Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Fludarabine with Myeloablative Timed-Sequential Busulfan in Older Patients:

Results of a Randomized Phase II Clinical Trial

Table S1: Microsatellite polymorphism analysis

	Myeloid						T cells					
	Day 30		Day 100		Day 180		Day 30		Day 100		Day 180	
	16K	20K	16K	20K	16K	20K	16K	20K	16K	20K	16K	20K
Median	100%	100%	100%	100%	100%	100%	81%	83%	99%	93%	100	99
IQR	100-100	100-100	100-100	100-100	100-100	100-100	61-100	58-100	86-100	81-100	97-100	89-100

IQR, interquartile range

Table S2 summarizes results from a multivariate Cox proportional hazards regression model for overall survival

Overall Survival: Results of Multivariate Model

	HR	Lower.95.CI	Upper.95.CI	P
Donor Relation: Child/Sibling	1.194	0.593	2.403	0.6202
Age at Transplant	1.012	0.980	1.046	0.4651
Cell Type: HPC-M	1.094	0.499	2.400	0.8218
HCT Comorbidity Index (Continuous)	1.124	0.989	1.277	0.0737
Treatment Arm: AUC=5000	0.668	0.366	1.220	0.1893
DRI: High/Very High	1.783	0.970	3.279	0.0628

Abbreviations: HPC-M: hematopoietic progenitor cells bone marrow graft; DRI, disease risk index; AUC 5000 = 20K arm; HCT = hematopoietic cell transplantation

Table S3 summarizes results from a multivariate Cox proportional hazards regression model for progression free survival

Progression-Free Survival: Results of Multivariate Model

	HR	Lower.95.CI	Upper.95.CI	P
Donor Relation: Child/Sibling	1.754	0.904	3.402	0.0966
Age at Transplant	1.012	0.983	1.042	0.4286
Cell Type: HPC-M	1.018	0.464	2.234	0.9645
HCT Comorbidity Index (Continuous)	1.124	0.993	1.272	0.0654
Treatment Arm: AUC=5000	0.747	0.427	1.306	0.3060
DRI: High/Very High	1.709	0.956	3.056	0.0705

Abbreviations: HPC-M: hematopoietic progenitor cells bone marrow graft; DRI, disease risk index; AUC 5000 = 20K arm; HCT = hematopoietic cell transplantation

Table S4 summarizes results from a multivariate Cox proportional hazards regression model for relapse.

Relapse: Results of Multivariate Model

	HR	Lower CI	Upper CI	P-value
Age	0.989	0.966	1.013	0.3800
HCT Comorbidity Index (Continuous)	0.925	0.783	1.093	0.3600
Donor Relation: Child/Sibling	2.247	0.955	5.286	0.0640
Cell Type: HPC-M	0.763	0.211	2.754	0.6800
Treatment Arm: AUC=5000	1.017	0.486	2.131	0.9600
DRI: High/Very High	2.659	1.250	5.656	0.0110

Abbreviations: HPC-M: hematopoietic progenitor cells bone marrow graft; DRI, disease risk index; AUC 5000 = 20K arm; HCT-CI = hematopoietic cell transplantation comorbidity index

Table S5 summarizes results from a multivariate Cox proportional hazards regression model for non-relapse mortality.

Non-Relapse Mortality: Results of Multivariate Model

	HR	Lower CI	Upper CI	P-value
Age	1.051	0.980	1.128	0.1600
HCT Comorbidity Index (Continuous)	1.320	1.091	1.597	0.0042
Donor Relation: Child/Sibling	0.917	0.286	2.942	0.8800
Cell Type: HPC-M	1.199	0.408	3.528	0.7400
Treatment Arm: AUC=5000	0.621	0.260	1.487	0.2900
DRI: High/Very High	0.684	0.273	1.714	0.4200

Abbreviations: HPC-M: hematopoietic progenitor cells bone marrow graft; DRI, disease risk index; AUC 5000 = 20K arm; HCT = hematopoietic cell transplantation

TABLE S6: TRIAL OPERATING CHARACTERISTICS

True NRM Rate Arm 1	True NRM Rate Arm 2	Arm 1			Arm 2			P (Neither Selected)
		P (selected)	P (stopped early)	Median N Patients	P (selected)	P (stopped early)	Median N Patients	
0.20	0.20	13.4%	9.2%	50	11.9%	9.4%	50	74.8%
0.05	0.05	11.5%	2.7%	50	11.6%	2.4%	50	77.0%
0.40	0.40	7.9%	85.4%	30	8.0%	84.1%	29	84.2%
0.05	0.20	87.4%	0%	60	12.6%	62.0%	39	12.6%
0.10	0.20	57.4%	1.0%	53	0.6%	33.7%	47	42.1%
0.05	0.30	99.0%	0.0%	60	0%	91.0%	23	1.1%

Abbreviations: Arm 1: AUC 16K; Arm 2: AUC 20K; NRM, non-relapse mortality

Table S7. GRADE III-IV ACUTE GVHD, BY ARM AND DONOR/GRAFT TYPE

	No. of patients	
	Arm 1 AUC = 16K (n=49)	**Arm 2 AUC = 20K (n=48)**
Grade III-IV acute GVHD	1	6
Donor type		
HLA-matched unrelated	1	4
HLA-matched sibling	0	2
Graft source		
Peripheral blood	1	5
Bone marrow	0	1

TABLE S8. CAUSES OF DEATH BY DAY 100 AND BETWEEN DAYS 101 AND 365

Cause	Day 100	Between days 101-365
Relapse	5	15
Acute GVHD	1	8
Chronic GVHD	-	1
Bacterial infection	4	4
Idiopathic pneumonia syndrome	-	1
Total	**10**	**29**

TABLE S9. OVERALL CAUSES OF DEATH, BY ARM

Cause	No. of patients	
	Arm 1 AUC = 16K	Arm 2 AUC = 20K
Relapse	14	12
Acute GVHD	3	6
Chronic GVHD	2	0
Bacterial infection	4	4
Idiopathic pneumonia syndrome	1	0
Hemorrhage	1	0
Total	25	22

Abbreviations: AUC, area under the curve; GVHD, graft-versus-host disease

Figure S 1. Outcomes by Age and Treatment Arm; Univariate estimates

(a) Non-Relapse Mortality by Treatment Arm Patients < 65 — AUC=16K, AUC=20K, P=0.84

(b) Non-Relapse Mortality by Treatment Arm Patients ≥ 65 — AUC=16K, AUC=20K, P=0.38

(c) Progression Free Survival by Treatment Arm Patients < 65 — AUC=16K, AUC=20K, P=0.94

Number at risk (number censored)
	0	5	10	15	20	25	30
16K	38 (0)	25 (0)	19 (3)	13 (6)	7 (12)	2 (16)	1 (17)
20K	28 (0)	19 (0)	14 (2)	9 (6)	6 (8)	1 (13)	

(d) Progression Free Survival by Treatment Arm Patients ≥ 65 — AUC=16K, AUC=20K, P=0.16

Number at risk (number censored)
	0	5	10	15	20	25
16K	11 (0)	6 (0)	1 (2)	1 (2)		
20K	20 (0)	14 (0)	9 (1)	6 (3)	2 (7)	1 (8)

(e) Overall Survival by Treatment Arm Patients < 65 — AUC=16K, AUC=20K, P=0.50

Number at risk (number censored)
	0	5	10	15	20	25	30
16K	38 (0)	30 (0)	22 (3)	16 (8)	8 (14)	2 (18)	1 (19)
20K	28 (0)	23 (0)	19 (2)	13 (6)	8 (10)	3 (15)	1 (16)

(f) Overall Survival by Treatment Arm Patients ≥ 65 — AUC=16K, AUC=20K, P=0.26

Number at risk (number censored)
	0	5	10	15	20	25
16K	11 (0)	6 (0)	2 (2)	2 (2)		
20K	20 (0)	17 (0)	11 (1)	6 (3)	2 (7)	1 (8)

Outcomes by age and treatment arm, univariate estimates:
Sub group analysis of patients younger or older than 65 years of age showed no difference in non-relapse mortality (a, b), progression free survival (c, d) or overall survival (e, f) in the 16K versus 20K arms.

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Data sharing statement:

Will individual participant data be available (including data dictionaries)? Yes, de-identified data can be shared, if approved by our institutional review board.

What data in particular will be shared? Individual participant data that underlie the results reported in this article, after de-identification (text, tables, figures, and appendices).

What other documents will be available? Study protocol.

When will data be available (start and end dates)? Beginning 9 months and ending 36 months following article publication.

With whom? Investigators whose proposed use of the data has been approved by an independent review committee ("learned intermediary") identified for this purpose and if approved by our institutional review board.

For what types of analyses? For individual participant data meta-analysis.

By what mechanism will data be made available? Proposals may be submitted up to 36 months following article publication. After 36 months the data will not be available.

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Protocol Page

Randomized Phase II Study of Timed Sequential Busulfan in Combination with
Fludarabine in Allogeneic Stem Cell Transplantation
2011-0958

Core Protocol Information

Short Title	Allogeneic Transplantation Using Timed Sequential Busulfan and Fludarabine Conditioning
Study Chair:	Uday Popat
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Full Title:	Randomized Phase II Study of Timed Sequential Busulfan in Combination with Fludarabine in Allogeneic Stem Cell Transplantation
Protocol Type:	Standard Protocol
Protocol Phase:	Phase II
Version Status:	Activated 01/27/2014
Version:	14
Submitted by:	Yadira L. Cortez--1/23/2014 9:15:06 AM
OPR Action:	Accepted by: Rosheta McCray -- 1/23/2014 3:22:30 PM

Which Committee will review this protocol?

◉ The Clinical Research Committee - (CRC)

Protocol Body

1.0 Objectives

Primary Objective:
This is a randomized study which primary objective is to compare the day 100 non-relapse mortality rates of two doses of timed sequential busulfan and fludarabine conditioning regimens.

Secondary Objective:
To obtain preliminary estimate of efficacy and differences between two dose levels by studying following endpoints:
  a. Overall Survival,
  b. Progression free survival,
  c. Neutrophil and Platelet engraftment, and
  d. Estimate acute and chronic GVHD.

Tertiary Objective:
To study impact of timed sequential busulfan therapy on:
  a. Gene expression in tumor cells.
  b. Cytokines (both in plasma and cells).
  c. The change in busulfan pharmacokinetics (PKs) between day -13 and day -6 dosing.

2.0 Background and Study Rationale

Reduced-intensity allogeneic transplantation(RIC) has been a major advance in the transplant field in the last decade, extending allogeneic transplantation to older patients and patients with comorbidities. Median age for development of AML, MDS, MF, and CLL, is in late 60s, but the median age for the patients undergoing conventional myeloablative transplantation is in 40s. No prospective randomized comparative trial has been done comparing RIC with myeloablative transplantation, but retrospective comparative trials have shown similar overall and progression free survival. With availability of better and high resolution HLA typing, results with unrelated donors are generally similar to those with matched siblings. Hence, RIC is considered for a patient with hematological malignancy, when a suitable matching related or unrelated donor is available, but fully ablative transplant is not feasible[1-18].
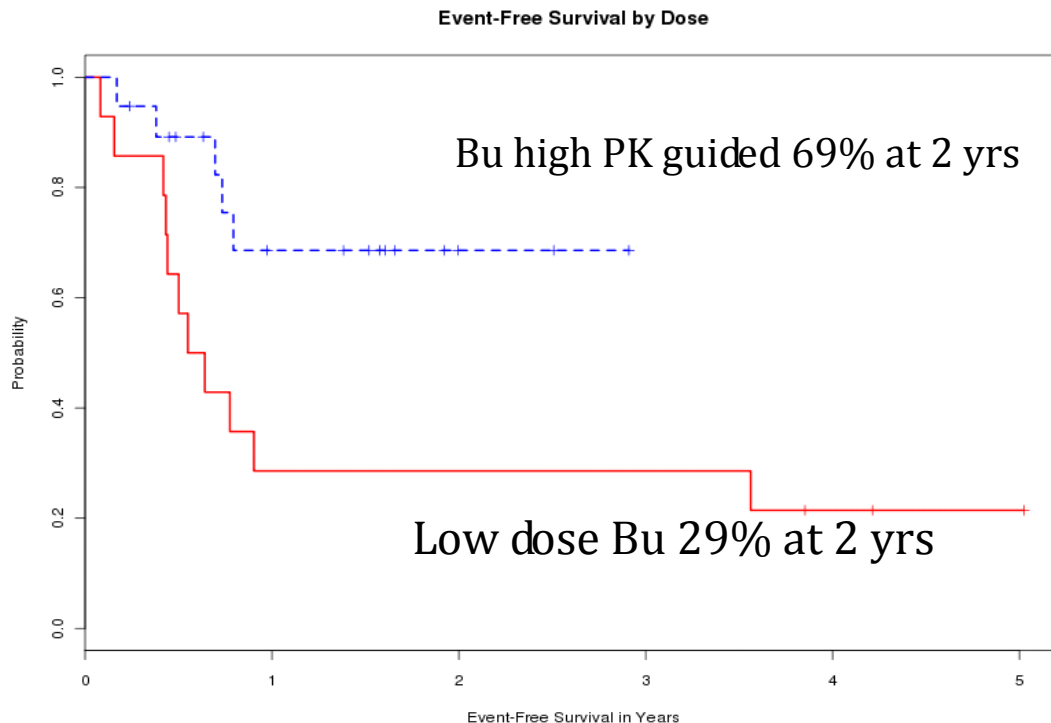
With RIC older patients in their 50s and 60s can be treated with a lower treatment related mortality of 15-30%, but the relapse rate of 20-40% is higher than that seen with myeloablative transplantation. For instance we recently analyzed transplant outcomes in 89 patients with MDS treated over a 7 year period between 2002 and 2008; non-relapse mortality and mortality due to disease recurrence were 26% and 31% respectively at 3 years. Similar results are seen in other hematological malignancies. To improve outcomes further, a safer conditioning regimen with low non-relapse mortality but with high antitumor efficacy is needed.

Busulfan and Fludarabine Conditioning.

Intravenous busulfan (Bu) developed at our institution has been a major advance over oral busulfan that was previously used to condition patients pretransplant. It has a better predictable pharmacological profile than oral busulfan negating the variability due to bioavailability of later. Combination of IV busulfan and fludarabine is significantly better than

busulfan and cyclophosphamide, which is commonly used regimen[19]. Further refinement was made by dosing IV busulfan based on pharmacokinetic studies. In a randomized study just completed (MD Anderson protocol 2005 -0366) in younger patients, PK guided IV busulfan targeting and area under the curve(AUC) of 6000 µMol/L was significantly superior to a fixed dose of IV busulfan 520mg/m^2. Progression free survival was 56% and 42% (p=0.03) in PK and fixed dose arm respectively.

In older patients and patients with comorbidities, our initial regimen consisted of half of fixed dose of busulfan (260 mg/m2) and fludarabine (bu-low). In a trial (MD Anderson protocol 2005-0726) conducted in patients with myelofibrosis, we observed high incidence of relapse in first 14 patients with this regimen. We therefore changed the dose of busulfan to PK guided target AUC of 4000 µMol/L (bu-high). As shown in the figure below bu-high was significantly superior to fixed dose busulfan with PFS of 69% and 29% (p=0.02) respectively, establishing fludarabine and PK guided busulfan with AUC of 4000 µMol/L as our current standard to improve upon for older patients.

Event-Free Survival by Dose

Bu high PK guided 69% at 2 yrs

Low dose Bu 29% at 2 yrs

Probability

Event-Free Survival in Years

Timed Sequential Therapy.

Timed sequential chemotherapy (TST)- delivery of a second course of chemotherapy 8-10 days after the previous one - was developed to enhance antitumor effect. Very promising efficacy was seen in clinical studies conducted to date. In vitro studies showed enhanced leukemia cell kill by recruiting a higher proportion of non cycling leukemic cells in to cell cycle after first course of chemo making them more susceptible to the second course of chemotherapy. Subsequent early clinical studies in patients with AML were promising and documented enhanced antileukemic activity[20-27]. Forty percent of patients with acute myeloid leukemia achieved long-term remission in these initial studies at Johns Hopkins[25]. German AML

group noted 68% complete remission (CR) rate in refractory patients and 84% remission rate as frontline therapy in a phase II study[28], leading to ongoing phase III study. French AML group likewise showed 61% CR rate in refractory patients, which led to a phase III study comparing this approach to standard therapy. While overall results were not significantly different, relapse rate was significantly lower in younger patients receiving TST compared with those receiving standard therapy, 36% vs. 50%[29]. Children's cancer group noted most impressive results in a phase III study enrolling 589 AML subjects. Patients were randomized to receive second course of therapy 6 days(TST) after the first one or at the standard interval of 14 – 28 days depending on bone marrow results. Event free survival was 42% in TST group and 27% in the standard group (p=0.0005) and overall survival was 52% and 42% in two groups respectively (p=0.04)[30]. This principle was further tested in a multicenter German phase II study in patients with refractory AML. In this study patients received a course of induction chemotherapy 4 days before reduced intensity conditioning allogeneic transplantation. Event free survival was 37% in these refractory patients, which is quite notable because expected EFS in this group of patients would be 15% - 20%[31].

We would like to apply these principles in our current study to treat patients with high-risk hematological malignancies. We would like to split busulfan in the conditioning regimen in two phases given 6 days apart. In the first phase we will give busulfan 80mg/m2 for two days. We will do PK analysis with first dose and adjust the dose of phase II busulfan to achieve a target AUC of 16000 or 20000 µmol/L over whole course of treatment. Effectively patients will receive the same dose as our standard but will receive a third or less of busulfan in first phase and about 2/3 in second phase 6 days later. Patients will be randomized between two groups with total AUC or 16000 and 20000 µmol/L. This will also enable us to define optimal dose of busulfan with this schedule of drug administration. We will also do correlative studies on blood samples obtained at set time point to study the cytokine profile, which was postulated in in vitro studies to cause increased anti tumor efficacy of this approach. A second correlative study will be to look at gene expression analysis of tumor cells at different time points to see if pretherapy at day 13 has any impact on gene expression.

Tertiary Endpoints and Correlative studies

Early in vitro studies of the concept of timed sequential therapy (TST) had indicated that the increased antitumor activity seen with this approach was due to a humoral substance in plasma[23,24]. Plasma samples obtained 8-10 days after first course of therapy from a patient increased leukemia cell kill in vitro. It therefore appears that antitumor efficacy of this approach is possibly due to a soluble factor released in response to a course of chemotherapy synergizing with a second course of chemotherapy delivered 8-10 days later. We hypothesize that this soluble factor is a cytokine and wish to study various cytokines including plasma levels of cytokines (IL-1β, IL-6, IL-8, IL-10, IL-12, and TNF-α) and IL-1 receptor antagonist (IL-1ra), soluble TNF- receptors (TNF-R1/R2 or p55/p75). Since the majority of inflammatory cytokines are secreted by monocytes and macrophages, we propose to assess the synthesis of inflammtory cytokines (IL-1b, IL-6, IL-10, TNF-α, and VEGF) by resting as well as by TLR4/LPS-activated monocytes by flow cytometry. This method measures the synthesis of cytokines by individual monocytes. Syntheses of inflammatory cytokines by M1 and M2 monocytes will be assessed. M1 and M2 monocytes can be distinguished based on the ability to differentially secrete TNF-a and VEGF, respectively. Furthermore, we will study the genetic changes in host cells induced by first course of chemotherapy and cytokines released by it. Mononuclear cells (MNCs) will be isolated from patient blood samples. Total RNA will be purified and analyzed by high throughput gene expression profiling and real-time PCR for genes

involved in DNA repair, cell cycle checkpoint, drug metabolism, apoptosis and survival. Proteins will also be isolated if enough MNCs are isolated. A correlation between time of drug exposure and expression of the above-mentioned genes will be determined. These studies will help us understand mechanism of anti tumor effect of this approach and help us fine tune it further.

3.0 Background Drug Information

Busulfan:
Therapeutic Classification: Antineoplastic Alkylating agent.

Pharmaceutical data: Busulfan injection is a sterile, pyrogen-free solution provided in a mixture of dimethylacetamide (DMA) and polyethyleneglycol 400 (PEG400). It is supplied in 10 ml single use ampoules at a concentration of six (6) mg busulfan per ml. Each ampoule contains 60 mg of busulfan in 3.3 ml of DMA and 6.7 ml of PEG400. When diluted in normal saline or D5W to a concentration of 0.5 mg/ml, the resulting solution must be administered within eight (8) hours of preparation including the three (3) hour infusion of the drug.

Stability and storage: Ampoules should be stored refrigerated at 2-8°C (35-46°F). Stable at 4°C for at least twelve (12) months. Additional stability studies are in progress. DO NOT use beyond the expiration date. DO NOT use if the solution is cloudy or if particulates are present.

Solution Preparation: Mix into normal saline to a final concentration of 0.5 mg/mL.

In each bag 6.0 mg busulfan (1.0 ml at 6 mg/ml and 11 ml saline) should be added to compensate for drug lost in the tubing with each infusion (approximately 12 ml at 0.5 mg/ml is lost in the tubing when using the controlled rate infusion pump).

Route of Administration: It is to be noted, that a sufficient amount of diluted busulfan should be added to compensate for the amount needed to prime the IV tubing; when hanging the infusate, the tubing should be primed with the busulfan solution and connected as close to the patient as possible. After completed infusion, the tubing with remaining busulfan (approximately 12 mL) should be disconnected and discarded. All busulfan infusions should be performed by programmable pump.

The high-dose busulfan will be given by slow intravenous infusion over three (3) hours into a central venous catheter.

CAUTION: DO NOT ADMINISTER AS AN INTRAVENOUS PUSH OR BOLUS.
An infusion pump will be used with the busulfan solutions as prepared above. A new infusion set must be used for administration of each dose. Prior to and following each infusion, flush the catheter line with normal saline or (approximately 5 ml). Start the three-hour infusion at the calculated flow rate. DO NOT infuse concomitantly with another intravenous solution of unknown compatibility.

If a delay in administration occurs after the infusion solution is prepared, the properly identified container should be kept at room temperature (20-25°C), but administration must be completed within eight (8) hours of preparation including the three (3) hour drug infusion.

Adverse Events: Dose limiting toxicity is expected to be hematological when used without

stem cell support. Other toxicities seen frequently following high-dose busulfan in preparative regimens for bone marrow transplantation include: VOD, nausea, vomiting, pulmonary fibrosis, seizures, rash, and an Addison's-like syndrome.

Mechanism of action: Interferes with DNA replication and transcription of RNA through DNA alkylation, and ultimately results in the disruption of nucleic acid function.

Animal Tumor Data: Busulfan has been shown to be active against a variety of animal neoplasm *in vivo*, including mouse sarcoma 180 and Ehrlich's mouse ascites tumor.

Human Pharmacology: Limited pharmacology data are available for the parenteral formulation to be used in this study and is detailed in the evaluation of IV Bu in a Phase II Trial using IV Bu at 0.8 mg/kg BW given over 2 hr every 6 hr for a total of 16 doses (Andersson et al, 2002) and when administered once daily for 4 days at a dose of 130 mg/m2 in combination with Flu (Madden et al, AHS 2003, de Lima et al, BLOOD 2004). The pharmacokinetic data suggests that the plasma decay of the formulation fits an open one-compartment model with linear pharmacokinetics in the dose range of 12 mg-130 mg/m2. Based on studies of oral Bu, the drug is reported to be extensively metabolized; twelve (12) metabolites have been isolated, but most have not been identified. The drug is slowly excreted in the urine, chiefly as methanesulfonic acid. Ten to fifty percent (10-50%) of a dose is excreted as metabolites within twenty-four (24) hours (Nadkarni 1959).

Fludarabine:
Therapeutic Classification: Antineoplastic agent; Antimetabolite (Purine Analaog).

Pharmaceutical Data: Each vial contains 50 mg lyophilized drug, to be reconstituted with 2 ml sterile water to a solution that is 25 mg/ml for IV administration.

Solution Preparation: Mix each vial with 2 ml sterile pyrogen-free water to a clear solution, which is 25 mg/ml for IV administration only. Reconstituted solution should be used within 8 hours. Fludarabine is diluted in 100mL NS for a final concentration to be infused over 1 hour.

Adverse Effects: Cardiovascular: Edema (8% to 19%); Central nervous system: Fever (11% to 69%), fatigue (10% to 38%), pain (5% to 22%), chills (11% to 19%); Dermatologic: Rash (4% to 15%); Gastrointestinal: Nausea/vomiting (1% to 36%), anorexia (·34%), diarrhea (5% to 15%), gastrointestinal bleeding (3% to 13%); Genitourinary: Urinary tract infection (2% to 15%); Hematologic: Myelosuppression (nadir: 10-14 days; recovery: 5-7 weeks; dose-limiting toxicity), anemia (14% to 60%), neutropenia (grade 4: 37% to 59%; nadir: ~13 days), thrombocytopenia (17% to 55%; nadir: ~16 days); Neuromuscular & skeletal: Weakness (9% to 65%), myalgia (4% to 16%), paresthesia (4% to 12%); Ocular: Visual disturbance (3% to 15%); Respiratory: Cough (·44%), pneumonia (3% to 22%), dyspnea (1% to 22%), upper respiratory infection (2% to 16%), rhinitis (·11%); Miscellaneous: Infection (12% to 44%), diaphoresis (·14%).

Mechanism of Action: Fludarabine inhibits DNA synthesis by inhibition of DNA polymerase and ribonucleotide reductase; also inhibits DNA primase and DNA ligase I.
Human Safety and Pharmacology: Distribution: Vd: 38-96 L/m2; widely with extensive tissue binding. Protein binding: 2-fluoro-ara-A: ~19% to 29%. Metabolism: I.V.: Fludarabine phosphate is rapidly dephosphorylated in the plasma to 2-fluoro-ara-A (active metabolite), which subsequently enters tumor cells and is phosphorylated by deoxycytidine kinase to the

active triphosphate derivative (2-fluoro-ara-ATP). Bioavailability: Oral: 2-fluoro-ara-A: 50% to 65%. Half-life elimination: 2-fluoro-ara-A: ~20 hours. Time to peak, plasma: Oral: 1-2 hours. Excretion: Urine (60%, 23% as 2-fluoro-ara-A) within 24 hours.

Dosing (in stem cell transplant): Stem cell transplant (allogeneic) conditioning regimen, reduced-intensity, (unlabeled use): 30 mg/m2/dose for 6 doses beginning 10 days prior to transplant or 30 mg/m2/dose for 5 days beginning 6 days prior to transplant (in combination with busulfan with or without antithymocyte globulin) (Schetelig, 2003) or 40 mg/m2/dose for 4 days.

Stem cell transplant (allogeneic) nonmyeloablative conditioning regimen (unlabeled use): 30 mg/m2/dose for 3 doses beginning 5 days prior to transplant (in combination with cyclophosphamide and rituximab) (Khouri, 2008) or 30 mg/m2/dose for 3 doses beginning 4 days prior to transplant (in combination with total body irradiation) (Rezvani, 2008).

Dose adjustment in renal and hepatic impairment: It appears that no adjustment is needed in hepatic impairment. Renal impairment dosing is **NOT** specific to stem cell transplant patients. In patients not receiving a stem cell transplant, doses are typically reduced by 20% for CrCl of 30-70 ml/min and not used for CrCl < 30 ml/min.

Monitoring Parameters: CBC with differential, platelet count, AST, ALT, serum creatinine, serum albumin, uric acid; monitor for signs of infection and neurotoxicity.

4.0 Patient Eligibility

Inclusion Criteria:
1. Patients with high-risk hematologic malignancies with anticipated poor prognosis with non transplant therapy, including those in remission or with induction failure and after treated or untreated relapse. Diagnoses to be included a) Acute myeloid leukemia; b) Acute lymphocytic leukemia; c) Chronic myeloid leukemia; d) Chronic lymphocytic leukemia; e) Myelodysplastic syndrome; f) Myeloproliferative syndromes; g) Non-Hodgkins lymphoma; h) Hodgkins Lymphoma; i) Multiple myeloma.
2. Patients must have a histocompatible stem cell donor. An HLA-identical related donor or an 8/8 matched unrelated donor.
3. Age 5 to 75 years old.
4. Performance score of >/= 70 by Karnofsky/Lansky or PS 0 to 1 (ECOG </=1).
5. Left ventricular ejection fraction at least 40%.
6. Adequate pulmonary function with FEV1, FVC and DLCO >/=50% of expected corrected for hemoglobin and/or volume. Children unable to perform pulmonary function tests (e.g., less than 7 years old) pulse oximetry of >/= 92% on room air.
7. Creatinine clearance (calculated creatinine clearance is permitted) should be >40 ml/min.
8. Bilirubin </= 2 x the upper limit of normal (except Gilbert's Syndrome). SGPT (ALT) < 200.
9. Negative Beta HCG test in a woman with child bearing potential, defined as not post-menopausal for 12 months or no previous surgical sterilization. Women of child bearing potential must be willing to use an effective contraceptive measure while on study.
10. Patient or patient's legal representative able to sign informed consent.

Exclusion Criteria:

1. HIV seropositivity.
2. Uncontrolled infections.

5.0 Treatment Plan

The transplant day is referred to as day zero (D0), treatment plan activities prior or after D0 are denominated as day minus (D-) or day plus (D+).

Patients will be equally randomized between two separate timed sequential doses of Busulfan based on AUC of 16000 and 20000 μmol/l.

Allogeneic graft.
Peripheral blood (PB) or bone marrow (BM) progenitor cells may be used in this study. The allogeneic blood stem cell collection is a standard procedure for which a separate consent will be signed. Donors (related or unrelated) must meet standard medical eligibility criteria for allogeneic blood stem cell donation.

Preparative Regimen.
Acetaminophen should not be used between D-14 (starting 24 hours before the first dose of IV Bu) and D0, since there is a major interference between these drugs and the metabolism of Bu which is likely to contribute in a major way to cause serious liver damage.

Other drugs known to interfere with the metabolism of Fludarabine and/or Busulfan should not to be concomitantly used during the chemotherapy administration up to and including the day of transplantation. In particular, this pertains to drugs that are know effective inhibitors of, or inducers of the hepatic cytochrome P450-system, such as primidone, voriconazole, itraconazole, and metronidazole as well as tyrosine-kinase inhibitors. Such agents must be omitted for at least 5 days prior to the test dose of IV Bu or to admission, whichever comes first, for transplantation on this program since these agents have well described interference with busulfan metabolism. They can be resumed starting one before the stem cell transplant procedure.

Busulfan first two doses.
The first two doses of Busulfan, 80 mg/m^2 can be administered as an outpatient or as an inpatient. If inpatient, patients will be admitted on a Monday, Tuesday or Wednesday to facilitate for this pharmacokinetically directed therapy.

The first Busulfan dose will be based on actual body weight and will be given IV over three hours by controlled-rate infusion pump.

Depending on randomization, Busulfan is administered at the dose calculated to achieve a **total** (including first two doses delivered on day -13 and -12) systemic exposure of 16,000 ± 12% μMol-min or 20000 ± 12% μMol-min based on the pharmacokinetic studies.

D-6 to D-3 Fludarabine/Busulfan administration.
Fludarabine will be dosed per actual body weight/actual body surface area. No arbitrary dose adjustment(s) based on a perceived need for using adjusted body weight/body surface area will be allowed for Fludarabine. Fludarabine must be administered IV once daily by a controlled-rate pump.

Fludarabine is administered IV at the dose of 40 mg/m2 in 100 ml of NS on each of four

(4) consecutive days.

Pharmacokinetic-guided (PK-guided) treatment:
The first two doses of Busulfan of 80 mg/m2 will be administered on day –13 and -12, it will be will be given IV by controlled-rate infusion pump. PK studies will be done with the first dose. The pharmacokinetic- guided daily high-dose busulfan dose(s) will be started immediately upon completion of the daily fludarabine on days -6 to -3. The busulfan doses will be diluted in normal saline and administered daily by controlled rate infusion pump starting immediately after the completion of Fludarabine.

Busulfan is administered at the dose calculated to achieve a **total** (including first two doses delivered on day -13 and -12) systemic exposure of 16,000 ± 12% µMol-min or 20000 ± 12% µMol-min based on the pharmacokinetic studies. (PK studies may also be done on day -6 if the physician deems it necessary for patient safety or in order to adjust the final two days of Busulfan on day -4 and day -3 to meet this total systemic exposure.) We will also analyze the percentage of patients who achieve the targeted systemic exposure (± 10%) based on first dose PK guided recommendation and characterize the variability and change between the two busulfan PKs in the patient population.

Stem Cell infusion.
Fresh or cryopreserved bone marrow or peripheral blood progenitor cells will be infused on day 0. Depending on arrival time, patients who receive a graft from an unrelated donor might have one day delayed from D0.

Prophylaxis and Supportive Care as per standard practice in patients receiving allogeneic transplant and SCTCT Guidelines.
GvHD with Tacrolimus and Mini Methotrexate with dose adjustment as clinically indicated. Tacrolimus will be administered at starting dose of 0.015 mg/kg (ideal body weight) as a 24 hour continuous infusion daily adjusted to achieve a therapeutic level of 5-15 ng/ml. Tacrolimus is changed to oral dosing when tolerated and can be tapered off after day +90 if no GVHD is present. Methotrexate 5 mg/m^2 will be administered intravenously on days 1, 3, 6 and 11 post transplant.   Day 11 methotrexate may be held if the patient has symptomatic mucositis.

G-CSF administered at a dose of 5 mcg/kg/day (rounded up the nearest vial size) subcutaneously beginning on D+7, and continuing until the absolute neutrophil count (ANC) is > 500 x 10/L for 3 consecutive days.

Antiseizure prophylaxis and other supportive care (allopurinol, menstrual suppression, prophylactic antibiotics, empiric antibiotics, IVIG, transfusions of blood products, hyperalimentation, etc.) as indicated.

6.0 Study Evaluations

Disease assessment prior to start treatment (baseline).
Disease assessment is done prior to study entry as part of diagnostic or routine pre-transplant workup.

Post transplant Evaluations:
A. To be performed around engraftment time:
1. Chimerism studies from peripheral blood performed on separated T-cells and myeloid cells.

2. Physical examination and adverse event documentation including GvHD assessment.

B. To be performed at approximately 1, 3, 6 and 12 months post transplant.
These evaluations follow our standard practice and are done to monitor engraftment and disease status. If clinically indicated these studies may be done at other time points which can replace the nearest planned timepoint.

1. Chimerism studies from peripheral blood performed on separated T-cells and myeloid cells.
2. Transplant (immunodeficiency) panel by flow cytometry to assess for immune reconstitution will be performed on peripheral blood.
3. At each visit, a physical examination and adverse event documentation including GvHD assessment.
4. Disease specific assessment as per standard of care and SCT&CT guidelines with bone marrow aspirate with cytogenetics.

C. After the first year per routine follow-up and standard of practice for bone marrow transplant. We recomend follow at at MDACC for the first 3 years post treatment completion. Patients will be follow up for disease status, presence of GVHD and survival.

The following lab tests are to be performed as frequently as clinically indicated:CBC, differential, platelets, SGPT, calcium, glucose, uric acid, magnesium, serum bilirubin, BUN and creatinine, serum protein, albumin, alkaline phosphatase, electrolytes, urinalysis, tacrolimus levels and CMV antigenemia.

D. Optional Research labs (participants 18 years old or older): 20 cc of blood will be collected for correlative research studies on day -13, -6, 0, +7, +14, +21, and +28 (+/- 3 days per time point) to check for tumor markers, to study the effect of chemotherapy on cancer cells and blood cells, and to check the status of the disease. This research blood will be sent to Dr. Borje Andersson's lab.

7.0 Study Definitions

Treatment periods.
   Active treatment administration is defined from the first day of treatment administration as outlined in the treatment plan through D0.
   Active treatment period is defined from the first day of treatment administration through Day +30.
   Follow-up period is defined from BMT Day +31 until 3 years post treatment completion.

Engraftment is defined as the evidence of donor derived cells (more than 95%) by chimerism studies in the presence of neutrophil recovery by day 28 post stem cell infusion.

Other definitions used to assess engraftment:
Neutrophil recovery is defined as a sustained absolute neutrophil count (ANC) > 0.5 x 10^9/L for 3 consecutive days.
Engraftment date is the first day of three (3) consecutive days that the ANC exceeds 0.5 x10^9/L.
Delayed engraftment is defined as the evidence of engraftment beyond day 28 post SC infusion achieved after the administration of therapeutic (high dose) hematopoietic growth

factors.

Primary Graft failure is defined as failure to achieve an ANC > 0.5 x 10^9/L for 3 consecutive days by day 28 post SC infusion, with no evidence of donor derived cells by bone marrow chimerism studies and no evidence of persistent or relapsing disease.

Secondary graft failure is defined as a sustained declined of ANC <0.5 x 10^9/L for 3 consecutive days after initial documented engraftment with no evidence of disease progression.

Autologous reconstitution is defined by the presence of ANC > 0.5 x 10^9/L without evidence of donor-derive cells by bone marrow chimerism studies. This can occur at initial engraftment or later after initial engraftment has been documented.

Disease Response as per CIBMTR criteria.
    A.   For AML/MDS:
<u>Complete remission</u> (CR):
BM < 5% blasts (absence of blasts with Auer rods).
ANC > 1000/ul.
Platelet count >100 x 10e9/ul (independent of red cell transfusions).
Absence of extramedullary disease.

<u>Marrow CR</u> (CRi) (incomplete hematologic recovery).
BM < 5% blasts (absence of blasts with Auer rods).
ANC < 1000/ul orPlatelet count <100 x 10e9/ul.
Absence of extramedullary disease.

<u>No Response</u> (NR) or <u>Disease Progression</u>
BM > 5% leukemia blasts
Persistent presence of blasts in peripheral blood.
Presence of extramedullary disease.

    B.   For CML:
<u>Cytogenetic Response</u>
Complete: No Ph positive metaphases.
Major: 0-35% Ph positive metaphases.
Partial: 1-34% Ph positive metaphases.
Minor: 35-90% Ph positive metaphases.

<u>Complete Hematologic Response</u>
Complete normalization of peripheral blood counts with leukocyte count <10x10^9/L.
Platelet count <450x10^9/L.
No immature cells in peripheral blood.
No signs and symptoms of disease with disappereance of palpable splenomegaly.

<u>Partial Hematologic Response</u>
Same as complete hematologic response, except for:
Presence of immature cells.
Platelet count < 50% of the pretreatment count, but >450x10^9/L..
Persistent splenomegaly, but < 50% of the pretreatment extent.

<u>Molecular Response</u>:
Complete molecular response: BCR-ABL mRNA undetectable by RT-PCR.
Major molecular response equal or more 3-log reduction of BCR-ABL mRNA.

C.   For CLL:

Complete Response (CR):
No lymphadenopathy; no organomegaly
Neutrophils >1.5 x10^9/L; platelets >100 x10^9/L; Hg > 11g/dL, lymphocytes;
Absence of constitutional symptoms.

Nodular Partial Response (NPR):
CR with persistent lymphoid nodules in the bone marrow.

Partial Response (PR):
Equal or more than 50% decrease in peripheral blood lymphocytes count from pre-treatment value.
Equal or more than 50% reduction in lymphadenopathy, liver and or spleen if abnormal at pre-treatment.
50% reduction from baseline in one or more of the following: Neutrophils, platelets, Hg and lymphocytes.

Stable Disease (SD):
No change in disease status from baseline, no progression.

Disease Progression:
More than 50% increase in one or more of the following:
The sum of the products of 2 or more lymph nodes or new nodes; the size of liver, spleen, in absolute lymphocyte count;
New hepatomegaly or splenomegaly.
Transformation to a more aggressive histology.

D.   For Multiple Myeloma

Stringent complete response (sCR) (all of the following):
CR as defined.
 Normal free light chain ratio
Absence of clonal cells in bone marrow by inmmunohistochemistry or immunofluorescence (defined by absence of abnormal ê/ë ratio of >4:1 or <1:2)

Complete response (CR) (all of the following):
Negative immunofixation in serum and urine.
£ 5% plasma cells in the bone marrow.

Disappearance of any soft tissue plasmacytomas.

Very good partial response (VGPR) (one of the following):
 Serum and urine M protein detectable by immunofixation but not by electrophoresis.
90% or greater reduction in serum M protein plus urine M protein level <100 mg per 4h.

Partial response (PR) (all of the following):
Reduction by > 50% in serum monoclonal protein.
Reduction of urinary monoclonal protein to < 200 mg/24h or >90%.

Stable disease:
Not meeting criteria for CR, VGPR, PR or PD.

Progressive disease (PD) (any one or more of the following):
Increase of >= 25% from baseline in:
Serum M protein (absolute increase must be >= 0.5 g/dL).
Urine M component (absolute increase must be >= 200 mg/24h).Only in patients without measurable serum and urine M protein levels.
Difference between involved and uninvolved FLC levels (absolute increase must be > 10 mg/dL).
Bone marrow plasma percentage (absolute % must be >=10%).

Definite development of new bone lesions or soft tissue plasmacytomas or definite increase in the size of existing bone lesions or soft tissue plasmacytomas.
Development of hypercalcemia (corrected serum calcium >11.5 mg/dL or 2.65 mol/L) that can be solely attributed to the myeloma.

Relapse from CR (any one or more of the following):
Reappearance of serum or urine M protein by immunofixation or electrophoresis.
Development >=5% plasma cells in the bone marrow.
Appearance of any other sign of progression (i.e., new plasmacytoma, lytic bone lesion or hypercalcemia).

Non-relapse mortality (NRM) is defined as death from any cause other than relapse disease.

Disease free survival (DFS) is defined as the interval between day of transplant and day of death or disease progression.

Overall Survival (OS) is defined as the interval between day of transplant and day of death.

8.0 Adverse Events and Reporting Requirements

Assessment of the Adverse Events Severity.
The severity of the adverse events (AEs) will be graded according to the Common Terminology Criteria v3.0 (CTCAE).
Events not included in the CTCAE chart will be scored as follows:

General grading:
Grade 1: Mild: discomfort present with no disruption of daily activity, no treatment required beyond prophylaxis.
Grade 2: Moderate: discomfort present with some disruption of daily activity, require treatment.
Grade 3: Severe: discomfort that interrupts normal daily activity, not responding to first line treatment.
Grade 4: Life Threatening: discomfort that represents immediate risk of death

Grading for specific syndromes:
Veno-occlusive disease (VOD):
Grade 3: Bili >2mg/dl with at least two of the following: increased weight >4% from baseline, ascites or hepatomegaly
Grade 4: pulmonary and or renal failure

Pulmonary events not caused by CHF (interstitial pneumonitis (IP), pulmonary hemorrhage (DAH):

Grade 1: CXR showing mild infiltrates or interstitial changes
Grade 2: mild SOB
Grade 3: requires supplemental oxygen, or is a documented infection
Grade 4: requires intubation

Transplant related microangiopathy:
Grade 1: No treatment required
Grade 2:Requires steroids and/or plasma transfusions
Grade 3: Requires plasma exchange

Cytokine storm or engraftment syndrome:
Grade 1: No treatment required
Grade 2: Treatment required
Grade 3: Organ dysfunction
Grade 4: Total Bilirubin >5

Hemorrhagic Cystitis:
Grade 1: minimal or microscopic bleeding/pain
Grade 2: gross bleeding/pain and spasms
Grade 3: transfusion/irrigation required
Grade 4: dialysis required

Casualty Assessment.
For the purpose of this study the treatment plan (preparative regimen followed by allogeneic stem cell transplantation) is defined as the "transplant package"; therefore adverse events known to be caused by components of the transplant package and its direct consequences will be scored as definitive related. Adverse events known to be related to drugs used for the treatment of GVHD and Infection episodes will be scored as probable related. When the relationship of the adverse event cannot be ruled out with certainty the AE may be considered possible related. Adverse events known to be related to drugs used for supportive treatment will be scored as unrelated.

The principal investigator will be the final arbiter in determining the casualty assessment.

List of most common expected adverse events.
1. Infections in the presence or absence of neutropenia: fungal, bacterial and or viral infections.
2. Fever: Non-neutropenic or neutropenic without infection
3. Acute graft versus host disease (aGVHD): most commonly manifested by skin rash, diarrhea and abnormal liver function tests could also present with some degree of fever, upper gastrointestinal symptoms (nausea and vomiting) mucositis and eye dryness.
4. Gastrointestinal (GI tract): the GI tract manifestations could be not only due to direct damage from the preparative regiment but also be a manifestation of GVHD or infections. Therefore, the time course and its presentation are crucial when assessing these as adverse events. Nausea/vomiting, mucositis, diarrhea when presented within first 7 to 10 days most likely will be related to the preparative regimen.
5. Skin rash: not related to GVHD could be caused by chemotherapy used for the preparative regimen or antibiotics used a supportive treatment.
6. Transaminitis: liver function test elevation.
7. Pulmonary events: not related to CHF most likely caused by drug injury or infection. These could present with a pneumonitis pattern manifested with shortness of breath, pulmonary

infiltrates on chest radiograph, sometimes accompanied by fever and cough and progress to acute respiratory insufficiency and a diffuse bilateral alveolar pattern.

8. Cytokine Storm/ engraftment syndrome: most likely caused by released cytokines.
9. Hemorrhagic cystitis: not related to chemotherapy agents used in the proposed preparative regimen is most likely caused by viral infection.
10. Thrombotic thrombocytopenic purpura (TTP).
11. Veno-occlusive Disease of the Liver (VOD): could be caused by busulfan. Some antimicrobial agents have been also incriminated in its development.
12. Fluid overload due to hydration required for conditioning regimen, blood product transfusions and or IV alimentation
13. Graft failure.
14. Chronic GVHD.
15. For the purpose of this study the following events would not be considered adverse events and would not be recorded in the database:
    1. Flu-like symptoms not associated with infection
    3. Abnormal laboratory findings considered associated to the original disease
    4. Isolated changes in laboratory parameters such as electrolyte, magnesium and metabolic imbalances, uric acid changes, elevations of ALT, AST, LDH and alkaline phosphatase.

Adverse events considered serious.

1. Prolonged hospitalization due to infections and/or organ failure requiring extensive supportive care (i.e. dialysis, mechanical ventilation).
2. Readmissions from any cause resulting in a prolonged hospitalization (>10 days).
3. Graft Failure/ rejection.
4. Any expected or unexpected event resulting in an irreversible condition and/or leading to death.

Adverse events data collection.

From the start of preparative regimen up to D+100 the collection of adverse events will reflect the onset and resolution date and maximum grade; beyond this point some events considered related to chronic GVHD or late complications post transplant might be recorded only with the first date of their awareness with no grade or resolution date.

Intermittent events should be labeled as such and followed until resolution.

If a patient is taken off study while an event is still ongoing, this will be followed until resolution unless another therapy is initiated. Pre-existing medical conditions will be recorded only if an exacerbation occurs during the active treatment period. Co-morbid events will not be scored separately.

As stated in the treatment plan, patients treated on this protocol will required supportive care treatment (concurrent medication). These medications are considered standard of care and have no scientific contributions to the protocol, therefore no data will be captured on the various medications needed or their sides effects.

AE and Protocol Deviations Reporting Requirements.

Adverse events will be reported accordingly to MDACC (HSRM chapter 15.001) and SCT&CT Department (HSRM chapter 15.053) policy and procedures.This study will be conducted in compliance however in the event of any protocol deviations or vilolations these will be reported accordingly to MDACC (HSRM chapter 25).

9.0 Off Study Criteria

Off Study Criteria
1. Patient's withdrawal of consent to participate.
2. Serious noncompliance with the protocol treatment which would compromise the study.
3. Graft failure requiring further treatment.
4. Relapse of the malignancy.
5. Not able to perform the pharmacokinetic-guided daily high-dose busulfan.
6. After 3 years post treatment completion.

10.0 Statistical Considerations

Overview

This is a randomized Phase II trial of two separate timed sequential busulfan/fludarabine conditioning regimens (Busulfan AUC of 16000 and 20000 µmol/l in patients with hematologic malignancies. The primary objective of the trial is to compare the non-relapse mortality (NRM) rate at day 100 between the two treatment arms. Secondary objectives include assessing overall and progression-free survival, time to platelet and neutrophil engraftment, the cumulative incidence of acute and chronic GVHD, and correlative studies of cytokines at serial time points. The primary endpoint will be non-relapse mortality at day 100. We will enroll 100 patients in this study, randomized fairly between the two arms. The expected accrual rate is 3 patients per month.

Patients will be equally randomized through CORE and the safety monitoring rules will be assessed monthly by the biostatistical collaborators using offline programs. If any stopping rule is met, the biostatistical collaborators will notify the study team such that CORE will stop randomizing patients and continue with only the superior arm if applicable.

Safety Monitoring

The primary endpoint of the study is non-relapse mortality (NRM) assessed at 100 days. We will use Bayesian monitoring rules[31] to monitor the 100-day NRM rate on this study. Let Arm 1 refer to the Busulfan AUC of 16000, and Arm 2 will refer to the Busulfan AUC of 20000. Denote theta 1 and theta 2 as the NRM rates in the two arms.

The following monitoring rules will be employed:

(1) An arm will be stopped early and a treatment will be selected as superior if the probability is 0.99 or more that the NRM rate for this arm is less than for the other arm. Formally, we will stop enrollment into the trial and select arm 1 as superior if at any time:

$$Pr\ (theta1 < theta2\ |\ Data) > 0.99$$

A similar rule applies for arm 2; i.e., we will stop the trial and select arm 2 as superior if at any time:

$$Pr\ (theta2 < theta1\ |\ Data) > 0.99$$

If a treatment arm is selected as superior by this rule during the study, we will continue to enroll patients in this arm until a total of 60 patients are reached (or the study

maximum of 100 is reached) so that we have additional data on NRM, overall survival, progression-free survival, and other secondary endpoints.

(2)   Additionally, the NRM rate in each arm will be monitored to assure that if it is likely that the rate is greater than 20%, the arm will be stopped. Formally, we will stop accrual to arm 1 if at any time:

$$Pr\ (theta1 > 0.20 \mid Data) > 0.99$$

A similar rule applies for arm 2.

If enrollment into a treatment arm is stopped, we will continue to accrue patients to the other arm until a total of 60 patients are accrued in this arm (or the study maximum of 100 is reached) so that we have additional data on NRM, overall survival, progression-free survival, and other secondary endpoints.

If accrual is stopped in both arms by rule 2, enrollment into the trials will stop.

If the trial is not stopped early by rule 1, we will select treatment #1 as being superior if Pr (theta1 < theta2 | Data) > 0.90 and treatment #2 as being superior if Pr (theta2 < theta1 | Data) > 0.90.

We assume a Beta (0.4, 1.6) prior distribution for the probability of NRM in each arm.

This trial design was simulated 2000 times for several scenarios, and the operating characteristics are summarized in the table below. This table was produced using software written by the biostatistical collaborators, which can be provided upon request. If the true NRM rates are 5% and 20% in Arms 1 and 2, respectively, then the probability that Arm 1 will be selected is 87.4%.

True NRM Rate Arm 1	True NRM Rate Arm 2	Arm 1			Arm 2			
		P (selected)	P (stopped early)	Median N Patients	P (selected)	P (stopped early)	Median N Patients	P (Neither Selected)
0.20	0.20	13.4%	9.2%	50	11.9%	9.4%	50	74.8%
0.05	0.05	11.5%	2.7%	50	11.6%	2.4%	50	77.0%
0.40	0.40	7.9%	85.4%	30	8.0%	84.1%	29	84.2%
0.05	0.20	87.4%	0%	60	12.6%	62.0%	39	12.6%
0.10	0.20	57.4%	1.0%	53	0.6%	33.7%	47	42.1%
0.05	0.30	99.0%	0.0%	60	0%	91.0%	23	1.1%

Analysis Methods

Continuous data will be summarized using descriptive statistics (mean, standard deviation, median, inter-quartile range, range). Categorical data will be summarized using frequency counts and percentages.

The proportion of patients with NRM will be reported for each treatment arm, along with 95% Bayesian credible intervals. A sample size of 100 patients ensures more than 80% probability of selecting Arm 1 when the NRM rates are 5% and 20%, respectively.

Overall survival and progression-free survival will be calculated from the time of

transplant by the method of Kaplan and Meier[32]Cox proportional hazards regression analysis[33] will be used to assess the association between these survival parameters and clinical and treatment covariates of interest.

The time to platelet and neutrophil engraftment will be calculated from the time of transplant and estimated by the Kaplan-Meier method. The cumulative incidence of acute and chronic GVHD with the competing risk of relapse will be estimated using the method of Gooley[35] and the method of Fine and Gray[36] will be used to model this incidence by disease and clinical characteristics of interest. Secondary analysis will also include analyzing NRM with these methods while taking into account the competing risk of deaths due to other causes within 100 days. Generalized linear mixed models will be used to assess the association between cytokines over time and treatment and other factors.

The change in busulfan PK between the first dose (day -13) and the third dose (day -6) will be compared using t test. Percentage of patients who achieve the targeted systemic busulfan exposure (±10%) based on first dose PK recommendation and overall population PK will be summarized using descriptive statistics.

Descriptive statistics will be used to summarize adverse events by treatment arm. The number (%) of subjects with treatment emergent adverse events will be reported. Frequency counts and percentages will also be presented of subjects with serious adverse events, adverse events leading to withdrawal.

All other safety parameters will be summarized using descriptive statistics or frequency counts. Graphical summaries will be used where appropriate.

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