UCB Version 1.0 05/07/2009
UCB Version 2.0 04/26/2010
UCB Version 3.0 02/16/2011
UCB Version 4.0 08/02/2011
UCB Version 5.0 12/07/2011
UCB Version 6.0 08/16/2012
UCB Version 7.0 06/14/2013
UCB Version 8.0 04/09/14
UCB Version 9.0 06/06/14
UCB Version 10.0 01/06/15
UCB Version 11.0 04/13/15
UCB Version 12.0 08/30/2017
UCB Version 13.0 11/01/2018
UCB Version 14.0 03/02/2020
UCB Version 15.0 06/01/2021

Umbilical Cord Blood Transplant for Congenital Pediatric Disorders (UCB)

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TABLE OF CONTENTS

CHECKLIST FOR PATIENT ELIGIBILITY AND NECESSARY INFORMATION

(Patient Eligibility Checklist)

Patient ID _____ Patient Name _____

YES NO VALUE/DATE

Any "**NO**" answers will make a patient ineligible for study participation:

____ ____ _____ Patients less than 18 years of age.

____ ____ _____ Patients with a congenital or acquired immunologic, metabolic or hematological pediatric disease (including SCID) in which stem cell transplantation has been beneficial.

____ ____ _____ Related or unrelated UCB units identified as the HSC source with 0-1 antigen mismatch (must be HLA matched at 5-6 HLA- A and B (at low to intermediate resolution) and DRB1 (at high resolution).

____ ____ _____ Total cryopreserved HSC graft cell dose must be $\geq 5 \times 10^7$ nucleated cells per kilogram recipient body weight.

____ ____ _____ Lansky/Karnofsky scores ≥ 60.

____ ____ _____ Patient has DLCO > 50% predicted or FEV1 > 50% predicted, if applicable.

____ ____ _____ Patient/Guardian able to give informed consent.

Any "**YES**" answers will make a patient ineligible for study participation:

____ ____ _____ Severe intercurrent infection.

____ ____ _____ Severe renal disease (Creatinine > 3X normal for age).

____ ____ _____ Severe hepatic disease (direct bilirubin > 3mg/dl, or SGOT > 500).

____ ____ _____ Patients with symptomatic cardiac failure unrelieved by medical therapy or evidence of significant cardiac dysfunction by echocardiogram (shortening fraction < 20%).

____ ____ _____ HIV positive.

Signature of MD _____ Date _____

To check eligibility of a patient, call Dr. Martinez at 832-824-4692.

1.0 OBJECTIVES

1.1 To determine the safety and overall survival at 100 days, 1 year, and 3 years after umbilical cord blood transplant in pediatric patients with congenital diseases.

1.2 To evaluate donor engraftment at 100 days, 6 months, and 12 months after transplant.

1.3 To determine neutrophil and platelet recovery at Day 42.

1.4 To estimate the risk of severe Grade III-IV acute GvHD at Day 100.

1.5 To estimate the risk of chronic GvHD at 1 year.

2.0 BACKGROUND AND RATIONALE

2.1 Introduction

Umbilical cord blood (UCB) is a readily available alternative source of HSCs that is capable of reconstituting hematopoiesis after myeloablative therapy. A recent survey by the Institute of Medicine found that more than 180,000 UCB units have been banked and more than 6,000 unrelated donor UCB transplantations have been performed[1-8]. In recent years, use of umbilical cord blood as an alternative source of transplantable hemopoietic stem cells has increased substantially, extending the availability of this treatment, especially for children.

2.2 Background of Congenital Diseases

Many children with rare congenital diseases such as immunodeficiencies, familial histiocytic disorders, bone marrow failure syndromes, lysosomal and peroxisomal inborn errors of metabolism characteristically present in the first decade of life with complications that prove debilitating and life threatening.

Primary cellular immunodeficiencies are a group of inherited disorders characterized by severe impairment of the innate or adaptive immune systems, which generally leads to early death from infectious complications. These disorders can be further categorized depending on the primary cell lineage affected, into two major groups: SCID (Severe Combined Immunodeficiency syndrome) with absent T and/or B lymphocyte function and non-SCID (defective T, B lymphocyte, or granulocytes). The most common non-SCID diseases include: Wiskott-Aldrich syndrome (WAS), T cell deficiencies (Omenn syndrome, HLA-class II deficiency, CD40 ligand deficiency), Phagocytic-cell disorders (agranulocytosis, Chronic granulomatous disorders, Leukocyte adhesion deficiency), and Hemophagocytic syndromes (Familial Lymphohistiocytosis, Chediak-Higashi syndrome, Griscelli syndrome). While improved supportive care has extended the life span of patients affected by these diseases and recent advances in gene therapy hold significant promise, definitive cure is generally only achieved by allogeneic hematopoietic stem cell transplant (HSCT).

The storage diseases represent a diverse group of lysosomal and peroxisomal disorders. Single gene defects involving a lysosomal hydrolytic enzyme or vital peroxisomal function lead to these devastating diseases and their systemic abnormalities affect multiple organs including the brain. Progressive loss of neurodevelopmental milestones and/or neurologic function is common. Shortened life-expectancy is often due to cardiopulmonary disease. Treatment options depend upon the stage of disease and rate of progression, and include HSCT, enzyme replacement (ERT), substrate depletion and gene therapy. The clinical response to ERT is variable, depending upon the disease, the organ or tissue involved the dose of enzyme, and the schedule of

administration. Of particular importance is the observation that there is limited-to-no penetration of exogenous, intravenous enzyme into the nervous system. These limitations make HSCT an attractive alternative to ameliorate/cure the disease, with best results obtained if the procedure is performed in the early stages of the disease.

2.3 Clinical Experience with Stem Cell Transplant for Congenital Diseases

The first successful allogeneic human HCT was performed in an infant with SCID who remains well more than 3 decades following an HLA-matched sibling transplant. Transplantation of unmodified BM from an HLA-identical sibling remains the treatment of choice for infants with SCID in whom overall survival now exceeds 90% if the transplant is performed early. Despite a later median age of transplant due to the time needed to perform a search, some groups report good outcomes (OS 63-67%) with unrelated donor transplants. Grunebaun et al.[23] reported that recipients of matched unrelated donor stem cells had superior 2 year overall survival (81%) compared to recipients of mismatched related donor grafts (53%). Antoine et al.[24] reported 3-year survival, with evidence of sustained engraftment and improvement of the immunodeficiency disorder, which was significantly better for HLA-identical than for HLA-mismatched transplantation (77% vs 54%; p=0.002). The main causes of death were infections; graft vs host disease, toxic effects of the conditioning regimen and rejection[24].

In selected lysosomal and peroxisomal inborn errors of metabolism (IEM) such as Hurlers syndrome (MPS I), Maroteaux-Lamy syndrome (MPS IV), α-mannosidosis, childhood onset cerebral X-linked adrenoleukodystrophy (COCALD), metachromatic leukodystrophy (MLD) and globoid-cell leukodystrophy (GLD), timely HSCT positively modifies the natural history of the disease by providing cells of hematopoietic origin capable of providing the missing gene product. Some benefit has also been described from engraftment of donor-derived glial cells in the brain. Stem cell transplant is currently the only method of providing a self-replenishing source of enzyme to these patients. Transplantation of marrow stem cell from a non-deficient donor will provide cells of the monocyte /macrophage system which are of donor origin and therefore competent to produce the target enzyme. These donor derived cells replace the host fixed tissue macrophages in the liver, bone, lung, lymph nodes, and skin. At least for some of the lysosomal and peroxisomal storage diseases, enzyme produced in the fixed tissue macrophage system is capable of uptake into recipient cells, where it is targeted to lysosomes. By this process, accumulated storage material can be hydrolyzed, reducing organomegaly and stabilizing or improving organ function. Since the marrow continuously replenishes the body with donor fixed tissue macrophages, the effects can be expected to be permanent. Over 200 marrow transplant have been performed in the past decade in patients with various lysosomal and peroxisomal storage diseases. Most of this experience is with matched related bone marrow transplantation. The rates of engraftment and 2 yr OS ranged between 60-65% and 50%, respectively.

In most of these congenital diseases, the outcomes of HSCT is affected mainly by (1) age at transplantation, (2) stem cell source, with best results using HLA-identical sibling donors, (3) timing of HSCT, in which delay can substantially compromise effectiveness of HSCT, (4) type of preparative regimen, and (5) cell dose.

2.4 Justification for Umbilical Cord Blood Transplantation

The major limiting step to HSCT in these hereditary disorders is the availability of a suitable

matched stem cell source. It is well known that delaying a HSCT in patients with congenital diseases increases the risk of infections, organ damage and progression of the disease. Recent reports demonstrate the successful use of unrelated cord blood transplantation for the treatment of malignant and non-malignant diseases. Currently, large inventories of UCB units are available in public banks for transplantation in those lacking bone marrow donors. In some experienced centers, unrelated umbilical cord blood (UCB) has become an acceptable and sometimes a preferred stem cell source because of its availability and lower incidence of graft-vs-host-disease (GvHD). Recent studies showed that patients transplanted at a very young age (2-3 yrs/old) before the onset of clinical symptoms can develop neurologically at a normal rate 3-5 yrs after transplant. The first umbilical cord transplantation (UCBT) was performed in 1988, in a child with Fanconi Anemia. Since then, several thousand UCBT has been performed for malignant and non-malignant disorders.

In a recent report by Prasad, Kutzberg et al.[19], 159 patients with inherited metabolic diseases were review after unrelated cord blood transplant. A total of 97% achieved high (> 90%) donor chimerism with normalization of serum enzyme levels. In a multivariate analysis, patient age of 2 years or younger at transplantation, more than 2.1 x 10^5/kg of infused CD34, and more than 5.7 x 10^4/kg of infused CFUs were favorable factors for neutrophil engraftment. Grade III-IV acute GvHD occurred in 10.3% of patients. Extensive cGvHD occurred in 10.8% of patients by 1 year. Overall survival at 1 and 5 years was 71.8% and 58% in all patients. 8 patients had graft failure for an incidence of 12.9%.[19] These findings were comparable and better than those with marrow products.

The total nucleated cell dose and CD34 cell dose are extremely important in the setting of umbilical cord blood transplant. In a report by Gluckman et al.[14], a graft nucleated cell dose > 3.7 x 10^7/kg was associated with shorter time to neutrophil recovery (25 days vs 35 days). Similarly, Rubinstein et al.[7,16] demonstrated that a step-wise increase in graft nucleated cell dose was associated with progressively shortened time to neutrophil recovery. Rubinstein et al.[16] suggested that the threshold was a cryopreserved nucleated cell dose $\geq$ 2.5 x 10^7/kg; while the Minnesota group observed a threshold for the infused CD34+ cell dose of $\geq$ 1.7 x 10^5/kg[5]. Other prognostic factors have also been reported but these have not been consistently observed. As one example, Rubinstein et al.[7,16] observed a relationship between HLA match and neutrophil recovery (23 days for HLA matched vs 28 days for HLA mismatched grafts, $p = 0.0027$); this association was not observed by Gluckman et al.[14] and Wagner et al.[5]

In larger series the neutrophil engraftment has been reported as high as 92%.[5] The incidence of acute GvHD reported in larger series ranges from 33-44% to 11-22% for grades II-IV and III-IV acute GvHD, respectively[9-12]. The incidence of chronic GvHD ranges from 0-25%.[9-12] These results are particularly notable since most UCB donor-recipient pairs are 1-2 HLA antigen mismatched.

There is little information regarding outcome of UCST for Primary Immunodeficiency Diseases (PID). Although PIDs, make up a small proportion of disorders amenable to treatment by HSCT, cord blood is an ideal source for many of these patients, who are often small, so that the limited stem cell dose in a cord blood unit is often adequate. Diaz de Heredia et al.[21] reported an engraftment rate of 100% in 15 patients with SCIDs after UCBT. Of these patients, 33%

developed Grade III-IV aGvHD and 9% developed cGvHD. The overall survival reported at 5 years was 73% with a follow-up post transplant of 64 months. The most common causes of death included grade IV aGvHD and interstitial viral pneumonitis. Nine of the fifteen patients received horse antithymocyte globulin (ATG). One of those patients died of aGvHD after receiving a 4/6 HLA match cord unit. Only one patient of 11 patients receiving a unit matched at 5-6 antigens developed aGvHD III-IV.[21]

Bhattacharya et al.[22] reported a very low incidence of GvHD after a 6/6 HLA antigen matched UCBT for PIDs in fourteen patients transplanted (2/14). They reported a median time of neutrophil engraftment of 22 days and a median time of 51 days to platelet independence. Patients with high risk diagnosis and increase risk of graft failure (MHC class II, Reticular dysgenesis, Omen syndrome) were successfully transplanted with 100% chimerism in all cell lineages after the use of a conditioning regimen. There was an increase mixed chimerism in patients transplanted without a conditioning regimen. The reported overall survival was 86%.[22]

Hence, UCB transplants offer several advantages over adult bone marrow or peripheral blood stem cell transplants, including: 1) rapid availability, 2) absence of donor risk, 3) low risk of transmissible infectious diseases, 4) low risk of acute GvHD in the setting of HLA mismatch (as compared to recipients of unrelated donor marrow and peripheral blood). UCB is particularly beneficial for patients of ethnic and racial minority descent for whom adult marrow and blood donors often cannot be identified.

2.5 Major Problems after Umbilical Cord Blood Transplant

The disadvantages of UCSCT include non-availability of the donor for booster stem cell infusion, lack of viral specific cytotoxic T cells, slower engraftment and small stem cell dose. Infection related treatment related mortality is still of concern after UCBT; rates of hemopoietic recovery are slower after UCBT; therefore infectious complications including viral infections or reactivations occur frequently. The two major causes of death after umbilical cord blood transplantation for congenital disorders reported in the literature are graft failure (20%) and infection (15%).

2.6 Protocol Proposal

In this study, we aim to address engraftment, incidence of acute and chronic Graft vs Host Disease, incidence of infections, immune reconstitution and overall survival in congenital childhood disorders after Umbilical Cord Stem Cell Transplant. With this study we are proposing an alternative conditioning regimen that will address the two major causes of death after UCBT for congenital diseases. To improve engraftment rate and reduce infections we will incorporate fludarabine in the conditioning regimen. Purine-analogs like fludarabine, have emerged as powerful immunosuppressive agents with minimal systemic toxicities. Fludarabine-based, preparative regimens have been shown to allow alloengraftment in the related and unrelated donor setting with acceptable systemic toxicity in patients with a variety of hematologic malignancies.[13-15]

Encouraging data on the fludarabine-cytoxan regimen have been reported. A fludarabine-based, conditioning regimen, with adequate immunosuppressive activity could conceivably allow engraftment of stem cells from alternative donors in hematologic malignancies patients with

acceptable engraftment rates and low transplant-related mortality. Regimen-related toxicity is believed to be a major contributing factor to GVHD. Therefore this approach may also lead to reduced GVHD, as some investigators have suggested.[16, 17]

We will attempt to decrease the rate of viral infection and reactivation by removing ATG (thymoglobulin) from our conditioning regimen and substituting mycophenolate mofetil (MMF). We anticipate that the removal of ATG will facilitate immune reconstitution after UCBT. The addition of fludarabine should compensate for any increased risk of graft failure due to removal of the ATG.

3.0 ELIGIBILITY

3.1 Inclusion Criteria

3.1.1 Patients less than 18 years of age.

3.1.2 Patients with a congenital or acquired immunologic, hematological or metabolic pediatric diseases (including SCID) in which stem cell transplantation has been beneficial.

3.1.3 Related or Unrelated Umbilical Cord Blood Unit with 0-1 antigen mismatch (5-6 HLA- A and B (at low to intermediate resolution) and DRB1 (at high resolution).

3.1.4 Total cryopreserved HSC graft cell dose must be $\geq 5x10^7$ nucleated cells per kilogram recipient body weight.

3.1.5 Lansky/Karnofsky scores ≥ 60.

3.1.6 Patient has DLCO > 50% predicted or FEV1 > 50%, if applicable.

3.1.7 Written informed consent and/or signed assent line from patient, parent or guardian.

3.2 Exclusion Criteria

3.2.1 Patients with uncontrolled infections as assessed by the principal investigator only. For bacterial infections, patients must be receiving definitive therapy and have no signs of progressing infection for 72 hours prior to starting conditioning. For fungal infections patients must be receiving definitive systemic antifungal therapy and have no signs of progressing infection for 1 week prior to enrollment. Progressing infection is defined as hemodynamic instability attributable to sepsis or new symptoms, worsening physical signs or radiographic findings attributable to infection. Persisting fever without other signs or symptoms will not be interpreted as progressing infection.

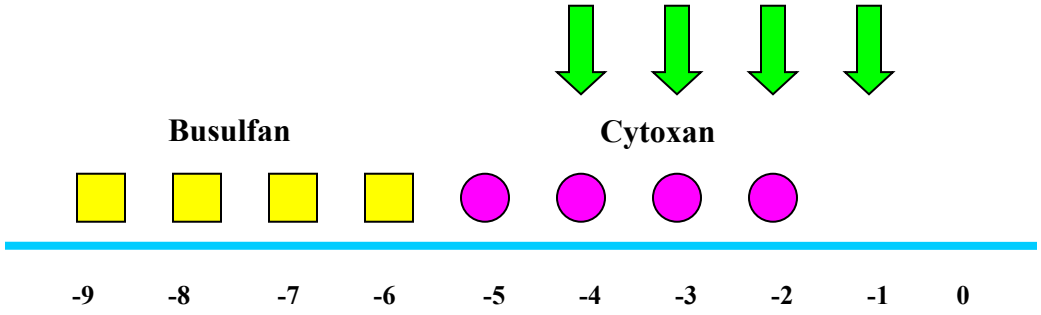
3.2.2 Severe renal disease (creatinine > 3X normal for age).

3.2.3 Severe hepatic disease (direct bilirubin > 3 mg/dL or SGOT > 500).

3.2.4 Patients with symptomatic cardiac failure unrelieved by medical therapy or evidence of significant cardiac dysfunction by echocardiogram (shortening fraction < 20%).

3.2.5 HIV-positive.

4.0 PREPARATIVE THERAPY/IMMUNOSUPPRESSIVE THERAPY



Day	Agent	
-9	Busulfan	
-8	Busulfan	
-7	Busulfan	
-6	Busulfan	
-5	Cytoxan	50 mg/kg/day IV over 2 hours. Can be given over 1 to 4 hours if needed (MESNA; continuous infusion or 5 times daily)
-4	Cytoxan Fludarabine	50 mg/kg/day IV over 2 hours. Can be given over 1 to 4 hours if needed (MESNA; continuous infusion or 5 times daily) 40 mg/m^2/day IV over 1 hour for patients > 10 kg, or 1.3 mg/kg/day for patients ≤ 10 kg

-3	Cytoxan Fludarabine	50 mg/kg/day IV over 2 hours. Can be given over 1 to 4 hours if needed (MESNA; continuous infusion or 5 times daily) 40 mg/m^2/day IV over 1 hour for patients > 10 kg, or 1.3 mg/kg/day for patients ≤ 10 kg
-2	Cytoxan Fludarabine	50 mg/kg/day IV over 2 hours. Can be given over 1 to 4 hours if needed (MESNA; continuous infusion or 5 times daily) 40 mg/m^2/day IV over 1 hour for patients > 10 kg, or 1.3 mg/kg/day for patients ≤ 10 kg
-1	Fludarabine	40 mg/m^2/day IV over 1 hour for patients > 10 kg, or 1.3 mg/kg/day for patients ≤ 10 kg
0		Stem Cell Infusion

4.1 Busulfan: Busulfan (intravenous BUSULFEX) dosing will be as follows:

Patients ≤ 12 kg: 1.1 mg/kg/dose IV every 6 hours for 16 doses total; patients > 12 kg: 0.8 mg/kg/dose IV every 6 hours for 16 doses. Administration and pharmacokinetic monitoring will be performed as per standard practice. Phenytoin/fosphenytoin will be given in accordance with standard TCH Formulary guidelines.

4.2 Cytoxan: Cytoxan (50 mg/kg/dose) will be given IV on Days -5, - 4, -3, and -2 over 2 hours. It can be given over 1 to 4 hours as needed and as determined by the treating physician. The total dose to be given over 4 days is 200 mg/kg. Mesna will be given in accordance with standard Blood and Marrow Transplant program recommendations.

4.3 Fludarabine: Fludarabine will be given IV daily over 1 hour for 4 days. Preparation, administration and monitoring will be according to standard practice procedure.

4.4 Post-Transplant Immunosuppression:

4.4.1 Cyclosporin A (CSA) will begin on Day -2. The initial dose will be 2.5 mg/kg IV over 2 hours every 12 hours. Dose adjustments will be made on the basis of toxicity and low CSA levels with a trough level of < 200 mg/L. Once the patient can tolerate oral medications and has a normal gastrointestinal transit time, CSA will be converted to an oral form.

4.4.2 MMF will begin on Day 0 at a dose of 15 mg/kg IV or orally TID and will be discontinued on Day +45 unless GvHD is present.

4.5 Supportive Care:

4.5.1 Supportive care will be provided as per standard operating procedures of the

Blood and Marrow Stem Cell Transplant program at the Texas Children's Hospital, including all prophylactic and therapeutic clinical care issues. These practices may be modified if necessary for any individual patient in order to optimize care.

4.5.2 IVIG (intravenous immunoglobulin 500 mg/kg per dose – pharmacy to round dose to nearest vial size) will be given as per CAGT SOP for infection prophylaxis.

4.5.3 G-CSF (Granulocyte-Colony Stimulating Factor: 10 mcg/kg IV/SC) to be given daily starting at Day +7 until ANC > 2500 for three consecutive days.

5.0 EVALUATIONS DURING THE STUDY

5.1 Screening Procedures; Pre-HCT:

5.1.1 Physical examination by Pediatric Bone Marrow Transplant physician.
5.1.2 Evaluation by pediatric neurologist as applicable.
5.1.3 Pulmonary consultation for MPS I (Hurler), Niemann-Pick B and others as applicable.
5.1.4 Pediatric Cardiology consultation for MPS I, MPS VI, MPS VII, and mannosidosis as applicable.
5.1.5 Evaluation with Pediatric Endocrinology as applicable.
5.1.6 Pediatric Ophthalmology consultation as applicable.
5.1.7 Audiology consultation with BAER/BVER as applicable.
5.1.8 Genetic Counseling unless previously done here or at another institution as applicable.
5.1.9 Cerebral MRI with high field strength volumetric acquisition and multiplanar diffusion tensor imaging unless performed at our institution within 1 month as applicable.
5.1.10 Chest radiograph.
5.1.11 Electrocardiogram.
5.1.12 Echocardiography.
5.1.13 Pulmonary Function Tests: DLCO and FEV1 as applicable.
5.1.14 Dual energy E-ray Absorptiometry (DXA) scan with bone age is to be performed on all patients greater than 5 years old as applicable.
5.1.15 Confirmation of diagnosis, if not clearly established. This may include enzyme/substrate quantization, genetic studies, or VLCFA determination for ALD.
5.1.16 CBC with differential, INR/PTT for all patients.
5.1.17 Serum chemistries, including liver function testing.
5.1.18 Endocrine testing including TSH, free thyroxine (FT4), vitamin D 25, vitamin D1,25, intact PTH, IGF-1 and IGF-BP3 for growth hormone deficiency screening as applicable.
5.1.19 For patients with ALD, a low-dose ACTH stimulation test along with renin activity will be performed as applicable.
5.1.20 Urinalysis.
5.1.21 Pregnancy test (serum) as applicable.
5.1.22 HIV testing.
5.1.23 IgG, IgA, and IgM levels.

5.2 Evaluations Between Day 0 and Day 100:

5.2.1 Peripheral blood for STRs or FISH analysis for molecular diagnostics on Days 21, 60, and 100.**5.2.2** For patients with measurable enzymes (including the lysosomal disorders), perform enzyme analysis at Day 42 and Day 100.

5.2.3 Lymphocyte phenotype testing (CD3, CD4, CD8, CD14, CD19 and CD56), other general viral immune reconstitution studies (ex. ELISPOT, spectrotyping, B cell panel) will be performed at Days 21-42, and Days 60-100.

5.2.4 IgG, IgA, and IgM levels at Day 100.

5.2.5 PHA stimulation assay to evaluate T cell function at Days 21-42, and Days 60-100.

5.2.6 History, physical exam, vital signs, and weight at Days 0, 21, 42, 60, and 100.

5.2.7 Performance Status on Day 100.

5.2.8 CBC with differential and platelets at Days 0, 21, 42, 60, and 100.

5.2.9 Electrolytes, BUN, Creatinine, AST, ALT, Bilirubin, Albumin and LDH at Days 0, 21, 42, 60, and 100.

5.3 Evaluations After Day 100:

5.3.1 Peripheral blood with assessment of engraftment by STRs or FISH analysis and enzyme levels will be done as applicable.

5.3.2 Lymphocyte phenotype testing (CD3, CD4, CD8, CD14, CD19 and CD56), other general viral immune reconstitution studies (ex. ELISPOT, spectrotyping, B cell panel) will be performed at 6, 9, 12, and 24 months and 3 years.

5.3.3 IgG, IgA, and IgM levels at 6, 9, 12, and 24 months and 3 years.

5.3.4 PHA stimulation assay to evaluate T cell function at 6, 9, 12, and 24 months and 3 years.

5.3.5 Neuropsychometric testing at 1 year, and yearly for three years.

5.3.6 Echocardiography with LVEF at 1 year, 2 years, and 3 years.

5.3.7 History, physical exam, vital signs, weight, performance status at months 6 and 9, and at 1 year, 2 years, and 3 years.

5.3.8 CBC with differential and platelets at months 6 and 9, and at 1 year, 2 years, 3 years and 5 years. INR/PTT at 2 years, and 3 years.

5.3.9 Electrolytes, BUN, Creatinine, AST, ALT, Bilirubin, Albumin and LDH at months 6 and 9, and at 1 year, 2 years, and 3 years.

5.3.10 Pulmonary Function Tests: DLCO and FEV1 as applicable at 1 year, 2 years and 3 years.

5.3.11 For patients that can return at Day 180 for evaluation, evaluations by the multidisciplinary team if applicable.

5.3.12 Yearly visits for evaluation by the multidisciplinary team (neurology, neuropsychology, endocrinology, ophthalmology, and others (cardiology, pulmonary, orthopedics, etc.) for three years.

5.4 Follow-Up Interval:

Patients will be seen in the hospital everyday until discharge. After discharge from the hospital, the patient will be followed in the BMT clinic on a regular basis as recommended by the primary physician.

5.5 Calendar of Study Evaluations:

	Pre-HCT	Day 0	Day 21	Day 42	Day 60	Day 100	M6	M9	M12	M24	M36
Infusion of cells		X									
Hx	X	X	X	X	X	X	X	X	X	X	X
PE	X	X	X	X	X	X	X	X	X	X	X
Weight	X	X	X	X	X	X	X	X	X	X	X
VS	X	X	X	X	X	X	X	X	X	X	X
Performance Status	X					X	X	X	X	X	X
Pregnancy Test	X										
Chest x-ray	X										
ECHO	X								X	X	X
ECG	X										
Pulmonary Function Tests	X									X	X
DXA Scan[2]	X										
Cerebral MRI [1]	X										
Genetic Counseling[1]	X										
Audiology Consultation[1]	X								X	X	X
Ophthalmology Consultation[1]	X								X	X	X
Endocrinology Evaluation[1]	X								X	X	X
Cardiology Consulation[1]	X								X	X	X
Pulmonary Consultation[1]	X								X	X	X
Neurology	X								X	X	X

	Pre-HCT	Day 0	Day 21	Day 42	Day 60	Day 100	M6	M9	M12	M24	M36
Evaluation[1]											
Neuropsychometric Testing									X	X	X
CBC d/p	X	X	X	X	X	X	X	X	X	X	X
INR/PTT	X									X	X
Anti-Xa and heparin cofactor II-thrombin complex	X										
Lytes/BUN/Cr	X	X	X	X	X	X	X	X	X	X	X
AST/ALT/Bili/Alb/ LDH	X	X	X	X	X	X	X	X	X	X	X
Low-dose ACTH per 5.1.19, if applicable	X										
Endocrine testing per 5.1.18	X										
Urinalysis	X										
Enzyme Analysis[3]				X		X					
PB STRs / FISH			X		X	X	X		X	X	X
Lymphocyte subset/PHA assay, other general viral immune reconstitution studies (ex. ELISPOT, spectrotyping, B cell panel)			X[4]		X[4]		X	X	X	X	X
Immunoglobulin levels	X					X	X	X	X	X	X

[1] **As applicable; these evaluations are also performed on Day 180 as applicable.**
[2] **On all patients greater than 5.**
[3] **For patients with measurable enzymes.**
[4] **To be performed between Days 21 and 42, and between Days 60 and 100.**

6.0 STUDY ENDPOINTS

6.1 Engraftment

6.2 Overall survival and organ function:
100 days, 1, and 3years after UCBT.

6.3 Acute and Chronic GvHD:
Assess severity based on standard criteria.

6.4 Systemic Infections:
Classified as infections documented microbiologically occurring within 6 months. Viral load will be monitored for CMV, EBV and AdV as per institutional protocols. If there is any evidence of infection with these viruses, the patient will be started on medical or cellular therapy.

6.5 Enzyme levels (when applicable):
Determine the replaced enzyme levels at Day 100, 6,12, 24, and 36months after transplant.

7.0 STUDY DRUGS

7.1 Busulfan

7.1.1 Therapeutic Classification: Bifunctional alkylating agent.

7.1.2 Pharmaceutical Data: Busulfan (Busulfex Injection® Orphan Medical) is supplied as a sterile solution in single-use ampules containing 60 mg at a concentration of 6 mg/mL. It is provided as a mixture of demethylacetamide (DMA) and polyethylene glycol 400 (PEG400).

7.1.3 Solution Preparation: Busulfan solution for injection must be diluted with 0.9% Sodium Chloride Injection (NS). The diluent quantity must be 10 times the volume of busulfan, ensuring that the final concentration is 0.5 mg/mL. Sample calculation for a 50 kg patient: (50 kg) x (0.8 mg/kg of busulfan) = 40 mg = 6.7 mL. 6.7 mL of busulfan + 67 mL of NS = 74 mL total volume. Final concentration: 0.54 mg/mL.

7.1.4 Stability and Storage Requirements: After dilution with NS or D5W, busulfan is stable at room temperature (25 degrees Celsius) for 8 hours. The infusion must be completed within that time. Prior to mixing: store under refrigeration (2 to 8 degrees Celsius). Busulfan for injection is stable at 4°C for at least 12 months.

7.1.5 Route of Administration: Busulfan should be administered intravenously via a central venous catheter as a two-hour infusion.

7.1.6 Usual Dosage Range: 0.8-1.1 mg/kg/dose given every 6 hours for a total of 16 doses. For patients ≤ 12 kg a dose of 1.1 mg/kg/dose will be used, and for patients > 12

kg the starting dose will be 0.8 mg/kg/dose. Doses are based on actual body weight, unless the patient's weight is greater than 30% of ideal body weight, then dosing will be based on adjusted weight of ideal plus 25%. Busulfan pharmacokinetics will be performed on all patients with dose adjustment as appropriate.

7.1.7 Pharmacokinetics: Doses will be adjusted to achieve the desired plasma area under the curve (AUC) of 800 – 1200 μmol/min/L. Doses will be adjusted as necessary pending the results of the first dose pharmacokinetics. For patients whose AUC values are greater than 5% outside the acceptable AUC range, the dose will be adjusted to achieve a target AUC of 1125 μmol/min/L (midpoint of acceptable range) not to exceed a maximum dose of 1.6 mg/kg per dose of busulfan.

7.1.8 Side-Effects: Myelosuppression, neurotoxicity (manifesting as seizures), mild to moderate nausea and vomiting, mild to moderate tachycardia, skin hyperpigmentation, sterility, and rarely hepatotoxicity (hepatic veno-occlusive disease) and pulmonary toxicity (interstitial fibrosis).

7.1.9 Special Precautions: Increased toxicity in obese patients unless dose is adjusted appropriately. Generalized seizures have been reported after use of high dose busulfan. Phenytoin/fosphenytoin will be given in accordance with TCH Formulary guidelines.

7.1.10 Mechanism of Action: Busulfan is a bifunctional alkylating agent in which two labile methanesulfonate groups are attached to opposite ends of a four-carbon alkyl chain. In aqueous media, busulfan hydrolyzes to release the methanesulfonate groups. This produces reactive carbonium ions that can alkylate DNA. DNA damage is thought to be responsible for much of the cytotoxicity of busulfan.

7.1.11 Human Pharmacology: Busulfan can be administered orally or intravenously. Busulfan achieves levels in cerebrospinal fluid similar to plasma levels. Busulfan is predominantly metabolized by conjugation with glutathione, both spontaneously and by glutathione S-transferase (GST) catalysis. This conjugate undergoes further extensive oxidative metabolism in the liver. Approximately 30% of busulfan and metabolites can be recovered in the urine within 48 hours after administration.

7.2 CYTOXAN (CTX, Cyclophosphamide) - Commercially Available

7.2.1 Formulation: Oral drug is supplied as 25 mg and 50 mg tablets. Injectable form is available as lyophilized cakes containing 100 mg, 200 mg, or 500 mg of active drug, and 75 mg of mannitol per 100 mg of active drug in single-use vials.

7.2.2 Storage: Cytoxan is to be kept dry and at room temperature until reconstitution and use.

7.2.3 Stability: All preparations are stable at room temperature (not to exceed 30 degrees Celsius). Discard reconstituted solutions after 24 hours at room temperature; stable up to 6 days if refrigerated (2-8 degrees Celsius). Since there is no preservative, precautions should be taken to insure sterility, or solution should be discarded within 8

hours. The parenteral form of cytoxan prepared as a liquid oral preparation diluted to < 2 mg/mL can be refrigerated (2-8 degrees Celsius) for up to 14 days.

7.2.4 Administration: 50 mg/kg will be given IV over 2 hours daily for a total of 4 doses. It can be given over 1 to 4 hours (as needed and as determined by the treating physician). Cyclophosphamide may be diluted in D5NS 200 mL/m^2.

7.2.5 Toxicities:

Known Toxicities:

	Common (21-100% Frequency)	**Occasional (5-20% Frequency)**	**Rare (<5% Frequency)**
Immediate:	Anorexia (L), nausea (L), vomiting (L)	Metallic taste (L), Inappropriate ADH secretion1	Transient blurred vision[1], cardiac toxicity with arrythmias[1], myocardial necrosis[2](L)
Prompt:	Myelosuppression (L), alopecia (L)	Hemorrhagic cystitis (L)	
Delayed:	Immunosuppression, gonadal dysfunction/sterility		Pulmonary fibrosis [3] (L)
Late:			Secondary malignancy, bladder fibrosis
Unknown Timing and Frequency	Fetal and teratogenic toxicities [4,5]		

(L) Toxicity may also occur later.
[1] Less common with lower doses.
[2] Only with very high doses.
[3] Risk increased with chest radiation.
[4] Fetal toxicities and teratogenic effects of cytoxan (alone or in combination with other anti-neoplastic agents) have been noted in humans. Toxicities include: chromosome abnormalities, multiple anomalies, pancytopenia, and low birth weight.
[5] Cytoxan is excreted into breast milk. Neutropenia has been reported in breast-fed infants.
Cytoxan is considered to be contraindicated during breast feeding because of the reported cases of neutropenia and because of the potential adverse effects relating to immune suppression, growth, and carcinogenesis.

7.3 FLUDARABINE

7.3.1 Therapeutic Classification: Purine antimetabolite.

7.3.2 Pharmaceutical Data: In vials of 50 mg.

7.3.3 Solution Preparation: Reconstituted with 2 mL of sterile water for injection. The resulting solution will contain 25 mg/mL. For infusion (maximum concentration of 10 mg/mL) intravenously in 60 mL/m^2 of D5W over 1 hour.

7.3.4 Stability and Storage Requirements: Prior to mixing: store under refrigeration at 2 to 8 degrees Celsius. After mixing: stable for 16 days at room temperature, but needs to be used in 8 hours because of the lack of antibacterial preservatives.

7.3.5 Routes of Administration: IV infusion.

7.3.6 Usual Dosage Range: For patients ≤ 10 kg: 1.3 mg/kg/day; for patients > 10 kg: 40 mg/m^2/day given IV over 1 hour for a total of 4 doses.

7.3.7 Side-Effects: Myelosuppression, exacerbation of hemolytic anemia, prolonged immunosuppression, opportunistic infection, and rare neurotoxicity.

7.3.8 Special Precautions: Increased myelosuppression in patients with creatinine clearances of less than 50 mL/min.

7.3.9 Mechanism of Action: A purine antimetabolite modified with fluorine and monophosphate to resist deamination by adenosine deaminase and to increased solubility. Dephosphorylation followed by cellular incorporation and conversion to active triphospahte, which is a competitive inhibitor of DNA synthesis.

7.3.10 Antitumor Data: The drug has greater activity to T cells than B cells, but clinical activity is observed in B cell malignancies.

7.3.11 Human Pharmacology: Fludarabine can only be given via intravenous route. Renal excretion accounts for 23%. Half-life is 10 hours.

8.0 EVALUATION OF TOXICITIES

The criteria listed in the NCI Common Toxicity Criteria Scale will be used in grading toxicity. (Version 4.0 located at http://ctep.cancer.gov). GVHD will be graded by the method of Przepiorka et al. (see Appendix I).

9.0 RISKS

9.1 Non-Engraftment

One major risk of patients undergoing stem cell transplant for non-malignant diseases is non-engraftment. We are anticipating lower incidence of graft failure or autologous recovery with the use of fludarabine in our protocol.

9.2 GvHD

Acute GvHD produces a skin rash, liver dysfunction, and enteritis. GvHD prophylaxis in most types of patients reduces the incidence of grade II-IV GvHD to about 35% with commonly used regimens of today. Recent reports showed an incidence of aGvHD grade III-IV of 10% (Prasad

et al., Blood 2008). Chronic GvHD is a multiorgan autoimmune disease usually requiring therapy with steroids and other immunosuppressives. It is seen in about 30% of adults undergoing transplant and lower percentage of children. The risk of cGvHD after UCBT for congenital pediatric diseases has been reported to be as low as 8% (unpublished data of the University of Minnesota; Martinez, C., Orchard, P. and Tolar, J.).

9.3 Additional Risks

The degree of immune suppression of marrow transplant patients leads to an increased risk of opportunistic infections, especially those due to CMV, HSV, EBV, Pneumocystis, and other viruses and fungi. Prophylaxis is given where possible (Acyclovir, Bactrim, Antifungal, and antibacterial mouth rinses, etc.), and treatment for suspected agents is initiated very promptly, usually before the infection is confirmed.

Partial or incomplete T cell function sometimes leads to immune dysregulation and has been associated with autoimmune phenomena in patients with primary immune deficiency. These phenomena include but are not limited to autoimmune hemolytic anemia, neutropenia and thrombocytopenia. These complications may occur as a result of the underlying immune deficiency or from incomplete reconstitution following allogeneic SCT.

Patients with SCID or PID may be at risk for malignancy, in particular lymphoreticular malignancies often associated with primary or reactivated EBV infection. The risk for these cancers remains high in patients until immune recovery has been attained.

Other complications of an unexpected nature may be seen. In fact, most every patient presents with some new or very rare complication during transplant. There is a chance the patient could die from this treatment or from side effects of the treatment such as bleeding or infection. Patients are made aware of this possibility.

10.0 STATISTICAL CONSIDERATIONS

10.1 Sample Size Considerations

The protocol is designed to demonstrate that pediatric patients with congenital diseases received umbilical cord blood transplant will have a 1-year overall survival rate of at least 60%. Accrual of pediatric patients with congenital diseases who are eligible to receive umbilical cord blood transplant is about 3 subjects per year. If we suppose that a true 1-year overall survival rate of 40% would not be unacceptable, then we would need to accrue 22 patients to have 80% power to detect the deficiency in overall survival (with alpha level 0.05 and one-sided testing). The accrual period is 6 year with follow-up of at least 3 years or till death for each patient.

10.2 Monitoring Overall Survival

The primary outcome of overall survival will be compared to a fixed null hypothesis 1-year survival rate of 40% by a one-sided. Survival data and toxicity data will be summarized and monitored every year by the TXCH Data Review and Monitoring Committee.

10.3 Statistical Analysis

In addition to routine summary statistics for the outcome data, we will compute Kaplan-Meier estimates and confidence intervals for time to overall survival, disease-free survival, graft failure and length of remission. Additionally, we will also calculate incidence rates and 95% confidence intervals for engraftment, acute GvHD, neutrophil and platelet recovery at Day 42. As requested by TXCH-DRC, these short-term secondary endpoints will also be analyzed according to the two cohorts defined by the timing of G-CSF (G-CSF starting at Day +7 vs. G-CSF starting after Day +7). It should be noted that these analyses are exploratory, because the timing of G-CSF is not the goal of the study, hence not powered.

11.0 RECORDS TO BE KEPT

The CAGT data manager will maintain a database documenting the clinical parameters as well as the chemistries and hematologic parameters. The clinical status and occurrence of any adverse events and subsequent interventions are to be kept on all patients.

- Imaging reports
- Surgical summaries
- Autopsy summaries, where appropriate
- Informed consent documents

All required clinical evaluation records will be the responsibility of Dr. Martinez, who will also be responsible for analysis of the clinical outcome and toxicity.

The laboratory evaluation of immunological efficacy will be the responsibility of Dr. Martinez.

12.0 REPORTING REQUIREMENTS

12.1 Register all patients with Cell and Gene Therapy Research Nurse.

12.2 Enter all patients by calling Drs. Martinez/Krance. The following forms should be completed:

- Eligibility Checklist
- Adverse Events Record
- Follow-Up Forms
- Off-Study Forms

12.3 Drug Toxicity and/or Adverse Reactions

Adverse events will be collected per SOP J 02.05.XX, J 02.06.XX and J 02.78.XX.

13.0 INFORMED CONSENT

All patients and/or their legal guardian must sign a document of informed consent consistent with local institutional and Federal guidelines stating that they are aware of the investigational nature of this protocol and of the possible side effects of treatment. Further, patients must be informed that no efficacy of this therapy is guaranteed, and that unforeseen toxicities may occur. Patients have the right to withdraw from this protocol at any time. No patient will be accepted

for treatment without such a document signed by him or his legal guardian. Full confidentiality of patients and patient records will be provided according to institutional guidelines.

14.0 DATA MONITORING PLAN

This protocol will be monitored in accordance with current TXCH Data Safety Monitoring Board Plan for investigator-initiated Phase I and II studies. The conduct of this clinical trial will be evaluated in accordance with the Texas Children's Cancer Center and the Center for Cell and Gene Therapy Quality Assurance Policy and Procedure Plan.

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APPENDIX I

GVHD STAGES AND GRADES

For skin:	Stage	Skin Involvement
	0	0
	1	greater than 0, less than 25%
	2	greater than or equal to 25%, less than or equal to 50%
	3	greater than 50%
	4	greater than 50% with blisters

For gut:	Stage	Stool Volume
	0	less than 7 cc/kg
	1	greater than or equal to 7 cc/kg, less than 14 cc/kg
	2	greater than or equal to 14 cc/kg, less than 21 cc/kg
	3	greater than or equal to 21 cc/kg, less than 28 cc/kg
	4	greater than or equal to 28 cc/kg

For liver:	Stage	Bilirubin (mg/dL)
	0	less than 2
	1	greater than or equal to 2, less than 3
	2	greater than or equal to 3, less than 6
	3	greater than or equal to 6, less than 15
	4	greater than or equal to 15

Overall:	Grade	Organ Stage
	0	0
	1	skin = 1 or 2
	2	skin = 3, or skin less than or equal to 3 and gut or liver equal to 1
	3	skin greater than or equal to 3 and gut or liver equal to 2 or 3
	4	skin, gut or liver equal to 4