN

Day	Dosage (HLH Patients)	Dosage (Non-HLH Patients)
-3	Cyclosporine dosed to maintain appropriate level. Given through Day +100, then taper to Day +180.	Cyclosporine dosed to maintain appropriate level. Given through Day +100, then taper to Day +180.
-2	Methylprednisolone 2 mg/kg/day IV (if patient is already on corticosteroids for HLH control, adjust to this amount)	--
-1	Methylprednisolone 2 mg/kg/day IV	--
0	Stem cell infusion Methylprednisolone 1 mg/kg/day (ongoing through Day +28, then taper over 1 month; may substitute PO Prednisone 1.2 mg/kg/day)	Stem cell infusion Methylprednisolone 1 mg/kg/day (ongoing through Day +28, then taper over 1 month; may substitute PO Prednisone 1.2 mg/kg/day)

2.5.1. Cyclosporine (CSA)

Cyclosporine administration will commence on Day -3 and dosing per institutional standard will be adjusted to maintain a level of 250-500 ng/mL by TDX method (or 200-350 ng/mL by Tandem MS or equivalent level for other CSA testing methods). CSA can be administered by continuous or intermittent infusion per institutional guidelines. Patients who are on CSA at the time of starting conditioning will continue on the pre-HCT dose, with adjustment to goal levels beginning on Day -3. Either brand name (Sandimmune) or generic cyclosporine will be allowed.

Dose adjustments will be made on the basis of toxicity and CSA levels. Once the patient can tolerate oral medications and has a normal gastro-intestinal transit time, CSA will be converted to an oral form per institutional standards. CSA dosing will be monitored and altered as clinically appropriate.

Patients will receive CSA until Day +100 and CSA will be tapered between Days +100 to +180. If there is no GVHD, the dose will be tapered by approximately 10% per week beginning on Day +100.

In the event of toxicity, tacrolimus may be substituted for cyclosporine. Tacrolimus administration will commence on Day –3, and doses will be adjusted to maintain a level of 8-12 ng/mL by the IMx immunoassay technique (Abbott Diagnostics) or a level of 5-8 ng/mL if measured by a LC-tandem mass-spectrometric assay. If using another method to measure levels then dose should be adjusted to maintain appropriate levels per institutional standards. Tacrolimus can be administered by intermittent infusion or by continuous infusion to maintain therapeutic levels per institutional guidelines.

Dose adjustments will be made on the basis of toxicity and tacrolimus levels. Once the patient can tolerate oral medications and has a normal gastro-intestinal transit time, tacrolimus will be converted to an oral form. Tacrolimus dosing will be monitored and altered as clinically appropriate.

From Day +100 to +180, tacrolimus will be gradually tapered in patients without significant acute or chronic GVHD (taper approximately 10% per week).

2.5.2. Methylprednisolone/Prednisone

On Day -2, methylprednisolone 2 mg/kg/day IV in two divided doses will be started as GVHD prophylaxis as well as prophylaxis against reactivation of HLH and adrenal insufficiency for patients with HLH. For patients who remain on corticosteroids for HLH control, this will be continued and unchanged through conditioning until Day -2, when previous corticosteroids will be replaced with methylprednisolone. On Day 0, the methylprednisolone dose will decrease to 1 mg/kg/day for patients with HLH and continue at that dose until Day +28. For patients without HLH, they will start methylprednisolone at Day 0 at 1mg/kg/day. All patients will continue at that dose until Day +28. In the absence of GVHD, methylprednisolone will be tapered and discontinued over 1 month. Oral prednisone may substitute for methylprednisolone at 1.2 mg/kg/day after Day 0.

2.6. Managing Chimerism

1. Donor chimerism studies (T cell and whole blood) are required monthly starting at Day +28 through Day +100, then at Day +180 and Day +365. An optional chimerism study at Day +21 is recommended if neutrophil engraftment occurs before Day +28. Additionally, donor chimerism studies are required at Day +42 if prior chimerism results are equivocal (donor chimerism < 20%). Additional donor chimerism studies are recommended at Day +70. Monitoring chimerism outside of the required studies can be performed by whole blood or T-cell chimerism or both, by center preference.

   Recommendation: If chimerism falls by > 25% after engraftment, we recommend that immune suppression be rapidly tapered (over 0-14 days) with more frequent (weekly) chimerism monitoring. If chimerism continues to fall despite withdrawal of immune suppression, DLI can be given according to center preference. If the patient has falling

chimerism in the face of clinically active, significant GVHD, full withdrawal of immune suppression may not be clinically feasible.

2. Patients with donor engraftment < 5% by Day +42 will be considered primary graft failures.

   Recommendation: It is recommended that these patients undergo a second transplant.

2.7. Supportive Care (Recommended)

Institutional standard care practice guidelines will be followed after transplantation for nutritional support, treatment of infections, and blood product support. Supportive guidelines are detailed below.

2.7.1. Engraftment Syndrome

Engraftment syndrome is a clinical diagnosis. The most frequently reported manifestations are transient fever, rash, and respiratory symptoms not attributable to infection or GVHD. The pathophysiology is multifactoral mediated by cellular, complement and cytokine components. Diagnostic criteria include fever (temperature > 38.5^{o} C) without an identifiable infectious cause within 4 days of the start of neutrophil recovery and/or an erythematous rash not attributable to GVHD or medications and/or capillary leak (weight gain, edema, ascites, effusions) or respiratory symptoms not attributable to IPS. Mild symptoms may not require therapy due to the self-limiting nature of this syndrome. For progressive symptoms, methylprednisolone at 2 mg/kg/day is recommended for 2-5 days. If recurrent, prolonged, or unusually severe, investigation for HLH reactivation and GVHD is recommended.

2.7.2. Venous Access

Recipients will have appropriate long-term central venous access placed, per institutional standard practice, prior to beginning the conditioning regimen. The placement of a double lumen tunneled catheter is recommended.

2.7.3. Growth Factor

Granulocyte colony stimulating factor (G-CSF) may be used at the treating physician's discretion. Granulocyte-monocyte colony stimulating factor (GM-CSF) is generally not recommended for patients with HLH.

2.7.4. Blood Products

The hemoglobin level is recommended to be maintained over 8.0 g/dL for at least 100 days post-transplant. Due to specific bleeding risks in HLH patients, the platelet count should be maintained > 50,000/ul, and transfused per institutional guidelines in patients with other immune defects. Irradiated blood products should be administered universally.

2.7.5. Treatment of Fever/Infections

Patients should be monitored closely for clinical manifestations of infection and treated per institutional guidelines with broad spectrum antibacterial, antiviral and antifungal agents. Since patients receive alemtuzumab, they are especially susceptible to bacterial and viral infections in the early post-transplant period.

2.7.6. Blood Pressure Monitoring and Control

Blood pressure should be strictly controlled to prevent CNS toxicity. Patients with HLH often have experienced renal toxicity prior to HSCT. This in combination with high doses of steroids and Calcineurin inhibitors used both before and after HSCT puts these patients at risk for posterior reversible encephalopathy syndrome (PRES). Blood pressure should be monitored closely and elevations in systolic and/or diastolic pressure(s) should be treated promptly to maintain blood pressure within 10% above the baseline age-related median systolic and diastolic pressure (see Appendix G).

PRES is characterized by headache, seizures, and visual loss, as well as an abrupt increase in blood pressure. If PRES is suspected or diagnosed, symptom-directed treatment should be maintained until the condition is reversed by control of hypertension or other instigating factors. A negative MRI is required to exclude PRES.

2.7.7. Infection Surveillance and Prophylaxis

2.7.7.1. HSV prophylaxis

Valacyclovir or acyclovir prophylaxis is recommended for 6 months for patients who are seropositive for HSV or VZV.

2.7.7.2. Pneumocystis jirovecii pneumonia (PJP) prophylaxis

Trimethoprim-sulfamethoxazole or an equivalent drug should be administered beginning after neutrophil recovery and continued post-transplant for 1 year.

2.7.7.3. Fungal prophylaxis

Due to the level of prolonged immune suppression, anti-fungal prophylaxis against *Aspergillus sp.* is recommended with agents such as itraconazole, voriconazole, posaconazole or other appropriate agents until Day +180.

2.7.7.4. Bacterial prophylaxis

May consider bacterial prophylaxis per institutional guidelines.

2.7.7.5. CMV surveillance

All recipients must be tested weekly for CMV from plasma using the PCR method beginning a week after commencing alemtuzumab (starting Day -7) until Day +100, then at Day +180 and Day +365. Antiviral therapy for CMV reactivation should commence if CMV testing reveals a high or rising viral load, according to institutional practice guidelines.

2.7.7.6. EBV surveillance

Patients will have EBV DNA quantitative PCR testing on plasma or peripheral blood cells every week after commencing alemtuzumab (starting Day -7) until Day +100, then at Day +180 and Day +365. In the event of persistent EBV viremia or signs/symptoms consistent with EBV-related PTLD, therapy is recommended according to institutional practice guidelines.

2.7.7.7. Adenovirus surveillance

Testing for adenovirus infection in the blood by PCR is recommended in the event of symptoms suspicious for an infection, including diarrhea, hepatic dysfunction or respiratory symptoms. If an active systemic infection is diagnosed, therapy should be instituted according to institutional practice guidelines.

2.7.7.8. Intravenous immune globulin

Intravenous immune globulin may be administered according to institutional practice guidelines.

2.7.7.9. HLH surveillance

a. HLH recurrence should be evaluated in patients who develop unexplained fever (> 2 days) or organ dysfunction. Markers of inflammation include sIL2Rα, ferritin, LDH and d-dimers. Recommendations for pre-transplant management of inflammation in HLH are discussed in Appendix E. Patients with HLH may have exaggerated engraftment syndrome. However, systemic HLH typically does not recur in the absence of graft loss.

b. All patients with history of CNS involvement by HLH (pleocytosis, elevated protein, abnormal MRI, or hemophagocytosis in CSF) should undergo diagnostic LP prior to transplant, at Day +28 ($\pm$ 3 days), at Day +100 ($\pm$ 14 days) or at any time of mental status change. Brain MRI may be substituted for LP if patients are not able to tolerate the procedure. CNS reactivation of HLH (as evidenced by pleocytosis or hemophagocytosis in CSF, or MRI consistent with HLH-associated inflammation) pre- or post-transplant may be treated with intrathecal therapy. Isolated CNS inflammation may be seen despite engraftment in patients without systemic symptoms of inflammation.

c. Patients who have reactivation of HLH, either isolated CNS or systemic (fever, highly elevated ferritin and soluble IL2Rα, end-organ damage) may receive salvage therapy per treating physician. Stable engraftment > 20% is typically sufficient to prevent symptoms of HLH, though isolated CNS inflammation is occasionally observed (Jordan et al., 2011). See Appendix E for recommendations for HLH salvage therapy.

2.7.7.10. Guidelines for infusing a second stem cell product or donor cellular infusion

Refer to Section 2.6 for guidelines for managing unstable chimerism and graft loss.

2.8. Toxicities

2.8.1. Conditioning: Pancytopenia

The administration of Flu/Mel/Alem is expected to produce pancytopenia for at least a week in most patients. Most patients will require transfusions of red blood cells and platelets during this period. In addition, many patients will develop fever and approximately 30% will develop a documented infection during the period of neutropenia. Complications related to pancytopenia may be life-threatening or fatal.

2.8.2. Alemtuzumab

Administration can cause fevers, rigors, nausea, vomiting, bleeding, hypotension, fatigue, rash, local irritation at injection site, urticaria, arrhythmias, dyspnea, headache, cough, pruritis, throat inflammation, diarrhea, pain, anorexia, increased perspiration, sepsis, myalgia, asthenia, hypertension, pharyngitis, abdominal pain, back pain, dizziness, anemia, infections, neutropenia and thrombocytopenia.

Fever and chills: These are regularly observed, particularly during initial doses. They probably result from breakdown of cells binding the antibody.

Skin rash and itching: A complication that is probably due to allergic reactions to the antibodies. These symptoms will usually be prevented by or controlled with anti-histamines as well as with concomitant administration of corticosteroids.

Anaphylaxis: A rare but severe allergic reaction which may cause a life-threatening drop in blood pressure, wheezing and difficulty breathing and severe hives or skin exfoliation. This complication may be treated with anti-histamines and steroids.

Platelet and white cell count depression: These are frequently observed and are probably caused by the binding of the antibody to human blood elements. Platelet transfusions should be administered to reduce the chance of bleeding or life-threatening hemorrhage.

2.8.3. Fludarabine

Administration can cause hemolytic anemia, neutropenia, thrombocytopenia, low blood counts secondary to bone marrow suppression, bleeding, infection, pneumonia, nausea, vomiting, anorexia, diarrhea, oral ulcers, stomatitis, pain, skin rash, pneumonitis, edema, fever, rigors, fatigue, weakness, blurred vision, peripheral neuropathy, agitation/nervousness, confusion, difficulty breathing, coma, renal damage, decreased immunity and rarely encephalopathy (in very high doses).

2.8.4. Melphalan

Administration can cause neutropenia, thrombocytopenia, anemia, infection, bleeding, nausea, vomiting, anorexia, weakness, weight loss, hypotension, diarrhea, oral ulcers, decreased immunity, sterility, interstitial pneumonitis, lung fibrosis, allergic reactions, skin breakdown (if drug leaks from vein), temporary hair loss, excessive perspiration, cancer of bone marrow cells, hepatic damage, cardiac arrest and rarely seizures (with very high doses).

2.8.5. Hematopoietic Cell Infusion

Infusion of allogeneic BM cells can result in shortness of breath, fever, hemolysis with renal dysfunction and back pain or anaphylaxis. To reduce the risk of reactions to product infusion, patients will be hydrated before and after administration of allogeneic BM, and will be monitored closely before, during and after infusion as per institutional practice.

2.8.6. Cyclosporine

Administration may cause nephrotoxicity, seizures, tremors, headaches, abdominal pain or indigestion, edema, confusion, muscle cramps, dizziness, hemolytic anemia, hypertension, hirsutism, thrombotic microangiopathy, electrolyte imbalances, paresthesia/neuropathy, gingival hyperplasia, hypertrygliceridemia, diarrhea, transient blindness, renal dysfunction and hepatic dysfunction.

2.8.7. Tacrolimus

Administration may cause hypertension, hyperglycemia, anemia, hyperkalemia, hypokalemia, hypomagnesemia, hypocalcemia, anorexia, diarrhea, nausea, fever, headache, hair loss, vomiting, peripheral neuropathy, pruritis, rash, abdominal pain, confusion, joint pain, increased light sensitivity, changes in vision, insomnia, infection, jaundice, renal injury, and seizures.

2.8.8. Dexamethasone, Methylprednisolone and Prednisone

Corticosteroids can cause predisposition to infection, overeating, abnormal hormone production, hyperglycemia, slowed growth, decreased bone density, headaches, poor wound healing, hypertension, stomach ulcers, muscle weakness, cataracts, increased intraocular pressure, worsening of diabetes, pancreatitis, disturbance of bone calcium, electrolyte disturbances, gastritis, GI bleeding, insomnia and mental status changes, fluid retention, edema, fat accumulation causing a change in facial appearance, and aseptic necrosis.

2.8.9. Filgrastim (G-CSF)

Administration can cause bone pain, increased levels of liver enzymes and uric acid in the blood, thrombocytopenia, headaches, fatigue, local irritation at injection site, nausea, bleeding, fever, allergic reaction, splenomegaly or rupture of the spleen, worsening of pre-existing skin rashes, temporary hair loss, and inflammation of a blood vessel in the skin.

CHAPTER 3

3. STUDY ENDPOINTS

3.1. Primary Endpoint

The primary endpoint will be overall survival at one year post-transplant in patients who receive at least one dose of the preparative regimen.

3.2. Secondary Endpoints

Secondary endpoints are evaluations of the effects of HCT on clinical and laboratory manifestations of immune deficiency at one year and evaluation of other transplant-related outcomes. These will include the following:

3.2.1. Sustained Engraftment (sustained donor lymphoid and myeloid chimerism)

Sustained engraftment will be considered to have occurred in the absence of:

1. Primary graft failure: < 5% donor chimerism by Day +42
2. Second stem cell infusion: DLI (except in the case of donor CTLs given for infection control) or second HCT following original HCT
3. Secondary graft failure: < 5% donor chimerism following original engraftment

Overall as well as T cell (CD3+) chimerism will be evaluated at least every month following engraftment until Day +100, then at Day +180 and Day +365, as well as when clinically indicated.

3.2.2. HLH and CAEBV Reactivation

3.2.2.1. Systemic HLH reactivation

HLH reactivations are difficult to define since the transplant process alters many of the diagnostic criteria (Henter et al., 2007). Post-transplant HLH reactivation is defined by clinical and lab evidence of pathologic inflammation (persistent fever, progressive cytopenias, rising ferritin and soluble IL2Rα, decreasing fibrinogen, hepatosplenomegaly, end-organ damage) not attributable to other causes.

3.2.2.2. CNS HLH reactivation

Reactivation of CNS inflammation in patients with HLH may present with or without altered mental status and is defined by pleocytosis in CSF or an MRI consistent with CNS inflammation not attributable to other causes.

3.2.2.3. CAEBV reactivation

In a patient with known CAEBV, CAEBV reactivation is defined as detection of recipient EBV+ lymphocytes in tissue (peripheral blood lymphocytes or tissue biopsy) following engraftment.

3.2.3. Immune Reconstitution and Functional Immune Recovery

3.2.3.1. Recovery of lymphocyte subpopulations

Absolute number of CD3, CD4, CD8, CD16+56 and CD19 cells will be measured by flow cytometry. Immunoglobulin levels (IgG, IgA and IgM) will also be quantified (at baseline prior to conditioning, Day +100 and Day +365). B cell reconstitution is defined as an IgG of > 500 mg/dL more than 12 weeks from the last IVIg infusion.

3.2.3.2. Correction of Immune Defects

The following will be tested prior to conditioning, on Day +100 and Day +365, per underlying diagnosis.

Disease-specific studies:

i. HLH: NK cell function
ii. HLH (with *PRF1* mutation): Perforin expression by flow cytometry
iii. XLP: SAP expression by flow cytometry
iv. CAEBV: EBV qPCR
v. HIGM: CD40L expression on activated CD4+ T cells by flow cytometry (if available)
vi. CGD: DHR assay
vii. IPEX: Quantitative Treg analysis by flow cytometry; Functional Treg assay (if available)
viii. LAD: CD18 expression on granulocytes by flow cytometry

3.2.4. Cumulative Incidence of Neutrophil and Platelet Engraftment

Neutrophil Engraftment: Time to ANC engraftment is defined as the first of three measurements on different days that the patient has an absolute neutrophil count of ≥ 500/uL following conditioning regimen induced nadir.

Platelet Engraftment: Platelet engraftment is defined as the first day of a minimum of three measurements on different days that the patient has achieved a platelet count > 20,000 AND the patient is platelet transfusion independent for a minimum of seven days following conditioning regimen induced nadir.

Subjects must not have had platelet transfusions during the preceding 7 days after the day of engraftment, unless the platelet transfusion is being given specifically to achieve a platelet threshold to allow an elective invasive procedure.

3.2.5. Grade II-IV and Grade III-IV Acute GVHD

The cumulative incidence of grade II-IV and grade III-IV acute GVHD will be assessed according to the BMT CTN Manual of Procedures (MOP).

3.2.6. Chronic GVHD

Incidence and severity of chronic GVHD will be scored according to the BMT CTN MOP.

3.2.7. Frequency of Transplant-related Complications

3.2.7.1. Veno-occlusive disease

Veno-occlusive disease (VOD) is diagnosed by the presence of two or more of the following with no other identifiable cause for liver disease (McDonald et al., 1993):

1. Jaundice (direct bilirubin > 2 mg/dL or > 34 umol/L)
2. Hepatomegaly with right upper quadrant pain
3. Ascites and/or weight gain (> 5% over baseline)

3.2.7.2. CNS toxicity

CNS toxicity is defined as a patient experiencing seizures, CNS hemorrhage, or posterior reversible encephalopathy syndrome (PRES).

3.2.7.3. Infection

Infection is defined as documented infection with or reactivation of viruses, including CMV reactivation, adenovirus infection, or EBV reactivation; invasive fungal infections; and systemic bacterial infections. CMV and EBV will be followed by PCR weekly. Other pathogens will be evaluated as clinically indicated.

3.2.7.4. Disease Group Specific Outcomes

The above endpoints will be analyzed within disease subgroups, specifically HLH patients versus patients entering with other primary immune deficiencies.

3.3. Correlative Biology Studies

Optional peripheral blood samples will be collected for correlative biology studies and shipped to the BMT CTN Research Sample Repository within 14 days prior to start of conditioning (Day -28 to Day -14), on Day -7, Day -1, Day +1, Day +14, Day +28, Day +42, Day +70, Day +100, Day +180 and Day +365. As samples must be collected and shipped the same day and only on Monday through Thursday, there is some flexibility in these specific days as shown below

(Table 3.3). Blood samples will be used in correlative biology studies, including correlation of clinical variables with alemtuzumab pharmacokinetics, engraftment, and immune reconstitution.

TABLE 3.3: OPTIONAL PERIPHERAL BLOOD SAMPLES FOLLOW-UP SCHEDULE

Visit	Window
Prior to start of conditioning	Day -28 to Day -14
Day -7	Day -8 to Day -6
Day -1	Day -2 to Day 0
Day +1	Day +1 to Day +3
Day +14	Day +12 to Day +16
Day +28	Day +26 to Day +30
Day +42	Day +39 to Day +45
Day +70	Day +60 to Day +80
Day +100	Day +90 to Day +110
Day +180	Day +160 to Day +200
Day +365	Day +335 to Day +395

CHAPTER 4

4. PATIENT ENROLLMENT AND EVALUATION

4.1. Enrollment Procedures

4.1.1. Screening and Eligibility Procedures

Patients will be registered using the BMT CTN Electronic Data Capture System (AdvantageEDC^{SM}). The following procedures should be followed:

1. The transplant coordinator at the transplant center should proceed with their institution's standard procedure to identify a sibling donor, if applicable. If an unrelated donor search is to be pursued, then the coordinator should inform the transplant center's assigned NMDP (or other appropriate donor search organization) coordinator through the standard procedure for any unrelated donor.
2. Within the 14 days prior to initiation of the conditioning regimen, an authorized user at the transplant center should enter the patient demographics and complete the Segment 0 Enrollment Form in AdvantageEDC. The Segment 0 form includes a question confirming that the patient (or legally authorized representative) signed the informed consent. The patient will be assigned a study number at this time. Additionally, Segment 0 requires completion of the HLA-typing form (patient and donor) to confirm that the HLA-typing meets protocol criteria. Upon successful completion of the two Segment 0 forms, the authorized user will proceed to Segment A and complete the Segment A enrollment form to verify eligibility and capture proposed start date of conditioning. The Segment A enrollment form must be completed prior to initiation of conditioning regimen.

4.2. Study Monitoring

4.2.1. Follow-up Schedule

The follow-up schedule for scheduled study visits is outlined in Table 4.2.1. A detailed description of each of the forms and the procedures required for forms completion and submission can be found in the Data Management Handbook and User's Guide. The Data Management Handbook, including the Forms Submission Schedule, is available on the homepage of the Internet data entry system.

4.2.1.1. Follow-up visits

Follow-up visits will begin as soon as patients are enrolled onto the study. The follow-up period is 12 months.

TABLE 4.2.1: FOLLOW-UP SCHEDULE

Study Visit	Target Day (± 3 Days to Day +42 Post-HCT) (± 7 Days to Day +60) (± 14 Days to Day +100) (± 28 Days After Day +100)
1 week	7 days
2 week	14 days
3 week	21 days
4 week	28 days
5 week	35 days
6 week	42 days
7 week	49 days
8 week	56 days
9 week	63 days
10 week	70 days
11 week	77 days
12 week	84 days
13 week	91 days
100 day	100 days
6 month	180 days
12 month	365 days

4.2.1.2. Criteria for forms submission

Criteria for timeliness of submission for all study forms are detailed in the Data Management Handbook and User's Guide. Forms that are not entered into AdvantageEDC^{SM} within the specified time will be considered delinquent. A missing form will continue to be requested either until the form is entered into AdvantageEDC^{SM} and integrated into the Data and Coordinating Center's (DCC) master database or until an exception is granted and entered into the Missing Form Exception File, as detailed in the Data Management Handbook.

4.2.1.3. Reporting patient deaths

Recipient Death Information must be entered into AdvantageEDC^{SM} within 24 hours of knowledge of the patient's death on the Death Form and the Unexpected, Grade 3-5 Adverse Event Form. If the cause of death is unknown at that time, the cause of death field may be left

blank. However, once the cause of death is determined, the form must be updated in AdvantageEDC[SM].

4.2.1.4. CIBMTR data reporting

Centers participating in BMT CTN trials must register pre- and post-transplant outcomes on all consecutive hematopoietic stem transplants done at their institution during their time of participation to the Center for International Blood and Marrow Transplant Research (CIBMTR). Registration is done using procedures and forms of the Stem Cell Transplant Outcomes Database (SCTOD) (Note: Federal legislation requires submission of these forms for all US allotransplant recipients). Enrollment of BMT CTN #1204 must be indicated on the SCTOD pre-transplant registration form. Additionally, CIBMTR pre- and post-transplant Report Forms must also be submitted for all patients enrolled on this trial. CIBMTR forms will be submitted directly to the CIBMTR at the times specified on the Form Submission Schedule.

4.2.1.5. Weekly GVHD monitoring

GVHD should be monitored in accordance with BMT CTN guidelines as specified in the Manual of Procedures. Patients should be assessed weekly until Day +100 post-transplant for GVHD. After Day +100, patients will be assessed at each follow-up visit for the presence of GVHD through 12 months. For scheduling, a target day range has been provided in Table 4.2.1.

4.2.2. Adverse Event Reporting

Unexpected, grade 3-5 adverse events (AE) and all deaths will be reported through an expedited AE reporting system via AdvantageEDC[SM]. Additionally, any occurrence of posterior reversible encephalopathy syndrome (PRES) or reversible posterior leukoencephalopathy syndrome (RPLS) requires reporting through the AE reporting system. Unexpected, grade 4-5 AEs must be reported within 24 hours of knowledge of the event. Unexpected, grade 3 AEs must be reported within three business days of knowledge of the event. Expected AEs will be reported using NCI's Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 at regular intervals as defined on the Form Submission Schedule.

4.2.3. Patient Evaluations Prior to Enrollment

Table 4.2.3 summarizes patient clinical assessments over the course of the study.

TABLE 4.2.3: CLINICAL STUDIES AND RESEARCH LABS

Week	**Pre-Conditioning[9]**	**-1**	**-1**	**0**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**26**	**52**
Day[2]		**-7**	**-1**	**0**	**1**	**7**	**14**	**21**	**28**	**35**	**42**	**49**	**56**	**63**	**70**	**77**	**84**	**91**	**100**	**180**	**365**
PE with performance status	x	x			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
CBC, chem10, LDH, AST, ALT, GGT, AP, bilirubin, INR, PTT, d-dimer, fibrinogen, triglycerides (See 4.2.4.9 for recommendations to screen for reactivation in patients with HLH)	x																				
Bone marrow aspirate[5]	x																				
GFR	x																				
HLA typing, ABO/Rh typing	x																				
PFT/oxygen saturation	x																				
Chest x-ray or CT scan	x																				
EKG/Echo	x																				
beta-HCG[4]	x																				
Bone marrow graft - total nucleated cells and CD34+ count				x																	
Ferritin	x																				
Hepatitis, HSV, syphilis, HIV, HTLV1, VZV tests	x																				
CMV and EBV qPCR	x	x			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Infection evaluation	x																				
HLH: LP and/or brain MRI[1]	x								x										x		
Immune reconstitution	x																		x		x
Immunoglobulin levels	x																		x		x
Chimerism (unfractionated and CD3+)[8]	x							x[3]	x		x[7]		x		x[1]		x		x	x	x
Optional research blood	x[10]	x	x		x		x		x		x				x				x	x	x
HLH & related disorders: NK function, Perforin expression[5], SAP expression[5]	x																		x		x
CGD: DHR assay	x																		x		x
HIGM: CD40L expression[6]	x																		x		x
IPEX: Treg analysis	x																		x		x
LAD: CD18 expression	x																		x		x

Notes:
[1] Recommended but not required
[2] Please follow the same windows as listed for follow-up visits in Table 4.2.1
[3] If neutrophil engraftment achieved before Day +28
[4] If patient is of childbearing potential
[5] As indicated (perforin study for PRF1 familial HLH; SAP expression for XLP1)
[6] If available
[7] Required if prior chimerism results are equivocal (donor chimerism < 20%)
[8] Monthly chimerism studies are standard of care
[9] ≤ 30 days prior to enrollment (unless noted otherwise)
[10] Within 14 days prior to onset of conditioning (Day -28 to Day -14)

The following observations must be performed within 30 days prior to enrollment (unless noted otherwise):

1. History, physical examination, height and weight.
2. Lansky/Karnofsky performance status.
3. Blood count with differential, chemistries (sodium, potassium, carbon dioxide, chloride, magnesium, calcium, phosphorous, BUN, creatinine), lactate dehydrogenase, AST, ALT, GGT, alkaline phosphatase, bilirubin (conjugated and unconjugated), INR, PTT, d-dimer, fibrinogen, triglycerides, ferritin, soluble IL2Rα (recommended for HLH patients), soluble CD163 (recommended for HLH patients).
4. Bone marrow aspirate (recommended in patients with cytopenias or other concerns for abnormal hematopoiesis).
5. Disease-specific studies:
   i. HLH: NK cell function, diagnostic lumbar puncture and/or brain MRI
   ii. HLH: *(*with *PRF1* mutation): Perforin expression by flow cytometry*
   iii. XLP: SAP expression by flow cytometry*
   iv. CAEBV: EBV qPCR, NK cell function (if available), diagnostic lumbar puncture and/or brain MRI
   v. HIGM: CD40L expression on activated CD4+ T cells by flow cytometry (if available)*
   vi. CGD: DHR assay*
   vii. IPEX: Quantitative Treg analysis by flow cytometry; Functional Treg assay (if available)*
   viii. LAD: CD18 expression on granulocytes by flow cytometry*

   *May be performed at any time prior to enrollment
6. Calculated or radionuclide GFR.
7. EBV antibody test and PCR, CMV antibody test and PCR, hepatitis panel (HepA Ab, HepB sAb, HepB sAg, HepB Core Ab, HepC Ab), herpes simplex, syphilis, HIV and HTLV1 I/II antibody, and varicella zoster virus antibody.
8. HLA typing, ABO and Rh typing, if not already performed. For sibling donors: 6/6 match for HLA–A, –B (intermediate or higher resolution) and –DRB1 (at high resolution using DNA-based typing); for related donors other than a sibling: 7/8 or 8/8 match for HLA–A, –B, –C (at intermediate or higher resolution), and –DRB1 (at high resolution using DNA-based typing). For unrelated donors: 7/8 or 8/8 match for HLA–A, –B, –C, and –DRB1 (at high resolution using DNA-based typing).
9. Baseline peripheral blood samples for chimerism analysis by molecular methods (STR/VNTR).
10. Baseline EKG.

11. Baseline echocardiography for left ventricular ejection fraction (LVEF) or left ventricular shortening fraction. If tricuspid regurgitation is present, it is recommended to evaluate presence of pulmonary hypertension. It is strongly recommended that these tests be performed ≤ 2 weeks prior to enrollment. However, they can be done up to 6 weeks prior to enrollment, provided the patient has been asymptomatic since the time of the tests.

12. Pulmonary function testing: FEV1 and DLCO if patient is able to undergo PFT testing. If not; record oxygen saturation by pulse oxymetry. It is strongly recommended that these tests be performed ≤ 2 weeks prior to enrollment. However they can be done up to 6 weeks prior to enrollment, provided the patient has been asymptomatic since the time of the tests.

13. β-HCG serum pregnancy test for females of childbearing potential.

14. Chest X-ray or CT scan: It is strongly recommended that the X-ray or scan be performed ≤ 2 weeks prior to enrollment in symptomatic patients.

15. Baseline immune profile: Absolute lymphocyte numbers by flow cytometry for lymphocyte subpopulations (CD3, CD4, CD8, CD19 and CD16+56 cell subsets), immunoglobulin levels (IgG, IgA and IgM).

16. Optional samples for correlative research studies: Peripheral blood 20 mL in Sodium Heparin tubes at three time points: within 14 days prior to start of conditioning (Day -28 to Day -14); Day -7 and Day -1. For patients that weigh less than 6.7 kg, 3 mL/kg will be collected at each time point.

17. Total nucleated cell count and CD34+ count of the infused product on Day 0.

4.2.4. Patient Evaluations Prior to Conditioning

If an infection occurs or a previously controlled infection worsens between the day of enrollment and the day the preparative regimen is scheduled to begin, the infection must be controlled and the patient clinically improved prior to initiating the preparative regimen. We recognize that patients with CAEBV may have ongoing EBV viremia at the time of initiating pre-transplant therapy, but other patients should have no uncontrolled bacterial, viral or fungal infections at the time of initiation of the transplant preparative regimen.

4.2.5. Required Post-Transplant Evaluations (Follow evaluation windows listed in Table 4.2.3)

1. History and physical exam to assess GVHD and other morbidity weekly until Day +100 post-transplant, then at Day +180 and Day +365. GVHD evaluation and grading to be in keeping with BMT CTN MOP.
2. Peripheral blood sample for post-transplant chimerism assay (unfractionated and CD3+) by molecular methods monthly beginning at Day +28, and at Day +42 if prior chimerism results are equivocal (donor chimerism < 20%), then at least monthly (recommended every 2 weeks) through Day +100, then at Day +180 and Day +365. An optional chimerism study at Day +21 is recommended if neutrophil engraftment occurs before Day +28.

3. EBV qPCR on plasma or blood cells weekly from Day +1 through Day +100, then at Day +180 and Day +365.
4. CMV qPCR on plasma weekly from Day +1 through Day +100, then at Day +180 and Day +365.
5. Immune reconstitution (absolute lymphocyte numbers) by flow cytometry for lymphocyte subpopulations (CD3, CD4, CD8, CD19 and CD16+56 cell subsets), immunoglobulin levels (IgG, IgA and IgM) at Day +100 and Day +365.
6. Disease-specific studies (Pre-therapy, Day +100, Day +365):
   i. HLH: NK cell function
   ii. HLH (*PRF1* mutation): Perforin expression by flow cytometry
   iii. XLP: SAP expression by flow cytometry
   iv. CAEBV: EBV qPCR, NK cell function (if available), diagnostic lumbar puncture and/or brain MRI
   v. HIGM: CD40L expression on activated CD4+ T cells by flow cytometry (if available)
   vi. CGD: DHR assay
   vii. IPEX: Quantitative Treg analysis by flow cytometry; Functional Treg assay (if available)
   viii. LAD: CD18 expression on granulocytes by flow cytometry
8. Optional samples for correlative research studies: Peripheral blood 20 mL in Sodium Heparin tubes collected on Day +1, Day +14, Day +28, Day +42, Day +70, Day +100, Day +180, and Day +365. For patients less than 6.7 kg, 3 mL/kg will be collected at each time point.
9. HLH Reactivation: For patients with HLH or hemophagocytic syndromes, regular surveillance labs (blood counts, ferritin, soluble IL2Rα, soluble CD163, d-dimer, fibrinogen, triglyercerides, LDH, AST/ALT/GGT and bilirubin) are recommended. If patients develop persistent fever of uncertain etiology, HLH reactivation should be re-evaluated completely according to the diagnostic criteria (Table 1.2), along with chimerism studies including CD3+ fraction chimerism. Resources to manage HLH reactivation (systemic or CNS) are outlined in Appendix E.

CHAPTER 5

5. STATISTICAL CONSIDERATIONS

5.1. Study Design

The study is a Phase II, non-randomized, multi-center trial. It is designed to assess overall survival 365 days after patients undergo reduced-intensity conditioning (RIC) HCT with fludarabine, melphalan, and intermediate timing of alemtuzumab for HLH or related conditions. The sample size is a minimum of 35 HLH patients.

5.2. Accrual

It is estimated that three years of accrual will be necessary to enroll the targeted sample size. Accrual will be reported by race, ethnicity, gender, and age.

5.3. Study Duration

Patients will be followed for one year post-transplant.

5.4. Randomization

There is no randomization in this trial.

5.5. Primary Objective

The primary endpoint is the proportion of patients who survive for one year after transplantation. The choice of this endpoint is based on CIBMTR registry data for patients receiving HCT for the above diseases between 2008 and 2010. In these data, the probability of one year survival was 70% for HLH patients. Recent single center data with the RIC strategy used in this protocol have shown survival probabilities closer to 90%. The primary analysis will include all patients who receive at least one dose of the preparative regimen. Death from any cause is the event for this endpoint. The study is designed to rule out survival percentages below 70%.

5.6. Sample Size and Power Considerations

Sample size calculations were based on an original sample size of 35 patients. After rapid accrual, the protocol was amended to include a minimum of 35 HLH patients to improve the precision of both the overall estimate and the secondary disease specific subgroup analysis.

Table 5.6.1 provides 90% confidence intervals for a variety of observed proportions. For example if 28 of the 35 patients survive (80% observed survival percentage), the length of the confidence interval is 22.2%. The percentages above and below 80% are intended to represent other plausible survival rates.

The probability to rule out survival percentages of a certain size is known as "power." Table 5.6.2 provides the probability (or power) that the lower bound of a 90% two-sided confidence interval for the overall survival probability will be greater than a threshold of 65%, 70%, 75%, or 80%. Based on the table below, there is 87% power at a one-sided $\alpha = .05$ to reject the null of 70% survival if the true percentage is 90%.

TABLE 5.6.1: CONFIDENCE INTERVAL LENGTHS AND POSSIBLE CONFIDENCE INTERVALS FOR VARIOUS OBSERVED OVERALL SURVIVAL PROBABILITIES

N	Overall Survival (OS) %	Length of 90% Confidence Interval	Possible 90% Confidence Intervals
35	70	25.4	(57.3, 82.7)
35	75	24	(63.0, 87.0)
35	80	22.2	(68.9, 91.1)
35	85	19.8	(75.1, 94.9)
35	90	16.6	(81.7, 98.3)

TABLE 5.6.2: PROBABILITY OF RULING OUT A THRESHOLD OF SIZE T FOR VARIOUS TRUE UNDERLYING OVERALL SURVIVAL PERCENTAGES

N	True Overall Survival %	Probability of Ruling Out Overall Survival Percentages of Size T			
		T=80%	T=75%	T=70%	T=65%
35	90	0.31	0.73	0.87	0.98
35	85	0.09	0.38	0.57	0.86
35	80		0.14	0.27	0.60
35	75			0.10	0.32
35	70				0.13

5.7. Interim Analysis and Stopping Guidelines

Interim analyses for safety will be conducted at times specified in the power and sample size calculations. Monitoring of a key safety endpoint (overall mortality) will be conducted monthly, and if rate significantly exceeds pre-set thresholds, the NHLBI will be notified in order that the

DSMB can be advised. Policies and composition of the DSMB are described in the BMT CTN's Manual of Procedures. The stopping guidelines serve as a trigger for consultation with the DSMB for additional review, and are not formal "stopping rules" that would mandate automatic closure of study enrollment.
The rate of overall mortality will be monitored up to 100 days post-transplant. Monitoring will be performed monthly beginning after the third month of enrollment until enrollment is closed. At least three events must be observed in order to trigger review. Each month, the null hypothesis that the 100-day overall mortality rate is less than or equal to 15% is tested. An extension of the sequential probability ratio test (SPRT) will be used to monitor overall mortality. A description of the SPRT is provided below.

The SPRT can be represented graphically. At each monthly interim analysis, the total time on study is plotted against the total number of patient deaths. The continuation region of the SPRT is defined by two parallel lines. Only the lower boundary will be used for monitoring to protect against excessive 100-day overall mortality. If the graph falls below the lower boundary, the SPRT rejects the null hypothesis, and concludes that there are more events than predicted by the observed time on study. Otherwise, the SPRT continues until enrollment reaches the maximum sample size.

This procedure assumes an exponential distribution for the time until death during the first 100 days, but censors follow-up time after 100 days. Only events that occur on or before the patient has been followed for 100 days are counted. Total time on study is computed as time from registration to event, or to 100 days, whichever comes first, summed for all evaluable patients.

The usual measures of performance of an SPRT are the error probabilities α and β of rejecting H_0 when $\theta = \theta_0$ and of accepting H_1 when $\theta = \theta_1$, respectively, and the expected sample size $E(N|\theta_i)$. The test to be used in this protocol was developed from the following SPRTs: A SPRT contrasting 15% versus 35% 100-day rate of overall mortality results in a common slope of 0.99 and the intercepts are -2.18 and 1.83.

The actual operating characteristics of the truncated test, shown in Table 5.7.1, were determined in a simulation study that assumed uniform accrual of 35 individuals over a 3 year time period and exponential time to failure after registration. Since 100,000 replications were used, the estimates have two digits of precision.

TABLE 5.7.1: OPERATING CHARACTERISTICS OF SEQUENTIAL TESTING PROCEDURE FROM A SIMULATION STUDY WITH 100,000 REPLICATIONS FOR OVERALL MORTALITY

True 100-Day Rate	15%	25%	30%	35%
Probability Reject Null	0.07	0.42	0.65	0.83
Mean Month Stopped	15.8	17.8	17.1	15.7
Mean # Endpoints in 100 Days	5.7	6.2	6.1	5.8
Mean # Patients Enrolled	16	18	17	16

The testing procedure for overall mortality rejects the null hypothesis in favor of the alternative 7% of the time when the true 100-day overall mortality rate for a treatment is 15%, and 83% of the time when the rate is 35%. This corresponds to a type I error rate of $\alpha = 0.07$ and a type II error rate of $\beta = 0.17$. When the true 100-day overall mortality rate is 35%, on average, the DSMB will be consulted 15.7 months after the opening, when approximately 6 events have been observed in 16 patients.

5.8. Demographic and Baseline Characteristics

Demographics and baseline characteristics will be summarized for all patients. Characteristics to be examined are: age, gender, race/ethnicity, performance status, HLA match, disease stage, serum bilirubin level, serum creatinine level, donor age, donor gender, and donor ethnicity.

5.9. Analysis of Primary Endpoint

The primary analysis will consist of estimating the one year overall survival probability based on the Kaplan-Meier product limit estimator. The one year overall survival probability and confidence interval will be calculated using Greenwood's Formula for the variance. All patients who receive at least one dose of the preparative regimen will be considered for this analysis.

5.10. Analysis of Secondary Endpoints

- **Rate of Sustained Engraftment:** The proportion of patients achieving sustained engraftment at one year will be estimated along with 95% confidence intervals.
- **Graft Failure:** To assess the incidence of primary and secondary graft failure (as outlined in Chapter 3), the proportion of subjects experiencing primarily graft failure will be estimated and the cumulative incidence of secondary graft failure will be computed along with 95% confidence intervals. Death prior to graft failure will be considered as a competing risk
- **HLH and CAEBV Reactivation**: To assess the incidence of reactivation, cumulative incidence curves will be computed along with 95% confidence intervals. Death prior to

reactivation will be considered as a competing risk. Death due to reactivation will be noted.

- **Immune Reconstitution:** Summary statistics for absolute number of CD3, CD4, CD8, CD16+56, and CD19 cells and immunoglobulin levels IgG, IgA and IgM will be reported at baseline, Day +100 and Day +365. Further proportion of subjects experiencing B cell reconstitution as defined in Chapter 3 will be estimated along with a 95% confidence interval.
- **Correction of Immune Defects:** Descriptive statistics for disease-specific immune measures will be reported by underlying disease for measurements at baseline, Day +100 and Day +365.
- **Neutrophil Engraftment:** To assess the incidence of neutrophil engraftment from day of transplant, a cumulative incidence curve will be computed along with a 95% confidence interval. Death prior to neutrophil engraftment will be considered as a competing risk.
- **Platelet Engraftment:** To assess the incidence of platelet engraftment (> 20,000) from day of transplant, a cumulative incidence curve will be computed along with a 95% confidence interval. Death prior to platelet engraftment will be considered as a competing risk.
- **Acute GVHD:** To assess the incidence and severity of grades II-IV and grades III-IV acute GVHD from day of transplant, the first day of acute GVHD onset at a certain grade will be used to calculate a cumulative incidence curve for that acute GVHD grade. An overall cumulative incidence curve will be computed along with a 95% confidence interval at one year post-transplant with death considered as a competing risk.
- **First Clinical Onset of Chronic GVHD:** To assess the incidence and severity of chronic GVHD from day of transplant, a cumulative incidence curve will be computed along with a 95% confidence interval at one year post-transplant. Death prior to occurrence of chronic GVHD will be considered as a competing risk.
- **Transplant-Related Complications:** The frequencies of transplant-related complications (veno-occlusive disease (VOD), CNS toxicity, and infection) will be described using proportions (95% confidence interval).
- **Disease Group Specific Outcomes:** In addition to the overall analysis of the primary and secondary endpoints, endpoints will be analyzed within disease subgroups, specifically HLH patients versus patients entering with primary immune deficiencies. Note that due to the small sample size no formal comparisons between the groups will be made.

5.11. Safety Analysis

The reporting of serious adverse events will be consistent with standard BMT CTN procedures. The type and severity of adverse events will be analyzed.

APPENDIX A

HUMAN SUBJECTS

APPENDIX A

HUMAN SUBJECTS

1. Subject Consent

A conference will be held with the patient and family to discuss this study and alternative treatments available for the treatment of the underlying disease. The Principal Investigator or another designated physician will conduct the conference. Potential risks associated with HCT should be discussed as objectively as possible.

The consent document should be reviewed with the patient and family prior to proceeding to ablative therapy.

Informed consent from the patient will be obtained using a form approved by the Institutional Review Board of the institution enrolling the patient.

2. Confidentiality

Confidentiality will be maintained by masking of individual names and assignment of a patient identifier code. The identifier code representing the patient's identity will be kept separately from the research file at the center. The ID code will be transmitted to the BMT CTN Data and Coordinating Center upon enrollment.

3. Participation of Women and Minorities and Other Populations

Women, ethnic minorities, and other populations will be included in this study. Accrual of women and minorities at each center will be monitored to determine whether their rates of enrollment are reflective of the distribution of potentially eligible women and minorities expected from data reported to the CIBMTR and from published data on the incidence of HLH, HLH-related disorders and the selected primary immunodeficiencies (CGD, hyper-IgM, IPEX, LAD-I). Centers will be notified if their rates differ significantly from those expected and asked to develop appropriate recruitment strategies.

APPENDIX B

INFORMED CONSENT AND ASSENT

Informed Consent to Participate in Research

Your Name: _____

Study Title: Reduced-Intensity Conditioning for Children and Adults With Hemophagocytic Syndromes or Selected Primary Immune Deficiencies (RICHI)

Protocol: BMT CTN #1204

Principal Co-Investigator: Carl Allen, MD, PhD
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Principal Co-Investigator: Michael Pulsipher, MD
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100 North Mario Capecchi Dr.
Salt Lake City, UT 84113
(801) 662-4732

Transplant Center Investigator: _____
(Insert contact information for PI at your site)

Sponsor: The National Institutes of Health (NIH) is sponsoring this study by providing financial support through the Blood and Marrow Transplant Clinical Trials Network (BMT CTN).

1. Introduction

We invite you to join this clinical trial, also known as a research study. We are doing this study because we want to learn if **reduced-intensity transplants** are safe and work well for people with **hemophagocytic syndromes** or immune disorders. A reduced-intensity transplant uses lower doses of chemotherapy. This type of transplant is also called a non-myeloablative, or 'mini' transplant.

An **allogeneic transplant** uses cells from a family member or an unrelated donor to remove and replace your diseased cells. An allogeneic transplant generally uses higher levels of chemotherapy and radiation (also known as high intensity treatments or myeloablative conditioning regimens) to destroy the diseased cells before receiving the donor cells. However, high intensity transplants can have more side effects during and after transplant. A reduced-intensity transplant can have fewer side effects, but may have more problems with **engraftment**. Engraftment is the ability of the donor cells to replace the patient's cells.

Your study participation will last for **1 year** post-transplant. This study will take about 3 years total and will include 35 HLH patients in addition to patients with other hemophagocytic syndromes or immune disorders from around the United States and Canada.

To be part of the study, you must:

- Be between the ages of 3 months and 45 years
- Have a hemophagocytic syndrome or an immune disorder including:
  - Hemophagocytic lymphohistiocytosis (HLH)
  - Griscelli syndrome
  - Chediak-Higashi syndrome
  - X-linked lymphoproliferative disease
  - Chronic active EBV (CAEBV), which is typically associated with HLH
  - Chronic granulomatous disease (CGD)
  - X-linked hyper IgM syndrome (HIGM1)
  - IPEX syndrome
  - Severe leukocyte adhesion deficiency (LAD-I)
- Have a matched related marrow donor or unrelated marrow donor available. The unrelated donor needs to be a close tissue match.
- Provide a signed consent for participation in the study

This Consent Form tells you about the purpose of the study, the possible risks and benefits, other treatment options available to you, and your rights as a participant in the study. Please take your time to make your decision.

Everyone who takes part in research at [*insert facility name*] should know that:

- Being in any research study is voluntary.
- You may or may not benefit from being in the study. Knowledge we gain from this study may benefit others.
- If you join the study, you can quit the study at any time.
- If you decide to quit the study, it will not affect your care at [*insert name of facility or institution*].
- Please ask the study staff questions about anything that you don't understand, or if you would like to have more information.
- You can ask questions now or any time during the study.
- Please take the time you need to talk about the study with your doctor, study staff, and your family and friends. It is your decision to be in the study. If you decide to join, please sign and date the end of the Consent Form.

You and your doctor will discuss other treatment options if you don't want to participate in this study.

2. Study Background

The National Institutes of Health (NIH), through the Blood and Marrow Transplant Clinical Trials Network (BMT CTN), are providing staff support and money for this research study. The BMT CTN will direct the research study. The BMT CTN and the NIH will make decisions about how to manage the study together.

Some of the immune system disorders included in this study are: Hemophagocytic lymphohistiocytosis, Griscelli syndrome, Chediak-Higashi syndrome, X-linked lymphoproliferative disease, chronic active EBV, chronic granulomatous disease, hyper IgM syndrome, IPEX syndrome, and severe leukocyte adhesion deficiency. These disorders prevent the immune system from working well. Your immune system fights off infections, so people with these disorders might develop deadly infections.

One way to treat these disorders is through a blood and bone marrow transplant. A transplant replaces your unhealthy blood cells with bone marrow cells from a family member or an unrelated donor. It requires a close tissue match between you and the donor. Your donor could be a sibling (a sister or brother) or an unrelated person. In the United States, we use the Be The Match® Registry to find unrelated donors.

A transplant first uses chemotherapy to destroy the unhealthy blood cells or stop them from growing. Then, we replace the destroyed cells with the new cells from your donor. In this study, doctors want to use lower doses of chemotherapy. This type of transplant is called a reduced-intensity transplant.

Your disease can be treated in other ways too. You and your doctor will decide on the best treatment for you.

3. Study Purpose

We are inviting you to join this study because you have an immune system disorder, a matched bone marrow donor, and a reduced-intensity transplant is a treatment option for you. The goal of this study is to learn if a reduced-intensity transplant is safe and works well to treat your disease. We also want to know how well your immune system responds to the transplant.

4. Right to Ask Questions and/or Withdraw

You have the right to ask questions about the study at any time. If you have questions about the study or you want to leave the study, please contact:

[*insert contact info for site PI*]

Being in this study is voluntary. You can choose not to be in this study or leave this study at any time. If you choose not to take part or to leave this study, it will not affect your regular medical care in any way.

Your study doctor and study staff will be available to answer any questions you may have about taking part in or leaving this study.

5. Study Treatment and Tests

We will check your health before your transplant. We will also check your health several times after your transplant for up to 1 year.

Before Your Transplant:

You will be admitted to the hospital 14 days before your transplant. Your doctor will put an intravenous (IV) catheter (a thin tube) in a large vein in your neck or chest. This is done to make giving you drugs and blood transfusions easier and less painful. This is also known as a central venous catheter.

When you are ready to get your catheter, your doctor will explain what will happen in more detail. Your anesthesiologist (the doctor who will give you pain medicine) will describe the risks and benefits of the pain medicine.

When the catheter is in your chest, it will need to be cleaned and flushed regularly to prevent infection and blood clots. Your nurse or caregiver will help you clean your catheter.

Reduced-Intensity Transplant:

To prepare your body for transplant and destroy the diseased cells, you will be treated with chemotherapy and other medicines. Your chemotherapy will start the day you are admitted to the hospital (14 days before your transplant).

Study chemotherapy drugs
The study drugs that will be used for your chemotherapy are called **alemtuzumab**, **fludarabine**, and **melphalan**.

We will start giving you alemtuzumab 14 days before your transplant. It will be given as a shot. You will get a dose of alemtuzumab once a day for 5 days. The first day will be a small test dose. The test dose is given to make sure you will not have a bad reaction to the full dose. If you have a bad reaction, your doctor will talk with you about other drug options to prepare for transplant.

The risks of alemtuzumab are listed in Section 6: Risks and Discomforts. These risks include serious allergic reactions. Patients are usually admitted to the hospital for their first dose of alemtuzumab. Your doctor will decide if you need to stay in the hospital for the other doses of alemtuzumab or if you can receive them as an outpatient.

If you stay in the hospital for all of the alemtuzumab shots, your doctor might discharge you after the shots are done if you respond well. If you are discharged, you will return to the hospital 8 days before your transplant to start receiving the other chemotherapy drugs (fludarabine and melphalan).

Fludarabine will be given through your central venous catheter, once a day for 5 days. A central venous catheter is a thin tube that is placed in a large vein in your neck or

chest. We will give you one dose of melphalan 3 days before the transplant. It will also be given through your central venous catheter.

You will have 2 days to rest before your transplant. We won't give you any chemotherapy drugs on these days.

Table 1 shows the timeline for the chemotherapy drugs and blood tests. This timeline does not show the drugs we will give you to help prevent Graft Versus Host Disease (below).

Drugs to prevent Graft-Versus-Host Disease (GVHD):
We will give you immune suppressing drugs 3 days before your transplant. We will continue to give you these drugs after your transplant. These drugs are important because they allow the donor cells to perform their new role in your body. They can also lower your chances of developing GVHD. GVHD happens when the donor cells attack your body (GVHD is discussed in more detail below).

You will receive 1 or more standard drugs (drugs that are not part of the study) to lower your chances of developing GVHD. We will give you these drugs for at least 6 months after the transplant. They are cyclosporine (also called Gengraf® or Neoral®) or tacrolimus (also called FK 506 or Prograf®). We will give them to you with methylprednisolone or prednisone daily starting 2 days prior to transplant and continuing until 28 days after transplant (both of these drugs work the same).

Donated marrow cells:
The donated marrow cells will come from a related donor or unrelated donor. The donor cells will be given through your catheter, just like a blood transfusion.

After Your Transplant:

The chemotherapy drugs will destroy the cells in your bone marrow that produce your blood and immune cells. The donor cells will produce new blood cells in your body. To speed up this process, we may give you a drug called Filgrastim (also called G-CSF or Neupogen). This drug helps protect against infections. It is given as a shot or IV.

Check-up appointments:
You will stay in the hospital after your transplant until your doctor feels you're well enough to go home. When you're in the hospital, we will watch you carefully for signs of infection and other problems. A physical exam and blood tests will be done every day to know how you're doing. Your doctor may need to give you other drugs, tests and treatments if you have problems.

After you leave the hospital, you will need to visit the transplant clinic at least once a week for check-ups to make sure you're still doing well. You will also need to visit us 100 days (3 months), 6 months, and 1 year after your transplant so we can check your progress and treat any problems. You will check in with us less and less over time.

At these visits, we will take 2 – 6 teaspoons of your blood (10 – 30 mL) from your catheter or from a vein in your arm if your catheter has been removed. We will test your blood to see how well your body is responding to the chemotherapy drugs and the donated cells.

Health Evaluations:

We will test (evaluate) your health during the study. These tests and how often they are scheduled are standard care for patients receiving an allogeneic transplant. They would be done even if you were not part of this study.

Health evaluations after treatment:

1) Physical exam to assess toxicities, and infections weekly until Day +56 and then at Days +180 and +365.

2) Physical exam to assess GVHD weekly until Day +100 and then at Days +180 and +365.

3) Routine blood tests (cell counts and liver and kidney function) weekly until Day +56 and then at Days +180 and +365.

4) Blood or bone marrow tests to find the amount of donor cells in your body monthly until Day +100 and then at Days +180 and +365. This is also called *chimerism*.

5) Disease-specific tests to see how much disease you have before treatment and after treatment on Days +100 and +365.

6) Optional blood samples for future research (see **Section 17: Blood Samples for Future Research**).

Your doctor may decide that you need other tests and treatments that are not part of this research study, but are necessary to take care of you.

Table 1: Timeline for Chemotherapy Drugs and Blood Tests

Day:	Before transplant															Transplant Day	After transplant							
	After you consent	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0	1	14	28	42	70	100 (3 mths)	180 (6 mths)	365 (1 yr)
Optional Blood Tests:	✓								✓						✓		✓	✓	✓	✓	✓	✓	✓	✓
Drugs*:		A	B	B	B	B		C	C	C	C	C	D											

***Legend for Chemotherapy Drugs**

A	Alemtuzumab test dose
B	Alemtuzumab
C	Fludarabine
D	Melphalan

6. Risks and Discomforts

You will have side effects while on the study. Side effects can range from mild to very serious.

The risks listed in this section might happen from transplant. These risks might happen if you have a transplant as part of this study or standard care. The chances of developing GVHD and infections are the same if you have a reduced-intensity or standard transplant.

Your doctor will give you drugs to help lower the side effects, such as feeling sick to your stomach (nausea). In some cases, side effects can be long lasting or may never go away.

The chemotherapy drugs can cause leukemia years later, but this is rare. These "secondary cancers" are often very hard to treat and can cause death.

Risks of Study Treatments and Drugs

Likely	What it means: This type of side effect is expected to occur in more than 20% of patients. This means that 21 or more patients out of 100 might get this side effect.
Less Likely	What it means: This type of side effect is expected to occur in 20% of patients or fewer. This means that 20 patients or fewer out of 100 might get this side effect.
Rare, but Serious	What it means: This type of side effect does not occur very often – in fewer than 2% of patients – but is serious when it occurs. This means that 1 or 2 patients (or fewer) out of 100 might get this side effect.

Possible Side Effects of Study Drugs

The most common side effects of the treatments used in this study are listed below. There is also the risk of very uncommon or unknown side effects. All chemotherapy drugs in this study are commonly used in transplant.

Alemtuzumab		
Likely	**Less Likely**	**Rare, but Serious**
• Fever • Chills • Anemia (decreased number of red cells) • Infection • Bleeding • Weakened immune system • Local irritation (skin) at injection site • Low number of white blood cells • Low number of platelets in the blood	• Nausea • Vomiting • Diarrhea • Rash • Headache • Sweating • Back pain • Severe itching • Allergic reaction of skin and blood vessels • Tiredness • Loss of appetite • Low blood pressure • Irregular heartbeat • Shortness of breath • Sore throat • Pain • Cough	• Abdominal pain • Dizziness • High blood pressure • Blisters • Pain in the muscles • Herpes simplex infection • Swelling of the throat • Bacterial infection in the bloodstream

Fludarabine		
Likely	**Less Likely**	**Rare, but Serious**
▪ Infection ▪ Anemia (low red blood cell count) ▪ Tiredness ▪ Nausea ▪ Vomiting ▪ Pneumonia ▪ Mouth sores ▪ Fever ▪ Swelling of hands and feet ▪ Weakened immune system ▪ Pain ▪ Low number of white blood cells ▪ Low number of platelets in the blood ▪ Electrolyte imbalances	▪ Diarrhea ▪ Numbness and tingling in hands and/or feet ▪ Changes in vision ▪ Skin rash ▪ Cough ▪ Changes in heartbeat ▪ Loss of appetite ▪ Chills ▪ Lung inflammation	▪ Changes in vision ▪ Agitation/nervousness ▪ Confusion ▪ Difficulty breathing ▪ Weakness ▪ Severe brain injury and death ▪ Bleeding due to decreased numbers of platelets ▪ Kidney damage that could require dialysis ▪ Coma

Melphalan		
Likely	**Less Likely**	**Rare, but Serious**
▪ Loss of appetite ▪ Nausea ▪ Vomiting ▪ Skin breakdown (if drug leaks from vein) ▪ Anemia (low red blood cell count) ▪ Infection ▪ Bleeding ▪ Mouth sores ▪ Temporary hair loss ▪ Decreased immunity ▪ Low number of white blood cells ▪ Low number of platelets in the blood	▪ Diarrhea ▪ Inflammation of the lung ▪ Weakness ▪ Weight loss	▪ Low blood pressure ▪ Excessive perspiration ▪ Allergic reaction ▪ Damage/scarring of lung tissue ▪ Sterility ▪ Seizure ▪ Cancer of bone marrow cells ▪ Heart stops beating ▪ Liver damage

Side Effects of Drugs Used To Prevent GVHD

The side effects of the GVHD drugs (listed below) usually stop when you're done taking them.

Cyclosporine (Gengraf® or Neoral®).

This drug may be used for all patients

Likely	Less Likely	Rare, but Serious
▪ Tremors ▪ High blood pressure ▪ Kidney problems ▪ Headaches ▪ Nausea ▪ Vomiting ▪ Stomach pain or indigestion ▪ Swelling of the hands or feet ▪ Increased hair growth ▪ Electrolyte imbalances	▪ Confusion ▪ High levels of triglycerides in the blood ▪ Diarrhea ▪ Gum enlargement ▪ Liver dysfunction ▪ RPLS/PRES[1]	▪ Muscle cramps ▪ Numbness and tingling of the hands or feet ▪ Seizures ▪ Dizziness ▪ Red blood cell destruction ▪ Temporary blindness

[1] Reversible posterior leukoencephalopathy syndrome (RPLS) also known as posterior reversible encephalopathy syndrome (PRES) – See text below for description

Tacrolimus (Prograf® or FK-506)

This drug may be used for all patients.

Likely	Less Likely	Rare, but Serious
▪ High blood pressure ▪ High blood sugar ▪ Anemia (low red blood cell count) ▪ High or low potassium levels ▪ Low magnesium and calcium levels ▪ Loss of appetite ▪ Diarrhea ▪ Nausea ▪ Fever ▪ Headache	▪ Hair loss ▪ Vomiting ▪ Tingling sensation in the extremities ▪ Itching ▪ Rash ▪ Abdominal pain ▪ RPLS/PRES[1]	▪ Confusion ▪ Painful joints ▪ Increased sensitivity to light ▪ Change in vision ▪ Insomnia (trouble sleeping) ▪ Infection ▪ Jaundice (skin yellowing) ▪ Kidney injury ▪ Seizures

[1] Reversible posterior leukoencephalopathy syndrome (RPLS) also known as posterior reversible encephalopathy syndrome (PRES) – See text below for description

Methylprednisolone and Prednisone

This drug will be used as part of GVHD prophylaxis for bone marrow recipients.

Likely	Less Likely	Rare, but Serious
▪ Water retention (storing of extra water) ▪ Overeating ▪ Weaker immune system ▪ Temporary personality changes ▪ Abnormal hormone production ▪ High blood sugar ▪ Slowed growth ▪ Decreased bone density ▪ Fat accumulation causing a change in facial appearance	▪ Headaches ▪ Poor wound healing ▪ Stomach swelling or pain ▪ Tissue swelling ▪ High blood pressure ▪ Stomach ulcer ▪ Muscle weakness ▪ Cataracts ▪ Bone cell death ▪ RPLS/PRES[1]	▪ Difficulty falling asleep ▪ Worsening of diabetes ▪ Inflammation of pancreas ▪ Personality disturbances ▪ Bleeding in the stomach and intestines ▪ Increased pressure within the eye ▪ Disturbance of bone calcium (might lead to possible broken bones or permanent bone damage)

[1] Reversible posterior leukoencephalopathy syndrome (RPLS) also known as posterior reversible encephalopathy syndrome (PRES) – See text below for description

Filgrastim (G-CSF)		
Likely	**Less Likely**	**Rare, but Serious**
▪ Ache or pain inside the bones ▪ Increased levels of liver enzymes and uric acid in the blood ▪ Low number of platelets in the blood ▪ Headache ▪ Tiredness	▪ Local irritation (skin) at injection site ▪ NauseaBleedingFever	▪ Allergic reaction ▪ Enlargement or rupture of the spleen ▪ Worsening of pre-existing skin rashes ▪ Temporary hair loss ▪ Inflammation of a blood vessel in the skin

Potential Risk of RPLS/PRES

The Data Safety and Monitoring Board (DSMB) of the Blood and Marrow Transplant Clinical Trials Network is a group of transplant, HLH and immune deficiency disease and other experts that ensure the safety of patients treated on this and other trials. This group carefully monitors the experience of patients to make sure that the side effects that they experience are not unusual or more frequent or more severe than would be expected.

The DSMB has noted that patients transplanted on the clinical trial BMT CTN 1204 have a higher than expected occurrence of a usually uncommon (<5%) complication called reversible posterior leukoencephalopathy syndrome (RPLS) also known as posterior reversible encephalopathy syndrome (PRES). Patients with RPLS/PRES have confusion and other changes in their ability to think. Sometimes, they experience seizures, sleepiness or, rarely, loss of consciousness. RPLS is diagnosed with an MRI of the brain. In transplant patients, it is usually caused by some of the drugs used to prevent or treat graft versus host disease. It can often, but not always, be prevented by very careful control of blood pressure. It is treated by changing graft versus host disease drugs, controlling blood pressure and/or giving anti-seizure medicines. Three out of thirty-five patients on BMT CTN 1204 have developed RPLS/PRES; all were successfully treated for this complication. Thus far, no RPLS/PRES has been observed in any patient more than 6 months from their date of transplant. We believe that children who are on prednisone or other corticosteroids, or immunosuppressive drugs such as cyclosporine or tacrolimus or have high blood pressure are more likely to develop RPLS/PRES.

If you/your child experience any of these side effects or changes in mental status, you should contact your/your child's transplant physician right away, since early treatment is important. It is also important that any blood pressure medication be taken as prescribed to decrease the risk of RPLS/PRES.

Risks of Transplant:

The following problems might happen from your transplant. These problems might happen if you have a transplant as part of the study or standard care:

Graft-versus-host disease (GVHD)
GVHD develops when the white blood cells, which are called T cells, in the donor's cells attack your body. You are more likely to get GVHD if your donor's tissue does not closely match your tissue.

There are 2 kinds of GVHD: acute and chronic. Acute GVHD usually develops in the first 3 months after transplant. Chronic GVHD usually develops later and lasts longer.

You may experience these side effects with acute GVHD:

- Skin rash
- Feel sick to your stomach (nausea)
- Throw up (vomit)
- Diarrhea
- Abdominal (stomach area) pain
- Problems with your liver (your doctor will run tests for this)
- Infection

You may experience these side effects with chronic GVHD:

- Skin rashes
- Hair loss
- Thickened skin
- Joint stiffness (knees, elbows, fingers)
- Dry eyes
- Dry mouth
- Liver disease (your doctor will run tests for this)
- Weight loss
- Diarrhea
- Infection

We don't know for sure if you will develop acute or chronic GVHD. The chance that you will get GVHD is 10-30%. This means that 10 to 30 people out of 100 might develop it. We will watch you closely for GVHD and treat it if it happens.

To know for sure if you have acute or chronic GVHD, we may do a biopsy of your skin. A biopsy is where we take a small piece of your skin and look at it under a microscope for signs of GVHD. There's a small chance that we might also do a biopsy of your intestine and liver. Risks of biopsy may include pain, infection, or bleeding.

In most cases, GVHD can be treated. If GVHD does not respond to the drugs, your doctor will talk with you about other treatment options. If you choose a different treatment option, we will give you information about the side effects.

You may need to be treated for GVHD for many months or years. GVHD treatments can cause your immune system to become very weak if it goes on for a long time. This means you may develop more infections and need to be admitted to the hospital often. GVHD can be very serious and hard to treat. It might also cause death.

Damage to your vital organs

Your vital organs include your heart, lungs, liver, intestines, kidneys, bladder and brain. The chemotherapy and GVHD drugs may hurt these organs. You may develop lung problems from chemotherapy or an infection.

Some patients can have veno-occlusive disease (VOD) of the liver even from a reduced-intensity transplant (lower doses of chemotherapy). Patients with VOD become jaundiced (yellow skin), have problems with their liver, retain too much water (feel swollen and uncomfortable), and have stomach swelling and pain.

If there is serious damage to your vital organs, you may have to stay in the hospital longer or return to the hospital after your transplant. Many patients get better, but these complications can cause permanent damage to your organs or death.

Serious infections

It may take many months for your immune system to recover from the chemotherapy and GVHD drugs. There is an increased risk of infection during this time when your body is healing. We will give you drugs to reduce the chance of infections, but they may not work. If you have an infection, you may have to stay in the hospital longer or return to the hospital after transplant. Many patients get better, but some infections can cause death.

Graft (donor cells) rejection

Some patients' bodies reject the donor cells (graft) with a standard (non-reduced-intensity) transplant. There is an increased risk of rejection with a reduced-intensity transplant. Also, a certain amount of your old blood and marrow cells will remain in your body.

If your body rejects the donor cells, your doctor may need to give you a donor lymphocyte infusion (DLI). A DLI is an extra dose of the donor cells. You may also get another transplant, but this is rare. If you need a DLI or second transplant, your doctor will explain the risks and benefits.

Central venous catheter

You may feel pain and bleed a little where the catheter is placed in your chest. The most common risks of a catheter are blood

clots and infections. If you get a clot, we will give you a drug to break it up. If the drug doesn't work, the catheter may need to be replaced. Infections will also be treated with drugs. Sometimes, the catheter has to be taken out and a new catheter put in. Also, the catheter could puncture (create a hole) in your lung and cause serious bleeding, but this is very rare.

We will do an X-ray or CT scan of your chest to make sure we know the best place to insert the catheter and reduce the risks from happening as much as possible.

Infertility and reduced sexual functioning
Chemotherapy can cause infertility (inability to have children). It may decrease your sexual desire and cause female vaginal dryness.

The chemotherapy doses used in this study are lower than what is used in standard transplants, so the risk of infertility may be lower. Some patients who received reduced-intensity transplants had children. It's difficult to know the exact risk of infertility from reduced-intensity transplants. We don't know for sure what your risk of infertility will be.

Risk to the unborn

The treatments in this study have not been proven to be safe at any stage of pregnancy. Therefore, if you are pregnant or nursing, you are not eligible for this study. Women who can become pregnant must use two forms of effective birth control while receiving chemotherapy and drugs to prevent GVHD. Effective birth control is defined as the following:

1. Refraining from all acts of vaginal sex (abstinence)
2. Consistent use of birth control pills
3. Injectable birth control methods (Depo-Provera, Norplant)
4. Tubal sterilization or male partner who has undergone a vasectomy
5. Placement of an IUD (intrauterine device)
6. Use of a diaphragm with contraceptive jelly and/or condoms with contraceptive foam every time you have sex

Reproductive Risks

The drugs used in this research study may damage your reproductive organs, affect your ability to have children or possibly cause birth defects if you take them while you are pregnant. It is important that a woman is not pregnant or breast-feeding and does not become pregnant during the course of the study.

If you are a woman and can become pregnant, you will need to take a pregnancy test before you start the study. You should discuss ways to prevent pregnancy while you are in the study. Women who have gone through puberty may find that their menstrual cycle becomes irregular or stops permanently. This does not mean that you cannot become pregnant. You must still use an effective method of birth control during

your transplant and continue until you are finished with your GVHD prevention treatment.

If you are a man, your body may not be able to produce sperm (become sterile). You should talk with your doctor about banking your sperm before having a transplant.

Please check with your doctor to understand more about these risks.

Risk of death
The side effects of a blood and bone marrow transplant might be very serious and cause death. Your doctor will do everything to make sure your transplant is as safe as possible, but there is still a risk of death.

7. Alternative Treatments

Participation in this study is optional. If you choose not to take part, you may still receive a blood or marrow transplant to treat your disease. The treatment and evaluations you would receive could be very similar to what would receive if you join this study.

Your study doctor will talk with you about your options. If you decide not to participate in this study, your medical care will not be affected in any way.

Your other choices may include:

- Treatment with other drugs, radiation, or a combination of drugs and radiation without a transplant
- A blood or marrow transplant that is not part of the study, or another type of transplant
- Participation in another clinical trial, if available (check with your doctor)
- No treatment for your blood disorder at this time
- Comfort care: This can occur at any stage of a disease. The goal is to make you comfortable by treating symptoms of the disease and improving your quality of life. You may still be receiving treatment for your disease. This is different from hospice care.

Every treatment option has benefits and risks. Talk with your doctor about your treatment choices before you decide if you will take part in this study.

It is important that you talk to your doctor about treatment choices before you decide to participate in this study.

8. Possible Benefits

We don't know if taking part in this study will make your health better. If the transplant works well, you may not have any more symptoms of your disorder. The information from this study will help doctors learn if reduced-intensity transplants are safe and work well for people with hemophagocytic syndromes or immune disorders.

9. New Information Available During the Study

During this study, the study doctors may learn new information about the study drug or the risks and benefits of the study. If this happens, they will tell you about the new information. The new information may mean that you can no longer take part in the study, or that you may not want to continue in the study. If this happens, the study doctor will stop your participation and will offer you all available care to meet your needs and medical conditions.

10. Privacy, Confidentiality and Use of Information

Your confidentiality is one of our main concerns. We will do our best to make sure that the personal information in your medical record is kept private. However, we cannot guarantee total privacy. All your medical and demographic (such as race and ethnicity, gender and household income) information will be kept private and confidential. *(Name of Transplant Center)* and the organizations listed below will not disclose your participation by any means of communication to any person or organization, except by your written request or permission, or unless required by federal, state or local laws, or regulatory agencies.

Individuals authorized by the organizations below will have access to your research and medical information. They may use this information for inspections or audits to study the outcomes of your treatment. In agreeing to participate, you consent to such inspections and to the copying of parts of your records, if required by these organizations.

We may give out your personal information if required by law. If information from this study is published or presented at scientific meetings, your name and other personal information will not be used.

Information about your transplant from your original medical records may be seen or sent to national and international transplant registries, including:

- /Institution/
- The National Institutes of Health (NIH)
- The National Heart, Lung, and Blood Institute (NHLBI)
- The National Cancer Institute (NCI)
- Office of Human Research Protection (OHRP)
- The Food and Drug Administration (FDA)
- Institutional Review Boards (IRBs) responsible for this study
- Data and Safety Monitoring Board (DSMB), not part of /Institution/
- Blood and Marrow Transplant Clinical Trials Network (BMT CTN), including the Center for International Blood and Marrow Transplant Research (CIBMTR), the National Marrow Donor Program (NMDP) and the EMMES Corporation who are coordinating the studies of the BMT CTN
- Study investigators

We will not identify you by name in any publications or reports that come from these organizations or groups.

Information that does not include personally identifiable information about this study has been or will be submitted, at the appropriate and required time, to the government-operated clinical trial registry data bank, which contains registration, results, and other information about registered studies.

This data bank can be accessed by you and the general public at:

www.ClinicalTrials.gov. Federal law requires study information for certain studies to be submitted to the data bank.

For questions about access to your medical records, please contact /name/ at /number/.

11. Ending Your Participation

The study doctor or the study sponsor may stop the study at any time, and we may ask you to leave the study. If we ask you to leave the study, the reasons will be discussed with you. Possible reasons to end your participation in this study include:

- You do not meet the study requirements.
- You need a medical treatment not allowed in this study.
- The study doctor decides that it would be harmful to you to stay in the study.
- You are having serious side effects.
- You become pregnant.
- You cannot keep appointments or take study drugs as directed.
- The study is stopped for any reason.

You could have serious health risks if you stop treatment during the conditioning process before you receive your transplant. If you stop taking the immune suppressing drugs (see **Section 6: Risks and Discomforts**) too soon after transplant, your body could reject the donor stem cells or you could develop serious complications and possibly die.

We ask that you talk with the research doctor and your regular doctor before you leave the study. Your doctors will tell you how to stop safely and talk with you about other treatment choices.

If you decide to leave this study after the study, or your doctor asks you to leave the study for medical reasons, you will need to come back to the doctor's office for tests for your safety. Even if you leave the study, the information collected from your participation will be included in the study results.

12. Physical Injury as a Result of Participation

It is important to tell your study doctor, _____ *[investigator's name(s)]*, or study staff if you feel that you have been injured from taking part in this study. You can tell the doctor in person or call him/her at _____ *[telephone number]*.

You will get all available medical treatment if you are injured from taking part in this study. You and/or your health plan will be charged for this treatment. The study will not pay for medical treatment.

In case you are injured in this study, you do not lose any of your legal rights to receive payment by signing this Consent Form.

13. Compensation or Payment

You will not be paid for taking part in this study. You will not be compensated or reimbursed for any extra costs (for example, travel and meals) from taking part in this study.

14. Costs and Reimbursement

Most of the tests for this study are standard medical care for your transplant and will be billed to your insurance company. You and/or your health plan/insurance company will need to pay for some or all of the costs of standard treatment in this study.

You or your insurance will not be charged for tests that are only done for research on this study. You will not pay for any extra tests that are being done for the study.

Some health plans will not pay the costs for people taking part in studies. Check with your health plan or insurance company to find out if they will pay.

For questions about your costs, financial responsibilities, and/or medical insurance coverage for your transplant and this study, please contact /Center/ Financial Counselor at /Number/.

For more information on clinical trials and insurance coverage, you can visit the National Cancer Institute's Web site at http://cancer.gov/clinicaltrials/understanding/insurance-coverage. You can print a copy of the "Clinical Trials and Insurance Coverage" information from this Web site.

Another way to get the information is to call 1-800-4-CANCER (1-800-422-6237) and ask them to send you a free copy.

15. For More Information

If you need more information about this study, or if you have problems while taking part in this study, you can contact the study doctor or his/her staff. They can be reached at the telephone numbers listed here:

[Insert name and contact detail]

16. Contact Someone about Your Rights

If you wish to speak to someone not directly involved in the study, or if you have any complaints about the project, or any questions about your rights as a research participant, then you may contact:

[Insert appropriate contact details]

The ethical aspects of this research study have been reviewed and approved by [name of IRB].

For questions about your rights while taking part in this study, call the _____[name of center] Institutional Review Board (a group of people who review the research to protect your rights) at _____ telephone number).

17. Blood Samples for Future Research (Optional)

This section of the informed consent form is about future research studies that will use blood samples from people who are taking part in the main study. You may choose to give blood samples for these future research studies if you want to. You can still be a part of the main study even if you say 'no' to give blood samples for future research studies.

Researchers are trying to learn more about how the human body processes the drugs used for transplant and how the body recovers after transplant. This research is meant to gain knowledge that may help people in the future and make transplants even more successful.

If you agree to provide blood samples, here is what will happen:

a) We will collect 11 extra blood samples from your catheter or by a vein in your arm at the same time you have routine blood tests done **(Table 1)**. We will take about 4 teaspoons (20 mL) of your blood each time. If you weigh less than 6.7 kg, the amount of blood will be based on your weight (3 mL/kg).

   We will collect your blood on these 11 days:

   - After you consent to giving blood samples, but before you start the chemotherapy drugs
   - 7 days and 1 day <u>before</u> transplant
   - 1, 14, 28, 42, 70, 100, 180, 365 days <u>after</u> transplant

b) The blood samples for future research will be sent to the BMT CTN Repository for processing and storage. A repository is a place that protects, stores and sends out samples for approved research studies. Only the repository will have the link between you and your research samples. All research samples will be given a bar code that cannot be linked to you by future researchers testing your samples.

c) Samples stored in the Repository will be used mainly by clinicians and researchers in the BMT CTN network. In the future, the unused research samples and clinical data will be made available outside of this network.

d) Researchers can apply to study the materials stored in the Repository. The BMT CTN Steering Committee and/or the BMT CTN Executive Committee must approve each request before they will share samples or information with researchers. This is to make sure that the investigators requesting the samples are qualified, and that the research is of high quality.

e) DNA from your stored blood samples might be used in genome-wide association (GWA) studies for a future project either done or supported by the National Institutes of Health (NIH).

Genome-wide association studies are a way for scientists to find genes that have a role in human disease or treatment. Each study can look at hundreds of thousands of genetic changes at the same time.

If your coded samples are used in such a study, the researcher is required to add your test results and sample information into a shared, public research database. This public database is called the NIH Genotype and Phenotype Database and it is managed by the National Center for Biotechnology Information (NCBI). The NCBI will never have any information that would identify you, or link you to your information or research samples although the results of genetic studies could theoretically include identifying information about you.

Your name and other information that could directly identify you (such as address or social security number) will not be placed into any scientific database. However, because your genetic information is unique to you, there is a small chance that someone could trace it back to you. The risk of this happening is small, but may grow in the future. Researchers have a duty to protect your privacy and to keep your information confidential.

Some general things you should know about letting us store your blood for research are:

- We will only store samples from people who give us permission.
- Research is meant to gain knowledge that may help people in the future. You will not get any direct benefit from taking part. Additionally, you or your doctor will not be given results and they will not be added to your medical record.
- A possible risk is the loss of confidentiality about your medical information. We will use safety measures with both your samples and clinical information to make sure that your personal information will be kept private. The chance that this information will be given to someone else is extremely small.
- Your blood will be used only for research and will not be sold. The research done with your blood may help to develop new products in the future. You will not get paid for any samples or for any products that may be developed from current or future research.

You can change your mind at any time about allowing us to use your samples and health information for research.

We ask that you contact [Principal Investigator] in writing and let him/her know you do not want us to use your research samples or health information for research. His/her mailing address is on the first page of this form. However, samples and information that have already been shared with other researchers cannot be taken back or destroyed.

Making Your Choice

Please read each sentence below and think about your choice. After reading each sentence, please indicate your choice below. If you have any questions, please talk to your doctor or nurse, or call our research review board at _____.

No matter what you decide to do, it will not affect your care.

Statement of Consent for Future Research Samples

The purpose of storing blood samples, the procedures involved, and the risks and benefits have been explained to me. I have asked all the questions I have at this time and I have been told whom to contact if I have more questions. I have been told that I will be given a signed copy of this consent form to keep.

I understand that I do not have to allow the use of my blood for research. If I decide to not let you store research samples now or in the future, it will not affect my medical care in any way.

I voluntarily agree that my blood and information can be stored indefinitely by the BMT CTN and/or NHLBI Repositories for research to learn about, prevent, or treat health problems. I also understand that my DNA and health information may or may not be used in genome-wide association studies.

The decision of whether to allow us to use the samples for future research and for your genetic code to be released onto a public database is completely up to you. There will be no penalty to you if you decide not to allow this, and your decision will in no way affect your participation in this research.

If you agree to allow your samples to be used for future research you also agree to have your genetic code released to a public database and made accessible to other researchers.

❑ I agree to allow my blood samples to be stored for future research.

❑ I <u>do not</u> agree to allow my blood samples to be stored for future research.

_____ _____

Signature Date

HIPAA[1] authorization to use and disclose individual health information for research purposes:

a. Purpose: As a research participant, I authorize the Principal Investigator and the researcher's staff to use and disclose my individual health information for the purpose of conducting the research study entitled *Reduced Intensity Conditioning for Children and Adults with Hemophagocytic Syndromes or Selected Primary Immune Deficiencies.*

b. Individual Health Information to be Used or Disclosed: My individual health information that may be used or disclosed to conduct this research includes: demographic information (e.g., age, date of birth, sex, weight), medical history (e.g., diagnosis, complications with prior treatment), physical examination findings, and laboratory test results obtained at the time of work-up and after transplantation (e.g. blood tests, biopsy results). The identities of individuals such as names and addresses will not be shared or de-identified to make sure information cannot be linked to you.

c. Parties Who May Disclose My Individual Health Information: The researcher and the researcher's staff may obtain my child's (my) individual health information from:
(list: hospitals, clinics or providers from which health care information can be requested)

_____

_____

_____

d. Parties Who May Receive or Use My Individual Health Information: The individual health information disclosed by parties listed in item c and information disclosed by my child (me) during the course of the research may be received and used by the following parties:

- Members of the BMT CTN Data and Coordinating Center and BMT CTN #1204 Protocol Team
- National Heart, Lung, and Blood Institute (NHLBI) and the National Cancer Institute (NCI), both of the National Institutes of Health (NIH), study sponsors
- The National Marrow Donor Program (NMDP) and the Center for International Blood and Marrow Transplant Research (CIBMTR)
- U.S. government agencies that are responsible for overseeing research such as the Food and Drug Administration (FDA) and the Office of Human Research Protections (OHRP)
- U.S. government agencies that are responsible for overseeing public health concerns such as the Centers for Disease Control (CDC) and federal, state and local health departments

1 HIPAA is the Health Insurance Portability and Accountability Act of 1996, a federal law related to privacy of health information

_____

_____

_____

e. Right to Refuse to Sign this Authorization: I do not have to sign this Authorization. If I decide not to sign the Authorization, I will not be allowed to participate in this study or receive any research-related treatment that is provided through the study. However, my decision not to sign this authorization will not affect any other treatment, payment, or enrollment in health plans or eligibility for benefits.

f. Right to Revoke: I can change my mind and withdraw this authorization at any time by sending a written notice to the Principal Investigator to inform the researcher of the decision. If I withdraw this authorization, the researcher may only use and disclose the protected health information already collected for this research study. No further health information about my child (me) will be collected by or disclosed to the researcher for this study.

g. Potential for Re-disclosure: My individual health information disclosed under this authorization may be subject to re-disclosure outside the research study and no longer protected. Examples include potential disclosures for law enforcement purposes, mandated reporting for abuse or neglect, judicial proceedings, health oversight activities and public health measures.

h. This authorization does not have an expiration date. However, you can elect at any time to withdraw your authorization to participate in the study.

You will receive a copy of this form. If you need more information about this study, ask the study doctor.

TITLE: Reduced-Intensity Conditioning for Children and Adults with Hemophagocytic Syndromes or Selected Primary Immune Deficiencies (RICHI)

PROTOCOL NUMBER: BMT CTN #1204

PRINCIPAL INVESTIGATOR(S):

Name:

Address:

Email:

Phone:

Fax:

I have read and understood this Consent Form. The nature and purpose of the research study has been explained to me.

- I have had the chance to ask questions, and understand the answers I have been given. I understand that I may ask questions at any time during the study.
- I freely agree to be a participant in the study.
- I understand that I may not directly benefit from taking part in the study.
- I understand that, while information gained during the study may be published, I will not be identified and my personal results will stay confidential.
- I have had the chance to discuss my participation in this research study with a family member or friend.
- I understand that I can leave this study at any time, and doing so will not affect my current care or prevent me from receiving future treatment.
- I understand that I will be given a copy of this signed consent form.

_____ _____
Participant Name Date

_____ _____
Signature Date

I certify that I have provided a verbal explanation of the details of the research study, including the procedures and risks. I believe the participant has understood the information provided.

_____ _____
Name of Counseling Physician Date

_____ _____
Signature of Counseling Physician Date

Pediatric Assent to Participate in Research

For Children Ages 7 to 17 years old

Study Title: Reduced-Intensity Conditioning for Children and Adults with Hemophagocytic Syndromes or Selected Primary Immune Deficiencies

Protocol: BMT CTN 1204

A. Why am I here?

We are inviting you to join our study because you will receive a bone marrow transplant to treat your disease. A transplant uses blood-making cells from another person (donor) to replace your cells that are not healthy. A donor is the name for a person who gives some of their blood-making cells for a transplant.

B. Why are you doing this study?

We want to learn if transplant works to cure your disease.

C. What will happen to me?

Before your transplant, you will have check-ups with the study doctors. Then, you will get a small tube put in your chest in the operating room (you will be asleep for this). The small tube makes it easier for you to get your medicines. It will also make it easier and less painful for drawing blood for tests.

We will give you medicines that will help make the cells from your donor grow in your body. These medicines might make you feel sick. You might throw up, lose your hair, or get sores in your mouth.

After you're done taking the medicines, you will get cells from your donor. This is your transplant. Your donor can be your sister or brother (related) or someone you don't know (unrelated). Your new cells will come from your donor's bone marrow. The cells will make new and healthy cells in your body.

Sometimes the donor cells can cause a problem called graft versus host disease (GVHD). GVHD happens when your body attacks the donor cells. It can give you diarrhea, a skin rash, make you feel sick and throw up, or make you not feel hungry. Your doctors will give you medicines to try to make sure you don't get GVHD.

You will stay in the hospital for several days before your transplant and for about 4 weeks after your transplant. After you go home, you will need to go back to see your doctor often.

It is possible that your disease will come back. If this happens, your doctor will find another way to treat you.

D. Will it hurt?

For your transplant, we will put a small tube in your chest. It might hurt a little and you might bleed a little. Your doctor and nurses will make sure you feel as little pain as possible.

E. Will the study help me?

We don't know if the study will help you or not.

F. What if I have questions?

You can ask any questions that you have about the study. If you forget to ask a question and think of it later, you can call me [*insert office number*]. You can also ask your question the next time you see me.

You can call the study office at any time to ask questions about the study.

G. Do I have to be in this study?

You don't have to be in this study. Your doctor and nurses will not be mad at you if you don't want to join. If you decide you don't want to be in this study, you should talk to your doctor, nurses and parents about other ways to treat your disease.

You can say yes now and change your mind later.

Be sure to talk this over with your parents before you decide if you want be in the study. We will also ask your parents to give their permission for you to join this study.

Writing your name on this page means that you agree to be in the study and know what will happen to you. If you decide to quit the study, all you have to do is tell your doctor.

You and your parent or guardian will get a copy of this form after you sign it.

Signature of Child	Date
Print Name of Child	Age of Child

Certification of Counseling Healthcare Professional: I certify that the nature and purpose, the potential benefits, and possible risks associated with participation in this study have been explained to the above individual and that any questions about this information have been answered.

Counseling Healthcare Professional	Date

APPENDIX C

LABORATORY PROCEDURES

APPENDIX C

LABORATORY PROCEDURES

Participants consenting to the optional future research will have samples collected for future research supporting the protocol. All research sample aliquots will be given unique bar code designations that generally cannot be linked back to the participant's name or other identifying information, though participants are given the opportunity to consent to research including genetic data, in which case the results could theoretically include identifying information. Laboratory test results, clinical information, etc., associated with the coded samples may be provided to the Investigators to associate biological findings with clinical outcomes. Investigators will be able to link results from serial aliquots (for example: Pre-conditioning, Day +1, Day +30) plasma samples. Similarly, investigators will be able to link data from different sample types (for example: protein data from plasma and gene expression data from peripheral mononuclear cells). All research samples will be collected and shipped same-day to the BMT CTN Repository for processing and sample aliquot storage. Priority for biological samples will be determined according to the BMT CTN# 1204 team, along with the BMT CTN Biomarker Committee.

The rationale for this sample collection strategy is to enable independently funded correlative biology studies. The sample collection schedule is designed to allow development of studies to test the role of early inflammation and alemtuzumab pharmacokinetics on clinical outcomes including immune reconstitution, engraftment and development of mixed chimerism, development and clearance of viral infections, and development of GVHD. Endpoints may be determined by clinical reporting, with requirements outlined in the body of the protocol, and/or by specific research tests. For example, chimerism (whole and CD3+ fractions) will be collected in clinical labs for all patients, though more refined lineage-specific chimerism may be tested on a research basis. Samples at all time points will also be banked for unspecified future research.

Biomarker Approach	**Sample Type**	**Pre-Conditioning**	**Day -7**	**Day -1**	**Day +1**	**Day +14**	**Day +28**	**Day +42**	**Day +70**	**Day +100**	**Day +180**	**Day +365**
Proteonomics	Plasma	X		X		X	X	X	X	X	X	X
Gene Expression	PBMC	X		X		X	X	X	X	X	X	X
Alemtuzumab Concentration	Plasma	X	X	X	X	X	X	X				
Clinical Endpoints	**Sample Type**	**Pre-Conditioning**	**Day -7**	**Day -1**	**Day +1**	**Day +14**	**Day +28**	**Day +42**	**Day +70**	**Day +100**	**Day +180**	**Day +365**
Early Inflammation		X	X	X								
Immune Reconstitution							X	X	X	X	X	X
Chimerism							X	X	X	X	X	X
Viral Infections		X	X	X	X	X	X	X	X	X	X	X
GVHD							X	X	X	X	X	X

Research sample collection and storage plan outlined below:

Subjects	Research Sample Type	Peripheral Blood Collection Volume	Stored Material	Aliquots Stored
Patients who consent to future research	Peripheral Blood	**20 mL (Sodium Heparin Tubes)** Maximum blood volume collection limit at each scheduled time point **Pediatric patients (< 6.7 kg):** 3 mL/kg	Plasma	**Maximum 12 aliquots** ~0.5 to 1.0 mL aliquots; stored at -80°C (If ≤ 2 mL plasma: stored in 0.25 mL aliquots)
			Viable PBMC	**Maximum 6 aliquots** 1.0 mL aliquots containing ~2.5-5.0 x 10^6 PBMC; controlled-rate frozen and stored in LN (When PBMC recovery is low: stored in 1.0 mL aliquots containing ~1.0 x 10^6 PBMC)

***** All blood samples will be collected and shipped same-day for next-day processing and storage at the BMT CTN Research Sample Repository.

APPENDIX D

LANSKY AND KARNOFSKY PERFORMANCE STATUS SCALES

APPENDIX D

LANSKY and KARNOFSKY PERFORMANCE STATUS SCALES

LANSKY SCALE (< 16 YEARS)

Percentage	
100	Fully active
90	Minor restriction in physically strenuous play
80	Restricted in strenuous play, tires more easily, otherwise active
70	Both greater restrictions of, and less time spent in, active play
60	Ambulatory up to 50% of time, limited active play with assistance/supervision
50	Considerable assistance required for any active play; fully able to engage in quiet play
40	Able to initiate quiet activities
30	Needs considerable assistance for quiet activity
20	Limited to very passive activity initiated by others (e.g., TV)
10	Completely disabled, not even passive play
0	Dead

KARNOFSKY SCALE (≥ 16 YEARS)

Percentage	
100	Normal health
90	Minor symptoms
80	Normal activity with some effort
70	Unable to carry on normal activity but able to care for oneself
60	Requires occasional help with personal needs
50	Disabled
40	Requires considerable assistance and medical care
30	Severely disabled, in hospital
20	Very sick, active support needed
10	Moribund
0	Dead

REFERENCE Karnofsky DA: Meaningful clinical classification of therapeutic responses to anti-cancer drugs. Editorial: Clin Pharmacol Ther 2:709-712, 1961.

APPENDIX E

RECOMMENDATIONS FOR HLH THERAPY PRIOR TO HCT AND FOR HLH SALVAGE THERAPY

APPENDIX E

RECOMMENDATIONS FOR HLH THERAPY PRIOR TO HCT AND FOR HLH SALVAGE THERAPY

1. Patients with active HLH should be treated according to the Histiocyte Society HLH-94 Protocol prior to HCT.[1]
2. For patients with evidence of refractory HLH despite treatment per HLH-94 (As determined by clinician, but may include persistent fever; rising ferritin, rising soluble IL2Rα, rising LDH; clinical and lab evidence of organ damage/failure(Jordan et al., 2011)
   a. Re-escalate dexamethasone and etoposide to maximum of etoposide 150 mg/m^2 twice weekly and dexamethasone 10 mg/m^2/day, then wean etoposide frequency and dexamethasone dose as tolerated.
   b. If HLH is refractory despite maximum etoposide and dexamethasone, consider trial of alemtuzumab "salvage" therapy:
      - Alemtuzumab: 0.1 mg/kg (IV or subcutaneous) x 1 day, then 0.3 mg/kg (IV or subcutaneous) x 3 days
      - Continue dexamethasone at 10 mg/m^2/day x 1 week, then wean as tolerated back to either "Induction" or "Continuation" per HLH-94
      - Discontinue cyclosporine while patient has symptoms of active/refractory HLH (Risk of PRES may be associated with cyclosporine and active HLH (Thompson et al., 2009))
3. All patients with history of CNS involvement by HLH (pleocytosis, elevated protein, abnormal MRI, or hemophagocytosis in CSF) should undergo diagnostic LP and/or at Day +30 (± 1 week), or at any time of unexplained mental status change. Brain MRI may be substituted for LP if patients are not able to tolerate the procedure. Intrathecal therapy is recommended, when clinically safe to administer in patients with pleocytosis or hemphagocytosis in CSF, or MRI consistent with HLH-associated inflammation.

   Recommended therapy for CNS inflammation in HLH: Weekly intrathecal therapy is generally continued until at least 1 week after resolution of CNS involvement (both clinical and CSF indices): age < 1 year: 6 mg MTX/8 mg hydrocortisone; 1-2 years, 8 mg MTX/10 mg hydrocortisone; 2-3 years, 10 mg MTX/12 mg hydrocortisone; > 3 years, 12 mg MTX/15 mg hydrocortisone (Jordan et al., 2011).

APPENDIX F

DERIVATION OF A SEQUENTIAL TEST STATISTIC FOR CENSORED EXPONENTIAL DATA

APPENDIX F

DERIVATION OF A SEQUENTIAL TEST STATISTIC FOR CENSORED EXPONENTIAL DATA

Background – The Sequential Probability Ratio Test

Let $f(.,\theta)$ be the density function for random variable X. According to Neyman and Pearson, the most powerful test of $H_0:\theta=\theta_o$ versus $H_1:\theta=\theta_1$ decides in favor of H_1 or H_0 if $L_n > c_\alpha$ or $L_n < c_\alpha$, respectively, where $L_n = \prod_i^n f(x_i;\theta_1)/f(x_i;\theta_0)$ is the likelihood ratio, and c_α is determined to have the size α. When the sample size is not fixed in advance, further improvement is possible by using Wald's Sequential Probability Ratio Test (SPRT). The SPRT continues to sample as long as $B < L_n < A$ for some constant $B<1<A$, stops sampling and decides in favor of H_1 as soon as $L_n > A$, and stops sampling and decides in favor of H_0 as soon as $L_n < B$.

The usual measures of performance of such a procedure are the error probabilities α and β of rejecting H_0 when $\theta=\theta_0$, and of accepting H_0 when $\theta=\theta_1$, respectively, and the expected sample size $E(N\mid\theta_j)\equiv E_j(N)$. Wald and Wolfowitz showed that among all tests, sequential or not, for which $\Pr_0(\text{reject } H_0)\le\alpha$ and $\Pr_1(\text{reject } H_0)\le\beta$, and for which $E_j(N)$ are finite, j=0,1, the SPRT with error probabilities α and β minimizes $E_0(N)$ and $E_1(N)$. If, in addition, the $x1, x2, ...$ are independent and identically distributed (i.i.d.) with density function $f(x,\theta)$, with monotone likelihood ratio in $\tau(x)$, then any SPRT for testing θ_0 against $\theta_1(>\theta_0)$ has non-decreasing power function.

For the SPRT with error probabilities α and β, the SPRT boundaries are given approximately by $A=(1-\beta)/\alpha$ and $B=\beta/(1-\alpha)$. The operating characteristics of the SPRT are given by $O(\theta,\alpha,\beta,\theta_0,\theta_1)=(A^{h(\theta)}-1)/(A^{h(\theta)}-B^{h(\theta)})$ where $h(\theta)$ is the non-trivial solution to the equation $\int(f(x;\theta_1)/f(x,\theta_2))^{h(\theta)}f(x;\theta)dx=1$.

The formula $E(N;\theta)=[[(1-O(\theta)]\log A+O(\theta)\log B]/E(z;\theta)$ provides the average sample number for an arbitrary θ. The sample size distribution is very highly skewed, $Var(N)\approx[E(N)]^2$. Thus we will consider a truncated test with maximum sample size of N_0 and simulate to obtain the operating characteristics of the test.

Derivation of the SPRT for Censored Exponential Survival Times

Suppose that we wish to construct a sequential test for the composite null hypothesis that the rate of overall mortality at an early time point t is less than or equal to p_0 versus the alternative hypothesis that it is greater than or equal to p_0. Let us assume that the survival times, $T_1, T_2, ..., T_n$, are i.i.d. with exponential density function $f(T,\theta) = \theta e^{-\theta T}$. Although an exponential model may not fit well for overall mortality, it usually provides a reasonable model over a short time frame for modeling toxicity, so in all discussion below we assume that exponential survival times are censored at time point t. In the exponential parameterization, a t-day survival rate of p_0 translates into a mean survival of μ_0=-t/ln(1-p_0) (rate parameter θ_0 = -ln(1-p_0)/t). .

The SPRT is derived with reference to a simple null and alternative hypothesis for the rate parameter, in this case, $H_0 : \theta = \theta_o$ versus $H_1 : \theta = \theta_1$. The log-likelihood ratio for the exponential in the presence of censoring is $\log \prod_i^n f(x_i;\theta_1) - \log \prod_i^n f(x_i,\theta_0) = d(\log(\theta_1) - \log(\theta_0)) - (\theta_1 - \theta_0)\sum_i^n T_i$, where d is the number of events. The SPRT can be represented graphically when plotting the number of deaths (d) on the y axis against the total time on study $\sum_i^n T_i$ on the x axis. The continuation region in terms of d is bounded by two parallel lines given by

$$\left[\frac{\log(B)}{(\log\theta_1 - \log\theta_0)}\right] + \left[\frac{(\theta_1 - \theta_0)}{(\log\theta_1 - \log\theta_0)}\right]\sum_i^n T_i < d < \left[\frac{\log(A)}{(\log\theta_1 - \log\theta_0)}\right] + \left[\frac{(\theta_1 - \theta_0)}{(\log\theta_1 - \log\theta_0)}\right]\sum_i^n T_i$$

with common slope $(\theta_1 - \theta_0)/(\log\theta_1 - \log\theta_0)$, and intercepts $\log A/(\ln\theta_1 - \ln\theta_0)$ and $\log B/(\ln\theta_1 - \ln\theta_0)$, for the upper and lower bounds, respectively. For monitoring purposes, at an interim analysis calendar time point s, suppose that d(s) events have occurred and that the total time on study is $\sum_i^n T_i(s)$.The cumulative number of events d(s) is plotted on the y axis against the total time on study, $\sum_i^n T_i(s)$. When this graph crosses the upper boundary, the null hypothesis is rejected. In practice, monitoring will be scheduled monthly after the start of enrollment to the study.

A truncated version of the SPRT can be obtained by specifying a maximum sample size. We truncate the SPRT by declaring that if the test has failed to terminate after the maximum sample size, that the null hypothesis will be accepted. Since the probability that the untruncated SPRT would reject the null at the maximum sample size is negligible, it makes little difference how the final boundary value is selected, and this rule is chosen for simplicity. The operating characteristics of this proposed truncated SPRT for censored exponential data can be estimated by simulation.

APPENDIX G

BLOOD PRESSURE LEVELS BY AGE AND HEIGHT PERCENTILE

APPENDIX G

BLOOD PRESSURE LEVELS BY AGE AND HEIGHT PERCENTILE

Blood Pressure Levels for Boys by Age and Height Percentile

Age (Year)	BP Percentile	Systolic BP (mmHg)							Diastolic BP (mmHg)						
		Percentile of Height							Percentile of Height						
		5th	10th	25th	50th	75th	90th	95th	5th	10th	25th	50th	75th	90th	95th
1	50th	80	81	83	85	87	88	89	34	35	36	37	38	39	39
	90th	94	95	97	99	100	102	103	49	50	51	52	53	53	54
	95th	98	99	101	103	104	106	106	54	54	55	56	57	58	58
	99th	105	106	108	110	112	113	114	61	62	63	64	65	66	66
2	50th	84	85	87	88	90	92	92	39	40	41	42	43	44	44
	90th	97	99	100	102	104	105	106	54	55	56	57	58	58	59
	95th	101	102	104	106	108	109	110	59	59	60	61	62	63	63
	99th	109	110	111	113	115	117	117	66	67	68	69	70	71	71
3	50th	86	87	89	91	93	94	95	44	44	45	46	47	48	48
	90th	100	101	103	105	107	108	109	59	59	60	61	62	63	63
	95th	104	105	107	109	110	112	113	63	63	64	65	66	67	67
	99th	111	112	114	116	118	119	120	71	71	72	73	74	75	75
4	50th	88	89	91	93	95	96	97	47	48	49	50	51	51	52
	90th	102	103	105	107	109	110	111	62	63	64	65	66	66	67
	95th	106	107	109	111	112	114	115	66	67	68	69	70	71	71
	99th	113	114	116	118	120	121	122	74	75	76	77	78	78	79
5	50th	90	91	93	95	96	98	98	50	51	52	53	54	55	55
	90th	104	105	106	108	110	111	112	65	66	67	68	69	69	70
	95th	108	109	110	112	114	115	116	69	70	71	72	73	74	74
	99th	115	116	118	120	121	123	123	77	78	79	80	81	81	82
6	50th	91	92	94	96	98	99	100	53	53	54	55	56	57	57
	90th	105	106	108	110	111	113	113	68	68	69	70	71	72	72
	95th	109	110	112	114	115	117	117	72	72	73	74	75	76	76
	99th	116	117	119	121	123	124	125	80	80	81	82	83	84	84
7	50th	92	94	95	97	99	100	101	55	55	56	57	58	59	59
	90th	106	107	109	111	113	114	115	70	70	71	72	73	74	74
	95th	110	111	113	115	117	118	119	74	74	75	76	77	78	78
	99th	117	118	120	122	124	125	126	82	82	83	84	85	86	86
8	50th	94	95	97	99	100	102	102	56	57	58	59	60	60	61
	90th	107	109	110	112	114	115	116	71	72	72	73	74	75	76
	95th	111	112	114	116	118	119	120	75	76	77	78	79	79	80
	99th	119	120	122	123	125	127	127	83	84	85	86	87	87	88
9	50th	95	96	98	100	102	103	104	57	58	59	60	61	61	62
	90th	109	110	112	114	115	117	118	72	73	74	75	76	76	77
	95th	113	114	116	118	119	121	121	76	77	78	79	80	81	81
	99th	120	121	123	125	127	128	129	84	85	86	87	88	88	89

Blood Pressure Levels for Boys by Age and Height Percentile (Continued)

Age (Year)	BP Percentile	Systolic BP (mmHg) Percentile of Height 5th	10th	25th	50th	75th	90th	95th	Diastolic BP (mmHg) Percentile of Height 5th	10th	25th	50th	75th	90th	95th
10	50th	97	98	100	102	103	105	106	58	59	60	61	61	62	63
	90th	111	112	114	115	117	119	119	73	73	74	75	76	77	78
	95th	115	116	117	119	121	122	123	77	78	79	80	81	81	82
	99th	122	123	125	127	128	130	130	85	86	86	88	88	89	90
11	50th	99	100	102	104	105	107	107	59	59	60	61	62	63	63
	90th	113	114	115	117	119	120	121	74	74	75	76	77	78	78
	95th	117	118	119	121	123	124	125	78	78	79	80	81	82	82
	99th	124	125	127	129	130	132	132	86	86	87	88	89	90	90
12	50th	101	102	104	106	108	109	110	59	60	61	62	63	63	64
	90th	115	116	118	120	121	123	123	74	75	75	76	77	78	79
	95th	119	120	122	123	125	127	127	78	79	80	81	82	82	83
	99th	126	127	129	131	133	134	135	86	87	88	89	90	90	91
13	50th	104	105	106	108	110	111	112	60	60	61	62	63	64	64
	90th	117	118	120	122	124	125	126	75	75	76	77	78	79	79
	95th	121	122	124	126	128	129	130	79	79	80	81	82	83	83
	99th	128	130	131	133	135	136	137	87	87	88	89	90	91	91
14	50th	106	107	109	111	113	114	115	60	61	62	63	64	65	65
	90th	120	121	123	125	126	128	128	75	76	77	78	79	79	80
	95th	124	125	127	128	130	132	132	80	80	81	82	83	84	84
	99th	131	132	134	136	138	139	140	87	88	89	90	91	92	92
15	50th	109	110	112	113	115	117	117	61	62	63	64	65	66	66
	90th	122	124	125	127	129	130	131	76	77	78	79	80	80	81
	95th	126	127	129	131	133	134	135	81	81	82	83	84	85	85
	99th	134	135	136	138	140	142	142	88	89	90	91	92	93	93
16	50th	111	112	114	116	118	119	120	63	63	64	65	66	67	67
	90th	125	126	128	130	131	133	134	78	78	79	80	81	82	82
	95th	129	130	132	134	135	137	137	82	83	83	84	85	86	87
	99th	136	137	139	141	143	144	145	90	90	91	92	93	94	94
17	50th	114	115	116	118	120	121	122	65	66	66	67	68	69	70
	90th	127	128	130	132	134	135	136	80	80	81	82	83	84	84
	95th	131	132	134	136	138	139	140	84	85	86	87	87	88	89
	99th	139	140	141	143	145	146	147	92	93	93	94	95	96	97

BP, blood pressure

* The 90th percentile is 1.28 SD, 95th percentile is 1.645 SD, and the 99th percentile is 2.326 SD over the mean.

For research purposes, the standard deviations in Appendix Table B–1 allow one to compute BP Z-scores and percentiles for boys with height percentiles given in Table 3 (i.e., the 5th,10th, 25th, 50th, 75th, 90th, and 95th percentiles). These height percentiles must be converted to height Z-scores given by (5% = -1.645; 10% = -1.28; 25% = -0.68; 50% = 0; 75% = 0.68; 90% = 1.28%; 95% = 1.645) and then computed according to the methodology in steps 2–4 described in Appendix B. For children with height percentiles other than these, follow steps 1–4 as described in Appendix B.

Blood Pressure Levels for Girls by Age and Height Percentile

Age (Year)	BP Percentile	Systolic BP (mmHg) Percentile of Height 5th	10th	25th	50th	75th	90th	95th	Diastolic BP (mmHg) Percentile of Height 5th	10th	25th	50th	75th	90th	95th
I	50th	83	84	85	86	88	89	90	38	39	39	40	41	41	42
	90th	97	97	98	100	101	102	103	52	53	53	54	55	55	56
	95th	100	101	102	104	105	106	107	56	57	57	58	59	59	60
	99th	108	108	109	111	112	113	114	64	64	65	65	66	67	67
2	50th	85	85	87	88	89	91	91	43	44	44	45	46	46	47
	90th	98	99	100	101	103	104	105	57	58	58	59	60	61	61
	95th	102	103	104	105	107	108	109	61	62	62	63	64	65	65
	99th	109	110	111	112	114	115	116	69	69	70	70	71	72	72
3	50th	86	87	88	89	91	92	93	47	48	48	49	50	50	51
	90th	100	100	102	103	104	106	106	61	62	62	63	64	64	65
	95th	104	104	105	107	108	109	110	65	66	66	67	68	68	69
	99th	111	111	113	114	115	116	117	73	73	74	74	75	76	76
4	50th	88	88	90	91	92	94	94	50	50	51	52	52	53	54
	90th	101	102	103	104	106	107	108	64	64	65	66	67	67	68
	95th	105	106	107	108	110	111	112	68	68	69	70	71	71	72
	99th	112	113	114	115	117	118	119	76	76	76	77	78	79	79
5	50th	89	90	91	93	94	95	96	52	53	53	54	55	55	56
	90th	103	103	105	106	107	109	109	66	67	67	68	69	69	70
	95th	107	107	108	110	111	112	113	70	71	71	72	73	73	74
	99th	114	114	116	117	118	120	120	78	78	79	79	80	81	81
6	50th	91	92	93	94	96	97	98	54	54	55	56	56	57	58
	90th	104	105	106	108	109	110	111	68	68	69	70	70	71	72
	95th	108	109	110	111	113	114	115	72	72	73	74	74	75	76
	99th	115	116	117	119	120	121	122	80	80	80	81	82	83	83
7	50th	93	93	95	96	97	99	99	55	56	56	57	58	58	59
	90th	106	107	108	109	111	112	113	69	70	70	71	72	72	73
	95th	110	111	112	113	115	116	116	73	74	74	75	76	76	77
	99th	117	118	119	120	122	123	124	81	81	82	82	83	84	84
8	50th	95	95	96	98	99	100	101	57	57	57	58	59	60	60
	90th	108	109	110	111	113	114	114	71	71	71	72	73	74	74
	95th	112	112	114	115	116	118	118	75	75	75	76	77	78	78
	99th	119	120	121	122	123	125	125	82	82	83	83	84	85	86
9	50th	96	97	98	100	101	102	103	58	58	58	59	60	61	61
	90th	110	110	112	113	114	116	116	72	72	72	73	74	75	75
	95th	114	114	115	117	118	119	120	76	76	76	77	78	79	79
	99th	121	121	123	124	125	127	127	83	83	84	84	85	86	87
10	50th	98	99	100	102	103	104	105	59	59	59	60	61	62	62
	90th	112	112	114	115	116	118	118	73	73	73	74	75	76	76
	95th	116	116	117	119	120	121	122	77	77	77	78	79	80	80
	99th	123	123	125	126	127	129	129	84	84	85	86	86	87	88

Blood Pressure Levels for Girls by Age and Height Percentile (Continued)

Age (Year)	BP Percentile	Systolic BP (mmHg) Percentile of Height							Diastolic BP (mmHg) Percentile of Height						
		5th	10th	25th	50th	75th	90th	95th	5th	10th	25th	50th	75th	90th	95th
11	50th	100	101	102	103	105	106	107	60	60	60	61	62	63	63
	90th	114	114	116	117	118	119	120	74	74	74	75	76	77	77
	95th	118	118	119	121	122	123	124	78	78	78	79	80	81	81
	99th	125	125	126	128	129	130	131	85	85	86	87	87	88	89
12	50th	102	103	104	105	107	108	109	61	61	61	62	63	64	64
	90th	116	116	117	119	120	121	122	75	75	75	76	77	78	78
	95th	119	120	121	123	124	125	126	79	79	79	80	81	82	82
	99th	127	127	128	130	131	132	133	86	86	87	88	88	89	90
13	50th	104	105	106	107	109	110	110	62	62	62	63	64	65	65
	90th	117	118	119	121	122	123	124	76	76	76	77	78	79	79
	95th	121	122	123	124	126	127	128	80	80	80	81	82	83	83
	99th	128	129	130	132	133	134	135	87	87	88	89	89	90	91
14	50th	106	106	107	109	110	111	112	63	63	63	64	65	66	66
	90th	119	120	121	122	124	125	125	77	77	77	78	79	80	80
	95th	123	123	125	126	127	129	129	81	81	81	82	83	84	84
	99th	130	131	132	133	135	136	136	88	88	89	90	90	91	92
15	50th	107	108	109	110	111	113	113	64	64	64	65	66	67	67
	90th	120	121	122	123	125	126	127	78	78	78	79	80	81	81
	95th	124	125	126	127	129	130	131	82	82	82	83	84	85	85
	99th	131	132	133	134	136	137	138	89	89	90	91	91	92	93
16	50th	108	108	110	111	112	114	114	64	64	65	66	66	67	68
	90th	121	122	123	124	126	127	128	78	78	79	80	81	81	82
	95th	125	126	127	128	130	131	132	82	82	83	84	85	85	86
	99th	132	133	134	135	137	138	139	90	90	90	91	92	93	93
17	50th	108	109	110	111	113	114	115	64	65	65	66	67	67	68
	90th	122	122	123	125	126	127	128	78	79	79	80	81	81	82
	95th	125	126	127	129	130	131	132	82	83	83	84	85	85	86
	99th	133	133	134	136	137	138	139	90	90	91	91	92	93	93

BP, blood pressure

* The 90th percentile is 1.28 SD, 95th percentile is 1.645 SD, and the 99th percentile is 2.326 SD over the mean.

For research purposes, the standard deviations in Appendix Table B–1 allow one to compute BP Z-scores and percentiles for girls with height percentiles given in Table 4 (i.e., the 5th,10th, 25th, 50th, 75th, 90th, and 95th percentiles). These height percentiles must be converted to height Z-scores given by (5% = -1.645; 10% = -1.28; 25% = -0.68; 50% = 0; 75% = 0.68; 90% = 1.28%; 95% = 1.645) and then computed according to the methodology in steps 2–4 described in Appendix B. For children with height percentiles other than these, follow steps 1–4 as described in Appendix B.

REFERENCE: http://www.nhlbi.nih.gov/files/docs/guidelines/child_tbl.pdf

APPENDIX H

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APPENDIX H

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