



**Memorial Sloan-Kettering Cancer Center
IRB Protocol**

IRB#: 08-031 A(10)

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

MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL

Principal Investigator/Department:	Farid Boulad, MD	Pediatrics, BMT
Co-Principal Investigator(s)/Department:	Susan Prockop, MD	Pediatrics, BMT
Investigator(s)/Department:	Kevin Curran, MD	Pediatrics, BMT
	Aisha Hasan, MD	Pediatrics, BMT
	Nancy Kernan, MD	Pediatrics, BMT
	Rachel Kobos, MD	Pediatrics, BMT
	Andromachi Scaradavou, MD	Pediatrics, BMT
	Richard O'Reilly, MD	Pediatrics, BMT
	Glenn Heller, PhD	Epidemiology and Biostatistics
	Heidi Abendroth, R.N., P.N.P.	Nursing, Pediatrics
	Catherine Copeland, R.N., P.N.P.	Nursing, Pediatrics
	Anne Casson, R.N., P.N.P.	Nursing, Pediatrics
	Maura Murphy, R.N., P.N.P.	Nursing, Pediatrics
	Joanne Torok-Castanza, R.N., P.N.P.	Nursing, Pediatrics
	Nicole Zakak, R.N., P.N.P.	Nursing, Pediatrics
Consenting Professional(s)/Department:	Kevin Curran, MD	Pediatrics, BMT
	Aisha Hasan, MD	Pediatrics, BMT
	Farid Boulad, MD	Pediatrics, BMT
	Susan Prockop, MD	Pediatrics, BMT
	Nancy Kernan, MD	Pediatrics, BMT
	Rachel Kobos, MD	Pediatrics, BMT
	Andromachi Scaradavou, MD	Pediatrics, BMT
	Richard O'Reilly, MD	Pediatrics, BMT

**Memorial Sloan-Kettering Cancer Center
1275 York Ave. New
York, NY 10021**

**IRB
PB**

Amended: 06/18/14



**Memorial Sloan-Kettering Cancer Center
IRB Protocol**

IRB#: 08-031 A(10)

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

**Collaborating
Institution(s):**

Boston Children's Hospital, Data Collection
Cincinnati Children's Hospital, Data Collection
Children's Hospital of Wisconsin, Data Collection
Rockefeller University, Data analysis and/or specimen analysis
Fred Hutchinson Cancer Research Center, Data Collection



Memorial Sloan-Kettering Cancer Center
IRB Protocol

IRB#: 08-031 A(10)

Table of Contents

MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL..... 1
1.0 PROTOCOL SUMMARY AND/OR SCHEMA 4
2.0 OBJECTIVES AND SCIENTIFIC AIMS 5
3.0 BACKGROUND AND RATIONALE 6
4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION 8
4.1 DESIGN 8
5.0 THERAPEUTIC/DIAGNOSTIC AGENTS 9
6.0 CRITERIA FOR SUBJECT ELIGIBILITY 15
6.1 SUBJECT INCLUSION CRITERIA 15
6.2 SUBJECT EXCLUSION CRITERIA 16
7.0 RECRUITMENT PLAN 17
8.0 PRETREATMENT EVALUATION 17
9.0 TREATMENT/INTERVENTION PLAN 18
9.1 PREPARATIVE CYTOREDUCTION 18
9.2 IMMUNOSUPPRESSIVE THERAPIES 20
9.3 STEM CELL COLLECTION 22
10.0 EVALUATION DURING TREATMENT/INTERVENTION 25
11.0 TOXICITIES/SIDE EFFECTS 26
12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT 30
13.0 CRITERIA FOR REMOVAL FROM STUDY 31
14.0 BIOSTATISTICS 32
15.1 RESEARCH PARTICIPANT REGISTRATION - MSKCC 33
CONFIRM ELIGIBILITY AS DEFINED IN THE SECTION ENTITLED CRITERIA
FOR PATIENT/SUBJECT ELIGIBILITY 33
15.2 RESEARCH PARTICIPANT REGISTRATION - OTHER CENTERS 34
16.0 DATA MANAGEMENT ISSUES 34
16.2 QUALITY ASSURANCE 35
16.3 DATA AND SAFETY MONITORING 36
17.0 PROTECTION OF HUMAN SUBJECTS 36
17.1 PRIVACY 37
FOR IND/IDE PROTOCOLS: 38
17.2.1 SERIOUS ADVERSE EVENT (SAE) REPORTING FOR PARTICIPATING
INSTITUTIONS 38
18.0 INFORMED CONSENT PROCEDURES 40
19.0 REFERENCE(S) 40
20.0 APPENDICES 44
APPENDIX 1 45
APPENDIX 2 46
APPENDIX 3 48
APPENDIX 4 50
APPENDIX 5 52



Amended: 06/18/14



**Memorial Sloan-Kettering Cancer Center
IRB Protocol**

IRB#: 08-031 A(10)

1.0 PROTOCOL SUMMARY AND/OR SCHEMA

The trial proposed is a single arm phase II multicenter treatment protocol designed to examine engraftment, toxicity, graft-versus-host disease, and ultimate 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), severe isolated single lineage cytopenia (SISLC), 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.

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

All patients 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 (35 mg/m²/day x 4 doses). All patients will also receive rabbit ATG (thymoglobulin[®]) (2.5 mg/Kg/dose x 4 doses) prior to transplant to promote engraftment. Cyclosporine will be used for prophylaxis against GvHD. All patients will also receive G-CSF post-transplant to foster engraftment.

Busulfan doses were adjusted based on Busulfan pharmacokinetics.

As of April 2012, patient accrual is nearly complete. As a result, after the completion of the planned 25 patient study, we have amended the protocol to accrue at most an additional 20 patients in order to gain clinical experience using alternative levels of Busulfan.

Previously, 25 patients were treated with 0.8-1.0 mg/kg of Busulfan. In this continuation study, 10 patients will be treated at 0.6-0.8 mg/kg of Busulfan. If no primary graft failures are observed in these 10 patients, then a further cohort of 10 patients will be treated at 0.4-0.6 mg/kg. If however, at any time during this accrual a graft failure is observed, then the Busulfan dose will be modified and a final cohort will be treated at the initial dose of 0.8-1.0 mg/kg.

Although no graft failure has been seen with the dose of 0.6-0.8 mg/kg of Busulfan, it was decided to enroll the remainder of the patients at this cohort, as a precautionary measure and in order to maintain the safety and effectiveness of the condition regimen. Some patients have had some evidence of mixed chimerism, and we therefore do not see any benefit in reducing the Busulfan dose any further.

The preferred source of stem cells 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 uniformly performed in all centers 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 chemotherapy-based cytoreductive regimen plus a CD34+ selected T-cell depleted peripheral blood stem cell (PBSC) stem cell



**Memorial Sloan-Kettering Cancer Center
IRB Protocol**

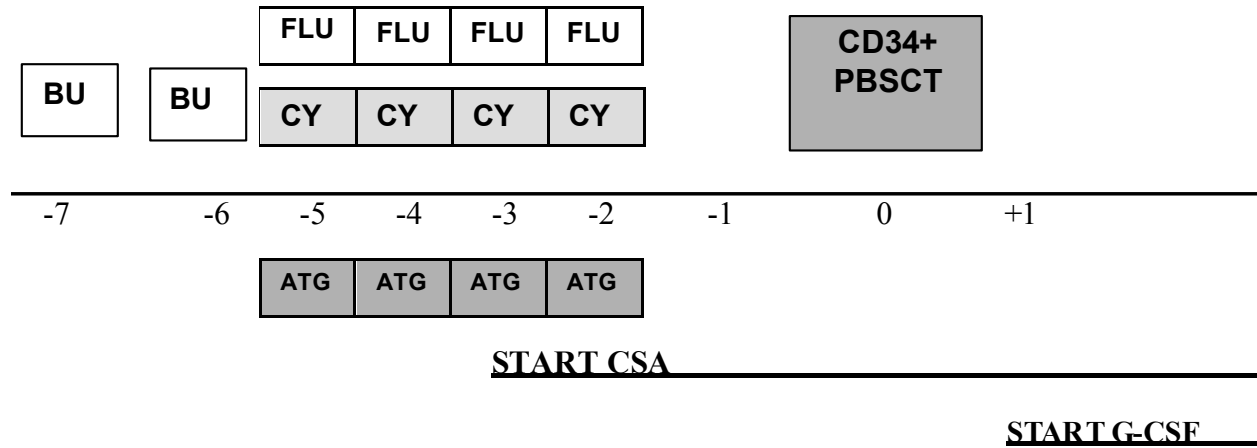
IRB#: 08-031 A(10)

transplant for the treatment of patients with Fanconi anemia and severe hematologic disease. The majority of patients will receive grafts derived from PBSC and will be the focus of the trial.

The study population will consist of one patient population with two types of donors: (1) HLA-compatible unrelated donors, and (2) HLA-mismatched related donors. Donor preference will be determined by each center. All patients eligible for this protocol by virtue of being seen at a participating center and having (1) hematologic disease AND (2) lack of genotypically matched related donor will be captured on the database of this protocol.

It is anticipated that the accrual will last 6 years and a total of approximately 45 PBSC patients will enter the study.

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 cyto-reduction and preparation for allogeneic PBSC:



BU	Busulfan	0.6-0.8 mg/Kg/dose IV Q 12H	X 4 doses
FLU	Fludarabine	35 mg/m2/dose IV once daily	X 4 doses
CY	Cyclophosphamide	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
CSA	Cyclosporine	2.5 mg/Kg/dose IV Q 8-12 H	
G-CSF	Filgrastim	as per institutional guidelines	

2.0 OBJECTIVES AND SCIENTIFIC AIMS

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



Memorial Sloan-Kettering Cancer Center IRB Protocol

IRB#: 08-031 A(10)

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) HLA-mismatched related donors.

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 initial estimates of:

4. the incidence of overall survival and disease-free survival over time

3.0 BACKGROUND AND RATIONALE

3.1. 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 (4-24). The chromosome fragility of FA and its hypersensitivity to DNA cross-linking agents and radiation has for some time limited the ability to perform marrow transplantation using donors other than HLA-matched siblings (25-27). In the context of alternative donors such as unrelated marrow donors or cord blood, the use of a similar cytoreduction has been associated with high rates of rejection/graft failure and GvHD, resulting in poor overall survival (28-37).

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 (4-24). More recent studies however have shown improved results (80%) when transplants have been performed in younger patients, with fewer prior transfusions (13).

Approximately 110 patients with FA were initially reported who received stem cell transplants from closely matched unrelated donors; 69 reported recently to the European Group for Blood and Marrow Transplantation and approximately 40 in the US (28-36). 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 cyclosporin 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 (30). Using elutriation to deplete T-cells the Minnesota group observed a 32% incidence of grade II-IV acute GvHD in 20 transplanted patients (35).

Guardiola et al. (33) 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% (29, 30,



Memorial Sloan-Kettering Cancer Center IRB Protocol

IRB#: 08-031 A(10)

35). The presence of lymphocyte mosaicism (i.e. presence of DEB insensitive cells) was associated with a significant increased risk of graft failure (35). 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 (35). 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 (35).

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 marrow 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 (37-39). 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 FA survivors of stem cell transplantation have developed solid tumors post transplant (40-43). 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 are aimed at decreasing these risks.

Based on these data, we propose to use a non-TBI based cytoreductive regimen of busulfan (instead of TBI), cyclophosphamide and fludarabine with ATG and cyclosporine followed by peripheral blood stem cell grafts, aggressively T-cell depleted.

3.2 RATIONALE

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

The use of the cyclophosphamide / fludarabine and ATG / CSA combination is because it has been successful in most recent trials with limited toxicity and decreased risks of rejection.

The purpose of peripheral blood stem cells is to achieve high doses of stem cells in order to prevent graft rejection. In addition, it could potentially decrease mucositis and possible related infectious morbidity by improving time to engraftment. The use of CD34selected stem cells (which are highly T cell depleted) is intended to minimize the risks of GVHD.



4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.1 Design

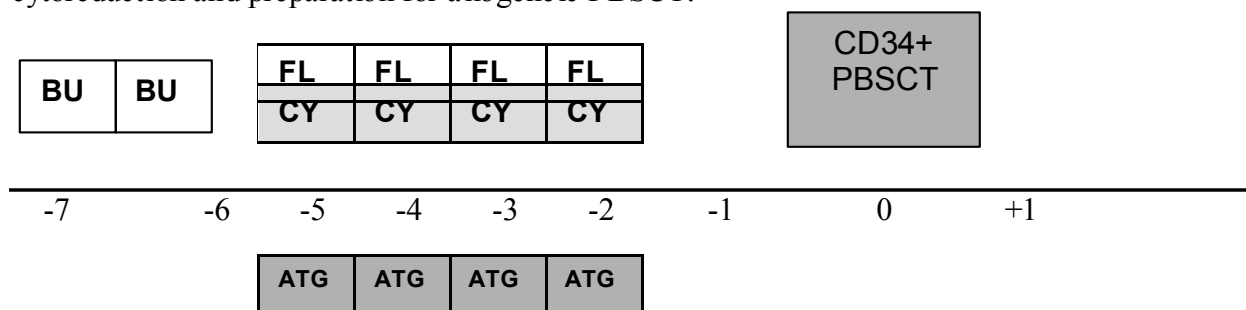
The trial proposed is a single arm phase II multicenter treatment protocol designed to examine engraftment, toxicity, graft-versus-host disease, and ultimate disease-free survival following a novel cytoreductive regimen including busulfan, cyclophosphamide and fludarabine and ATG for the treatment of 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), severe isolated single lineage cytopenia, 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 a 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 (2) 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.

4.2 Intervention

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



START CSA

START G-CSF

BU	Busulfan	0.6-0.8 mg/Kg/dose IV Q 12H	X 4 doses
FLU	Fludarabine	35 mg/m2/dose IV once daily	X 4 doses
CY	Cyclophosphamide	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
CSA	Cyclosporine	2.5 mg/Kg/dose IV Q 8-12 H	
G-CSF	Filgrastim	as per institutional guidelines	

nended: 06/18/14

**IRB
PB**



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 $\text{CH}_3\text{SO}_2\text{O}(\text{CH}_2)_4\text{OSO}_2\text{CH}_3$ 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 ampoules 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 INJECTION contains fludarabine phosphate, a fluorinated nucleotide analog of the antiviral agent vidarabine, 9-β-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-β-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.



**Memorial Sloan-Kettering Cancer Center
IRB Protocol**

IRB#: 08-031 A(10)

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.



**Memorial Sloan-Kettering Cancer Center
IRB Protocol**

IRB#: 08-031 A(10)

- 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 Cyclosporine (Sandimmune)

- a. Source and Pharmacology:** Supplier: Novartis. Cyclosporine
- b. Formulation and Stability:**
SUPPLIED AS: 50 mg/ml; 5 ml ampule (Protect from light)
- c. Solution Preparation:**
1. Dilute cyclosporine concentrate for injection in 20 -100 ml of NS or D5W injection. Prepare injections in glass containers to avoid possible leaching of diethylhexylphthalate (DEHP) from polyvinyl chloride (PVC) containers into injections of cyclosporine.
 2. Diluted solutions are stable for 24 hours in D5W injection and 12 hours in NS in glass containers.
 3. Visually inspect parenteral products for particulate matter and discoloration prior to administration whenever solution and container permit.
- d. Storage and Stability:**
72 hours under refrigeration or at room temperature.
- e. Administration:**
- I.V. PUSH contraindicated



**Memorial Sloan-Kettering Cancer Center
IRB Protocol**

IRB#: 08-031 A(10)

- I.V. PIGGYBACK: Dilute in D5W or NS to make a 2.5 mg/ml solution. Infuse slowly over approximately 2 hours (intermittent infusion) or 24 hours for continuous infusion. Prepare in a glass bottle only. The bottle should be spiked with vented tubing and primed with either D5W or NS.
- Do not administer rapidly; rapid administration may cause acute nephrotoxicity, flushing, and nausea.

LARGE VOLUME INFUSION: PRECAUTIONS AND COMMENTS:

1. Patients should be under close observation for possible allergic manifestations including facial flushing, respiratory distress, with dyspnea and wheezing, blood pressure changes and tachycardia.
2. Prior to infusion solution should be inspected visually for particulate matter and discoloration.
3. Concomitant use of immunosuppressive agents (steroids, ATG) require aggressive monitoring for infection.
4. Nephrotoxic agents will increase the risk of nephrotoxicity (amphotericin B, aminoglycosides, and acyclovir) high doses.
5. Plasma concentration of cyclosporine may be affected by the following drugs:
 - a. Increased cyclosporin levels: ketoconazole, erythromycin, cimetidine, calcium channel blockers, fluconazole, itraconazole, norfloxacin, imipenem/cisplatin
 - b. Decreased cyclosporin levels: rifampin, phenytoin, phenobarbital, imipenem/cilastin.
6. The IV to oral dose conversion is 1:3.
7. The target serum level of 200 - 400 is desirable; 800 is considered toxic.
8. Renal and hepatic parameters should be monitored routinely with dosage adjustments in the case of serum creatinine or LFT elevations.

5.6 The CliniMACS device

Drug Formulation and Procurement

5.6.1. 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.

- 5.6.1.1. A software controlled instrument that processes the blood sample, CliniMACS Instrument
- 5.6.1.2. A single-use, sterile disposable tubing set with two proprietary cell selection columns, CliniMACS Tubing Set
- 5.6.1.3. A monoclonal antibody reagent specific for CD34+ cells, CliniMACS CD34 Reagent
- 5.6.1.4. 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.

5.6.2 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



Memorial Sloan-Kettering Cancer Center IRB Protocol

IRB#: 08-031 A(10)

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.

5.6.3. CliniMACS Tubing Set

5.6.3.1. 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.

5.6.3.2. 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.

5.6.3.3. The 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.

5.6.4. 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 $\mu\text{g}/\text{mL}$ of dextran and 800 $\mu\text{g}/\text{mL}$ 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.



**Memorial Sloan-Kettering Cancer Center
IRB Protocol**

IRB#: 08-031 A(10)

5.6.5. 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
Na ₂ HPO ₄ anhy.	Ph. Eur.	1.15 g/L
KH ₂ PO ₄	Ph. Eur.	0.19 g/L
Na ₂ EDTA	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.

5.6.6. Principles of Operation

5.6.6.1.1. 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.

5.6.6.1.2. 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.

5.6.6.1.3. 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.

6.0 CRITERIA FOR SUBJECT ELIGIBILITY

6.1 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 an 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 10⁹/L or platelet transfusion dependence*
2. ANC <1000 x 10⁹/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

3. **Acute Myelogenous Leukemia**

Patients with acute leukemia are included in this trial in remission, 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 at each of the centers according to their own institutional criteria. All patients evaluated at trial sites and eligible for this trial by virtue of disease and



**Memorial Sloan-Kettering Cancer Center
IRB Protocol**

IRB#: 08-031 A(10)

lack of an HLA-genotypically matched related donor will be captured in the database of this trial. 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 1/8 loci (A, B, C or DRB1) (7/8) 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.

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. At the time of referral for transplantation, patients must have no co-existing medical problems that would significantly increase the risk of the transplant procedure.

6.1.7. 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:* $< 5 \times$ ULN SGOT and < 2.0 mg/dl total serum bilirubin.
- c) *Renal:* serum creatinine ≤ 1.5 mg/dl or if serum creatinine is outside the normal range, then $CrCl > 60$ -ml/min/ 1.73 m^2
- d) *Pulmonary:* asymptomatic or if symptomatic, DLCO $> 50\%$ of predicted (corrected for hemoglobin)

6.1.8. 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 age appropriate.

6.1.9. 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.

6.2 Subject Exclusion Criteria

1. Active CNS leukemic involvement



Memorial Sloan-Kettering Cancer Center IRB Protocol

IRB#: 08-031 A(10)

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.
3. Active uncontrolled viral, bacterial or fungal infection
4. Patient seropositive for HIV-I/II; HTLV -I/II

7.0 RECRUITMENT PLAN

One of the BMT attendings will see the patient in consultation; typically, this is the outpatient BMT attending. As part of the consultation, the BMT attending will present the patient with the risks and benefits of different types of cytoreduction and transplants. The BMT attending will then recruit patients who fulfill the eligibility criteria as listed in Section 6.0 for this study. After confirmation of patient eligibility by the medical or Protocol Participant Registration office, one of the participating investigators authorized to obtain consent will obtain informed consent. A copy of the signed informed consent will be placed in the chart, as well as in the research file.

8.0 PRETREATMENT EVALUATION

8.1. Pretreatment Evaluation of the patient

Prior to instituting preparatory cytoreduction, the patient will receive an extensive medical evaluation:

This evaluation should ideally include the following:

- Complete physical examination
- Detailed history with special attention to prior medical history, allergies, previous therapies, and response to treatment
- Dental evaluation.

Laboratory studies performed may include:

- Complete Blood Count with differential count and reticulocyte count
- Bone marrow aspiration and biopsy will be performed within approximately 1 month from starting cytoreduction. Marrow aspiration will be studied by morphology. It will also be studied by cytogenetic analysis by karyotype and FISH studies as per the discretion of the each institution's treating physician.* 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 if indicated.
- Chest and other X rays as clinically indicated
- Electrocardiogram
- Gated pool scan or echocardiogram
- Urinalysis
- Infectious Disease Markers will be tested as per each institutional guidelines as well as the discretion of the treating physician
- In addition, all patients will undergo pre-transplant complete immunologic evaluation as per each Center's standard testing and at the discretion of each institution's treating physician.
- Blood or marrow will be sent for diagnostic molecular pathology for DNA extraction for the definition of donor host differences.



**Memorial Sloan-Kettering Cancer Center
IRB Protocol**

IRB#: 08-031 A(10)

*Cytogenetic studies will be obtained from marrow and/or peripheral blood cells. In cases of "same sex" donor recipient combinations, restriction fragment length polymorphisms (RFLP) or other institutional standard CLIA approved analyses will be carried out in an attempt to define donor/host differences.

8.2. Pretransplant Evaluation of the Donor

Family Donor

Any 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 as a standard of care or per other study. 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.

The donor must also provide signed informed consent to receive a 4-6 day course of G-CSF, and to undergo 1-3 leukaphereses.

In preparation for the stem cell donation, the donor will provide informed consent and then 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.
- Cytogenetic studies (RFLP) will be performed on peripheral blood if the donor is of matched sex and may, per institutional practice, be obtained regardless of sex match.
- Type and cross match
- Infectious disease markers will be tested per each institutional guidelines or at the discretion of the treating physician
- Urinalysis
- 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 for postpubertal and premenopausal female donors

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 a 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



**Memorial Sloan-Kettering Cancer Center
IRB Protocol**

IRB#: 08-031 A(10)

The below outlines the approximate schema of cyto-reduction for allogeneic PBSCT:

Day	Treatment - Doses - Infusion guidelines			
-7	Busulfan	0.6-0.8 mg/Kg IV	Q 12H	over 2 hours
-6	Busulfan	<i>Dose adjusted per PK studies</i>		Q 12H 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 Antithymocyte globulin	2.5 mg/kg IV.	Once daily	over 6-9 hours
-4	Cyclophosphamide	10 mg/kg IV,	Once daily	over 1 hour
	Fludarabine	35 mg/m ² IV	Once daily	over 30 minutes
	Rabbit Antithymocyte globulin	2.5 mg/kg IV.	Once daily	over 6-9 hours
-3	Cyclophosphamide	10 mg/kg IV,	Once daily	over 1 hour
	Fludarabine	35 mg/m ² IV	Once daily	over 30 minutes
	Rabbit Antithymocyte globulin	2.5 mg/kg IV.	Once daily	over 6-9 hours
	<i>Start Cyclosporine</i>			
-2	Cyclophosphamide	10 mg/kg IV,	Once daily	over 1 hour
	Fludarabine	35 mg/m ² IV	Once daily	over 30 minutes
	Rabbit Antithymocyte globulin	2.5 mg/kg IV.	Once daily	over 6-9 hours
-1	Rest day			
0**	CD34+ PBSCT			
+1	Start G-CSF			

** Day 0 could extend to two days

Busulfan

As of April 2012, patient accrual is nearly complete. As a result, after the completion of the planned 25 patient study, we have amended the protocol to accrue at most an additional 20 patients in order to gain clinical experience using alternative levels of Busulfan. Previously, patients were treated with 0.8-1.0 mg/kg of Busulfan.

Of the 25 patients previously treated on this protocol, at doses of 0.8-1.0 mg/Kg of busulfan, 12 patients had adequate busulfan levels, and TEN patients had HIGH busulfan levels requiring a dose reduction, with no patients with low levels requiring an increase in the dose.

Toxicity included: oral mucositis – grade 3 for 10 patients and grade 4 for one patient. Five patients had hyperbilirubinemia; seven patients had transaminitis and one patient veno-occlusive disease. One patient developed renal failure. Two patients developed pulmonary hemorrhage and one patient developed a gastrointestinal hemorrhage.

Based on this data, in this continuation study, the first 10 additional patients will be treated at 0.6-0.8 mg/kg of Busulfan. If no primary graft failures are observed in these 10 patients, then a second cohort of 10 patients will be treated at 0.4-0.6 mg/kg.

If however, at any time during this accrual a graft failure is observed, then the Busulfan dose will be modified and a final cohort of patients will be treated at the initial dose of 0.8-1.0 mg/kg.



Memorial Sloan-Kettering Cancer Center
IRB Protocol

IRB#: 08-031 A(10)

Although no graft failure has been seen with the dose of .6-.8 mg/kg of Busulfan, it was decided to enroll the remainder of the patients at this cohort, as a precautionary measure and in order to maintain the safety and effectiveness of the condition regimen. Some patients have had some evidence of mixed chimerism, and we therefore do not see any benefit in reducing the Busulfan dose any further.

- **Busulfan pharmacokinetics will be done and dose adjustments will be made according to “institutional” standard clinical practice as indicated. The optimal desired steady state CSS target should be 300-350.**

Research participants will have busulfan levels drawn whenever possible after the first dose on day 1, with adjustments in dosing based on the pharmacokinetics of the first dose according to institutional standard practice as indicated. The doses administered on days -7 and -6 should be adjusted if patient is > 125% ideal body weight and should be calculated on adjusted ideal body weight per MSKCC standard of care guidelines. Once pharmacokinetics are available, subsequent dosing will be adjusted as per pharmacokinetics.

- Levetiracetam or other antiseizure medications may be administered for seizure prophylaxis with Busulfan from day -8 until day -4
- Patients should not receive any acetaminophen or azoles during busulfan administration.

Cyclophosphamide

Cyclophosphamide 10 mg/kg is to be given as an approximately 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. Mesna may be given per institutional practice. The dose will be adjusted according to patients ideal body weight for obese patients.

Fludarabine

Fludarabine 35 mg/m² will be given IV over approximately 30 minutes daily for 4 days for a total dose of 140 mg/m². The dose will be adjusted according to renal function according to Institutional guidelines.

9.2 IMMUNOSUPPRESSIVE THERAPIES

Graft Failure Prophylaxis

Methylprednisolone 1 mg/kg/day intravenously approximately every 12 hours will be given on days -5, -4, -3, and -2 immediately prior to the infusion of antithymocyte globulin. It will be discontinued thereafter. 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). 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)



**Memorial Sloan-Kettering Cancer Center
IRB Protocol**

IRB#: 08-031 A(10)

at 10 mg/m² IV once daily may be administered for each remaining day of ATG on the schema.

GVHD Prophylaxis

Cyclosporine- Patients will receive cyclosporin A (CSA) as GVHD prophylaxis. CSA therapy will begin on day -3 with a taper commencing on day +100, to be completed by day +180 unless the patient develops graft-versus-host disease. For adults with normal baseline renal function (creatinine <1.2 mg/dL), the initial CSA dose is recommended to be 2.5 mg/kg IV approximately over 2 hours every 12 hours; for children <40 kg, the initial dose is recommended to be 2.5 mg/kg IV approximately over 2 hours every 8 hours. Initial and subsequent doses of CSA may be adjusted and administered as per institutional guidelines. Trough levels will be obtained, and doses will be adjusted according to each Center's standard of care guidelines.

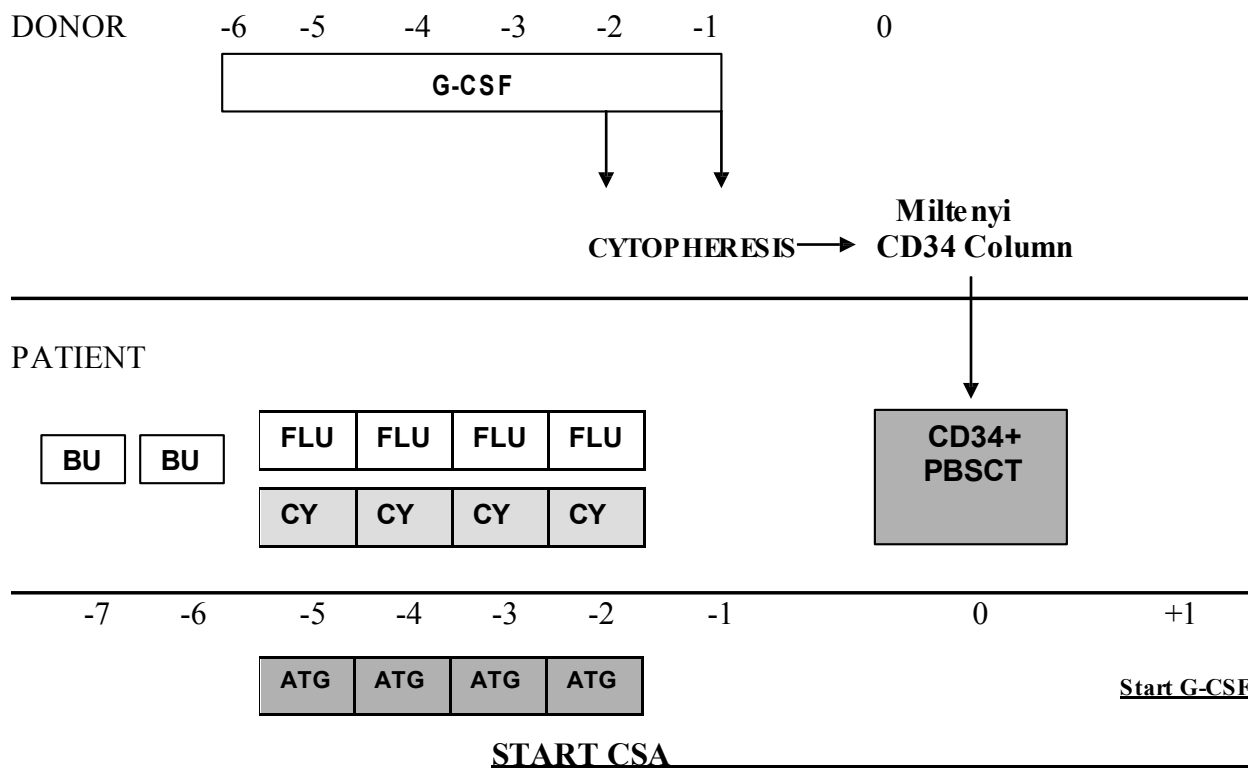
Additional GVHD Prophylaxis will be administered to patients as per guidelines outlined in *Table 9.3.2.2, Guidelines for achieving target allograft cell doses.*

Other

G-CSF will be administered to all patients post transplant starting day +1 as per institutional guidelines. 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 G-CSF as per each institution's guidelines.

Unrelated Donors

For Unrelated donors, G-CSF will be administered and the leukaphereses obtained according to the National Marrow Donor Program protocol and IND. Mononuclear cell fractions collected on the fifth and sixth days will be pooled. CD34⁺ progenitor cells in the mononuclear cell rich leukapheresis cell fraction will be positively selected.

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 as per each institution's guidelines.

The target allograft cell doses following processing on the CliniMACS device are a CD34⁺ cell dose of $> 5.0 \times 10^6$ cells/Kg recipient weight with a maximum CD3⁺ cell dose of $< 5.0 \times 10^4$ cells/Kg of recipient weight. A minimum CD34⁺ cell dose of 2.5×10^6 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 2.0×10^6 cells/Kg in order to be evaluable for all study endpoints. If the post CD34⁺ selection allograft content is $< 2.0 \times 10^6$ CD34⁺ cells/Kg after 2 leukapheresis procedures, the Transplant Center should perform a third leukapheresis collection without CD34⁺ selection 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) in place and validated at the study sites.

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



Memorial Sloan-Kettering Cancer Center IRB Protocol

IRB#: 08-031 A(10)

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 $< 2.0 \times 10^8$ 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 at either 6°C or 2-8°C depending upon the 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.

Cell processing personnel will receive training by Miltenyi on the CliniMACS system prior to initiation of clinical product selection. The site will provide documentation to Miltenyi on competency in the processing procedures including the results of validation runs on the CliniMACS System.

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)
 - 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, done locally or sent to an authorized lab
 - Flow cytometric analysis for CD34⁺ cells pre- and post-selection done locally using validated SOPs
 - Flow cytometric analysis for CD3⁺ cells pre- and post-selection and log depletion on each leukapheresis product done locally using validated SOPs
 - 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 $\geq 70\%$ after selection
 - Negative gram stain
 - CD34⁺ cell count of product

As noted above, the target optimal allograft cell doses following processing on the CliniMACS device include both a CD34⁺ cell count of $> 5.0 \times 10^6$ /kg recipient weight and a CD3⁺ cell dose of $< 5.0 \times 10^4$ /kg recipient weight. The targeted minimum CD34⁺ cell dose following CD34⁺ selection is 2.5×10^6 /kg. If after two leukapheresis collections followed by CD34⁺ selection the total allograft contains $2.5-5.0 \times 10^6$ CD34⁺ cells/kg and $< 5.0 \times 10^4$ 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 5.0×10^4 /kg. That is, a graft containing $2.5-5.0 \times 10^6$ CD34⁺



**Memorial Sloan-Kettering Cancer Center
IRB Protocol**

IRB#: 08-031 A(10)

cells/kg and $< 5.0 \times 10^4$ CD3⁺ cells/kg is acceptable whereas a graft containing 5.0×10^6 CD34⁺ cells/kg but $> 5.0 \times 10^4$ CD3⁺ cells/kg is not. A major goal is to administer a CD34⁺ cell dose $> 5.0 \times 10^6$ /kg while limiting the CD3⁺ cell dose to $< 5.0 \times 10^4$ but as long as a minimum of 2.0×10^6 /kg CD34⁺ cells are infused, the CD3⁺ cell dose should be kept to $< 5.0 \times 10^4$ /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 $< 5.0 \times 10^4$ /kg.

It is also possible that doses of CD34⁺ cells far exceeding 5.0×10^6 /kg can be given without exceeding the maximum T cell dose of 5.0×10^4 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 5.0×10^4 /kg. Furthermore, a minimum of 2 leukaphereses should be obtained from each donor even if the first leukapheresis provides 5.0×10^6 CD34⁺ cells/kg, if the anticipated cumulative CD3⁺ cell dose is expected to be $< 5.0 \times 10^4$ /kg of recipient weight.

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 at the site. The attached table outlines the various scenarios that may be encountered after each CD34⁺ cell selection and indicates appropriate steps for achieving target allograft cell doses.

Table 9.3.2.2 – Guidelines for achieving target allograft cell doses

Leuka-Pheresis Attempt	Total CD34 ⁺ dose after Leukapheresis ¹ (X10 ⁶ /Kg)				Total CD3 ⁺ dose after Leukapheresis ¹ (x10 ⁴ /Kg)		ACTION				
	<1.0	1.0-2.0	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. Maintain CD34 dose >2.0	Begin GvHD Prophylaxis
1				X	X		X	X ²			
1				X		X		X		X ³	
1	X ⁴	X ⁴	X ⁴		X		X	X			
1	X ⁴	X ⁴	X ⁴			X		X		X ³	
2				X	X		X		X		
2				X		X				X ⁵	
2		X ⁴	X ⁴		X		X	X			
2		X ⁴	X ⁴			X				X ⁶	
2	X				X ⁷	X ⁷	X	X ⁸			X ⁹
3				X	X		X		X		
3			X		X		X		X		
3				X		X				X ¹⁰	
3			X			X				X ¹⁰	
3		X			X		X		X		
3		X				X					X ¹¹
3	X ¹²				X ⁷	X ⁷					

- This number refers to CD34⁺ (x 10⁶/Kg) or CD3⁺ (x 10⁴/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
- If unsuccessful in reducing CD3⁺ cell dose to $< 5.0 \times 10^4$ 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 $> 2.0 \times 10^6$ cells/Kg with CD3⁺ cell dose of $< 5.0 \times 10^4$ 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 $< 5.0 \times 10^4$ cells/Kg while maintaining the total CD34⁺ cell dose to $> 2.0 \times 10^6$ cells/Kg.



Amended: 06/18/14



Memorial Sloan-Kettering Cancer Center IRB Protocol

IRB#: 08-031 A(10)

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 x 10⁴ 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 10⁴ cells/Kg while maintaining CD34+ cell dose ≥ 2.0 x 10⁶ cells/Kg, proceed to leukapheresis #3. If unsuccessful in reducing CD3+ cell dose to < 5.0 x 10⁴/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 10⁴ /Kg, begin 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 10⁶/kg CD34+ cell dose and < 5.0 x 10⁴/kg CD3+ cell dose, hold allograft of leukapheresis #3. If the patient has received total allograft containing < 2.0 x 10⁶/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 one of the Protocol Chairs to discuss options.

* 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 day of the transplant 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 peritransplant infections by mold must be used in all centers according to Institutional guidelines.

10.0 EVALUATION DURING TREATMENT/INTERVENTION

Post-transplant Evaluation

a. Clinical Assessments

Patients will undergo 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 abnormal new findings will be recorded. Following discharge, physical examination, will be performed at least every 2-4 weeks or as clinically indicated.



**Memorial Sloan-Kettering Cancer Center
IRB Protocol**

IRB#: 08-031 A(10)

All timelines are dose 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 abnormal new findings will be recorded.

- Complete hemograms:
 - daily until successful discontinuation of GCSF - then three times/week until discharge;
 - and at least every two weeks post-discharge to 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 or peripheral blood will be obtained to monitor engraftment by FISH for sex-mismatched donor and host or by RFLP or VNTR for sex matched donor or sex- mismatched donor and host, at approximately 1,3,6,12 months. Further engraftment studies after the 1-year mark will be done as clinically indicated.
- Bone Marrow aspirates for morphology, and cytogenetics will be done at the Investigator's discretion. Proposed time points are at approximately 1,3,6,12 months for patients with MDS or AML. Additional analyses will be done as clinically indicated.
- Immunologic reconstitution will be monitored by in vitro assays, possibly including but not limited to phenotypic analysis of circulating lymphocytes, lymphocyte transformation responses to T-cell mitogens, and to viruses, and immunoglobulin levels and antibody responses.

11.0 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. All patients entered into our studies provide written informed consent. In the case of minors, consent of the parent or guardian and assent of the child are obtained. All protocols and consent forms are reviewed and approved by the Institutional Review Board.

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 well being of the patient can be summarized as follows.

11.1. General Description of Risks to Recipients

**IRB
PB**

Amended: 06/18/14



**Memorial Sloan-Kettering Cancer Center
IRB Protocol**

IRB#: 08-031 A(10)

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 IDE 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 phenytoin therapy or other antiseizure medications such as Keppra, 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



Memorial Sloan-Kettering Cancer Center
IRB Protocol

IRB#: 08-031 A(10)

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 antihistamines. 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. **Cyclosporine**

- Renal dysfunction
- Increased blood pressure
- Hirsutism
- Neurological side effects: Tremor (common), seizures (rare), ataxia, cortical blindness (rare), and peripheral neuropathies.
- Liver toxicity
- Hyperkalemia or hypokalemia
- Low serum magnesium
- Gastrointestinal complaints including anorexia, nausea, and ileus
- Gingival hyperplasia
- Skin pigmentation changes
- Depression



Memorial Sloan-Kettering Cancer Center
IRB Protocol

IRB#: 08-031 A(10)

- Capillary leak syndrome (rare)
- Lymphoproliferative disorders (malignant lymphoma) (rare)
- Hemolytic anemia (rare)
- Thrombotic thrombocytopenic purpura (rare)

g ***G-CSF***

- Fever
- Fatigue
- Bone pain
- Allergic reaction, mild to severe
- Splenomegaly

h. ***Pneumocystis carinii prophylaxis***

The risk of trimethoprim and sulfamethoxazole in the doses given are primarily hypersensitivity reactions and signs of folate deficiency. Any patient with known hypersensitivity to these compounds will not receive these drugs. The risks of parenteral pentamidine are primarily hypotension and hypoglycemia both of which will be monitored during and following administration of the drug. Hypokalemia or hypomagnesemia associated with prolonged QT syndrome or Torsade de pointe necessitates strict electrolyte monitoring. The risks of aerosolized pentamidine are mild bronchospasm primarily observed in (prior) tobacco abusers and easily managed with bronchodilator therapy.

i. ***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.

j. ***Graft Failures***

The risk of rejection of a T-cell depleted related, HLA-disparate marrow and peripheral blood progenitor graft may be 20% or greater. 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.

k. ***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



Memorial Sloan-Kettering Cancer Center IRB Protocol

IRB#: 08-031 A(10)

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.

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.

1. Blood product 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 marrow graft.

Infusions of fractionated blood products may produce volume overload, which can be managed with diuretics. They 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 as per each institution's 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

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 $\geq 500/\mu\text{l}$ at any time in the first 28 days post-transplant. If (1) after achievement of an ANC $\geq 500/\text{mm}^3$, the ANC declines to $< 500/\text{mm}^3$ 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 assay in the absence of relapse, a diagnosis of secondary graft failure is made. If, however, recurrence of host MDS is detected concurrently, the patient is not evaluable for graft failure or rejection. 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



Memorial Sloan-Kettering Cancer Center IRB Protocol

IRB#: 08-031 A(10)

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 3.0.

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 will be followed indefinitely through annual contact with their treatment center in order to track the risk of developing a secondary malignancy.

13.0 CRITERIA FOR REMOVAL FROM STUDY

Patients may be removed from the study at any point deemed appropriate by the principle investigator. However, once the busulfan is given, patients will continue on study until after administration of the stem



**Memorial Sloan-Kettering Cancer Center
IRB Protocol**

IRB#: 08-031 A(10)

cells. Failure to rescue the patient with stem cells at this point in the cytoreduction would most likely be fatal.

14.0 BIOSTATISTICS

This phase II trial is designed to investigate the safety and efficacy of hematopoietic stem cell transplantation for the treatment of patients with Fanconi Anemia lacking a genetically identical donor. A maximum of 25 patients will be accrued onto the study. It is anticipated that the accrual will last 3 years, and will include patients from 6 different centers.

The primary objectives of this study are to assess the incidence of engraftment, graft versus host disease, and treatment related morbidity and mortality (treatment related morbidity will be assessed through the incidence of grade 1 to 3 toxicity). At the conclusion of the study, the probability of each these events will be computed along with their attendant 95% confidence interval. In addition, Kaplan-Meier estimates of overall and disease free survival will be computed over time for the entire cohort and by disease (AA and early MDS / advanced MDS and AML).

In order to reduce patient risk, the study design includes early termination in the event of excessive graft failure, graft versus host disease, or early treatment related mortality during the accrual period. The stopping rules are provided in the table below and consider only the marginal failure probabilities.

Failure type	# of failures needed to stop the study	Projected probability of failure in population	Probability of study completion (based on projection)
Graft failure	2 within the first 11 patients	0.20	0.10
	3 within the first 24 patients 4 in 25 patients	0.04	0.89
Acute Graft Versus Host Disease Grades 2-4	2 within the first 8 patients	0.25	0.06
	3 within the first 19 patients 4 in 25 patients	0.05	0.90
Treatment Related Mortality	4 within the first 10 patients	0.40	0.08
	5 within the first 14 patients 6 within the first 19 patients 7 within the first 23 patients 8 in 25 patients	0.15	0.90

As of April 2012, patient accrual is nearly complete in the HSCT Fanconi Anemia study. As a result, after the completion of the planned 25 patient study, we have amended the protocol to accrue at most an additional 20 patients in order to gain clinical experience using alternative levels of Busulfan. Previously, patients were treated with 0.8-1.0 mg/kg of Busulfan. In this continuation study, 10 patients will be treated at 0.6-0.8 mg/kg of Busulfan. If no primary graft failures are observed in these 10 patients, then a further cohort of 10 patients will be treated at 0.4-0.6 mg/kg. If however, at any time during this accrual a



**Memorial Sloan-Kettering Cancer Center
IRB Protocol**

IRB#: 08-031 A(10)

graft failure is observed, then the Busulfan dose will be modified and a final cohort of patients will be treated at the initial dose of 0.8-1.0 mg/kg. If no graft failures are observed in 10 patients at a given dose level, then we are confident that the probability of graft failure in this population is less than 0.20. In addition to monitoring for graft failure, the stopping rules below will be used to monitor the additional 20 patients for acute GvHD and treatment related mortality.

Failure endpoint	# of failures needed to stop the study	Failure rate in the population	Probability boundary is crossed
Acute graft versus host disease	2 in the first 13 patients 3 within 20 patients	0.04	0.10
		0.25	0.94
Treatment related mortality	3 in the first 9 patients 4 in the first 14 patients 5 within 20 patients	0.10	0.09
		0.40	0.96

Although no graft failure has been seen with the dose of .6-.8 mg/kg of Busulfan, it was decided to enroll the remainder of the patients at this cohort, as a precautionary measure and in order to maintain the safety and effectiveness of the condition regimen. These patients have had some evidence of mixed chimerism, and we therefore do not see any benefit in reducing the Busulfan dose any further.

15.0 Research Participant registration and randomization procedures

15.1 Research Participant Registration - MSKCC

Confirm eligibility as defined in the section entitled Criteria for Patient/Subject Eligibility.

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.

All participants must be registered through the Protocol Participant Registration (PPR) Office at Memorial Sloan-Kettering Cancer Center. PPR is available Monday through Friday from 8:30am – 5:30pm at 646-735-8000. Registrations must be submitted via the PPR Electronic Registration System (<http://ppr/>). The completed signature page of the written consent/RA or verbal script/RA, a completed Eligibility Checklist and other relevant documents must be uploaded via the PPR Electronic Registration System.



**Memorial Sloan-Kettering Cancer Center
IRB Protocol**

IRB#: 08-031 A(10)

15.2 Research Participant Registration - Other Centers

Study coordinators at other institutions will contact the Research Study Assistant (RSA) at Memorial Sloan Kettering Cancer Center within 48 hours of patients signing the informed consent to notify him/her of the patient registration and send registration/eligibility documents to the Department of Pediatrics Clinical Trials Office at MSKCC.

All patients must be registered at the site where they are being treated. Each participating institution must have an independent patient registration process and the procedures by which patients are registered onto the protocol should be followed in parallel.

Once eligibility has been established the patient will be assigned a unique ID number. This number is unique to the patient and must be written on all data and correspondence for the patient. This protocol patient number will be relayed to the sites via e-mail and will serve as the enrollment confirmation.

16.0 DATA MANAGEMENT ISSUES

16.1. Data Management at Participating Centers

The PI at each site will be responsible for the conduct of the study and the monitoring of the progress and will review all case report forms.

Research staff will be assigned at Boston Children's Hospital, Cincinnati Children's Hospital, Children's Hospital of Wisconsin, Rockefeller University, and Fred Hutchinson Cancer Research Center.

Their responsibilities will include maintaining file documentation of data for the clinical trial on each patient enrolled on study and all regulatory documents. A designated data manager at each institution will be responsible for submitting the data on a weekly basis to the RSA at MSKCC. Contact Information:

Pediatric Clinical Trials Office
Memorial Sloan-Kettering Cancer Center
405 Lexington Avenue
3rd Floor
New York, New York 10174
Telephone: (646) 888-5721
Fax: (646) 888-5727

The research staff at each site is responsible for upholding their institutional data management guidelines.

All sites are using the same protocol, therefore all amendments will originate from Memorial Sloan Kettering Cancer Center and will be communicated to the other sites.



Memorial Sloan-Kettering Cancer Center IRB Protocol

IRB#: 08-031 A(10)

16.1.1 Data Management at MSKCC

A Research Study Assistant (RSA) at Memorial Sloan-Kettering Cancer Center, will be assigned to the study. The responsibilities of the RSA include project compliance, data collection, abstraction and entry, data reporting regulatory monitoring, problems and prioritization.

MSKCC will be the data collection site for all 5 sites.

Data collection forms will be drafted in a standard format and will be provided to each participating institution by MSKCC. Case Report Forms (paper) will be submitted with the protocol. Completed data collection forms will be submitted to Memorial Sloan-Kettering Cancer Center on a weekly basis.

The data collected for this study will be entered into a secure database by the MSKCC RSA. Data will be collected, stored, and monitored at an institutional level via the Clinical Record Database (CRDB version 3.3) system.

Source documentation will be available to support the computerized patient record. Variables that will be recorded include the patient's birth date, date of diagnosis, date of study entry and histologic diagnosis. The results of the pretreatment and end of therapy evaluations, including the extent of disease evaluation (history, physical examination and imaging studies), baseline laboratory values, renal and hepatic function, will be recorded. The presence of toxicity during and after administration of the transplant will be monitored and recorded by each institution. On this study, we will be capturing and tracking Grade 3-4 toxicities which occur within 30 days following a transplant and are potentially attributable to treatment on study. Hematologic toxicities which are attributable to underlying hematologic disease will not be tracked. The results of the extent of disease evaluation (history, physical examination and imaging studies) following each course of treatment will be collected by the participating institutions and recorded. The patient's disease status and last follow-up will be collected by the participating institutions and recorded. If disease progresses or recurs, the results of the repeat extent of disease evaluation will be collected by the participating institutions and recorded. Any secondary malignancy arising in any organ will also need to be captured in database. Data collected for secondary malignancies at annual visits will include: (1) site, (2) pathology, (3) grade, (4) treatment and (5) outcome.

Standard baseline information will also be collected for donors.

The protocol will be conducted as a single research study effort and data from each participating institution will be included in the analysis of results.

16.2 Quality Assurance

Registration reports will be generated to monitor patient accruals and completeness of 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



Memorial Sloan-Kettering Cancer Center IRB Protocol

IRB#: 08-031 A(10)

Random-sample data quality and protocol compliance audits will be conducted by the study team, at a minimum of once a 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 IDE. 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 assessments scans, x-rays, etc. available for the audit

Response Review

Patients participating on trials at MSKCC where therapeutic efficacy is a stated primary objective, typically phase II and III trials, are subject to review by MSKCC's Therapeutic Response Review Committee (TRRC). In these cases, radiology, additional lab reports and possibly bone marrow biopsies and/or aspirates may need to be obtained from the outside sites for MSKCC TRRC review and confirmation of response assessment. These materials must be obtained within sixty days of request to the site.

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: <http://mskweb2.mskcc.org/irb/index.htm>

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.

Data from all participating sites will be monitored by the MSK DSMC on a quarterly basis.

17.0 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

**IRB
PB**

Amended: 06/18/14



Memorial Sloan-Kettering Cancer Center IRB Protocol

IRB#: 08-031 A(10)

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 conform to MSKCC 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.

17.1 Privacy

All institutional, FDA, and NCI requirements for human subjects must be met. This study will be carried out in compliance with the regulations of the Health Insurance Portability and Accountability Act (HIPAA). Each participating institution will have an appropriate assurance on file with the Office for Human Research Protection (OHRP), NIH.

The risks and benefits of participation in this study will be reviewed with the patient and/or parent/legal guardian.

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.

The following section is a Mandatory Section that pertains to MSKCC only:

MSKCC'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.



Memorial Sloan-Kettering Cancer Center IRB Protocol

IRB#: 08-031 A(10)

17.2 Serious Adverse Event (SAE) Reporting Guidelines for MSKCC

Any SAE must be reported to the IRB/PB as soon as possible but no later than 5 calendar days. The IRB/PB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office at sae@mskcc.org containing the following information:

Fields populated from the CRDB:

- Subject's name
- Medical record number
- Disease/histology (if applicable)
- Protocol number

Data needing to be entered:

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

The PI's signature and the date it was signed are required on the completed report.

For IND/IDE protocols:

The CRDB AE report should be completed as above and the FDA assigned IND/IDE number written at the top of the report. If appropriate, the report will be forwarded to the FDA by the SAE staff through the IND Office.

Each participating center will follow their institutional guidelines in regards to SAE submission to the FDA.

The MSKCC PI is responsible for reporting all grade 3-5 unexpected, definite, probable, possible related SAEs on protocol to all participating sites within 5 calendar days of receipt.

All SAEs must be entered into the CRDB SAE form page.

17.2.1 Serious Adverse Event (SAE) Reporting for Participating Institutions

Participating sites are responsible for reporting all SAEs to their IRB, the FDA and the MSKCC PI via fax or e-mail within 5 calendar days. Each participating center will follow their institutional guidelines in regards to SAE submission to their IRB.

17.2.2 Definition of SAE



Amended: 06/18/14



Memorial Sloan-Kettering Cancer Center IRB Protocol

IRB#: 08-031 A(10)

An SAE is an undesirable experience that meets one of the following criteria:

- Is fatal or life-threatening
- Is disabling
- Results in hospitalization or prolongation of hospitalization
- Results in a congenital anomaly or occurrence of malignancy
- Important medical event that jeopardizes the participant AND requires medical or surgical intervention to prevent one of the outcomes above *Note: Hospital admission for a planned procedure/disease treatment is not considered an SAE.*

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.

17.2.4 SAE Reporting Requirements to Miltenyi

A courtesy copy of the SAE report will be provided to Miltenyi:



Amended: 06/18/14



**Memorial Sloan-Kettering Cancer Center
IRB Protocol**

IRB#: 08-031 A(10)

ATTN: Normand Pilon
Clinical Application Manager
North East USA, Canada
Miltenyi Biotec Inc.
26 Des Bolets
Blainville, Quebec, Canada,
J7C 5T8
Cell: 514-451-7275
Email: Normand@miltenyibiotec.com
Fax: 450-419-9628.

18.0 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.

19.0 REFERENCE(S)

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. Gluckman E. Auerbach.
2. Auerbach AD, Woman SR. Susceptibility of Fanconi Anemia fibroblasts to chromosome damage by



Amended: 06/18/14



**Memorial Sloan-Kettering Cancer Center
IRB Protocol**

IRB#: 08-031 A(10)

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., Sohler J., Bernard J. Bone marrow transplantation for Fanconi Anemia. *Brit 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 Transpl.* 9:167-173, 1992.
11. Di Bartolomeo P. Di Girolamo G. Oliosio P. Angrilli F. Dragani A. Palka G. Guanciali-Franchi P. Ciancarelli M. Papalinetti G. Fioritoni G. et al. Allogeneic bone marrow transplantation for Fanconi anemia. *Bone Marrow Transplantation*. 10(1):53-6, 1992
12. Yabe M., Yabe H., Matsuda M., Hinohara T., Oh Y., Hattori K., Ishikawa K., Ohshima T., Yamamoto H., Kato S. Bone marrow transplantation for Fanconi Anemia. Adjustment of the dose of cyclophosphamide for preconditioning. *Amer. J. Pediatr. Hematol. Oncol.* 15:377-382, 1993.
13. 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.



Amended: 06/18/14



**Memorial Sloan-Kettering Cancer Center
IRB Protocol**

IRB#: 08-031 A(10)

14. Zanis-Neto J., Ribeiro RC., Medeiros C., Andrade RJ., Ogasawara V., Hush ., Magdalena N., Friedrich ML., Bitencourt MA., Bonfim C., Pasquini R. Bone marrow transplantation for patients with Fanconi Anemia: a study of 24 cases from a single institution. *Bone Marrow Transpl.* 15:293-298, 1995.
15. 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.
16. 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.
17. 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.
18. Tezcan I., Tuncer M., Uckan D., Cetin M., Alikasifoglu M. Ersoy F., Altay C. Allogeneic bone marrow transplantation in Fanconi anemia from Turkey: a report of four cases. *Pediatric Transplantation.* 2(3):236-9, 1998.
19. 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
20. 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. Vermynen 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
21. Mital MK. Curtis A. Spencer V. Barge D. Skinner R. Delayed engraftment and mixed chimerism after HLA-identical sibling donor BMT in Fanconi anaemia. *Bone Marrow Transplantation.* 24(2):201-4, 1999
22. Harris RE. Reduction of toxicity of marrow transplantation in children with Fanconi anemia. *Journal of Pediatric Hematology/Oncology.* 21(3):175-6, 1999
23. 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



**Memorial Sloan-Kettering Cancer Center
IRB Protocol**

IRB#: 08-031 A(10)

24. Hows JM, Chapple M, Marsh JC, Durrant S, Yin JL, Swirsky D, Gordon-Smith EC. Bone marrow transplantation for Fanconi's anaemia: the Hammersmith experience 1977-89. *Bone Marrow Transplantation*. 4(6):629-34, 1989
25. 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
26. 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.
27. 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.
28. Hows JM. Unrelated donor bone marrow transplantation for severe aplastic anaemia and Fanconi's anaemia. *Bone Marrow Transplantation*. 4 Suppl 4:126-8, 1989
29. 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
30. 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.
31. 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.
32. 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
33. Guardiola P, Locatelli F, Pasquini R, Socie G, Devergie A, Riband P, Esperou H, Gardenet L, Bittencourt M, Marollese 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.
34. 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 Marrow Transplantation. *Blood* 95: 422-429, 2000.
35. MacMillan ML, Davies SM, Defor TE, Auerbach AD, Gillio A, Giller R, Harris R, Cairo M, Dusenberk 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.



**Memorial Sloan-Kettering Cancer Center
IRB Protocol**

IRB#: 08-031 A(10)

36. Fischer A, Friedrich W, Fasth A, Blanche S, Le Deist F, Girault D, Veber F, Vossen J, Lopez M, Griscelli C, et al. Reduction of graft failure by a monoclonal antibody (anti-LFA-1 CD11a) after HLA nonidentical bone marrow transplantation in children with immunodeficiencies, osteopetrosis, and Fanconi's anemia: a European Group for Immunodeficiency/European Group for Bone Marrow Transplantation report. *Blood*. 77(2):249-56, 1991
37. 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.
38. 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.
39. Boulad F, Auerbach AD, Kernan NA, Small TN, Prockop SE, Chaudhury S, Castanza J, Casson A, Copeland C, Abendroth H, Collins N, O'Reilly RJ. Fludarabine (Flu) based cytoreductive regimen and T-cell depleted grafts from unrelated or mismatched related donors for the treatment of high risk patients with Fanconi anemia (FA). *Blood* 2004; 2152;592a.
40. 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
41. 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
42. 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
43. 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



Amended: 06/18/14



**Memorial Sloan-Kettering Cancer Center
IRB Protocol**

IRB#: 08-031 A(10)

APPENDIX 1

MDS Classification

WHO Classification

	BLOOD	BONE MARROW
Refractory Anemia RA	<ul style="list-style-type: none"> - 0 or rare blasts - Anemia - $< 1 \times 10^9/L$ monocytes 	<ul style="list-style-type: none"> - $< 5\%$ blasts - Dysplasia only Erythroid - Myeloid-MegaK dysplasia $< 10\%$ of cells
Refractory cytopenia with multilineage dysplasia RCMD	<ul style="list-style-type: none"> - 0 or rare blasts - Cytopenias (2-3 lineages) - No Auer rods - $< 1 \times 10^9/L$ monocytes 	<ul style="list-style-type: none"> - $< 5\%$ blasts - Dysplasia in 2-3 lineages - Dysplasia in $\geq 10\%$ of cells - No Auer rods
Refractory anemia with excess blasts – 1 RAEB-1	<ul style="list-style-type: none"> - $< 5\%$ blasts - Cytopenias (2-3 lineages) - No Auer rods - $< 1 \times 10^9/L$ monocytes 	<ul style="list-style-type: none"> - 5-9% blasts - Dysplasia in 1-3 lineages - No Auer rods
Refractory anemia with excess blasts – 2	<ul style="list-style-type: none"> - 5-19% blasts - Cytopenias (2-3 lineages) 	<ul style="list-style-type: none"> - 10-19% blasts - Dysplasia in 1-3 lineages



**Memorial Sloan-Kettering Cancer Center
IRB Protocol**

IRB#: 08-031 A(10)

RAEB-2	- Auer rods +/- - $< 1 \times 10^9/L$ monocytes	- 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

LANSKYSCALE (< 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)



Amended: 06/18/14



**Memorial Sloan-Kettering Cancer Center
IRB Protocol**

IRB#: 08-031 A(10)

10 Completely disabled; not even passive play

KARNOFSKYS 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.



**Memorial Sloan-Kettering Cancer Center
IRB Protocol**

IRB#: 08-031 A(10)

**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	<i>Diarrhea</i> 500-1000 ml/d (children: 280-555 ml/m ² /d) OR <i>persistent nausea</i>
2	maculopapular rash 25-50 % of body surface	Bili 3.1 – 6.0 mg/dl	<i>Diarrhea</i> >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	<i>Diarrhea</i> >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.*



Memorial Sloan-Kettering Cancer Center IRB Protocol

IRB#: 08-031 A(10)

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

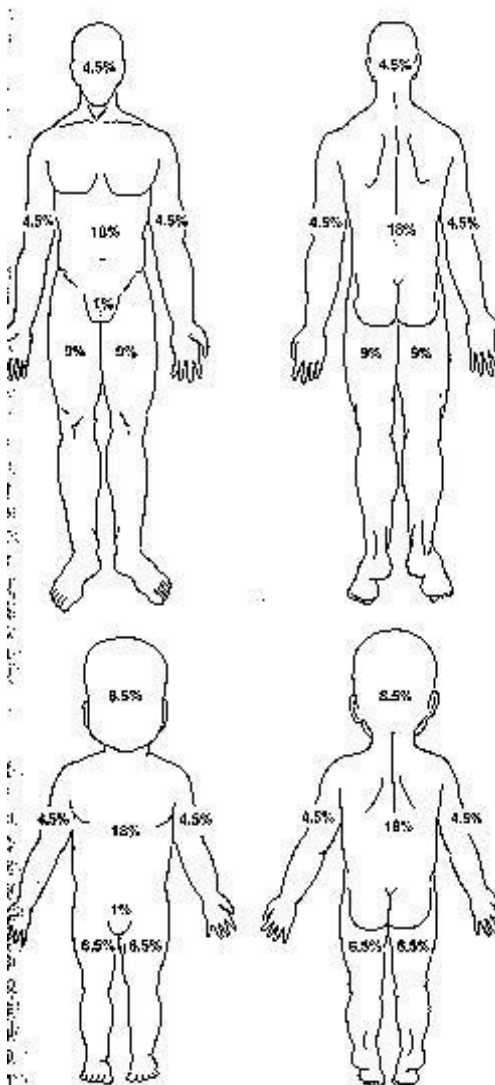


FIGURE 26-9. Rule of Nines to determine percentage of body surface area burnt in adults (A) and children (B).



**Memorial Sloan-Kettering Cancer Center
IRB Protocol**

IRB#: 08-031 A(10)

APPENDIX 4

Chronic GvHD

Chronic GvHD Scoring

<i>Limited</i>	<p>Either or both:</p> <ol style="list-style-type: none"> 1. localized skin involvement 2. hepatic dysfunction due to chronic GvHD
Extensive	<p>Either</p> <ol style="list-style-type: none"> 1. generalized skin involvement, OR 2. localized skin involvement AND/OR hepatic dysfunction due to chronic GvHD <p>PLUS</p> <ol style="list-style-type: none"> 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		Regular dental evaluation (with appropriate endocarditis prophylaxis). Viral and fungal cultures at diagnosis and at any worsening. tis prophylaxis. Topical steroid rinses followed by



**Memorial Sloan-Kettering Cancer Center
IRB Protocol**

IRB#: 08-031 A(10)

	scleroderma with decreased sensitivity to temperature can also happen.		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; endoscopic/ 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 immunocompromised 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.	



**Memorial Sloan-Kettering Cancer Center
IRB Protocol**

IRB#: 08-031 A(10)

APPENDIX 5

Toxicity

Refer to NCI Common Toxicity Criteria 3.0

Website:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcae3.pdf

**IRB
PB**

Amended: 06/18/14