A Phase II Trial of Hematopoietic Stem Cell Transplantation for the Treatment of Patients with Fanconi Anemia Lacking a Genotypically Identical Donor, Using a Risk-Adjusted Chemotherapy Only Cytoreduction with Busulfan, Cyclophosphamide and Fludarabine

PROTOCOL FACE PAGE FOR
MSK THERAPEUTIC/DIAGNOSTIC PROTOCOL

Principal Investigator/Department:	Farid Boulad, M.D.	Pediatrics
Co-Principal Investigator(s)/Department:	Maria Cancio, M.D.	Pediatrics
Investigator(s)/Department:	Jaap Jan Boelens, M.D., Ph.D. Susan Prockop, M.D. Kevin Curran, M.D. Nancy Kernan, M.D. Andrew Kung, M.D., Ph.D Andromachi Scaradavou, M.D. Barbara Spitzer, M.D. Cheryl Goss, M.D. Jennifer Bieler, RN Victoria Szenes, NP Adlynn Parado, RN Melissa Varda, RN	Pediatrics Pediatrics Pediatrics Pediatrics Pediatrics Pediatrics Pediatrics Laboratory Medicine Nursing Nursing Nursing Nursing
Consenting Professional(s)/Department:	Farid Boulad, M.D. Maria Cancio, M.D. Jaap Jan Boelens, M.D., Ph.D. Susan Prockop, M.D. Kevin Curran, M.D. Nancy Kernan, M.D. Andrew Kung, M.D., Ph.D Andromachi Scaradavou, M.D. Barbara Spitzer, M.D.	Pediatrics Pediatrics Pediatrics Pediatrics Pediatrics Pediatrics Pediatrics Pediatrics Pediatrics

Please Note: A Consenting Professional must have completed the mandatory Human Subjects Education and Certification Program.

Participating Institutions:	PI's Name	Site's Role

Cincinnati Children's Hospital Medical Center	Parinda Mehta, MD	Data Collection/Data Coordinating Center
FRED HUTCHINSON CANCER CENTER	K. Scott Baker, MD	Data Collection

OneMSK Sites
Manhattan

Memorial Sloan Kettering Cancer Center
1275 York Avenue
New York, New York 10065

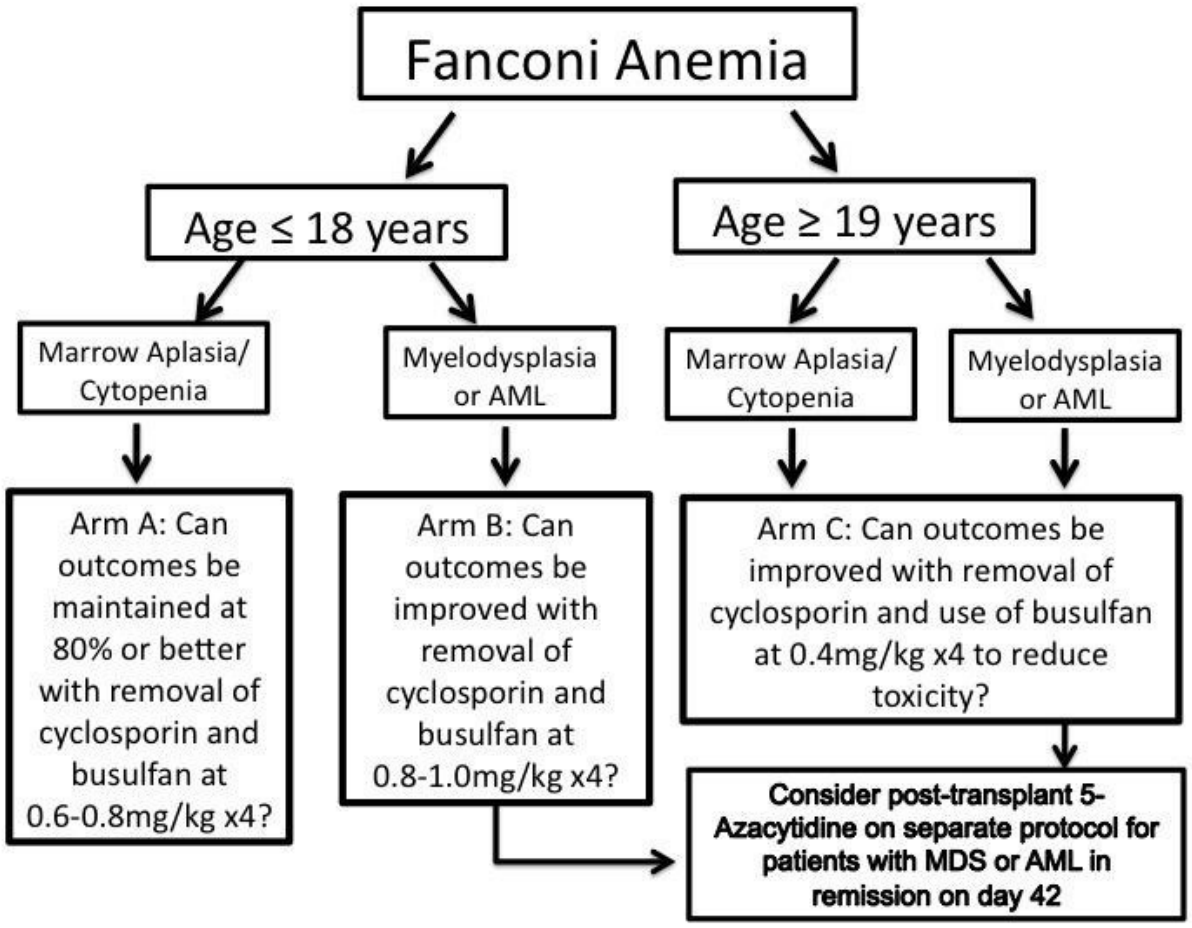
Table of Contents

1.0 PROTOCOL SUMMARY AND/OR SCHEMA

The trial proposed is a three-arm phase II treatment protocol designed to examine engraftment, toxicity, graft-versus-host disease, and disease-free survival following a novel cytoreductive regimen including busulfan, cyclophosphamide and fludarabine and ATG for the treatment of patients with Fanconi anemia who have severe aplastic anemia (SAA), or myelodysplastic syndrome (MDS) or acute myelogenous leukemia (AML), lacking HLA-genotypically identical donors using stem cell transplants derived from HLA-compatible unrelated donors or HLA haplotype-mismatched related donors. Therapy will be risk-adapted according to recipient age and stage of marrow disease.

Candidates for this trial will include patients with Fanconi anemia who have severe aplastic anemia (transfusion dependent) or myelodysplastic syndrome, or acute myelogenous leukemia for whom an allogeneic stem cell transplant is indicated.

SCHEMA



Good Risk Patients: Arm A: Patients 18 years old or younger with marrow aplasia or single lineage cytopenias will be conditioned for transplantation with intravenous busulfan (busulfex®) (0.6-0.8 mg/Kg/dose q 12 hours x 4 doses), cyclophosphamide (10 mg/Kg/dose x 4 doses) and fludarabine (35mg/m2/day x 4 doses). This strategy lead to survival rates > 90% in our prior study.

Age and weight-based Busulfan dosing:

< 10 kg: 0.6 mg/kg/dose as a starting dose q 12 hours x 4 doses
≥ 10 kg but ≤ 4 years old: 0.8 mg/kg/dose as a starting dose q 12 hours x 4 doses
> 4 years: 0.6 mg/kg/dose as a starting dose q 12hours x 4 doses

Busulfan pharmacokinetics will not be used in this good risk arm receiving a low dose of Busulfan.

Intermediate risk patients: Arm B: Patients 18 years old or younger with MDS or AML will be will be conditioned for transplantation with intravenous busulfan (busulfex®) (0.8-1.0mg/Kg/dose q 12 hours x 4 doses), cyclophosphamide (10 mg/Kg/dose x 4 doses) and fludarabine (35mg/m2/day x 4 doses). A higher dose of busulfan will be used as this has been well-tolerated in younger patients with FA when used in conjunction with PK, and we will test whether this higher dose improves disease control, as these patients are at risk of relapse.

The busulfan dose will be modified by recipient age and weight as described below:

Age and weight-based Busulfan dosing:

< 10 kg: 0.8 mg/kg/dose as a starting dose q 12 hours x 4 doses
≥ 10 kg but ≤ 4 years old: 1.0 mg/kg/dose as a starting dose q 12 hours x 4 doses
> 4 years: 0.8 mg/kg/dose as a starting dose q 12hours x 4 doses

Busulfan pharmacokinetics WILL be used in this arm, as the dose of busulfan is higher, to ensure that the Css after the first dose of busulfan does not exceed 350.

High risk patients: Arm C: Patients 19 years old or older with marrow aplasia or MDS or AML will be will be conditioned for transplantation with intravenous busulfan (busulfex®) (0.4mg/Kg/dose q 12 hours x 4 doses), cyclophosphamide (10 mg/Kg/dose x 4 doses) and fludarabine (35mg/m2/day x 4 doses). In our previous studies older patients had increased toxicity from transplant, and in this study we will test whether outcomes can be improved, yet engraftment maintained, with a reduced dose of busulfan. No age or weight adjustment is needed for arm C patients as all cases will be over 4 years old and weigh more than 10kg.

All Patients (Arms A, B and C) All patients will also receive rabbit ATG (thymoglobulin®) (2.5 mg/Kg/dose x 4 doses) prior to transplant to promote engraftment. Cyclosporine will NOT be used for prophylaxis against GvHD. All patients will also receive G-CSF post-transplant to foster engraftment. The source of stem cells for all patients will be peripheral blood stem cells (PBSC) induced and mobilized by treatment of the donor with G-CSF as per institutional guidelines. T-cell depletion will be performed by positive CD34 selection with the use of the Miltenyi system (CliniMACS device). The CD34+peripheral blood progenitors will then be administered to the patients after they have completed cytoreduction.

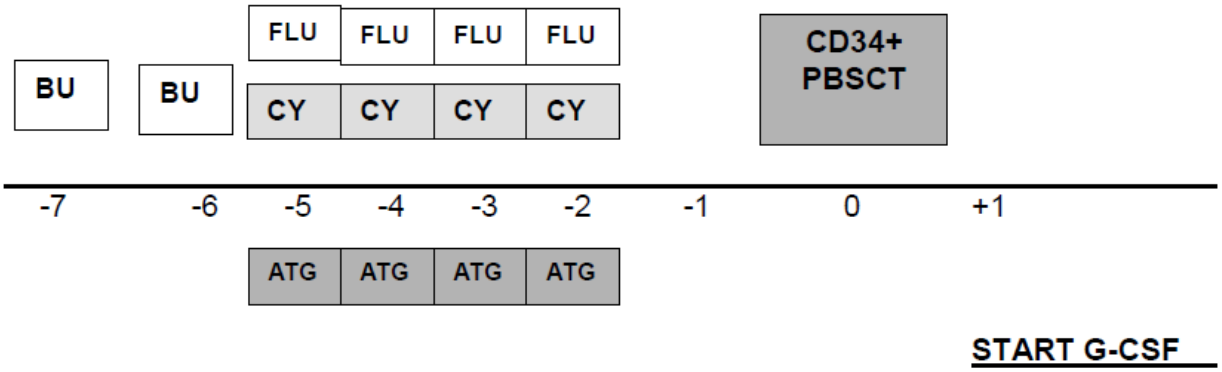
Patients will be carefully monitored for engraftment, chimerism, incidence and severity of acute and chronic GvHD, regimen-related toxicity, characteristics of hematopoietic and immune reconstitution and survival and disease-free survival.

This phase II trial is designed to investigate the safety and efficacy of a risk-adjusted chemotherapy-based cytoreductive regimen plus a CD34+ selected T-cell depleted peripheral blood stem cell (PBSC) stem cell transplant for the treatment of patients with Fanconi anemia and severe hematologic disease. The study population will include two types of donors: (1) HLA-compatible unrelated donors, and (2) HLA–mismatched related donors. Donor preference will be determined as per institutional practice.

A total of up to approximately 10 participants at MSK will enter into this study over a period of 4 years.

In order to reduce patient risk, the study design includes early termination in the event of excessive graft failure, grade 3-4 acute graft-versus-host disease or severe chronic graft-versus host disease, or early transplant related mortality during the accrual period. Stopping rules will be utilized.

See below for approximate schema of cytoreduction and preparation for allogeneic PBSCT:



BU	Busulfan	Arm A: 0.6-0.8 mg/Kg/dose IV Q 12hrs	x 4 doses
		Arm B: 0.8-1.0 mg/kg/dose IV Q12 hrs	x 4 doses
		Arm C: 0.4 mg/kg IV Q12 hrs	x 4 doses
FLU	Fludarabine	35 mg/m2/dose IV once daily	x 4 doses
CY	Cyclophosphamide (Mesna)	10 mg/Kg/dose IV once daily	x 4 doses
ATG	Anti-thymocyte globulin - Thymoglobulin	2.5 mg/Kg/dose IV daily	x 4 doses
G-SCF	Filgrastim	5 mcg/Kg/dose IV Q 12H	

2.1 OBJECTIVES AND SCIENTIFIC AIMS

Objectives

The primary objectives of this trial are to establish:

1. The incidence and quality of engraftment and hematopoietic reconstitution.
2. The incidence of early transplant related mortality
3. The incidence and severity of acute GvHD and chronic GVHD.

Secondary objectives of this study are to establish estimates of:

1. The incidences of overall survival and disease-free survival 2 years after transplant

3.0 BACKGROUND AND RATIONALE

Background

Fanconi anemia (FA) is a genetic disease characterized by chromosome fragility, multiple birth defects, and an increased risk for hematopoietic disorders and solid tumors (1-2). Progressive bone marrow failure results in aplasia, myelodysplasia and/or leukemia (3). When an HLA-matched sibling is identified, stem cell transplantation using cytoreduction with low doses of radiation and chemotherapy has been successful in curing the hematologic manifestations of FA (4-17). The chromosome fragility of FA and its hypersensitivity to DNA cross-linking agents and radiation had for

some time limited the ability to perform marrow transplantation using donors other than HLA-matched siblings (18-20). In the context of alternative donors such as unrelated marrow donors or cord blood, the use of a similar cytoreduction was associated with high rates of rejection/graft failure and GvHD, resulting in poor overall survival (21-29).

Since the first report of marrow transplantation for FA in 1977 (4), approximately 200+ recipients of marrow transplants from HLA-matched siblings have been reported; the risk of rejection has been less than 10%, the risk of developing acute and/or chronic GvHD has been approximately 45%, and 69% of patients are alive disease-free (4-17). More recent studies however have shown improved results (80%) when transplants have been performed in younger patients, with fewer prior transfusions (11).

Approximately 110 patients with FA were initially reported who received stem cell transplants from closely matched unrelated donors; 69 reported to the European Group for Blood and Marrow Transplantation and approximately 40 in the US (21-28). A proportion of these transplants were T-cell depleted for the prevention of GvHD. Overall, there was an increased risk of rejection of 17-37%, risks of grade II-IV GvHD and of chronic GvHD were as high as 50%, and only one third of the patients were alive at the time of the reports. The cytoreduction used in the majority of these transplants consisted of low dose TBI (450-600 cGy) (TBI), and cyclophosphamide (Cy) (10 mg/Kg x 4) with the addition of ATG, steroids, and cyclosporine A. In an analysis of 49 patients with FA who received stem cell transplants from unrelated donors reported by 12 centers, the incidence of acute GvHD grades II-IV was 52% in conventional grafts and 29% in T-cell-depleted grafts (23). Using elutriation to deplete T-cells the Minnesota group observed a 32% incidence of grade II-IV acute GvHD in 20 transplanted patients (28).

Guardiola et al. (26) using CD34+ selection (ISOLEX) to T-cell deplete FA unrelated bone marrow, observed an incidence of only 15% II-IV grade acute GvHD. However, two of the 16 patients died of acute GvHD. Graft rejection has also been a major problem for unrelated donor transplants for FA. In those patients receiving T-cell-depleted transplants, the incidence of graft failure was 20-37% (22, 23, 28). The presence of lymphocyte mosaicism (i.e. presence of DEB insensitive cells) was associated with a significant increased risk of graft failure (28). Escalation of TBI from 450 cGy (standard FA dose) to 600 cGy did not decrease the risk of graft rejection in the most recent Minnesota trial (28). In all of the unrelated donor trials for patients with FA, infectious mortality has been significant. In a Minnesota trial of 29 FA MUD transplants, eight patients succumbed to invasive Aspergillus infection despite aggressive antifungal therapy (28).

Based on the results cited above, the problems associated with stem cell transplants for FA were: immune complications, including graft-versus-host disease (GvHD), graft rejection, infections, and organ toxicity. The challenges to the optimization of stem cell transplants for Fanconi anemia using alternative donors were thus based on the need for (1) a regimen immunosuppressive enough to allow engraftment, with a low risk of rejection, (2) a regimen for stem cell processing (T-cell depletion) that can decrease the risk of GvHD, and (3) a regimen that does not give rise to excessive toxicity in children with FA.

Three groups in the US including the University of Minnesota, Cincinnati Children's Hospital and Memorial Sloan-Kettering Cancer Center have pioneered new regimens that included low dose total body irradiation, cyclophosphamide, ATG and the addition of fludarabine followed by T-cell depleted marrow or peripheral blood stem cell transplants (29-31). All three centers have had promising results with minimal risks of graft rejection, low rates of graft-versus-host disease and overall promising disease-free survival. However, with the small numbers of patients with FA who undergo allogeneic stem cell transplants, results of single center trials are difficult to interpret in terms of optimal

cytoreductive regimens and dosing. In addition, T-cell depletion methods were also different in all three centers.

Moreover, the use of TBI in these studies also poses additional concerns with respect to widespread adoption of one of these approaches. It is possible (based on risks of secondary malignancies in non-FA patients) that irradiation plays some role in the increased risk of secondary malignancies in FA patients. To date, a significant number of patients with FA surviving stem cell transplantation have developed solid tumors (32-35). These solid tumors are mostly squamous cell carcinomas. The relative contributions of TBI and chronic GvHD to the development of such tumors are unknown. However, the elimination of TBI coupled with T-cell depletion to prevent GVHD is an attractive approach to reducing long term adverse effects.

In our prior multi-center study of transplant for FA we used a regimen of busulfan, fludarabine, ATG and cyclophosphamide together with a T-cell depleted graft to treat adults and children with Fanconi anemia. The study included matched and mismatched unrelated donors and non-genoidentical related donors. In this study we treated an initial 25 cases using a dose of busulfan of 0.8-1.0 mg/kg/dose. Outcomes at this dose level were good and the dose of busulfan was decreased to 0.6-0.8mg/kg/dose with the goal of reducing toxicity in the next 20 cases enrolled.

The outcomes of this study, as of September 2013 are summarized below. Notably, persons aged less than 18 years with aplastic anemia had a survival rate of 91%, an outstanding result. As a result of these good outcomes, our goal for this good risk population in this successor study is toxicity reduction, using a lower dose of busulfan than we have previously studied, and omitting cyclosporine post-transplant.

Survival	%
OS and DFS	74.40%
DFS by age (p = .026)	
< 10 years old (N=26)	88.50%
> 10 years old (N=11 incl pts older than 20)	46.80%
DFS by Hematologic disease (p = .190)	
MDS (N=9)	61.00%
SAA/SISLC (N=28)	79.30%
DFS by Donor (p = .240)	
Matched Unrelated (N=21)	84.00%
Mismatched related or unrelated (N=15)	56.10%
DFS by BU dose given (p = .856)	
0. 8 mg/kg	73.40%
0.6 mg/kg	80.00%

Children with MDS remained at risk of relapse. In this successor study we will give the intial higher dose of busulfan that we know is tolerable from our first cohort of patients (0.8-1.0 mg/kg/dose), and will also omit cyclosporine post-transplant to maximize donor T-cell recovery.

In our first study, we have observed that brisk host lymphocyte expansion can occur in the immediate post-transplant period, with an associated fall in donor chimerism, raising the possibility of graft rejection. Lymphocyte expansion can be remedied and full donor chimerism restored with subcutaneous (SC) Campath administration. Weekly chimerism studies will be performed in this successor study to monitor for this complication.

Rationale

The purpose of the use of busulfan is to avoid the use of TBI and possibly decrease the risk of secondary malignancies.

Cyclophosphamide / fludarabine and ATG combination is used because these drugs were successful in our recent trial, with limited toxicity and decreased risks of rejection.

We are using peripheral blood stem cells as the stem cell source to achieve high doses of stem cells in order to prevent graft rejection and maximize immune reconstitution. CD34-selected stem cells (which are highly T cell depleted) are used to minimize the risks of GVHD.

4.1 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.2 Design

The trial proposed is a three arm phase II treatment protocol designed to examine engraftment, toxicity, graft-versus-host disease, and ultimate disease-free survival following a novel risk-adjusted cytoreductive regimen including busulfan, cyclophosphamide and fludarabine and ATG for the treatment of patients with Fanconi anemia lacking HLA-genotypically identical donors using stem cell transplants derived from HLA-compatible unrelated donors or HLA haplotype-mismatched related donors. Candidates for this trial will include patients with Fanconi anemia and severe aplastic anemia (transfusion dependent) or myelodysplastic syndrome, or acute myelogenous leukemia for whom an allogeneic stem cell transplant is indicated.

This phase II trial is designed to investigate the safety and efficacy of risk-adjusted chemotherapy-based cytoreductive regimen plus a CD34+ selected T-cell depleted peripheral blood stem cell (PBSC) stem cell transplant for the treatment of patients with Fanconi anemia and severe hematologic disease. The majority of patients will receive grafts derived from PBSC from HLA-compatible unrelated donors and HLA–mismatched related donors and will be the focus of the trial.

The primary objectives of this trial are to establish (1) the incidence and quality of engraftment and hematopoietic reconstitution, (2) the incidence of early transplant related mortality, (3) the incidence and severity of acute GvHD and chronic GVHD, and (4) the probability of overall survival and disease-free survival over time.

4.3 Intervention

A prospective phase II trial is proposed. Patients with FA who lack an HLA-genotypically matched related donor and have severe hematologic disorders will be eligible to receive CD34+ selected, T cell depleted allogeneic hematopoietic stem cell transplants after chemotherapy-based cytoreduction

using intravenous busulfan, cyclophosphamide, fludarabine and rabbit ATG. Stem cells will be collected from (1) HLA-compatible unrelated donors, or (2) non genoidentical related donors.

Busulfan dosing will be risk-adapted:

A lower dose of busulfan (0.4 mg/kg/dose) will be used in older patients (arm C) to minimize toxicity.

A standard dose of busulfan (0.6-0.8 mg/kg/dose), associated with excellent outcomes in our previous trial will be used for young patients with marrow aplasia (arm A).

A higher dose of busulfan (0.8-1.0 mg/kg/dose) will be used in younger patients with MDS and AML (arm B) to maximize disease control.

Cyclosporine will not be used post-transplant as no GVHD was seen in our previous trial and cyclosporine can cause significant toxicity.

Patients will receive cytoreduction with busulfan, fludarabine, and cyclophosphamide with the addition of immunosuppression with ATG. They will receive a CD34+ T-cell depleted peripheral blood stem cell transplant. See below for approximate schema of cytoreduction and preparation for allogeneic PBSCT:

Good Risk Patients: Arm A: Patients 18 years old or younger with marrow aplasia or single lineage cytopenias (Arm A) will be conditioned for transplantation with intravenous busulfan (busulfex®) (0.6-0.8 mg/Kg/dose q 12 hours x 4 doses), cyclophosphamide (10 mg/Kg/dose x 4 doses) and fludarabine (35mg/m^2/day x 4 doses). This strategy lead to survival rates > 90% in our prior study.

Age and weight-based Busulfan dosing:

< 10 kg: 0.6 mg/kg/dose as a starting dose q 12 hours x 4 doses

≥ 10 kg but ≤ 4 years old: 0.8 mg/kg/dose as a starting dose q 12 hours x 4 doses

> 4 years: 0.6 mg/kg/dose as a starting dose q 12hours x 4 doses

Busulfan pharmacokinetics will not be used in this good risk arm receiving a low dose of busulfan.

Intermediate risk patients: Arm B: Patients 18 years old or younger with MDS or AML will be will be conditioned for transplantation with intravenous busulfan (busulfex®) (0.8-1.0mg/Kg/dose q 12 hours x 4 doses), cyclophosphamide (10 mg/Kg/dose x 4 doses) and fludarabine (35mg/m2/day x 4 doses). A higher dose of busulfan will be used as this has been well-tolerated in younger patients with FA when used in conjunction with PK, and we will test whether this higher dose improves disease control, as these patients are at risk of relapse.

The busulfan dose will be modified by recipient age and weight as described below:

Age and weight-based Busulfan dosing:

< 10 kg: 0.8 mg/kg/dose as a starting dose q 12 hours x 4 doses

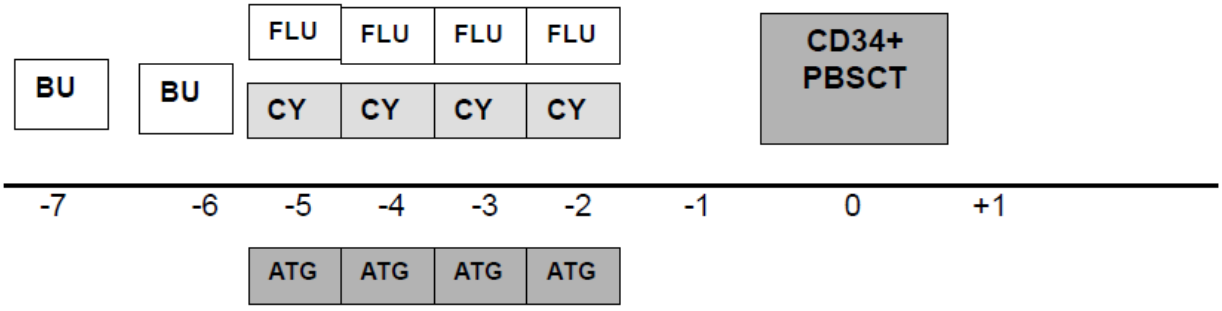
≥ 10 kg but ≤ 4 years old: 1.0 mg/kg/dose as a starting dose q 12 hours x 4 doses

> 4 years: 0.8 mg/kg/dose as a starting dose q 12hours x 4 doses

Busulfan pharmacokinetics WILL be used in this arm, as the dose of busulfan is higher, to ensure that the Css after the first dose of busulfan does not exceed 350.

High risk patients: Arm C: Patients 19 years old or older with marrow aplasia or MDS or AML will be will be conditioned for transplantation with intravenous busulfan (busulfex®) (0.4mg/Kg/dose q 12 hours x 4 doses), cyclophosphamide (10 mg/Kg/dose x 4 doses) and fludarabine (35mg/m2/day x 4 doses). In our previous studies older patients had increased toxicity from transplant, and in this study we will test whether outcomes can be improved, yet engraftment maintained, with a reduced dose of busulfan. No age or weight adjustment is needed for arm C patients as all cases will be over 4 years old and weigh more than 10kg.

All patients will also receive rabbit ATG (thymoglobulin®) (2.5 mg/Kg/dose x 4 doses) prior to transplant to promote engraftment. Cyclosporine will NOT be used for prophylaxis against GvHD. All patients will also receive G-CSF post-transplant to foster engraftment. The source of stem cells for all patients will be peripheral blood stem cells (PBSC) induced and mobilized by treatment of the donor with G-CSF for 4-6 days. T-cell depletion will be performed by positive CD34 selection with the use of the Miltenyi system (CliniMACS device). The CD34+peripheral blood progenitors will then be administered to the patients after they have completed cytoreduction.



BU	Busulfan	Arm A: 0.6-0.8 mg/Kg/dose IV Q 12hrs	x 4 doses
		Arm B: 0.8-1.0 mg/kg/dose IV Q12 hrs	x 4 doses
		Arm C: 0.4 mg/kg IV Q12 hrs	x 4 doses
FLU	Fludarabine	35 mg/m2/dose IV once daily	x 4 doses
CY	Cyclophosphamide (Mesna)	10 mg/Kg/dose IV once daily	x 4 doses
ATG	Anti-thymocyte globulin - Thymoglobulin	2.5 mg/Kg/dose IV daily	x 4 doses
G-SCF	Filgrastim	5 mcg/Kg/dose IV Q 12H	

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

5.1. Busulfan (busulfex®)

a. Source and Pharmacology: Supplier: Orphan Medical Company; Busulfan is a bifunctional alkylating agent known chemically as 1,4-butanediol, dimethanesulfonate. BUSULFEX® (busulfan). This is an 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.

b. Formulation and Stability: It is supplied as a clear, colorless, sterile, solution in 10 mL single use ampoules. Each ampoule of BUSULFEX contains 60 mg (6 mg/mL) of busulfan, the active ingredient,

a white crystalline powder with a molecular formula of $CH_3SO_2O(CH_2)_4OSO_2CH_3$ and a molecular weight of 246 g/mole. Busulfan is dissolved in N,N-dimethylacetamide (DMA) 33% wt/wt and polyethylene glycol 400, 67% wt/wt. Busulfan's solubility in water is 0.1 g/L and the pH of a >0.5% solution in 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP as recommended for infusion reflects the pH of the diluent used and ranges from 3.4 to 3.9.

c. Solution Preparation: BUSULFEX is supplied as a sterile solution in 10 mL single-use clear glass ampoules each containing 60 mg of busulfan at a concentration of 6 mg/mL for intravenous use. BUSULFEX must be diluted prior to use with either 0.9% Sodium Chloride Injection, USP (normal saline) or 5% Dextrose Injection, USP (D5W). The diluent quantity should be 10 times the volume of BUSULFEX, ensuring that the final concentration of busulfan is approximately 0.5 mg/mL.

d. Storage and Stability: Unopened ampules of BUSULFEX must be stored under refrigerated conditions between 2° -8° C (36° -46° F).

e. Administration: Intravenous, over 2 hours.

5.2. Fludarabine (FLUDARA®)

a. Source and Pharmacology: Supplier: Berlex Laboratories, Inc. FLUDARA FOR INJECTIO N contains fludarabine phosphate, a fluorinated nucleotide analog of the antiviral agent vidarabine, 9-ß-D-arabinofuranosyladenine (ara-A) that is relatively resistant to deamination by adenosine deaminase. The chemical name for fludarabine phosphate is 9H-Purin-6-amine, 2-fluoro-9-(5-O-phosphono-ß-D-arabinofuranosyl). Fludarabine phosphate is rapidly dephosphorylated to 2-fluoro-ara-A and then phosphorylated intracellularly by deoxycytidine kinase to the active triphosphate, 2-fluoro-ara-ATP. This metabolite appears to act by inhibiting DNA polymerase alpha, ribonucleotide reductase and DNA primase, thus inhibiting DNA synthesis. The mechanism of action of this antimetabolite is not completely characterized and may be multi-faceted.

b. Formulation and Stability: Each vial of sterile lyophilized solid cake contains 50 mg of the active ingredient fludarabine phosphate, 50 mg of mannitol, and sodium hydroxide to adjust pH to 7.7. The pH range for the final product is 7.2-8.2. Reconstitution with 2 mL of Sterile Water for Injection USP results in a solution containing 25 mg/mL of fludarabine phosphate intended for intravenous administration. FLUDARA FOR INJECTION is supplied in a clear glass single dose vial (6 mL capacity) and packaged in a single dose vial carton in a shelf pack of five.

c. Solution Preparation: FLUDARA should be prepared for parenteral use by aseptically adding Sterile Water for Injection USP. When reconstituted with 2 mL of Sterile Water for Injection, USP, the solid cake should fully dissolve in 15 seconds or less; each mL of the resulting solution will contain 25 mg of fludarabine phosphate, 25 mg of mannitol, and sodium hydroxide to adjust the pH to 7.7. The pH range for the final product is 7.2-8.2. In clinical studies, the product has been diluted in 100 cc or 125 cc of 5% Dextrose Injection USP or 0.9% Sodium Chloride USP.

d. Storage and Stability: FLUDARA is supplied as a white, lyophilized solid cake. Each vial contains 50 mg of fludarabine phosphate, 50 mg of mannitol and sodium hydroxide to adjust pH to 7.7. The pH range for the final product is 7.2-8.2. Store under refrigeration, between 2°-8° C (36°-46° F).

e. Administration: Intravenous, over thirty minutes.

5.3.Cyclophosphamide (Cytoxan®)

a. Source and Pharmacology: Supplier: Bristol-Meyers. Cytoxan®,. Cyclophosphamide is an alkylating agent that is initially inactive but then is activated by metabolism in the liver by the mixed-function oxidase system of the smooth endoplasmic reticulum. The hepatic cytochrome P-450 mixed-function converts cyclophosphamide to 4-hydroxycyclophosphamide, which is in a steady state with the acyclic tautomer, aldophosphamide.

b. Formulation and Stability: Injections: Each lyophilized vial contains: cyclophosphamide USP 500, 1000 or 2000 mg with mannitol. Cartons of 6 (1 000 and 2 000 mg) and 12 (500 mg).

c. Solution Preparation: Prepare Cytoxan for Injection for parenteral use by adding Sterile Water for Injection USP or Bacteriostatic Water for Injection USP (paraben preserved only) to the vial and shaking to dissolve to produce a clear colorless solution. Heating should not be used to facilitate dissolution. Solutions of Cytoxan for Injection may be infused i.v. in Dextrose Injection USP (5% Dextrose, 5% Dextrose and 0.9% Sodium Chloride) Dextrose 5% and Ringers Injection, Lactated Ringers Injection USP, Sodium Chloride Injection USP (0.45% sodium chloride) and Sodium Lactate Injection USP (1/6 molar sodium lactate).

d. Storage and Stability: Reconstituted Cytoxan for Injection is chemically and physically stable at room temperature for 24 hours and for 6 days in the refrigerator. For solutions further diluted for i.v. infusion, it is recommended that the solutions be used within 24 hours at room temperature or 72 hours under refrigeration. Solutions prepared with Sterile Water for Injection should be used for single dose administration and any unused solution discarded.

e. Administration: Intravenous, over thirty-sixty minutes.

5.4. Anti-Thymocyte Globulin (Rabbit) (Thymoglobulin®)

a. Source and Pharmacology: Supplier: Sangstat, The Transplant Company®. Thymoglobulin® [Anti-thymocyte Globulin (Rabbit)] is a purified, pasteurized, gamma immune globulin, obtained by immunization of rabbits with human thymocytes. This immunosuppressive product contains cytotoxic antibodies directed against antigens expressed on human T-lymphocytes.

b. Formulation and Stability: Thymoglobulin is a sterile, freeze-dried product for intravenous administration after reconstitution with sterile Water for Injection, USP (WFI). Each package contains two 7 mL vials: Vial 1: Freeze-Dried Thymoglobulin Formulation Active ingredient: Anti-thymocyte Globulin (Rabbit) 25 mg - Inactive ingredients: Glycine (50 mg), mannitol (50 mg), sodium chloride (10 mg); Vial 2: Diluent Sterile Water for Injection, USP 5 mL. The reconstituted preparation contains approximately 5 mg/mL of Thymoglobulin, of which >90% is rabbit gamma immune globulin (IgG). The reconstituted solution has a pH of 7.0± 0.4. Human red blood cells are used in the manufacturing process to deplete cross-reactive antibodies to non-T-cell antigens. The manufacturing process is validated to remove or inactivate potential exogenous viruses. All human red blood cells are from US registered or FDA licensed blood banks. A viral inactivation step (pasteurization, i.e., heat treatment of active ingredient at 60°C/10 hr) is performed for each lot. Each Thymoglobulin lot is released following potency testing (lymphocytotoxicity and E-rosette inhibition assays), and cross-reactive antibody testing (hemagglutination, platelet agglutination, anti-human serum protein antibody, antiglomerular basement membrane antibody, and fibroblast toxicity assays on every 5th lot).

c. Solution Preparation: Each reconstituted vial contains 25 mg or 5 mg/mL of Thymoglobulin. Transfer the contents of the calculated number of Thymoglobulin vials into the bag of infusion solution (saline or dextrose). Recommended volume: per one vial of Thymoglobulin use 50 mL of infusion solution (total volume usually between 50 to 500 mL). Mix the solution by inverting the bag gently only once or twice.

d. Storage and Stability: Store in refrigerator between +2° C to +8° C (36° F to 46° F). Protect from light. Do not freeze. Do not use after the expiration date indicated on the label. Reconstituted vials of Thymoglobulin should be used within 4 hours. Infusion solutions of Thymoglobulin must be used immediately. Any unused drug remaining after infusion must be discarded.

e. Administration: Infuse through a 0.22-micron filter into a high-flow vein. Set the flow rate to deliver the dose over a minimum of 6 hours for the first dose and over at least 4 hours for subsequent doses.

5.5. The CliniMACS device

Drug Formulation and Procurement

5.5.1. The CliniMACs device (Miltenyi Biotec, Auburn, CA) will be employed for the CD34 selection procedure. It consists of tubing, bags, and a pair of columns, placed at appropriate locations in the Tubing Set to facilitate the cell selection process. The CliniMACS System intended for selection of CD34+ cells is comprised of four primary components listed here. A brief description of each component is provided below.

- A software controlled instrument that processes the blood sample, CliniMACS Instrument
- A single-use, sterile disposable tubing set with two proprietary cell selection columns, CliniMACS Tubing Set
- A monoclonal antibody reagent specific for CD34+ cells, CliniMACS CD34 Reagent
- A sterile, isotonic phosphate buffered, 1 mM EDTA, saline solution, used as external wash and transport fluid for the in vitro preparation of blood cells, CliniMACS PBS/EDTA Buffer.

5.5.2. CliniMACS Instrument

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

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

5.5.3. *CliniMACS Tubing Set*

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

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

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

5.5.4. *CliniMACS CD34 Reagent*

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

5.5.5. *The CliniMACS PBS/EDTA Buffer*

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

Table 1. Formulations of the CliniMACS PBS/EDTA Buffer

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

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

Mononuclear cells for separation are collected. After incubating the cells with CD34 Reagent in PBS/EDTA Buffer the excess of unbound reagent is removed by washing with the PBS/EDTA Buffer. Prior to and during incubation of the antiCD34 beads with the mobilized PBSC, intravenous gammaglobulin is added to the incubation fluid at a concentration of 1.5 mg IVIG/ml.

During the following automated selection, using PBS/EDTA Buffer supplemented with 0.5 % human serum albumin (HSA), the unwanted cells are removed and, in the final step, the selected cells are eluted from the column by the means of the PBS/EDTA Buffer. The HSA is not a component of the buffer and should be supplied by the clinical site.

5.5.6. *Principles of Operation*

The function of the CliniMACS System with the CliniMACS CD34 reagent is to select CD34+ cells from heterogeneous cell populations. The detailed procedures are provided in the CliniMACS User Manual that accompanies the CliniMACS Instrument. The selection process involves two phases; cell labeling prior to selection (phase 1) and the automated cell selection process (phase 2). Provided below is a brief summary of the procedure.

Phase 1 – Labeling. This phase is referred to as the cell-labeling step. This step involves combining the antibody reagent with the cellular harvest from the donor or patient. The antibody reagent is incubated for 30 minutes at room temperature with the heterogeneous cell population during which the antibody selectively binds to cells expressing the CD34 antigen. The mixture is then washed twice in PBS/EDTA separation buffer. Following centrifugation, the resulting cell pellet is resuspended in the separation buffer and the labeled product is ready for cell selection.

Phase 2 – Cell Selection. The labeled cell product is attached to the sterile tubing set of the CliniMACS system. Following a series of automated priming steps, the cell product is passed through a blood filter to remove any cell aggregates that may be present. The cell suspension is then passed through the first column, which serves as a pre-column separation step to eliminate cells that bind non-specifically. The labeled product then passes through the second column positioned within the magnetic field. The CD34+ cells, to which the antibody reagent has been bound, are selected and retained in the column matrix. All other cells flow through the column and are collected in the negative fraction bag. After automated buffer washes of the column containing the isolated CD34+ cells, the column is removed from the magnetic field, and the CD34+ cells are eluted into a positive fraction bag. The selected cells can be used immediately after analysis or cryopreserved for later infusion into the patient. The device is non-invasive in all aspects that involve processing of the cellular harvest and is not connected to the patient at any time.

The CliniMACS device is currently pending FDA approval.

6.1 CRITERIA FOR SUBJECT ELIGIBILITY

6.2 Subject Inclusion Criteria

6.1.1. Diagnosis
Patients must have a diagnosis of Fanconi anemia (confirmed by mitomycin C or diepoxybutane [DEB] chromosomal breakage testing at a CLIA approved laboratory).

6.1.2. Hematologic Diagnosis and Status
Patients must have one of the following hematologic diagnoses:

1. Severe Aplastic Anemia (SAA), with bone marrow cellularity of <25%

   OR

   Severe Isolated Single lineage Cytopenia

   AND at least one of the following features:
   1. Platelet count <20 x 10^9/L or platelet transfusion dependence*
   2. ANC <1000 x 10^9/L
   3. Hgb <8 gm/dl or red cell transfusion dependence*

2. Myelodysplastic Syndrome (MDS) (Appendix 1: MDS Classification)

   MDS at any stage, based on either one of the following classifications:
   - *WHO Classification*
   - Refractory anemia and transfusion dependence*
   - Any of other stages
   - *IPSS Classification*
   - Low risk (score 0) and transfusion dependence*
   - Any other risk groups Score ≥ 0.5

   Note that patients with chromosome 1q cytogenetic abnormalities in the absence of morphologic dysplasia will not be considered to have MDS.

3. Acute Myelogenous Leukemia

   Patients with acute leukemia are included in this trial untreated, in remission or with refractory or relapsed disease.

* Transfusion dependence will be defined as greater than ONE transfusion of platelets or red blood cells in the last year prior to evaluation on protocol.

6.1.3. Donor
Donor choices will be determined by the investigators according to institutional criteria. Patients who will be enrolled on this protocol must have one of the following donor choices:

HLA-compatible unrelated volunteer donors
Patients who do not have a related HLA-matched donor but have an unrelated donor who is either matched at all A, B, C and DRB1 (8/8) loci or who is mismatched at no more than 2/8 loci (A, B, C or DRB1) (6/8) as tested by DNA analysis (high resolution), will be eligible for entry on this protocol.

HLA-mismatched Related donors
Patients who do not have a related or unrelated HLA-compatible donor must have a healthy family member who is at least HLA-haplotype identical to the recipient. First degree related donors must have a normal DEB test.

The donor must be healthy and willing and able to receive a 4-6 day course of G-CSF and undergo 1-3 daily leukaphereses, as per institutional guidelines.

Related and unrelated donors must be medically evaluated and fulfill the criteria for collection of PBSCs as per institutional guidelines.

6.1.4. Patients and donors may be of either gender or any ethnic background.

6.1.5. Patients must have a Karnofsky adult, or Lansky pediatric performance scale status $\geq$ 70%.

6.1.6. Patients must have adequate physical function measured by :

a) *Cardiac*: asymptomatic or if symptomatic then 1) LVEF at rest must be $\geq$ 50% and must improve with exercise or 2) Shortening Fraction $\geq$ 29%
b) *Hepatic:* < 5 x ULN alanine transaminase (ALT) and < 2.0 mg/dl total serum bilirubin.
c) *Renal:* serum creatinine $\leq$1.5 mg/dl or if serum creatinine is outside the normal range, then CrCl > 50 ml/min/1.73 m^2
d) *Pulmonary:* asymptomatic or if symptomatic, DLCO > 50% of predicted (corrected for hemoglobin)

6.1.7. Each patient must be willing to participate as a research subject and must sign an informed consent form. Parent or legal guardians of patients who are minors will sign the informed consent form. Assents will be obtained as per institutional guidelines.

6.1.8. Female patients and donors must not be pregnant or breastfeeding at the time of signing consent. Women must be willing to undergo a pregnancy test prior to transplant and avoid becoming pregnant while on study. Positive pregnancy test results will be reported to the parent(s) or guardian of minor participants, as required per institutional guidelines.

6.2 Subject Exclusion Criteria

6.2.1. Active CNS leukemia

6.2.2. Female patients who are pregnant (positive serum or urine HCG) or breast-feeding. Women of childbearing age must avoid becoming pregnant while on study.

6.2.3. Active uncontrolled viral, bacterial or fungal infection

6.2.4. Patient seropositive for HIV-I/II; HTLV -I/II

7.0 RECRUITMENT PLAN

One of the Pediatric BMT attendings will see the patient in consultation. As part of the consultation, the attending will present the patient and/or parent/legal guardian with the risks and benefits of different types of cytoreduction and transplants. One of the participating investigators authorized to obtain consent will obtain informed consent. A copy of the signed informed consent will be provided to the family, and the original will be scanned into the electronic medical record.

Related donors will undergo HLA-antigen testing. If related donors are identified and are 4-7/8 antigen matched with the patient, they will be candidates to become a stem cell donor. Donors will sign a separate institutional consent form for stem cell collection using marrow or peripheral blood.

8.0 PRETREATMENT EVALUATION

8.1. Pretreatment Evaluation of the patient

Prior to instituting preparatory cytoreduction, the patient will receive an extensive medical evaluation according to standard institutional practice.

This evaluation may include the following:
- Complete physical examination
- Detailed history with special attention to prior medical history, allergies, previous therapies, and response to treatment
- Complete Blood Count with differential count and reticulocyte count.
- Bone marrow aspiration and biopsy, performed within approximately 1 month from starting cytoreduction. Marrow aspirations will be studied by morphology. It will also be studied by cytogenetic analysis by karyotype and FISH studies for chromosomes 1, 3, and 7. Bone marrow biopsy should be performed for the determination of cellularity.
- Coagulation profile
- Serum chemistries including BUN, creatinine, electrolytes, Calcium, magnesium, phosphorus, glucose, total protein, albumin, liver function tests (AST, ALT, bilirubin, alkaline phosphatase) and LDH. Patients with advanced MDS or AML may also have uric acid determination.
- Chest and other X rays as clinically indicated
- Electrocardiogram
- Gated pool scan or echocardiogram
- Urinalysis if clinically indicated
- Infectious Disease Markers will be tested as per institutional guidelines as well as at the discretion of the treating physician
- In addition, all patients will undergo pre-transplant complete immunologic evaluation as per institutional standards and at the discretion of treating physician.
- Blood or marrow will be sent for diagnostic molecular pathology for DNA extraction for the definition of donor host differences.

8.2. Pretransplant Evaluation of the Donor

The pre-transplant evaluation of the donor will be performed as per institutional guidelines, and is not specific to this protocol:

Family Donor

A consenting healthy family donor who is HLA compatible with the recipient will be given highest priority as a potential donor for PBSC or marrow transplant according to institutional

standard of care. A prospective HLA-non-identical related donor must be at least genotypically HLA-A, B, C, DRB1 haploidentical to the patient, but can differ for 1-4 HLA alleles on the unshared haplotype. Selection of the histocompatible donor will be based on high resolution typings of HLA A, B, C and DRB1 loci to be carried out on the recipient, and his/her siblings, parents or other family members.

In preparation for the stem cell donation, the donor will undergo a detailed medical history and physical examination. Clinical studies may include:

- DEB testing (for first degree related donors) to be performed.
- Full hemogram
- Coagulation profile
- Complete screening biochemical profile.
- Type and cross match
- Infectious Disease Markers will be tested per all FDA, AABB, NY State, and FACT standards.
- A chest x-ray and/or EKG or other additional medical evaluation will be obtained as clinically indicated by the evaluating physician.
- Blood or marrow will be sent for diagnostic molecular pathology for DNA extraction for the definition of donor host differences.
- Pregnancy testing will be performed as per institutional guidelines

Unrelated donor

Unrelated donors will undergo preparation for peripheral blood harvest as per the standards of the National Marrow Donor Program (NMDP). The donor will undergo pretransplant work-up and will sign consent for G-CSF administration and leukapheresis at the donor center, also according to standard procedure as dictated by the NMDP.

9.0 TREATMENT/INTERVENTION PLAN

9.1. Preparative Cytoreduction

The schemata below outlines cytoreduction for allogeneic PBSCT for arms A, B, and C:

Arm A: Standard dose busulfan with no PK study

Day	Treatment	Doses	Infusion guidelines
-7	Busulfan	0.6-0.8 mg/kg/dose q12	over 2 hours
-6	Busulfan	0.6-0.8 mg/kg/dose q12	over 2 hours
-5	Cyclophosphamide*	10 mg/kg IV, once daily	over 1 hour
	Fludarabine	35 mg/m^2 IV once daily	over 30 minutes
	Rabbit ATG	2.5 mg/kg IV once daily	over 8 hours
-4	Cyclophosphamide*	10 mg/kg IV once daily	over 1 hour
	Fludarabine	35 mg/m^2 IV once daily	over 30 minutes
	Rabbit ATG	2.5 mg/kg IV once daily	over 8 hours
-3	Cyclophosphamide*	10 mg/kg IV once daily	over 1 hour
	Fludarabine	35 mg/m^2 IV once daily	over 30 minutes
	Rabbit ATG	2.5 mg/kg IV once daily	over 8 hours

-2	Cyclophosphamide*	10 mg/kg IV once daily	over 1 hour
	Fludarabine	35 mg/m^2 IV once daily	over 30 minutes
	Rabbit ATG	2.5 mg/kg IV once daily	over 8 hours
-1	Rest day		
0**	CD34+ PBSCT		
+1	Start G-CSF		

* With Mesna per Institutional practice
** Day 0 could extend to two days if collection requires two separation procedures.

Arm B: Higher dose busulfan with PK study

For ARM B patients Busulfan pharmacokinetics will be done with the first dose of busulfan and dose adjustments will be made according to institutional standard clinical practice as indicated. Busulfan concentrations will be determined by the Department of Laboratory Medicine Clinical Chemistry Laboratory and pharmacokinetic analysis by the Department of Pharmacy. The optimal desired steady state CSS target should be 300-350. The second dose of busulfan will be given after the results of the PK study are available, and the dose reduced if necessary. The dose of busulfan will NOT be increased per the PK study, but will be decreased if the Css is >350.

Day	Treatment	Doses	Infusion guidelines
- 8	Busulfan PK study	0.8-1.0 mg/kg/dose	over 2 hours
-7	Busulfan	PK adjusted dose *x1*	over 2 hours
-6	Busulfan	PK adjusted dose x2	over 2 hours
-5	Cyclophosphamide*	10 mg/kg IV once daily	over 1 hour
	Fludarabine	35 mg/m^2 IV once daily	over 30 minutes
	Rabbit ATG	2.5 mg/kg IV once daily	over 8 hours
-4	Cyclophosphamide*	10 mg/kg IV once daily	over 1 hour
	Fludarabine	35 mg/m^2 IV once daily	over 30 minutes
	Rabbit ATG	2.5 mg/kg IV once daily	over 8 hours
-3	Cyclophosphamide*	10 mg/kg IV once daily	over 1 hour
	Fludarabine	35 mg/m^2 IV once daily	over 30 minutes
	Rabbit ATG	2.5 mg/kg IV once daily	over 8 hours
-2	Cyclophosphamide*	10 mg/kg IV once daily	over 1 hour
	Fludarabine	35 mg/m^2 IV once daily	over 30 minutes
	Rabbit ATG	2.5 mg/kg IV once daily	over 8 hours
-1	Rest day		
0**	CD34+ PBSCT		
+1	Start G-CSF		

* With Mesna per Institutional practice
** Day 0 could extend to two days if collection requires two separation procedures

Arm C: Low dose busulfan for adult patients 19 years or older

For ARM C patients a low dose of busulfan (0.4mg/kg/dose) will be used. There will be no PK study required.

Day	Treatment	Doses	Infusion guidelines
-7	Busulfan	0.4 mg/kg/dose q12	over 2 hours
-6	Busulfan	0.4 mg/kg/dose q12	over 2 hours
-5	Cyclophosphamide*	10 mg/kg IV, once daily	over 1 hour
	Fludarabine	35 mg/m^2 IV once daily	over 30 minutes
	Rabbit ATG	2.5 mg/kg IV once daily	over 8 hours
-4	Cyclophosphamide*	10 mg/kg IV once daily	over 1 hour
	Fludarabine	35 mg/m^2 IV once daily	over 30 minutes
	Rabbit ATG	2.5 mg/kg IV once daily	over 8 hours
-3	Cyclophosphamide*	10 mg/kg IV once daily	over 1 hour
	Fludarabine	35 mg/m^2 IV once daily	over 30 minutes
	Rabbit ATG	2.5 mg/kg IV once daily	over 8 hours
-2	Cyclophosphamide*	10 mg/kg IV once daily	over 1 hour
	Fludarabine	35 mg/m^2 IV once daily	over 30 minutes
	Rabbit ATG	2.5 mg/kg IV once daily	over 8 hours
-1	Rest day		
0**	CD34+ PBSCT		
+1	Start G-CSF		

* With Mesna per Institutional practice
** Day 0 could extend to two days if collection requires two separation procedures

An anti-convulsant may be administered during the busulfan per institutional guidelines.

Cyclophosphamide
Cyclophosphamide 10 mg/kg is to be given as a 1-2 hour infusion for 4 days for a total dose of 40 mg/kg. Strict attention should be made to vigorous hydration, fluid balance and maintenance of good urine output. It is recommended that Mesna be administered prior to and after each dose of cyclophosphamide per institutional practice. The dose will be adjusted according to patients ideal body weight for obese patients.

Fludarabine
Fludarabine 35 mg/m^2 will be given IV over 30 minutes daily for 4 days for a total dose of 140 mg/m^2. The dose will be adjusted according to renal function according to Institutional guidelines.

9.2 Immunosuppressive Therapies

Failure Prophylaxis

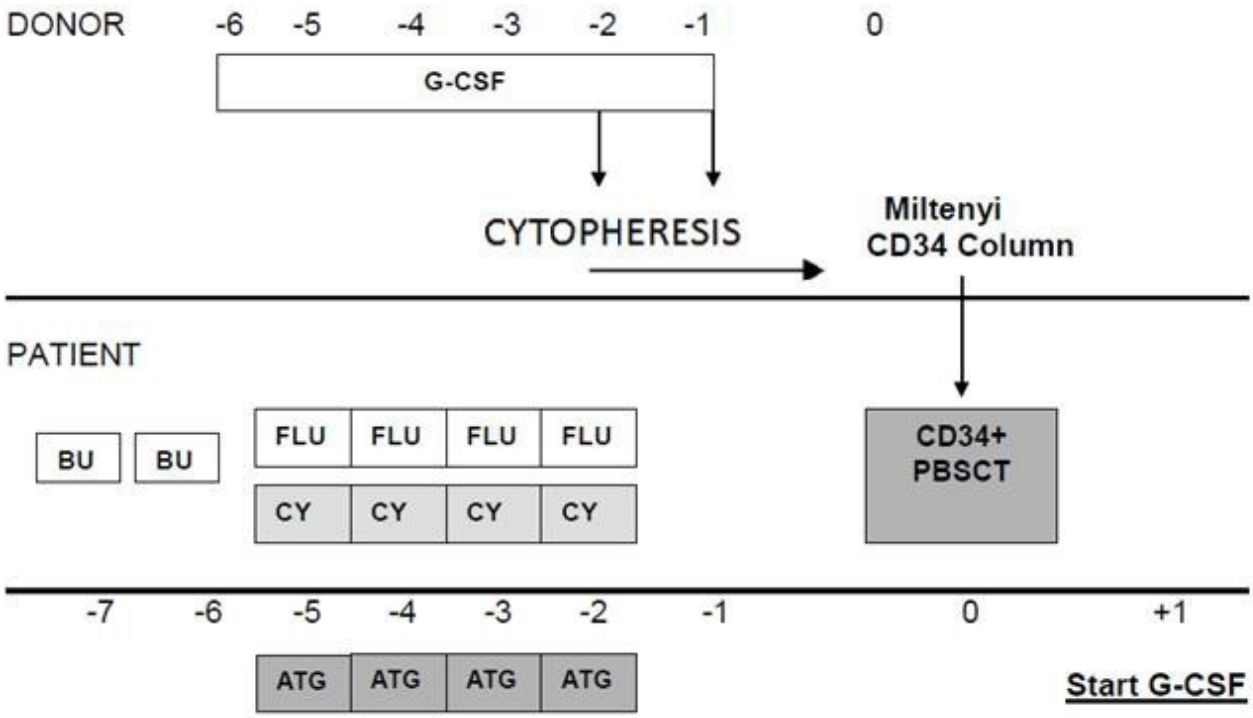
Methylprednisolone Methylprednisolone will be given with the infusion of ATG as per institutional guidelines, and will be discontinued after the completion of the ATG infusion.

Rabbit antithymocyte globulin (Thymoglobulin) 2.5 mg/kg will be given once daily on days -5, -4, -3 and -2. Premedication will include the use of methylprednisolone (as defined above). The first dose of

Rabbit ATG is to be infused over 8 hours. Subsequent doses can be given over 4-6 hours based on institutional guidelines or at the treating physician's discretion. In the event of a severe reaction to antithymocyte globulin, further doses of the agent will not be administered. In this event, Equine antithymocyte globulin (ATGAM, Upjohn) 15 mg/kg IV once daily can be substituted for each remaining day of ATG on the schema. In the event of reaction to ATGAM, Alemtuzumab (Campath 1H) at 10 mg/m2 IV once daily may be administered for each remaining day of ATG on the schema.

Other
G-CSF will be administered to all patients post-transplant starting day +1 as per institutional guidelines. It will be rapidly tapered when the ANC reaches levels ≥ 2.0 x 10^9/L and stabilizes thereafter. G-CSF doses will be rounded according to institutional guidelines.

APPROXIMATE SCHEMA OF CYTOREDUCTION AND PREPARATION FOR ALLOGENEIC PBSCT:



9.3 Stem Cell Collection

9.3.1 Mobilization of Donor

Related Donors
Following screening and enrollment, the donor will receive mobilization therapy with once daily subcutaneous G-CSF as per institutional guidelines. Mobilization will begin on prior to the conditioning regimen at a G-CSF dose of 10 µg/Kg/day of actual body weight (rounded off to a multiple of the nearest vial size of either 300 or 480 µg). Based on the volume, the dose may be split into 2 or 3 injection sites. The mobilization phase starts on the first day of administration of G-CSF and continues until the final day of leukapheresis.

Unrelated Donors
For Unrelated donors, G-CSF will be administered and the leukaphereses obtained according to the National Marrow Donor Program protocol. If more than one leukapheresis is required, mononuclear cell fractions collected on the day 1 and day 2 of leukapheresis may either be pooled prior to CD34 selection or may be CD34 selected individually, per institutional practice.

9.3.2. Progenitor Cell Collection and Processing

9.3.2.1 Leukapheresis – for Related Donors

Leukapheresis will be performed on a continuous flow cell separator according to Institutional standards and will commence on the morning of the 5th day of G-CSF treatment. The anti-coagulant used for the procedure will be acid citrate dextrose (ACD). No additional anti-coagulants or additives (heparin etc.) should be added beyond those normally used during leukapheresis. The volume of blood processed per leukapheresis session should be approximately 3-4 times the total blood volume as tolerated by the donor. A unique identification and labeling system shall be used to track the leukapheresis product from collection to infusion according to institutional guidelines.

The target allograft cell doses following processing on the CliniMACS device are a CD34+ cell dose of > 5.0 x 10^6 cells/Kg recipient weight with a maximum CD3+ cell dose of < 5.0 x 10^4 cells/Kg of recipient weight. A minimum CD34+ cell dose of 2.5 x 10^6 cells/Kg will be targeted. If this minimum target CD34+ cell dose is not achieved after 2 leukaphereses, a third leukapheresis may be planned after discussion with the principal investigator of the Institution.

This protocol requires an absolute minimum CD34+ cell dose of 2.0 x 10^6 cells/Kg in order to be evaluable for all study endpoints. If the post CD34+ selection allograft content is < 2.0 x 10^6 CD34+ cells/Kg after 2 leukapheresis procedures, a third leukapheresis collection without CD34+ selection should be performed in order to ensure that an adequate CD34+ cell dose is transplanted. Since this graft will contain a high number of CD3+ cells, such patients must also receive the addition of pharmacologic GvHD prophylaxis per Institutional standards.

During mobilization and leukapheresis, Institutional Standards for donor supportive care should be maintained.

9.3.2.2. CD34+Selection with the CliniMACS Device – Related or Unrelated Donors

CD34$^+$ cell selection will be performed according to procedures given in the CliniMACS Users Operating Manual and institutional Standard Operating Procedures (SOPs).

Products will be received into the cell processing laboratories and will be either processed that day, or stored overnight. Products stored overnight must be processed the following day. At receipt the product will be accessioned and assigned a unique product identifier if not already assigned during collection. The product will be inspected at the time of receipt and the label information regarding donor name, medical record number (or other identifier) and ABO and Rh group will be compared for consistency to the information in the laboratory chart record and physicians order form.

The product will be sampled for total nucleated cell count (TNC), and if processing is to be performed on the day of receipt, additional sample will be obtained for starting CD34+ cell and starting T cell content and product sterility. Products may be stored overnight for one of two reasons, 1) late arrival preventing selection and infusion the same day, or 2) low TNC content that would permit pooling of two collections for a single column selection. If the product is to be stored overnight, the cells will be diluted to < 2.0 x 10^8 cells/mL using either autologous plasma or the CliniMACS PBS/EDTA-1.0% Human Serum Albumin (HSA) (CliniMACS buffer) and stored in a monitored refrigerator as per institutional policy. Products that are stored overnight will be sampled the following day, prior to processing, for TNC, starting CD34+ cell and starting T cell content, and for sterility.

Analysis of allograft

Samples will be taken from each leukapheresis product pre- and post-$CD34^+$ selection and characterized as follows:

- Graft Evaluation (all of these tests are standard of care and performed as per institutional guidelines)
  - Gram stain (done locally) post-selection on each leukapheresis product
  - Total nucleated cell count (done locally) pre- and post-selection on each leukapheresis product
  - Endotoxin testing post-selection on each leukapheresis product
  - Flow cytometric analysis for CD34+ cells pre- and post-selection Flow cytometric analysis for CD3+ cells pre- and post-selection and log depletion on each leukapheresis product done
  - Viability testing (trypan blue dye exclusion assay) post-selection
  - 14 day sterility cultures post-selection on each leukapheresis product

- Criteria for release of product
  - Viability ≥70% after selection
  - Negative gram stain
  - $CD34^+$ cell count of product

In regards to the 14 day sterility cultures performed post-selection on each leukapheresis product, a Stat Gram stain is used for release as well as performing endotoxin testing. In the event that a sterility test is found to be positive, post infusion the following will occur:

a. The Medical Director for the laboratory is immediately informed, and this information will be conveyed to the clinical care team to evaluate the patient clinically.
b. If any residual specimen is available, it will be submitted for confirmatory sterility testing.
c. An investigation review will be performed by the Medical Director to assess all process steps to attempt to identify a cause if possible. This includes a review of the environmental monitoring data that is generated periodically.

d. If a source of the contamination can be identified, corrective action steps will be devised and implemented as appropriate to prevent recurrence.
e. The investigator will be provided with the investigation review and will notify the FDA and IRB as appropriate.

As noted above, the target optimal allograft cell doses following processing on the CliniMACS device include both a $CD34^+$ cell count of > 5.0 x 10^6/kg recipient weight and a $CD3^+$ cell dose of < 5.0 x 10^4/kg recipient weight. The targeted minimum $CD34^+$ cell dose following $CD34^+$ selection is 2.5 x 10^6/kg. If after two leukapheresis collections followed by $CD34^+$ selection the total allograft contains 2.5-5.0 x10^6 $CD34^+$ cells/kg and < 5.0 x10^4 $CD3^+$ cells/kg, a total of three leukapheresis collections followed by $CD34^+$ selection are allowed as long as the total $CD3^+$ cell dose transplanted does not exceed 5.0 x 10^4/kg. That is, a graft containing 2.5-5.0x10^6 $CD34^+$ cells/kg and < 5.0 x10^4 $CD3^+$ cells/kg is acceptable whereas a graft containing 5.0x10^6 $CD34^+$ cells/kg but > 5.0 x10^4 $CD3^+$ cells/kg is not. A major goal is to administer a $CD34^+$ cell dose > 5.0x10^6/kg while limiting the $CD3^+$ cell dose to < 5.0 x10^4 but as long as a minimum of 2.0 x10^6/kg $CD34^+$ cells are infused, the $CD3^+$ cell dose should be kept to < 5.0 x10^4 /kg. It may be possible to give only a proportion of the $CD34^+$ selected product in order to maintain an adequate $CD34^+$ cell dose while limiting the $CD3^+$ cell dose to < 5.0 x10^4 /kg.

It is also possible that doses of $CD34^+$ cells far exceeding 5.0 x 10^6/kg can be given without exceeding the maximum T cell dose of 5.0 x10^4 $CD3^+$ cells/kg. High doses of $CD34^+$ cells in extensively T cell depleted transplants are postulated to hasten immune reconstitution, without altering the low risk of GVHD. Consequently, there is no upper limit for the dose of $CD34^+$ cells/kg as long as the dose of $CD3^+$ cells does not exceed 5.0 x10^4 /kg.

Decisions concerning the release of samples from the $CD34^+$ selected product will be based on $CD34^+$ and $CD3^+$ cell analysis done by the flow cytometry laboratory. The attached table outlines the various scenarios that may be encountered after each CD34+ cell selection and indicates steps that may be used to achieve target allograft cell doses. The steps below are recommendations only, as the requirement for a second or third leukapheresis procedure will be determined by the treating attending and patient/donor requirements.

Table 9.3.2.2 – Guidelines for achieving target allograft cell doses

Leuka-Pheresis Attempt	Total CD34+ dose after Leukapheresis1 (X 106/Kg)				Total CD3+ after Leukapheresis 1 (x 104/Kg)		ACTION				
	< 1.0	1.0 - 2	2.0 - 4.9	≥ 5.0	< 5.0	≥ 5.0	Administer Allograft	Proceed To next Leuka-Pheresis	Leuka-Pheresis Completed	Reduce CD3 dose to< 5.0 Maintain CD 34 dose > 2.0	Begin GvHD Prophylaxis
1				X	X		X	X2			
1				X		X		X		X3	
1	X	X4	X4		X		X	X			
1	X	X4	X4			X		X		X3	
2				X	X		X		X		
2				X		X				X5	
2		X4	X4		X		X	X			
2		X4	X4			X				X6	
2	X				X7	X7	X	X8			X9
3				X	X		X		X		
3			X		X		X		X		
3				X		X				X10	
3			X			X				X10	
3		X			X		X		X		
3		X				X					X11
3	X				X7	X7					

1. This number refers to CD34+ (x 10^6/Kg) or CD3+ (x 10^4/Kg) doses after CD34+ selection. If after leukapheresis #2 or #3, this number refers to total pooled CD34+ and CD3+ cell doses following all CD34+ selections
2. If unsuccessful in reducing CD3+ cell dose to < 5.0 x 10^4 cells/Kg at any CD34+ cell dose, hold allograft overnight and proceed to leukapheresis #2. If leukapheresis #2 results in a post selection CD34+ cell count of > 2.0 x 10^6 cells/Kg with CD3+ cell dose of < 5.0 x10^4 cells/Kg, give that allograft, proceed to leukapheresis #3 and discard or cryopreserved graft from leukapheresis #1. The primary goal is to reduce the total CD3+ cell dose to < 5.0 x 10^4 cells/Kg while maintaining the total CD34+ cell dose to > 2.0 x 10^6 cells/Kg.
3. Refers to any one of these three possible CD34+ cell doses (<1.0 – 4.9)

4. If unsuccessful in reducing CD3+ cell dose to < 5.0 x10^4 cells/Kg at any CD34+ cell dose, begin GvHD prophylaxis and administer allograft within 24 hours
5. If able to reduce CD3+ cell dose to < 5.0 x 10^4 cells/Kg while maintaining CD34+ cell dose ≥ 2.0 x 10^6 cells/Kg, proceed to leukapheresis #3. If unsuccessful in reducing CD3+ cell dose to < 5.0 x 10^4/Kg at any CD34+ cell dose, begin additional GvHD prophylaxis with steroids at 2.0 mg/Kg/day or Mycophenolate mofetil (Cellcept®)* and then administer allograft.
6. Refers to either one of the two possible CD3+ cell doses.
7. Proceed to leukapheresis #3, but do not perform CD34+ selection on product from leukapheresis #3
8. If the CD3+ cell dose is ≥ 5.0 x 10^4 /Kg, consider additional GvHD prophylaxis with steroids at 2.0 mg/Kg/day or Mycophenolate mofetil (Cellcept®)* and then administer product from leukapheresis #2.
9. If the patient has already received total allograft containing > 2.0 x 10^6/kg CD34+ cell dose and < 5.0 x 10^4/kg CD3+ cell dose, hold allograft of leukapheresis #3. If the patient has received total allograft containing < 2.0 x 10^6/kg CD34+ dose, administer product from leukapheresis #3 and begin additional GVHD prophylaxis with steroids at 2.0 mg/kg/day or Mycophenolate mofetil (Cellcept®)*.
10. Begin additional GvHD prophylaxis with steroids at 2.0 mg/Kg/day or Mycophenolate mofetil (Cellcept®)* and then administer product from leukapheresis #3.
11. Contact the study PI to discuss options.
12. A third leukapheresis collection without CD34+ selection should be performed in order to ensure that an adequate CD34+ cell dose is transplanted

* Mycophenolate mofetil (Cellcept®) will be administered as per institutional guidelines and if needed, Mycophenolate sodium (Myfortic®) may be substituted for Mycophenolate mofetil (Cellcept®) after appropriate dose conversion.

9.4. Stem Cell Transplantation

The final CD34+ T-cell depleted product will be infused into the patient intravenously according to Institutional standards. The first day of infusion will be designated as day 0 of transplant.

9.5. Supportive Care

Institutional standard of care guidelines will be followed for transplant related supportive care, including monitoring for CMV reactivation and provision of PCP prophylaxis. CMV safe blood products will be administered and prophylaxis against peri-transplant infections will be used according to Institutional guidelines.

10.0 EVALUATION DURING TREATMENT/INTERVENTION

Post-transplant Evaluation

a. Clinical Assessments

Patients will receive physical examinations daily until discharge with particular attention to assessments of potential toxicities induced by preparatory cytoreductive therapy, including mucositis, cystitis, sepsis, pneumonia, veno-occlusive disease (VOD) and transplant-associated complications including graft failure, acute and chronic GvHD, and transplant associated infections. Patients are

closely monitored for alterations in vital signs, weight, oral and intravenous intake, and intestinal and urinary output. Cardiac assessments are obtained prior to admission to assess cardiac function and thereafter when clinically indicated. Pulmonary status is closely monitored when clinically indicated by radiographic and functional analysis. Baseline physical examinations and any subsequent, clinically significant abnormal new findings will be recorded.

Following discharge, physical examination, will be performed at least every 2-4 weeks or as clinically indicated until day 100 after stem cell infusion.

All timelines are approximations and adhere to good clinical practice. Certain tests may be held at the discretion of the treating physician and/or if deemed in the best clinical interest of the patient.

b. Laboratory Evaluations

Baseline laboratory evaluations and any subsequent clinically significant, abnormal new findings will be recorded.

- Complete blood count daily until count recovery, then 3 times weekly until hospital discharge; at least every 1-2 weeks after discharge through day 100.

- Comprehensive metabolic panel with liver function tests
  - twice weekly for the first 30 days
  - then weekly to discharge
  - more frequently if clinically indicated.

- Bone marrow aspirates will be obtained as clinically indicated. Proposed time points are at approximately 1, 3, 6, and 12 months for patients with MDS or AML. Additional analyses will be done as clinically indicated.

- Chimerism studies will be performed approximately weekly as clinically indicated until at least day 100. Additional total engraftment studies are recommended at approximately 1, 3, 6, 12, 18 and 24 months. Sorted engraftment studies may also be performed according to institutional guidelines and treating physician's discretion at above time points as clinically indicated.

- Immunologic reconstitution will be monitored by in vitro assays and should include lymphocyte subpops, response to mitogens, and immunoglobulin levels. Immune studies are recommended at approximately 3, 6, 9, and 12 months. If immune reconstitution has not been demonstrated by 12 months, immune studies are recommended at 15, 18, and 24 months as well.

11.1 TOXICITIES/SIDE EFFECTS

Patients recruited to this transplantation trial are individuals who are either referred by physicians or self-referred for marrow transplantation as a potentially curative treatment for their malignancy. Prior to consideration for transplant, all patients undergo a series of 1-3 hour consultations discussing the risks and potential benefits of an allogeneic marrow transplant and the different procedures, which will be a normal part of the transplant course. The risks and potential benefits of the transplant procedure, as well as the participation in any given research, experimental, or therapeutic protocol are also discussed.

The potential risks of an allogeneic transplant from an HLA-compatible unrelated donor or HLA-mismatched related donor are significant. These risks along with approaches to circumvent or minimize their effects on the health and wellbeing of the patient can be summarized as follows.

11.1. General Description of Risks to Recipients

Infections and hemorrhage constitute major and continuing risks throughout the period of marrow aplasia. These are, however, also the major risks associated with the primary disease. Certain opportunistic infections remain a risk in bone marrow transplant recipients beyond recovery of circulating leukocytes, for at least 9-12 months post-transplant, e.g. Pneumocystis carinii and cytomegalovirus.

The above risks are carefully considered both by the physicians and by the patients prior to admission to a study protocol. The patients who are being considered for allogeneic peripheral blood stem cell transplantation are afflicted with Fanconi anemia and aplastic anemia, myelodysplastic syndrome or leukemias which are lethal diseases for whom a hematopoietic stem cell transplant from a an HLA-compatible unrelated donor or HLA-mismatched related donor currently constitutes the only curative option in the treatment of this disease. In the protocol proposed, use is made of an experimental device produced by Miltenyi Biotec, Auburn, CA. This device is approved for experimental applications under an IND from the Food and Drug Administration.

All patients undergoing cytoreduction in the protocol will be treated with busulfan, cyclophosphamide and fludarabine. They will also receive anti-human thymocyte globulin to prevent graft rejection. All of the treatments as well as other drugs used before and during the transplant may produce side effects. These risks are listed below.

a. Busulfan

- Myelosuppression, complete ablation is expected.
- Gastrointestinal: nausea and vomiting, anorexia, diarrhea
- Alopecia
- Mucositis
- Seizures: generally preventable by anti-convulsant therapy started 24 hours prior to administration and continued for 24 hours post busulfan
- Abnormal liver function
- Pulmonary fibrosis
- Gonadal dysfunction
- Hyperpigmentation

b. Cyclophosphamide

- Bone marrow suppression, complete bone marrow ablation is expected.
- Gastrointestinal: Nausea, vomiting, anorexia, diarrhea, stomatitis, esophagitis, colitis
- Alopecia
- Fever
- Serious hypersensitivity reactions: Edema, rash, anaphylaxis
- Water retention: inappropriate secretion of ADH, usually manifested 4-8 hours after IV administration
- Cardiomyopathy:Cyclophosphamide may cause a severe, sometimes lethal cardiomyopathy.
- Hemorrhagic cystitis: serious complication related to the interaction of cyclophosphamide metabolites
- Skin rash: This may develop 1-3 days post infusion and subsides thereafter
- Hemolytic anemia
- Sterility: This is a likely complication after puberty. Risk may be reduced in young children.

- Late effects: Late effects may occur with varying degree following cyclophosphamide and include growth failure, gonadal failure and sterility, hypothyroidism, and secondary malignancies.

c. Fludarabine
- Nausea, vomiting
- Mouth sores, stomach cramps and diarrhea
- Jaundice, and elevations of liver enzymes
- Scaling and redness of the skin, which is usually of short duration.
- Effects on the nervous system are not usually seen at the fludarabine dose used in this protocol, but, when they occur, can include cerebellar dysfunction with loss of balance and trouble walking.

d. *Methylprednisolone*
- Increased risk of infection
- Increased blood pressure
- Stomach upset including ulcers. Prophylaxis (such as antacids) will be administered as protection
- Hyperlipidemia
- Hyperglycemia

e. Anti-Thymocyte Globulin
- ATG is a rabbit protein, which may induce an immune response in humans. Prior exposure to rabbit proteins may predispose to serious allergic reactions such as anaphylaxis, generalized urticaria, or bronchospasm. This risk remains significant. Such reactions will be treated with epinephrine and anti-histamines. In the event of a severe systemic allergic reaction, a trial of an alternative equine ATG will be administered. If a similar reaction occurs with the equine ATG, no further ATG will be administered.

- Serum sickness - approximately 10-30% of patients treated with rabbit ATG will develop a late immune reaction to the globulin resulting in serum sickness 3-10 days after administration. This is manifested by fever, rash, and arthralgias. Renal toxicity is rare. Prednisone for one week at 1 mg/kg has been shown to be effective therapy.

- Fever and chills - are managed by antipyretics and meperidine but are usually only severe after first dose.

- Leukopenia/thrombocytopenia - ATG may also cause further decrease in leukocyte and platelet counts, which will be managed by transfusion therapy.

f. G-CSF
- Fever
- Fatigue
- Bone pain
- Allergic reaction, mild to severe
- Splenomegaly

g. Subsequent Malignancies
a. Patients transplanted for leukemia are at significant risk of recurrence of their original disease.

b. Patients receiving T-cell depleted marrow grafts with ATG are at significant risk of developing an EBV lymphoproliferative disease. For patients treated with horse ATG, this risk is approximately 10-15%. However, for those treated with rabbit ATG, it is less than 4%. This complication might be

prevented with Acyclovir prophylaxis and may be treatable by infusions of peripheral blood leukocytes from the marrow donor.

c. Secondary malignancies - there is a possibility that secondary malignancies may develop. The age-adjusted incidence of secondary cancers in transplant patients after radiation and chemotherapy has recently been estimated to be 6.7 times higher than that of first cancers in the general population. Most of these were non-Hodgkin's lymphomas.

h. Graft Failures

Rejection of a T-cell depleted related, HLA-disparate marrow and peripheral blood progenitor graft can occur. Patients rejecting their grafts may receive a secondary transplant from the same or an alternative donor, or may receive a reinfusion of autologous cryopreserved marrow whenever available. In our prior study using T-cell depleted grafts for Fanconi anemia, graft rejection leading to marrow aplasia did not occur.

Alternatively, mixed chimerism (>10% donor cells) with lymphocytosis may occur. Mixed chimerism as determined by the treating physician may be treated with Campath 0.2 mg/kg/dose SC given daily until the absolute lymphocyte count (ALC) is <200, to a maximum of 5 doses. A rapidly expanding lymphocytosis may be treated with Campath prior to the availability of chimerism data at the discretion of the treating physician.

i. Graft Versus Host Disease

Acute GvHD is manifested by skin rash; hepatitis; ulceration of the surfaces of the oral cavity, esophagus, and intestines; and suppressed or delayed recovery of the hematopoietic and immune system. In patients transplanted and engrafted with $SBA^{-}E^{-}$ T-cell depleted marrow from HLA 1-3 allele disparate related donors, this complication has been observed in fewer than 20% of patients and has rarely been severe. It may be fatal in at least 20-50% of cases and may also predispose to lethal infections which contribute to an additional mortality of 10-25%. Severe acute GvHD will be treated with intense immunosuppressive therapy according to standard clinical practice or other experimental protocol. In our prior study using T-cell depleted grafts for Fanconi anemia severe GVHD did not occur.

Approximately 50% of patients with acute GvHD may also develop chronic GvHD, manifested to varying degrees by scleroderma-like changes of the skin, cirrhosis of the liver, sclerosis of lacrimal and salivary ducts, chronic inflammation and scarring of the gastrointestinal tract with consequent malabsorption and diarrhea, chronic bronchitis, and suppression of the immune system. This can be treated with standard or protocol-based experimental immunosuppression, but may be refractory.

j. Stem cell infusions

The volume of the T-cell depleted peripheral blood stem cells infused is approximately 30-50 cc. They may induce allergic reactions. Small, subclinical pulmonary emboli may occur, but these rarely if ever require any intervention. Standard pre-medications for blood products may be used before administration of the stem cell graft.

Infusions of fractionated blood products may also induce allergic reactions of variable severity, many of which can be prevented or mitigated by premedication with antipyretics, antihistamines, and narcotics. These products may also serve as vectors of serious infection (e.g., CMV, hepatitis, AIDS). To circumvent this, prospective blood and marrow donors will be screened per institutional guidelines.

CMV antibody (-) blood products will be used in CMV (-) individuals, whenever possible, regardless of the antibody status of the marrow donor. ALL blood products are irradiated (3000r, ^{137}Cs) to circumvent the risk of GvHD caused by contaminating lymphocytes in the transfused fractions.

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

Primary Outcomes

Definition of events in the post-transplant course important for analysis and treatment

12.1. Graft Failure or Rejection

Graft failure or rejection will be the primary endpoint of this study:
Primary non-engraftment is diagnosed when the patient fails to achieve an ANC ≥500/μl at any time in the first 28 days post-transplant. If (1) after achievement of an ANC ≥500/mm^3, the ANC declines to <500/mm^3 for more than 3 consecutive days in the absence of relapse, or, (2) there is absence of donor cells in the marrow and/or blood as demonstrated by chimerism assayin the absence of relapse, a diagnosis of secondary graft failure is made. The patient is not evaluable for graft failure or rejection if recurrence of host MDS is detected concurrently. Patients with evidence of graft failure without evidence of recurrence of host MDS will have additional studies drawn to ascertain cause and define relevant histoincompatibilities.

These analyses may include (1) Evaluation of bone marrow aspirates and biopsies for residual or recurrent MDS/AML, when indicated, and (2) Culture and/or molecular analyses of marrow and/or blood for viral pathogens potentially causing graft failure including CMV, HHV6 and parvovirus B 19.

Patients who suffer graft failure will be considered for a secondary transplant. The need for additional immunosuppression or treatment for viral infection prior to the secondary transplant will be determined by the results obtained from chimerism and viral studies. We have performed secondary transplants for patients with graft failure and have published our experience on rescuing patients with graft failure with fludarabine based regimens – including one patient with Fanconi anemia (Chewning JH, et al. Fludarabine-based conditioning secures engraftment of second hematopoietic stem cell allografts in the treatment of initial graft failure. *Biol Blood Marrow Transplant. 13(11):1313-23; 2007)*

12.2. Early post-transplant severe morbidity and mortality

The occurrence of severe post-transplant regimen-related severe morbidity (grade IV toxicity) and/or mortality will be the second endpoint of this study. In the context of the agents or agent-combination used for cytoreduction used, particular attention will be given to toxicity involving (1) the liver, (2) the lungs, (3) the oral mucosa and gastrointestinal tract, and (4) the central nervous system. The grading for monitoring the morbidity and mortality will be based on the NCI/CTEP common toxicity criteria version 4.0.

12.3. Graft Versus Host Disease

Patients will be observed for acute and/or chronic GvHD. Standard clinical criteria for the grading of acute and chronic GvHD will be done according to IBMTR guidelines See Appendices 3 and 4.

To determine the severity of acute GvHD, data may be collected approximately weekly to characterize the severity of symptoms and signs caused by GvHD and to evaluate possible confounding factors. Real time data collection of GvHD will include grades for each organ involved and overall grade.

Patients with moderate to severe acute GvHD (grade II-IV) will be treated with immunosuppressive therapy according to standard clinical practice or other experimental protocol.

Treatment of chronic GvHD will consist of corticosteroids, and other agents or modalities, according to Institutional standard of care guidelines and protocols.

12.4. Leukemic Relapse

For patients with MDS or AML, relapse will be analyzed as to type and genetic origin of the MDS/leukemic cells. These will be defined by an increasing number of blasts in the marrow over 5%, by the presence of circulating peripheral blasts, or by the presence of blasts in any extramedullary site. Cytogenetic analysis of the marrow and/or peripheral blood will also be obtained for the diagnosis of relapse.

12.5. Secondary malignancies

Patients may be followed indefinitely through contact with MSKCC in order to track the risk of developing a secondary malignancy. All clinical outcomes will be followed per standard institutional practice.

13.0 CRITERIA FOR REMOVAL FROM STUDY

Patients may be removed from study therapy at any point deemed appropriate by the principal investigator. However, once the busulfan is given, patients will continue on study until after administration of the stem cells. Failure to rescue the patient with stem cells at this point in the cytoreduction would most likely be fatal.

14.0 BIOSTATISTICS

The primary objectives of this phase II trial are to assess the incidence of engraftment, graft versus host disease, and treatment related mortality and morbidity (treatment related morbidity will is defined as any attributable grade 4 toxicity) in patients with Fanconi Anemia lacking genetically identical donor. A total of 10 MSKCC patients will be enrolled. 60 additional patients will be enrolled onto separate but identical studies conducted outside of MSKCC. Patient data from these sites will be aggregated and reviewed in total for safety and data analysis.

Stopping rules and biostatistical calculations below are derived using a total of 70 patients treated at all 3 centers.

<u>Stopping Rules:</u>
Risk related to treatment regimen differs for each therapeutic arm. The recommended treatment for patients in Arm A is the standard of care and therefore no stopping rule is required. Stopping rules are:

- Arm B: 4 out of the first 5 patients or 6 out of 10 patients demonstrate any attributable grade 4 toxicity in the first 100 days.
- Arm C: 4 out of the first 5 patients or 6 out of 10 patients experience graft failure within the first 42 days of transplant as defined in section 12.1.

The data coordinating center is constantly in communication with participating sites, and will inform sites of any updates to stopping rules in real time.

Sample Size Consideration:
In Arm B the maximum number of patients enrolled will be 10. The increased dose is not expected to increase treatment related mortality, therefore the primary risk of interest is treatment related morbidity. Assuming treatment related morbidity is 40% the probability of the completion of Arm B is 80%.

In Arm C the maximum number of patients enrolled will be 10. The decreased dose is not expected to increase treatment related morbidity, therefore the primary risk of interest is treatment related mortality. Assuming treatment related mortality is 40% the probability of the completion of Arm C is 80%.

Interpretation of the Study Results:
For patients in Arm A, the primary risk is any treatment related death. In our previous study 85% (4/16) of patients who were less than 18 years, had SAA and an unrelated or mismatched related donor survived. In this present version of the protocol, enrollment on this arm will include up to 50 patients. If the study in Arm A is completed with full enrollment per original study design (i.e. 20 patients, as per an earlier version of this protocol) and 14 or more patients survive past 100 days this will be considered supporting evidence for the previous study results. Based on a conservative estimate of 80% overall survival the probability of 14 or more patients surviving past 100 days is 91%. The following table contains the estimates of overall survival and exact 95% confidence intervals for such events.

Observed Overall Survival	Estimated Survival Rate	95% Exact Confidence Interval
20	1.00	(0.861 - 1.00)
19	0.95	(0.751 - 0.999)
18	0.90	(0.683 – 0.988)
17	0.85	(0.621 – 0.968)
16	0.80	(0.563 – 0.943)
15	0.75	(0.509 – 0.913)
14	0.70	(0.457 – 0.881)

If the study in Arm B (or Arm C) is completed with maximum enrollment then the following table represents the estimated treatment related morbidity (or mortality in Arm C) along with its 95% exact confidence interval.

Number of treatment related morbidities (or mortalities) in 10 patients	Estimated treatment related morbidities (or mortalities)	Exact 95% Confidence Interval
5	0.5	(0.186 - 0.813)
4	0.4	(0.122 - 0.738)
3	0.3	(0.067 - 0.651)
2	0.2	(0.025 - 0.556)
1	0.1	(0.003 - 0.445)
0	0.0	(0.0 - 0.259)

Statistical analyses:

Analyses will include Fisher's exact test for categorical variables or Wilcoxon's rank sum test for continuous variables. Disease free survival will be defined as time from date of transplant to relapse, graft rejection or graft failure, or death. Survival and cumulative incidence curves will be plotted using the Kaplan-Meier method. Survival will be compared for the entire study duration by group using log-rank tests. Confidence intervals and p-values for the difference in 1 year survival by group will be computed using Wald's method with Greenwoods variance approximation.

Quality of engraftment will be evaluated by linear specific chimerism analysis studies at 3, 6, 9 months post-transplant. Hematopoietic reconstitution will be evaluated by 1) neutrophil, red cell, and platelet counts at regular times post-transplant, 2) time to red cell and platelet transfusion independence, and 3) time to normalization of cell counts for all three lineages.

15.1 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.2 Research Participant Registration

Confirm eligibility as defined in the section entitled Inclusion/Exclusion Criteria. Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures. During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist. The individual signing the Eligibility Checklist is confirming whether or not the participant is eligible to enroll in the study. Study staff are responsible for ensuring that all institutional requirements necessary to enroll a participant to the study have been completed. See related Clinical Research Policy and Procedure #401 (Protocol Participant Registration).

15.3 Randomization

N/A

16.1 DATA MANAGEMENT ISSUES

The PI will be responsible for the conduct of the study, monitoring of study progress, review and validation of data entry, and the conduct of data analysis for MSK patients. De-identified MSKCC data and source documentation will be shared with Cincinnati Children's Hospital Medical Center and Fred Hutchinson Cancer Research Center for combined data analysis.

A Clinical Research Coordinator (CRC)will be assigned to this study. The responsibilities of the CRC include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordinate the activities of the protocol study team. The data manager will also monitor laboratory compliance throughout the study. Laboratory data will be tabulated and summarized based on MSKCC normal ranges.

The data for MSKCC patients collected for this study will be entered into Medidata.

16.2 Quality Assurance

Registration reports will be generated by the CRC on a regular basis to monitor patient accruals and completeness of the registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period and potential problems will be brought to the attention of the study team for discussion and action.

Random-sample data quality and protocol compliance audits will be conducted by the study team, at a minimum of two times per year, more frequently if indicated.

In case of an FDA audit, the FDA will audit each participating site individually as each site will hold their own IND. Thus each center will be responsible for having all source documents, research records, all IRB approval documents, Drug Accountability Record forms, patient registration lists, response assessment scans, x-rays, etc. available for the audit.
There will not be external monitoring of this study by CCHMC as this study is being conducted under a separate IND.

16.3 Data and Safety Monitoring

The Data and Safety Monitoring (DSM) Plans at Memorial Sloan-Kettering Cancer Center were approved by the National Cancer Institute in September 2001. The plans address the new policies set forth by the NCI in the document entitled “Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials” which can be found at: http://cancertrials.nci.nih.gov/researchers/dsm/index.html. The DSM Plans at MSKCC were established and are monitored by the Office of Clinical Research. The MSKCC Data and Safety Monitoring Plans can be found on the MSKCC Intranet at: https://one.mskcc.org/sites/pub/clinresearch/Documents/MSKCC%20Data%20and%20Safety%20Monitoring%20Plans.pdf

There are several different mechanisms by which clinical trials are monitored for data, safety and quality. There are institutional processes in place for quality assurance (e.g., protocol monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research QA) and departmental procedures for quality control, plus there are two institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees: Data and Safety Monitoring Committee (DSMC) for Phase I and II clinical trials, and the Data and Safety Monitoring Board (DSMB) for Phase III clinical trials, report to the Center’s Research Council and Institutional Review Board.

During the protocol development and review process, each protocol will be assessed for its level of risk and degree of monitoring required. Every type of protocol (e.g., NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group, etc.) Will be addressed and the monitoring procedures will be established at the time of protocol activation.

MSK will submit safety monitoring reports for patients accrued at our institutions to MSK's DSMC.

17.1 PROTECTION OF HUMAN SUBJECTS

Risks: From the studies that have been done so far there appears to be no increase in risk to the patients. However, given this is a new treatment, it is possible that there are side effects that have not yet been seen.

Benefits: The information from this study will help future patients.

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

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

Alternatives: Alternative treatment options will be presented to the patient prior to taking part in this study. Alternative treatment options may include getting a transplant from a volunteer unrelated donor, if one is available; getting treatment for your cancer with either chemotherapy or a transplant without being on a study; taking part in another study; or getting no treatment.

Costs: The patient's health plan/insurance company will need to pay for all of the costs of treatment in this study. The patient will be responsible for the costs of standard medical care, all hospitalizations and any transplant complications. Pre-authorization for the transplant will be cleared with the health plan/insurance company prior to admission. Patients will not be paid for taking part in this study. Research tests will be done at no cost to the patient.

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

17.2 Privacy

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

The consent indicates that individualized de identified information collected for the purposes of this study may be shared with other qualified researchers. Only researchers who have received approval from MSK will be allowed to access this information which will not include protected health information, such as the participant's name, except for dates. It is also stated in the Research Authorization that their research data may be shared with others at the time of study publication.

Enrollment on this study is on a voluntary basis and every effort will be made to maintain privacy and confidentiality. The patient's records will be confidential. Only authorized individuals or agencies may inspect the records. No identifying information will be used in reports or publications resulting from this study.

17.3 Serious Adverse Event (SAE) Reporting

An adverse event is considered serious if it results in ANY of the following outcomes:

- Death
- A life-threatening adverse event
- An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

Note: Hospital admission for a planned procedure/disease treatment is not considered an SAE.

SAE reporting is required as soon as the participant signs consent. SAE reporting is required for 30-days after the participant's last investigational treatment or intervention. Any events that occur after the 30-day period and that are at least possibly related to protocol treatment must be reported.

Please note: Any SAE that occurs prior to the start of investigational treatment/intervention and is related to a screening test or procedure (i.e., a screening biopsy) must be reported.

All SAEs must be submitted in PIMS. If an SAE requires submission to the HRPP office per IRB SOP RR-408 'Reporting of Serious Adverse Events', the SAE report must be submitted within 5 calendar days of the event. All other SAEs must be submitted within 30 calendar days of the event.
The report should contain the following information:

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

- If the SAE is an Unanticipated Problem

The SAE report should be completed as per above instructions. If appropriate, the report will be forwarded to the FDA by the IND Office

17.2.1 Serious Adverse Event (SAE) Reporting Additional Information

Attribution:

- Unrelated: The AE is clearly NOT related to the intervention
- Unlikely: The AE is doubtfully related to the intervention
- Possible: The AE may be related to the intervention
- Probable: The AE is likely related to the intervention
- Definite: The AE is clearly related to the intervention

Expected and Unexpected Event:

- Expected: Any experience *previously reported* (in nature, severity, or incidence) in the current Investigator's Brochure or general investigational plan
- Unexpected: Any experience *not previously reported* (in nature, severity, or incidence) in the current Investigator's Brochure or general investigational plan

UNEXPECTED EVENT:

- Grades 1-2: Adverse Event Reporting NOT required.
- Grades 3: Possible, Probable or Definite attribution to the drug and/or device.
- Grades 4 and 5: Regardless of Attribution. This includes all deaths within 30 days of the last dose of treatment with an investigational agent regardless of attribution.

EXPECTED EVENT

- Grades 1 – 3: Adverse Event Reporting NOT required.
- Grades 4 and 5: Regardless of Attribution. This includes all deaths within 30 days of the last dose of treatment with an investigational agent regardless of attribution.

*Reportable events are those which occur within 30 days of the last dose of treatment on protocol. Events beyond 30 days will be reported at the discretion of the PI.

18.1 INFORMED CONSENT PROCEDURES

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

1. The nature and objectives, potential risks and benefits of the intended study.

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

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

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form. Signed copies of the consent will be forwarded to CCHMC at the time of patient registration.

19.0 REFERENCES

1. Young, N.S., Alter B.P. Clinical features of Fanconi's Anemia. In: Young NS, Alter BP, eds. (1994). Aplastic Anemia acquired and inherited. Philadelphia, PA: Saunders; pp275-309, 1994.

2. Auerbach AD, Woman SR. Susceptibility of Fanconi Anemia fibroblasts to chromosome damage by carcinogens. Nature 261: 494-496, 1976.

3. Butturini A, Gale RP, Verlander PC, Adler-Brecher B, Gillio AP, Auerbach AD. Hematologic abnormalities in Fanconi Anemia: an International Fanconi Anemia Registry study. Blood 84:1650-1655, 1994.

4. Barrett AJ, Brigden WD, Hobbs JR, Hugh-Jones K, Humble JG, James DC, Retsas S, Rogers TR, Selwyn S, Sneath P, Watson JG. Successful bone marrow transplant for Fanconi's anaemia. British Medical Journal. 1(6058):420-2, 1977.

5. Gluckman E., Devergie A., Schaison G., Bussel A., Berger R., Sohier J., Bernard J. Bone marrow transplantation for Fanconi Anemia. Br J. Haematol. 45:557-564, 1980.

6. Deeg HJ. Storb R. Thomas ED. Appelbaum F. Buckner CD. Clift RA. Doney K. Johnson L. Sanders JE. Stewart P. Sullivan KM. Witherspoon RP. Fanconi's anemia treated by allogeneic marrow transplantation. Blood. 61(5):954-9, 1983.

7. Gluckman E. Auerbach AD. Horowitz MM. Sobocinski KA. Ash RC. Bortin MM. Butturini A. Camitta BM. Champlin RE. Friedrich W. et al. Bone marrow transplantation for Fanconi anemia. Blood. 86(7):2856-62, 1995.

8. Gluckman E. Auerbach A. Ash RC. Biggs JC. Bortin MM. Camitta BM. Champlin RE. Friedrich W. Gale RP. Good RA. et al. Allogeneic bone marrow transplants for Fanconi anemia. A preliminary report from the International Bone Marrow Transplant Registry. Bone Marrow Transplantation. 10 Suppl 1:53-7, 1992.

9. Socie G., Gluckman E., Raynal B., Petit T., Landman J., Devergie A., Brison O. Bone marrow transplantation for Fanconi anemia using low-dose cyclophosphamide/ thoracoabdominal irradiation as conditioning regimen: chimerism study by the polymerase chain reaction. Blood. 82(7):2249-56, 1993.

10. Flowers MED., Doney KC., Storb R., Sanders JE., Sullivan KM., Bryant E., Witherspoon RP., Appelbaum F., Buckner CD., Hansen JA., Thomas ED., Marrow transplantation for Fanconi Anemia with or without leukemic transformation: an update of the Seattle experience. Bone Marrow Transplantation 9:167-173, 1992.

11. Kohli-Kumar M., Morris C., DeLaat C., Sambrano J., Masterson M., Mueller R., Shahidi N., Yanik G., Desantes K., Friedman DJ., Auerbach AD., Harris RE. Bone marrow transplantation in Fanconi Anemia using matched sibling donors. Blood 84:2050-2054, 1994.

12. Flowers MED., Zanis J., Pasquini R., Deeg JH., Ribeiro R., Longton G., Medeiros CR., Doney K., Sanders J., Bryant E., Hansen J., Sullivan KM., Appelbaum F., Thomas ED., Storb R. Marrow transplantation for Fanconi Anaemia: conditioning regimen with reduced doses of cyclophosphamide without radiation. Brit. J. Haematol. 92:699-706, 1996.

13. Dokal IS., Roberts IAG. Bone marrow transplantation for Fanconi's Anaemia: conditioning regimen with reduced doses of cyclophosphamide without radiation. Brit. J. Haematol. 94:423-425, 1996.

14. Solh H., Rao K., Martins da Cumba AM., Padmos A., Sackey K., Ernst P., Spence D., Clink H. Bone marrow transplantation in patients with Fanconi Anemia: experience with cyclophosphamide and total body irradiation conditioning regimen. J. Pediatr. Hematol. Oncol. 14:67-72, 1997.

15. Socie G. Devergie A. Girinski T. Piel G. Ribaud P. Esperou H. Parquet N. Maarek O. Noguera MH. Richard P. Brison O. Gluckman E. Transplantation for Fanconi's anaemia: long-term follow-up of fifty patients transplanted from a sibling donor after low-dose cyclophosphamide and thoraco-abdominal irradiation for conditioning. British Journal of Haematology. 103(1):249-55, 1998.

16. Guardiola P. Socie G. Pasquini R. Dokal I. Ortega JJ. van Weel-Sipman M. Marsh J. Locatelli F. Souillet G. Cahn JY. Ljungman P. Miniero R. Shaw J. Vermylen C. Archimbaud E. Bekassy AN.

Krivan G. Di Bartolomeo P. Bacigalupo A. Gluckman E. Allogeneic stem cell transplantation for Fanconi Anaemia. Severe Aplastic Anaemia Working Party of the EBMT and EUFAR. European Group for Blood and Marrow Transplantation. Bone Marrow Transplantation. 21 Suppl 2:S24-7, 1998.

17. Maschan AA. Kryzanovskii OI. Yourlova MI. Skorobogatova EV. Pashanov ED. Potapova YE. Timonova LA. Bogatcheva NY. Samochatova EV. Roumjantzev AG. Intermediate-dose busulfan and cyclophosphamide as a conditioning regimen for bone marrow transplantation in a case of Fanconi anemia in myelodysplastic transformation. Bone Marrow Transplantation. 19(4):385-7, 1997

18. Auerbach AD. Adler B. O'Reilly RJ. Kirkpatrick D. Chaganti RS. Effect of procarbazine and cyclophosphamide on chromosome breakage in Fanconi anemia cells: relevance to bone marrow transplantation. Cancer Genetics & Cytogenetics. 9(1):25-36, 1983

19. Gluckman E., Devergie A., Dutreix J. Radiosensitivity in Fanconi Anaemia: application to the conditioning regimen for bone marrow transplantation. Brit. J. Haematol. 54:431-440, 1983.

20. Yule SM., Price L., Cole M., Pearson AD., Boddy AV. Cyclophosphamide metabolism in children with Fanconi's anaemia. Bone Marrow Transplantation. 24(2):123-8, 1999.

21. Hows JM. Unrelated donor bone marrow transplantation for severe aplastic anaemia and Fanconi's anaemia. Bone Marrow Transplantation. 4 Suppl 4:126-8, 1989

22. Davies SM. Khan S. Wagner JE. Arthur DC. Auerbach AD. Ramsay NK. Weisdorf DJ. Unrelated donor bone marrow transplantation for Fanconi anemia. Bone Marrow Transplantation. 17(1):43-7, 1996

23. Davies SM, Harris RE, van Weel-Sipman, Dokal J, Skinner R, Casper JT, Kurtzberg J, Smith F, Karanes C, Chen A, Cairo A, Yeaker AM, Defor T, Wagner JE: Unrelated donor stem cell transplant for Fanconi anemia: Multicenter report of 49 cases. Blood 90:108a, 1997.

24. Massumoto C, Moraes JR, Moraes ME, Macedo MC, Medeiros R, Chamone D, et al. Unrelated peripheral blood stem cell transplantation for Fanconi Anemia. Bone Marrow Transpl. 19:299-300, 1997.

25. Zwaan CM. Van Weel-Sipman MH. Fibbe WE. Oudshoorn M. Vossen JM. Unrelated donor bone marrow transplantation in Fanconi anaemia: the Leiden experience. Bone Marrow Transplantation. 21(5):447-53, 1998

26. Guardiola P, Locatelli F, Pasquini R, Socie G, Devergie A, Riband P, Esperou H, Gardenet L, Bittencourt M, Marollease J, Gluckman E. Results of a phase I/II multicenter prospective study using CD34+ selection for HLA matched unrelated transplants in Fanconi anemia. Blood 92:290a, 1998.

27. Guardiola P, Pasquini R, Dokal I, Ortega JJ, van Weel-Sipman M, Warsh JC, Ball S, Locatelli F, Vermylen C, Skinner R, Ljungman P, Miniero R, Shaw PJ, Souillet G, Michalet M, Bekassy AN, Di Bartolomeo P, Heilmann C, Zanesco L, Cahn JY, Arcese W, Bacigalupo A, Gluckman E. Outcome of 69 allogeneic stem cell transplantations for Fanconi Anemia using HLA-matched unrelated donors: A study on behalf of the European Group for Blood and Marro Transplantation. Blood 95: 422-429, 2000.

28. MacMillan ML, Davies SM, Defor TE, Auerbach AD, Gillio A, Giller R, Harris R, Cairo M, Dusenberg K, Hirsch B, Ramsey NKC, Weisdorf DJ, Wagner JE: Hematopoietic cell transplantation in patients with Fanconi anemia using non-genotypically identical donors: Results of a TBI dose escalation trial. Br J Haematol. 2000 Apr;109(1):121-9.

29. Tan PL, Wagner JE, Auerbach AD, Defor TE, Slungaard A, Macmillan ML. Successful engraftment without radiation after fludarabine-based regimen in Fanconi anemia patients undergoing genotypically identical donor hematopoietic cell transplantation. Pediatr Blood Cancer. 2006 May 1;46(5):630-6.

30. Boyer MW, Gross TG, Loechelt B, Leemhuis T, Filipovich A, Harris RE. Low risk of graft-versus-host disease with transplantation of CD34 selected peripheral blood progenitor cells from alternative donors for Fanconi anemia. J Pediatr Hematol Oncol. 2003 Nov;25(11):890-5.

31. Mehta PA, Davies SM, Leemhuis T, Myers K, Kernan NA, Prockop SE, Scaradavou A, O'Reilly RJ, Williams DA, Lehmann L, Guinan E, Margolis D, Baker KS, Lane A, Boulad F. Radiation-free, alternative-donor HCT for Fanconi anemia patients: results from a prospective multi-institutional study. Blood. 2017 Apr 20;129(16):2308-2315.

32. Somers GR. Tabrizi SN. Tiedemann K. Chow CW. Garland SM. Venter DJ. Squamous cell carcinoma of the tongue in a child with Fanconi anemia: a case report and review of the literature. Pediatr Pathol Lab Med. 15(4):597-607, 1995

33. Millen FJ. Rainey MG. Hows JM. Burton PA. Irvine GH. Swirsky D. Oral squamous cell carcinoma after allogeneic bone marrow transplantation for Fanconi anaemia. British Journal of Haematology. 99(2):410-4, 1997

34. Socie G. Scieux C. Gluckman E. Soussi T. Clavel C. Saulnier P. Birembault P. Bosq J. Morinet F. Janin A. Squamous cell carcinomas after allogeneic bone marrow transplantation for aplastic anemia: further evidence of a multistep process. Transplantation. 66(5):667-70, 1998

35. Witherspoon, R.P., Fisher, L.D., Schoch, G., Martin, P., Sullivan, K.M., Sanders, J., Deeg, H.J., Doney, J., Thomas, D. & Storb, R. Secondary cancers after bone marrow transplantation for leukemia or aplastic anemia. New England Journal of Medicine, 321, 784-789, 1989

20.0 APPENDICES

Appendix 1 – WHO Classification
Appendix 2 – Performance Scores
Appendix 3 – Acute GVHD
Appendix 4 – Chronic GVHD
Appendix 5 – Toxicity

Appendix 1
MDS Classification

WHO Classification

	BLOOD	BONE MARROW
Refractory Anemia RA	- 0 or rare blasts - Anemia - < 1 x 10^9/L monocytes	- <5% blasts - Dysplasia only Erythroid - Myeloid-MegaK dysplasia < 10% of cells
Refractory cytopenia with multilineage dysplasia RCMD	- 0 or rare blasts - Cytopenias (2-3 lineages) - No Auer rods - < 1 x 10^9/L monocytes	- <5% blasts - Dysplasia in 2-3 lineages - Dysplasia in ≥10% of cells - No Auer rods
Refractory anemia with excess blasts – 1 RAEB-1	- <5% blasts - Cytopenias (2-3 lineages) - No Auer rods - < 1 x 10^9/L monocytes	- 5-9% blasts - Dysplasia in 1-3 lineages - No Auer rods
Refractory anemia with excess blasts – 2 RAEB-2	- 5-19% blasts - Cytopenias (2-3 lineages) - Auer rods +/- - < 1 x 10^9/L monocytes	- 10-19% blasts - Dysplasia in 1-3 lineages - Auer rods +/–
Myelodysplastic syndrome, unclassified MDS-U	- 0 or rare blasts - Cytopenias (2-3 lineages) - No Auer rods	- <5% blasts - Myeloid-MegaK dysplasia - No Auer rods

IPSS Classification*

1. Single Lineage Score

	BM Blasts	Cytogenetics**	Cytopenia
0	<5%	Good	0-1
0.5	5-10%	Intermediate	2-3
1	-	Poor	-
1.5	11-20%	-	-
2	21-30	-	-

**Good: normal, -Y, del(5q), del (20q);
Poor: complex (≥ 3 abnormalities) or chromosome 7 anomalies;
Intermediate: other abnormalities.

2. Overall IPSS Risk Group

Risk Group	Combined IPSS Score
Low	0
Intermediate 1	0.5-1.0
Intermediate 2	1.5-2.0
High	> 2.0

Appendix 2
Performance Scores

LANSKY SCALE (<16 y.o.)

The score is defined by the phrase which best describes the activity status of the recipient.
Able to carry on normal activity; no special care is needed.

100 Fully active
90 Minor restriction in physically strenuous play
80 Restricted in strenuous play, tires more easily, otherwise active

Mild to moderate restriction

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

Moderate to severe restriction

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

KARNOFSKY SCALE (≥16 y.o.)

The score is defined by the phrase which best describes the activity status of the recipient.

Able to carry on normal activity; no special care is needed.

100 Normal; no complaints; no evidence of disease
90 Able to carry on normal activity
80 Normal activity with effort

Unable to work; able to live at home, care for most personal needs; a varying amount of assistance is needed.

70 Cares for self; unable to carry on normal activity or to do active work
60 Requires occasional assistance but is able to care for most needs
50 Requires considerable assistance and frequent medical care

. Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly.

40 Disabled; requires special care and assistance
30 Severely disabled; hospitalization indicated, although death not imminent
20 Very sick; hospitalization necessary
10 Moribund; fatal process progressing rapidly.

Appendix 3
Acute GvHD

CLINICAL STAGING AND GRADING OF ACUTE GRAFT VERSUS HOST DISEASE

	ORGAN INVOLVEMENT		
STAGE	Skin	Liver	Gut
1	maculopapular rash <25% body surface	Bili 2.0 – 3.0 mg/dl	*Diarrhea* 500-1000 ml/d (children: 280-555 ml/m^2/d) OR *persistent nausea*
2	maculopapular rash 25-50 % of body surface	Bili 3.1 – 6.0 mg/dl	*Diarrhea* >1000 but ≤ 1500 ml/d (children: 556-833 ml/m^2/d)
3	maculopapular rash >50 % of body surface	Bili 6.1 - 15 mg/dl	*Diarrhea* >1500 ml/d (children: >834 ml/m^2/d)
4	generalized erythroderma with bullous formation	Bili > 15 mg/dl	Severe abdominal pain ± Ileus

	GRADING		
GRADE	Skin	Liver	Gut
0	**NONE**	None AND	**NONE**
I	Stage 1-2 AND	None AND	**NONE**
II	Stage 3 AND/OR	Stage 1 AND/OR	**Stage 1**
III	None OR Stage 3 AND	Stage 2-3 OR	**Stage 2-4**
IV	Stage 4 OR	**Stage 4**	NA

STAGING

- For skin GvHD: - Use "Rule of Nines or burn chart to determine extent of rash
- For liver GvHD: - Range of bilirubin given as total bilirubin.
  - Downgrade one stage if an additional cause of hyperbilirubinemia is documented
- For gut GvHD: - Downgrade one stage if an additional cause of diarrhea is documented
  - St 1: Persistent nausea, vomiting and anorexia in the absence of other known cause – Unless histology is negative

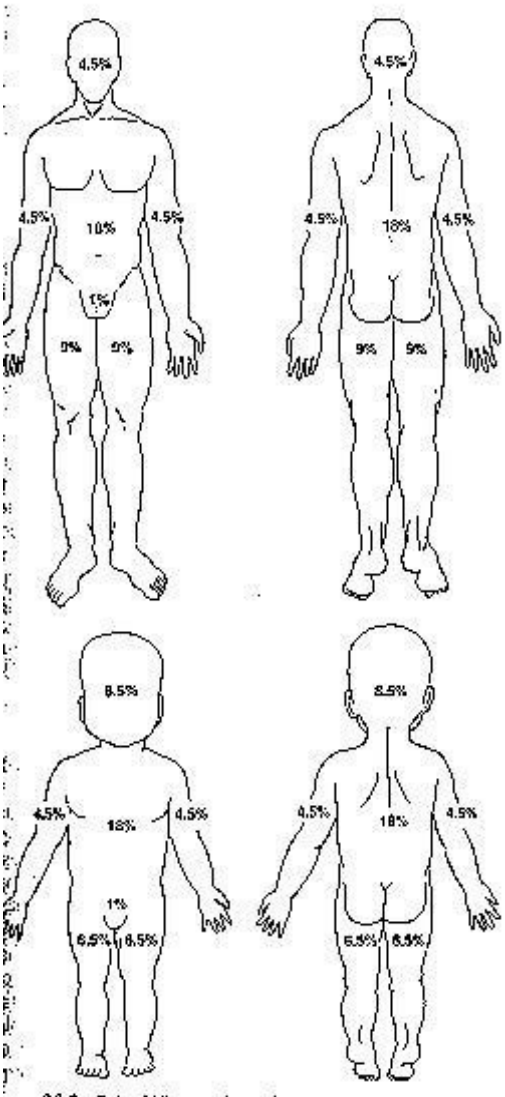
GRADING- Criteria for grading given as minimum degree of organ involvement required to confer that grade

REF: Przepiorka et al. 1994 Consensus Conference on Acute GVHD Grading ("Keystone Criteria"). Bone Marrow Transplant 1995; 15:825-8.

ADULT Rule of Nines (for GvHD scoring)

(see diagram for children – varies with age)

4.5%	front of head
4.5%	back of head
4.5%	front of arm x 2 arms, max 9%
4.5%	back of arm x 2 arms, max 9%
18%	chest/trunk
18%	back/trunk
9%	front of leg x 2 legs, max 18%
9%	back of leg x 2 legs, max 18%
1%	Perineum



ure 26-9. Rule of Nines to determine percentage of body surface ea burn in adults (A) and children (B).

Appendix 4
Chronic GvHD

Chronic GvHD Scoring

Limited	Either or both: 1. localized skin involvement 2. hepatic dysfunction due to chronic GvHD
Extensive	Either 1. generalized skin involvement, OR 2. localized skin involvement AND/OR hepatic dysfunction due to chronic GvHD PLUS 3a. liver histology showing chronic aggressive hepatitis, bridging necrosis, or cirrhosis, OR 3b. involvement of eye (Schirmer's <5mm wetting) 3c. involvement of minor salivary glands or oral mucosa demonstrated on labial bx 3d. involvement of any other target organ.

REF: Forman et al. Bone Marrow Transplantation (text) 1994, page 350.

Clinical Manifestations of Chronic GvHD

Organ	Clinical Manifestation	Evaluation	Intervention
Skin	Erythematous papular rash (lichenoid) or thickened, tight, fragile skin (sclerodermatous).	Clinical and biopsy to confirm the diagnosis of GVHD.	Moisturize (petroleum jelly), treat local infections, protect from further trauma. Topical steroid ointment may be used if it gives symptomatic relief to localized areas.
Nails	Vertical ridging, fragile. damage.	Clinical.	Nail polish may help to decrease further
Sweat glands	Destruction leading to risk of hyperthermia.		Avoid excessive heat.
Hair	Scalp and body hair is thin and fragile, can be partially or completely lost.	Clinical.	

Eyes	Dryness, photophobia, and burning. Progression to corneal abrasion.	Regular ophthalmologic evaluation including Schirmer's test.	Preservative free tears during the day and preservative free ointment at night.
Mouth	Dry; sensitivity to mint, spicy food, tomato. Whitish lace-like plaques Avoid foods which are not tolerated. Regular in the cheeks and tongue identical to lichen planus. Erythema and dental care preceded by appropriate endocardi-painful ulcerations, mucosal scleroderma with decreased sensitivity to temperature can also happen.		Regular dental evaluation (with appropriate endocarditis prophylaxis). Viral and fungal cultures at diagnosis and at any worsening. tis prophylaxis. Topical steroid rinses followed by an antifungal agent for symptomatic relief.
Respiratory tract	Bronchiolitis Obliterans can manifest as dyspnea, wheezing, cough with normal CT scan and marked obstruction at pulmonary function tests. Chronic sinopulmonary symptoms and/or infections are also common.With abnormal chest CT, must rule out infections. Lung biopsy if clinically indicated.	Pulmonary function tests including FEV^1, FVC, DLCO, helium lung volumes. CT scan in symptomatic patients.	Investigational therapy.
Gastrointestinal	Abnormal motility and strictures. Weight loss.	Swallowing studies, endoscopy if clinically indicated. Nutritional evaluation. intervention.	Systemic treatment of GVHD; endoscopical/ surgical treatment of strictures. Nutritional
Liver	Cholestasis (increased bilirubin, alkaline phosphatase). Isolated liver involvement needs histologic confirmation.	Liver function tests. Liver biopsy if clinically indicated.	No specific therapy is proven superior. FK506 may concentrate in the liver.

Musculoskeletal	Fasciitis. Myositis is rare. Osteoporosis may occur secondary to hormonal deficits, use of steroids, decreased activity.	Periodical physical therapy evaluation to document the range of motion. Bone density evaluation especially in patients using steroids.	Aggressive physical therapy program.
Immune system Variable IgG levels.	Profound immunodeficiency. Functional asplenia. High risk of pneumococcal sepsis, PCP, and invasive fungal infections. GVHD has resolved.	Assume all patients as severely immuno-compromised and asplenic to 6 months after vaccinations.	PCP prophylaxis (until 6 months after no GVHD) and Pneumococcal prophylaxis (lifetime). Delay
Hematopoietic system	Cytopenias. Occasional eosinophilia.	Counts. Bone marrow aspirate and biopsy, anti-neutrophil and anti-platelet antibodies when indicated.	Systemic treatment of GVHD.
Others	Virtually all autoimmune disease manifestations have been described in association with chronic GVHD.	As clinically indicated.	

Appendix 5
Toxicity

Refer to NCI Common Toxicity Criteria 4.0

Website:
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40