A Two Step Approach to Reduced Intensity Allogeneic Hematopoietic Stem Cell Transplantation for High Risk Hematologic Malignancies

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1.0 Objectives

Thirty-four patients in the TJU Blood and Marrow Transplantation Program were treated on the research protocol, *A Two Step Approach To Reduced Intensity Allogeneic Hematopoietic Stem Cell Transplantation for Hematologic Malignancies from HLA Partially-Matched Related Donors (TJU 2 Step RIC, IRB # 06U.328),* closed to enrollment as of December, 2010. While treatment on this protocol has resulted in durable responses for many older patients and younger, heavily pretreated patients with hematological malignancies, relapse-related mortality after hematopoietic stem cell transplantation (HSCT) was the major cause of mortality in this trial, primarily affecting those patients with disease at the time of transplant.

The objective of this study is to decrease post HSCT relapse rates in patients with high risk hematological diseases characterized by the presence of disease at the time of HSCT or by high risk features such as adverse cytogenetics. A strategy of immunological reduction of disease within the conditioning regimen will be employed to achieve this end. The specific objectives are:

Primary Objective

1. To compare the rate of disease-free survival (DFS) at 1 year post HSCT in patients undergoing HSCT treated on this successor TJU 2 Step RIC haploidentical regimen with that of the initial 2 Step RIC regimen

Secondary Objectives

2. To assess the 100 day regimen-related mortality (RRM) in patients undergoing HSCT on this treatment protocol.

3. To determine the incidence and severity of graft-versus-host disease (GVHD) in patients undergoing treatment on this regimen

4. To evaluate engraftment rates and lymphoid reconstitution in patients treated on this trial.

5. To assess overall survival at 1 and 3 years past HSCT in patients treated on this trial.

2.0 Introduction and Rationale

Allogeneic HSCT is a curative therapy for many disorders of lymphohematopoiesis.[1-5] While allogeneic transplants are often associated with lower rates of relapse than autografts or conventional dose treatment, this

advantage is partially offset by higher regimen related mortality.[6-24] Much of this increase can be traced to the toxicities of the conditioning regimen, GVHD, and the immunosuppressive measures required for the prevention and/or treatment of GVHD.[24-30] For years it has been understood that conditioning regimen intensity is not the primary basis of long-term disease control after allogeneic HSCT. Donor lymphocytes can also eradicate host tumor cells through graft versus tumor (GVT) effects, even in the absence of overt GVHD.[31] In AML and CML for example, relapse rates are higher in recipients of twin transplants than in GVH-free recipients of matched sibling grafts.[32] Moreover, in CML, many patients who relapse after BMT can be rendered disease free through the infusion of additional lymphocytes from the marrow donor without any additional chemoradiotherapy.[33-37]

Development of less intensive conditioning regimens

With the recognition that GVT effects, not conditioning regimen intensity are responsible for long term control of many disease states, transplant regimens that are not lethally myeloablative (NM HSCT) have been developed over the last decade. These approaches do not rely dose intensity to eradicate malignancy. Rather they use immunosuppressive agents, irrespective of their anti-neoplastic properties, to facilitate donor lymphoid and stem cell engraftment. The donor lymphoid elements then destroy the residual normal and in some cases malignant lymphohematopoietic elements allowing the transition to donor chimerism. These regimens rely less heavily on the conditioning regimen for disease control by exploiting the graft versus tumor effects of the donor immune system. They are associated with less treatment-related mortality[38] and have allowed older and heavily pretreated patients who otherwise would not tolerate the rigors of a fully myeloablative HSCT, to undergo transplant successfully.[39] Nonmyeloablative (NM) HSCT has been dramatically effective in CML, chronic lymphocytic leukemia (CLL), and follicular lymphoma in its original application and has proven efficacious in other diseases as well.[40-43]

A spectrum of intensity exists among the different NM regimens. Many of the non-myeloablative regimens are minimally myelosuppressive, while others are more immunosuppressive and are associated with prompter engraftment of donor cells than their less intensive counterparts. The former approach has been associated with less TRM but with incomplete initial chimerism and increased rates of relapse.[44-46] The latter approach, alternately referred to as “reduced intensity” (RIC) HSCT, has been associated with more TRM but less relapse.[47, 48] A NM HSCT approach that is not an “either/or” proposition has not been clearly identified.

Approaches to Alternative Donor Reduced Intensity Allogeneic HSCT

A barrier to the application of NM transplantation in hematologic malignancies is the availability of donors. Only 30% of patients in North America or Europe who may benefit from allogeneic HSCT will have an HLA-matched sibling donor. This percentage is lower for the older patient who may benefit from NM HSCT, but

whose HLA-identical sibling donor may be too old or have comorbidities that would preclude their use as a donor. Registries can provide a matched unrelated allogeneic stem cell graft for an additional 30 % of patients. However this is not an option for patients who do not have a match in the registry, or whose disease status precludes them from waiting to identify an appropriate unrelated donor. In this setting, it is easier and faster to identify a partially HLA-matched (haploidentical) family member as a stem cell donor. There is little data regarding the use of reduced intensity approaches in haploidentical HSCT**,** although a few recent trials have formed the basis for a growing application of this therapeutic modality.

RIC Approaches in Haploidentical HSCT

Ciurea et al.[49] treated 22 patients (median age 36 years) with AML/MDS on a RIC T-cell depleted HSCT regimen consisting of fludarabine, melphalan, thiotepa and antithymocyte globulin (ATG) followed by mobilized stem cells from a haploidentical donor (≥2 antigen mismatch). While no patients with advanced disease at transplant survived, 5 of 12 (42%) with better risk disease have become long-term survivors. NRM at 100 days in this very young sample group was 18%. Bethge and colleagues[50] infused a CD3/CD19 depleted peripheral blood product from haploidentical donors into 29 patients (median age 42 years), primarily with acute leukemia, after conditioning with fludarabine, thiotepa, melphalan, and OKT-3. Overall survival was 31%, with 7 deaths due to infection, 1 from GVHD, and 12 due to relapse, median follow-up of 241 days (range, 112–1271). The NRM at day 100 for this younger aged population was 20%. Dodero et al.[51] conditioned 28 patients (median age 38 years) primarily with advanced lymphoproliferative disorders with thiotepa, fludarabine, cyclophosphamide, and 2 Gy total body irradiation followed by an infusion of a T cell depleted stem cell product from a haploidentical donor. After transplant, the patients were given CD 8+ depleted T cells from their donors to support immune reconstitution. Six of 28 patients (21%) died of NRM at a median of 250 days post HSCT. The 2-year cumulative incidence of nonrelapse mortality was 26% and the 2-year overall survival (OS) was 44%, with a better outcome for patients with chemosensitive disease (OS of 75%). These trials are proof of principal that RIC haploidentical HSCT is a feasible approach to transplant and when applied to patients with earlier stage disease results in better survival rates.

T-Cell Depletion in Haploidentical HSCT

Early transplant approaches utilizing haploidentical donors employed soy bean agglutinin, E-rosetting and later $T_{10}B_9$ to deplete the donor product of T cells to avoid the catastrophic GVHD which would have occurred as a result of crossing the major histocompatibility barrier in this type of HSCT. Transplantation with products T cell depleted to this degree is associated with post-transplant immunodeficiency[52-55] and mortality from infection and relapse.[56-63] More recently, techniques have been developed to avoid profound T cell depletion of the donor product in order to avoid the negative consequences associated with this type of approach.

In two of the RIC trials discussed on page 5, strategies to attenuate rather than completely deplete T cells were employed to augment immune reconstitution post HSCT and resulted in highly acceptable rates of GVHD and infectious mortality. Another successful methodology used to avoid T cell depletion in RIC haploidentical HSCT was developed by the transplant group at Hopkins who employed cyclophosphamide (CY) both as part of the conditioning regimen and to tolerize T-cells for GVHD prophylaxis.

The Use of Cyclophosphamide (CY) for T cell Tolerization in Haploidentical NM HSCT

One of the earliest human NM trials using haploidentical donors and CY tolerization was conducted by O'Donnell and colleagues and published in 2002.[64] In this less intensive NM regimen, ten patients were treated with CY, total body irradiation and fludarabine. In the second group of treated patients, CY was given before transplant as part of the conditioning regimen and after the infusion of the donor product to establish bidirectional T cell tolerization and avoid rejection. Mycophenolate mofetil and tacrolimus were added on day 4. Two of the ten patients rejected the graft. At the time of the publication, 6/8 patients were alive and 4/8 were without evidence of disease (range 157-423 days). Of the 4 without evidence of disease, 2 had grade II GVHD, and 2 had no GVHD at all. The authors reported excellent immune reconstitution, and the primary causes of treatment failure were rejection and relapsed disease. A later publication by the same group[65] reported on outcomes of a larger sample size of 67 patients with advanced hematological malignancies treated with this same approach. Again, mortality from GVHD and infection was very low at 3% and 6% respectively. The primary cause of treatment failure was relapsed disease. Interestingly, patients with lymphoid disease fared better in terms of event free survival as did a subgroup of patients with Hodgkin disease analyzed separately.[66] These trials demonstrated the efficacy of CY tolerization for avoiding significant rates of GVHD and infection post haploidentical HSCT. Relapsed disease, whether due to the CY tolerization combined with a NM conditioning regimen or the late stage of disease of the patients treated or both, was a significant barrier to long term survival.

Jefferson 2 Step Approach to RIC HSCT

Based on murine studies and human clinical trials with CY, as well as promising results from our myeloablative trial using CY tolerization, we developed an RIC approach to haploidentical HSCT. The conditioning regimen is better characterized as a reduced intensity one in that the chemoradiotherapy is more intensive than the approach initially developed at Hopkins. We reasoned that because the patients being treated at our institution were primarily older or more heavily pretreated, they required a higher degree of regimen intensity to control the resistant diseases associated with this type of patient population.

A Two Step Approach to Reduced Intensity Allogeneic Hematopoietic Stem Cell Transplantation for Hematologic Malignancies from HLA Partially-Matched Related Donors (TJU 2 Step RIC, IRB # 06U.328) began enrollment in 2006 and met its accrual goal in 2010. This protocol was specifically designed to treat older patients (≥ 66 years) or patients who underwent previous transplants and could not tolerate the rigors of a second myeloablative conditioning regimen. In this 2 step transplant regimen, patients received fludarabine and either ARA-C or Thiotepa for 4 days. The patients then received one day of TBI (2 Gy), followed by an exact dose of 2.0 x $10e^8$ of their donor's T cells (step 1 of the transplant). After the donor T cell infusion (DLI) the patients consistently developed fevers and in many cases a skin rash and diarrhea, consistent with an immune reaction. On the 3rd day after the DLI the patients received the first of two daily doses of CY in order to tolerize the reactive lymphocytes. Twenty-four hours after the last dose of CY, the patients received a stem cell product from their donor (step 2 of the transplant). The conditioning regimen is shown in Table 1.

Table 1 2 Step Reduced Intensity HSCT-Conditioning Regimen

	Fri -12	Sat -11	Sun -10	Mon -9	Tues -8	Wed -7	Thur -6	Fri -5	Sat -4	Sun -3	Mon -2	Tues -1	Wed 0
AM	Admit	Fludara 30 mg/m^2	Fludara 30 mg/m^2	Fludara 30 mg/m^2	Fludara 30 mg/m^2	Rest	TBI 2 Gy	Rest	Rest	**CY 60 mg/kg**	**CY 60 mg/kg**	Rest	CD-34$^+$ PBSC Infu-sion
PM		Ara-C 2 gm/m^2 **OR** Thiotepa 5 mg/kg	Ara-C 2 gm/m^2 **OR** Thiotepa 5 mg/kg	Ara-C 2 gm/m^2 **OR** Thiotepa 5 mg/kg	Ara-C 2 gm/m^2 **OR** No Chemo-therapy		DLI					Start FK 506 & MMF	→

In general, patients received Thiotepa if there was evidence of an active malignancy where therapy with an alkylating agent was thought to be more beneficial than therapy with an anti-metabolite. We considered this especially important in our patient population which contains a preponderance of patients with acute leukemia who have already received front-line therapy with ARA-C. ARA-C was given to patients who did not have evidence of disease at the time of HSCT or had diseases in which ARA-C is thought to be beneficial such as CLL or follicular NHL. Therefore, the conditioning regimen was developed in this manner to allow for patient-specific adjustments within a consistent regimen. Patients were treated with ARA-C versus Thiotepa based on defined programmatic guidelines which were part of a greater policy governing patient placement on the various TJU 2 Step protocols.

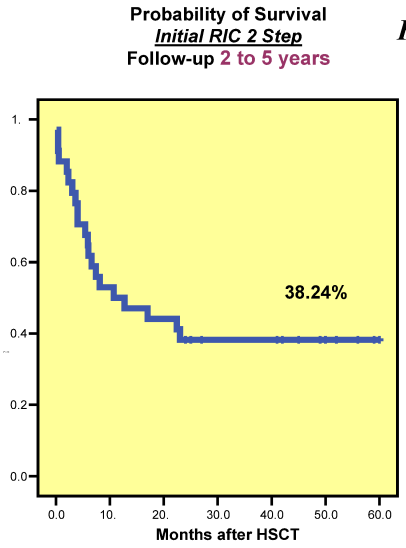


Figure 1

Overall Survival

This reduced intensity successor trial had accrued 34 patients at the time of closure in December, 2010.

The probability of OS in May, 2012, for the 34 patients who have been treated is 38%. At this time, the follow-up is almost 2 to 5 years (Figure 1).

Table 2 below contains patient information and outcomes for this trial.

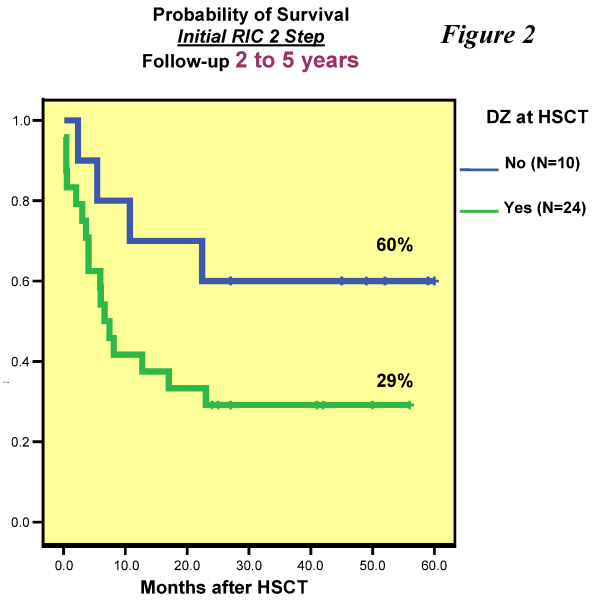
Table 2 **Initial TJU 2 Step RIC HSCT Summary (Follow Up 2 to 5 Years) TJUH 2 Step Approach to Reduced Intensity Haploidentical HSCT 2006-2009**	
Sample Size	**34**
Median Age	**67 years**
Diagnoses	**MDS-2 AML-18 ALL-3** **CLL-3 NHL-3 MPD-1** **Myeloma-2 Biphenotypical Leukemia-1** **Aplastic Anemia-1** **5 patients were S/P previous allogeneic transplants**
Disease at the time of HSCT	**24/34 (71%)**
Probability OS-Whole Cohort	**38%**
Probability OS-Disease at HSCT (N=24)	**29%**
Probability OS-No Disease at HSCT (N=10)	**60%**
Alive	**12/34 (35%)**
Mortality From Relapsed Disease	**12/34 (35%)**

Mortality Related to the Regimen	**8/34 (24%)**
Mortality from GVHD	**2/34 (6%)**
1 Year DFS	**12/33 (36%)** (Patient with Aplastic Anemia not counted in denominator for this category)

Sources of Mortality after the Initial TJU 2 Step Approach to NM HSCT

Despite the promising results of this initial reduced intensity trial (comparable to the NM haploidentical trials reviewed earlier) for patients without disease at HSCT, our goal was to optimize the treatment in order to increase survival rates further in this population. With 2 to 5 years of follow-up, relapsed disease is the major source of mortality as reflected in the above table. However, at the half-way point of this trial's accrual, (n=17 patients), the primary cause of death was toxicity (6/17) not relapse (4/17). This was not completely unexpected for two reasons. First, the backbone of this approach is based on reduced intensity as opposed to NM conditioning, to better treat the resistant disease of the older and/or heavily pretreated population presenting to our transplant program. As previously noted, the goal of many of the NM regimens is to create a niche for the donor immune system which in turn exerts GVT effects. In contrast, a reduced intensity approach applies both intensity and allo effects to control disease, and thus may be associated with more toxicity but less relapse. Secondly, older patients with more age-related comorbidities fare poorer in terms of regimen related death in HSCT,[67] and the median age of patients treated on this trial was 67 years. Therefore, although the higher incidence of toxicity was not unexpected, improvement was required in order to increase OS rates after this therapy.

Figure 2



To address this issue, we examined characteristics of the population and noted that older patients with a Karnofsky Performance Status (KPS) of 90% or lower fared poorly after this therapy.[68] For this analysis, we used the original KPS modified to be more detailed than the original scale. In addition, the assessment

was performed by a third party and not the primary team. This "modified KPS" was stringently applied during the latter half of the trial resulting in the selection of patients who were better able to tolerate the rigors of HSCT. Consequently, the application of this screening resulted in a decrease in mortality related to toxicity such that by the end of the trial, relapsed disease and not toxicity was the major cause of death. (See Figure 2). In addition, assessment of organ function based on the hematopoietic cell transplant comorbidity index (HCT CI)[69] scores was added although this assessment has not yet proven to be as predictive as the KPS, at least for older patients, in the original trial.

Based on these adjustments, a second generation RIC 2 Step HSCT regimen was opened in 2011 with an identical conditioning regimen to that of the initial trial (TJU IRB #11D.247). The primary difference in the trials is that only patients with better risk disease meeting stringent KPS and HCT CI criteria are eligible. The scientific aim of the successor trial is to demonstrate that despite older age, patients in good health with responsive disease can be safely transplanted using the 2 Step approach. Of 10 patients who have undergone haploidentical HSCT on this successor trial (1 to 12 months follow-up), no patients have died, reflecting a dramatic improvement in toxicity based on only more targeted screening and earlier treatment with HSCT prior to the development of comorbid conditions or resistant disease.

Unfortunately, a large group of patients who could potentially benefit from RIC HSCT in the initial trial had resistant diseases at HSCT. This group includes patients with high-risk diseases associated with aging.[70] To address this issue, an additional second generation 2 Step RIC was opened for patients with disease at HSCT with the strategy of substituting Melphalan (Mel) for CY to tolerize T cells. It was hypothesized that Mel would be less cardiotoxic in older patients and for many diseases, have more anti-tumor activity. The use of Mel in conditioning regimens have been associated with excellent rates of DFS in MDS, AML and AML arising from MDS, secondary leukemia,[71-77] myelofibrosis with transformation to acute leukemia,[49] CML,[78] ALL,[79-81] high-risk CLL,[82] Hodgkin Disease,[83] and NHL.[84-87] In 2010, *A Two Step Approach to Reduced Intensity Allogeneic Hematopoietic Stem Cell Transplantation for Hematologic Malignancies Using Melphalan for T Cell Tolerization* was opened (TJU IRB #10D.535). This phase I/II trial utilized a duplicate conditioning regimen to the initial 2 Step RIC approach (fluadarabine/thiotepa/TBI), except for a substitution of Mel 70 mg/m^2/day x 2 days for CY 60 mg.kg/day x 2 days for T cell tolerization. The dose of Mel was to be optimized based on outcomes in the phase I part of the trial. Eight patients were treated prior to its closure in mid-2012 for excessive toxicity. The initial Mel dose of 70 mg/m^2/day x2 resulted in acceptable rates of GVHD and robust immune reconstitution. Unfortunately, there was an increased incidence of gut and hepatic toxicity at this dose. Based on these findings, the dose of Mel was decreased to 60 mg/ m^2/day x2. In addition, cytarabine was substituted for thiotepa in the condition regimen to prevent the additive effects of two alkylating agents. Both

patients treated on this updated regimen experienced excessive gut toxicity AND grade 4 GVHD and the protocol was closed. Because higher doses of Mel are routinely used in autologous HSCT without this high grade of toxicity, it was hypothesized that the proximity of Mel to the cytokine storm associated with the alloreaction was primarily responsible for the high degree of gut injury, although the substitution of cytarabine for thiotepa successfully resulted in much less hepatic toxicity in these two patients. The trial was ultimately closed because the higher dose of Mel required to tolerize T cells and the lower dose of Mel required to avoid excessive toxicity were mutually exclusive. Table 3 summarizes the outcomes of patients on this trial. To date 33% of the patients undergoing haploidentical HSCT on this trial are alive without evidence of disease. This figure is close to the DFS of the initial trial and therefore does not represent improvement, especially in the context of the short follow-up time (2-12 months).

Table 3

Patient	Melphalan Dose	Donor Source	100 Day Grades 4-5 Regimen Related Toxicity	100 Day Grade 3-4 GVHD	Outcome
#1	70 mg/m2	Matched Related	No	No	Alive and Well 1 year post HSCT
#2	70 mg/m2	Haplo Related	*** Grade 4 Respiratory Failure**	No	Alive and Well 10 months post HSCT
#3	70 mg/m2	Haplo Related	No	No	Alive and Well 8 Months post HSCT (In Rehab)
#4	70 mg/m2	Haplo Related	Not evaluable	Not evaluable	Died early in course from neutropenic sepsis
#5	70 mg/m2	Haplo Related	***Grade 4 Gut Toxicity**	No	Alive 6 months after HSCT, recently readmitted for pneumonia and possible drug toxicity
Melphalan Dose Reduced Based on Two Grade 4 CTCAE Toxicities					
#6	60 mg/m2	Matched Related	No (81 days after HSCT Bili rose to grade 4 levels not 2° regimen)	Grade 4 Gut	Died of gram negative sepsis 95 days after HSCT
Remove 1 Alkylator from Regimen					
#7	60 mg/m2	Haplo Related	***Grade 4 Gut Toxicity**	Grade 4 Gut	Died 2 months after HSCT of infection

# 8	60 mg/m2	Haplo Related	***Grade 4 Gut Toxicity**	Grade 4 Gut	2 months after HSCT and stable, being treated for gut GVHD

While agents that are preferentially cytotoxic to activated T cells, such as thiotepa could also be substituted for CY in the 2 Step RIC approach to increase anti-tumor activity, little data exists regarding appropriate dosing of these other agents in the context of T cell tolerization. Therefore, other strategies are needed to decrease relapse rates in RIC HSCT for high risk patients. To address this issue, we hypothesized that immunological reduction of tumor burden during conditioning may represent a potent new strategy to address this issue.

Immunological Tumor Reduction at the Time of Conditioning

After treatment with 2 x 10^8/kg T cells in the DLI, all patients undergoing haploidentical HSCT on the TJU 2 Step approach experience high fevers, and in many cases rash and diarrhea, within 24 hours of the infusion which universally abates after the second dose of CY or Mel. This "alloreaction" is essentially an in-vivo mixed lymphocyte reaction with symptoms consistent with those reported by Colvin et al.[88] who demonstrated antitumor effects in patients receiving haploidentical T cells at the same doses used in the 2 Step trial, but who were without long-term engraftment. Skin and gut biopsies performed in two different patients at the time of the alloreaction were consistent with GVHD in the initial myeloablative TJU 2 Step trial, contrary to the lack of evidence for GVHD in the Colvin et al. study. Whether this difference is due to histopathological findings that differ in a setting of engraftment versus rejection, or the avoidance of type 2 polarization of T cells in the 2 Step approach versus the Colvin et al. approach is not known. However, based on our finding of graft versus host responses and the association of anti-tumor effects with haploidentical DLI in the Colvin et al. study, we hypothesize that the post DLI alloreaction may enhance the GVT effects of the regimen and furthermore, that the timing of the alloreaction could be optimized to increase these GVT effects.

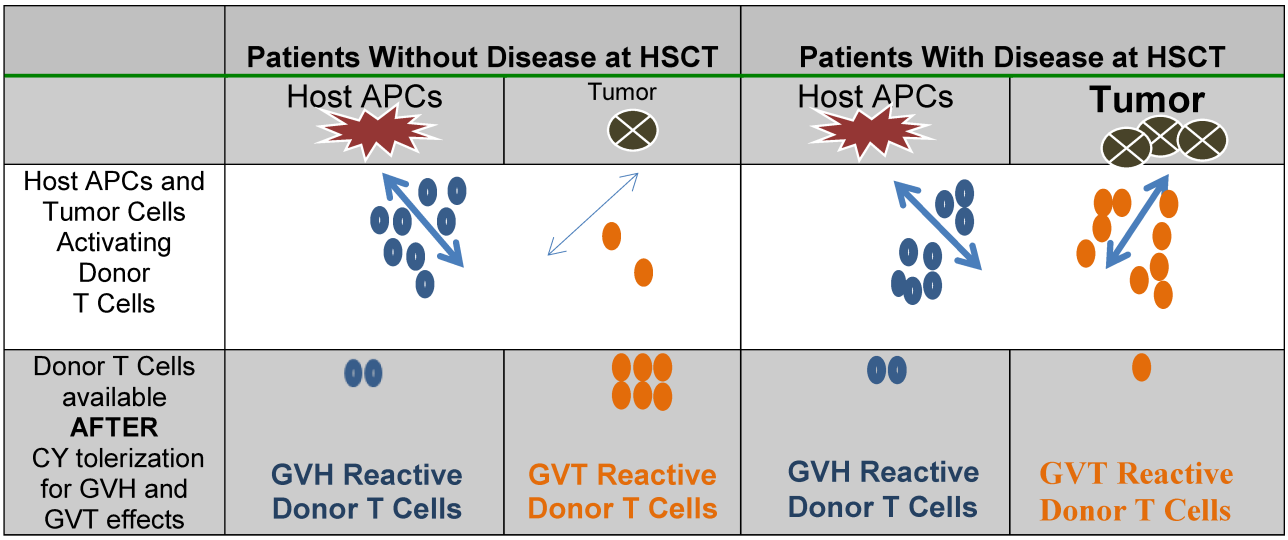
Hypothesis for the Current 2nd GenerationTrial-Immunological Reduction of Tumor During Conditioning Through Optimization of DLI Timing for Patients with High-Risk Disease at HSCT

A potentially important difference between remission and relapsed patients undergoing transplant is the percentage of GVT versus GVH reactive T cells that are likely to be rapidly activated in vivo. In both remission and relapsed patients, the majority of GVH reactive T cells are likely to encounter an antigen presenting cell capable of activating them, thus rendering them more susceptible to the tolerogenic effects of CY. In the remission patient, with a small tumor burden, many GVT reactive T cells may not encounter a tumor target in the first few days after infusion and therefore will potentially avoid activation and tolerization by CY.

This creates a preferential reduction in GVH versus GVT when CY is administered. As the tumor burden progressively increases, more and more GVT reactive T cells will encounter tumor cells during the first few days after infusion, thus becoming activated and subsequently eliminated by CY as well. The larger the tumor burden at the time of lymphocyte administration, the more the potential of CY to blunt the GVT effect, ultimately eliminating the differential impact compared to GVHD which may occur in remission patients. This phenomenon is diagramed in Figure 3.

FIGURE 3
Immunological Tumor Reduction at the Time of Conditioning

Differential T Cell Tolerization in the Presence of Detectable Disease at HSCT



One solution to this problem would be to administer the lymphocytes at the time of the true immune system nadir following the conditioning chemoradiotherapy. In the 2 Step RIC regimen described above, fludarabine and thiotepa or cytarabine were administered on days -11 to -8, prior to 2 Gy of TBI and the DLI on day – 6. Using the white cell count as a marker for immune system response to chemotherapy, a review of white count trends of 26 patients who have been transplanted to date on this approach (patients receiving thiotepa not cytarabine) revealed that the DLI was being given when the white cell count was at a median of 0.85 on day -6. This is 3-4 days before the white cell count nadir which occurred for the group on days -3 or -2.

In Figure 4, the median white cell counts of these 26 patients treated on the initial 2 Step RIC regimen have been added to the bottom of the regimen to demonstrate that on the old approach, the DLI was not given at the time of immune system nadir.

Figure 4

	-11	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	0
AM	Fludara 30 mg/m^2	Fludara 30 mg/m^2	Fludara 30 mg/m^2	Fludara 30 mg/m^2	Rest	**TBI 2 Gy**	Rest	Rest	CY 60 mg/kg	CY 60 mg/kg	Rest	CD-34$^+$ PBSC Infu-sion
PM	Thiotepa 5 mg/kg	Thiotepa 5 mg/kg	Thiotepa 5 mg/kg			**DLI** ↓					Start FK 506 & MMF	→
Median White Cell Counts of 26 Patients Treated To Date												
	3.95	3.95	2.5	2.05	1.45	0.85	0.6	0.25	0.1	0.1	**	**

* Counts are AM counts before that day's therapy
** Counts for ALL patients stayed at 0.0 or 0.1 until engraftment about 10 days later.

Therefore, in this current, 2nd generation trial, the DLI is given on the 4th day after the TBI at the time of the immune system nadir. This timing difference will potentially result in a decrease in the quantity of tumor cells available to react with GVT reactive T cells. Therefore, there will be less activated GVT reactive T cells available for elimination by CY. Ten patients have been treated to date on this 2nd generation protocol. In Figure 5, the median white cell counts of these 10 patients have been added to the schema to demonstrate that the DLI is now successfully given closer to the immune system nadir.

Figure 5

	-15	-14	-13	-12	-11	-10	-9	-8	-7	-6	-5 & -4	-3	-2	-1	0
AM	Fludar 30 mg/m^2	Fludar 30 mg/m^2	Fludar 30 mg/m^2	Fludar 30 mg/m^2	Rest	**TBI 2 Gy**	Rest	Rest	Rest		Rest	CY 60 mg/kg	CY 60 mg/kg		Stem Cell Infus
PM	Thio 5 mg/kg	Thio 5 mg/kg	Thio 5 mg/kg							**DLI** ↓					→
Median White Cell Counts of 10 Patients Treated To Date*															
	4.9	3.95	3.2	2.25	1.95	0.95	0.7	0.4	0.2	0.05	**	**			

* Counts are AM counts before that day's therapy
** Counts for ALL patients stayed at 0.0 or 0.1 until engraftment about 10 days later.

The median age of the 10 patients treated to date on the current, 2nd generation trial is 60 years old, and all have had resistant disease (AML-4, MDS-2, Hodgkin-1, NHL-1, Ph+ALL-1, Myeloma-1) at the time of HSCT. While follow-up time on this protocol is very short, we are pleased with the probability of OS rate thus far

which is 77% in this older, higher risk population. There has been 1 death from relapse and 1 death from toxicity.

April 19, 2014 Update

The Substitution of Alkylating Agents

Thiotepa has been used in the 2 step RIC approach for approximately 5 years and in cancer chemotherapy for over 50 years. It was designated as an orphan drug in 2007, and a critical shortage of Thiotepa was identified in 2013, [89] originating from market forces which have affected many oncology medications.[90] The drug is no longer manufactured in the United States[91] and purchasing the drug from overseas has been associated with increasing cost.[92] Therefore, the current expense of the drug makes its use in HSCT at TJUH no longer feasible, and a drug substitution in the regimen is required. The toxicities of the regimen to date have been minimal, allowing the assessment of the primary scientific question: Do the extra days in the regimen result in greater control of malignancy and by extension disease free survival? Therefore, when substituting for Thiotepa, the goal is to come as close as possible to the original regimen as both the toxicity rate and the ability to deliver the DLI at the immune system nadir have been achieved, at least in the first 10 patients treated on this study. Towards that end, we will use Busulfan 3.2 mg/m^2 IV daily x 2 days. This drug has been used in HSCT for over 30 years and is commonly used in RIC HSCT.[93-95] The majority of patients being enrolled on this trial will have AML or MDS. The IV formulation of the drug was compared to total body irradiation for conditioning in patients with AML and found to have excellent activity in the disease.[96] A comprehensive review of recent data regarding Busulfan in HSCT was written by Champlin in 2013[97] and supports the efficacy of the drug in this setting. It serves as a reasonable substitute for Thiotepa because like Thiotepa, its efficacy in hematopoietic diseases and HSCT, as well as its side effect profile are well known. Because there are only two low doses of Busulfan will be used in this regimen, dosing based pharmacokinetic analysis is not necessary.

We will use the dose of Busulfan that is most commonly used with Fludarabine in other RIC regimens. Typically, 3-4 doses of Busulfan are administered in these approaches.[98-101] CY is an additional alkylating drug used in the 2 step regimen to further treat malignancy and tolerize lymphocytes. Other regimens using Busulfan do not typically contain a second alkylator. Therefore, we will administer only 2 doses of Busulfan. Thiotepa is an alkylating agent that was successfully paired with CY, and so this will be a class for class substitution.

In the proposed approach, patients will receive Fludarabine, Busulfan, and TBI on a similar schedule to that of the patients treated on the 2 step trials using Thiotepa. Therefore, based on the median white counts of the patients on the approach using Thiotepa in the conditioning regimen, it is estimated that the

median WBC will be 0.05 at the time of the T cell infusion in the proposed trial using Busulfan. See Table 6.

Table 6

	-15	-14	-13	-12	-11	-10	-9	-8	-7	-6	-5 & -4	-3	-2	-1	0
AM	Fludar 30 mg/m^2	Fludar 30 mg/m^2	Fludar 30 mg/m^2	Fludar 30 mg/m^2	Rest	**TBI 2 Gy**	Rest	Rest	Rest		Rest	CY 60 mg/kg	CY 60 mg/kg		Stem Cell Infus
PM		Bu 3.2 mg/kg	Bu 3.2 mg/kg							**DLI**					→
Median White Cell Counts of 10 Patients Treated To Date*															
	4.9	3.95	3.2	2.25	1.95	0.95	0.7	0.4	0.2	0.05	**	**			

The updated regimen is shown is section 5.0.

The earlier administration of the conditioning regimen does not affect the principles of T cell tolerization as outlined by Mayumi et al. in the "cells-followed-by-CY system," [102-104] in which CY is given 1 to 3 days after antigenic stimulation with allogeneic cells resulting in the preferential bidirectional destruction of alloreactive clones leading to T cell tolerization. In this protocol as in all of the other 2 Step approaches, the CY will be given two days after allogeneic stimulation with the DLI satisfying the requirement for alloantigenicity to establish tolerization.

In addition to offering this therapy to patients with disease at the time of HSCT who have haploidentical donor options, two other groups of patients will be eligible for this trial.

Patients with high-risk disease, *without evidence of disease at the time of HSCT*, who have haploidentical donors and would benefit from a deeper reduction of tumor undetectable by current methodology, will be offered therapy on this high-risk protocol. In addition, the historical number of patients with available matched sibling donors presenting for RIC HSCT at our institution is very small precluding our ability to develop effective clinical trials for this group. Thus, patients with matched sibling donors who have evidence of disease at HSCT or who have high-risk disease and would potentially benefit from a deeper reduction of tumor, undetectable by current methodology will be offered therapy on this high-risk protocol. In this matched related donor group, alloreactivity of T cells is potentially based on minor histocompatibility differences between donor and recipient.

Because the outcomes of the current trial will be compared to that of the first 2 Step RIC trial in which only haploidentical donors were used, the outcomes of the patient group undergoing HSCT from haploidentical donors (2, 3, or 4 antigen mismatches in the GVH direction) will be exclusively used in the analysis of outcomes for the statistical ends of the trial. The outcomes of patients undergoing matched related donor HSCT will be reported descriptively.

3.0 Patient and Donor Selection

Patient Selection

Inclusion Criteria

1) By definition, patients with hematological malignancies or dyscrasias that require HSCT as part of cure-directed therapy are by definition high-risk and can be treated on this protocol. Examples of high risk patients include but are not limited to: .

    a. Acute myeloid leukemia with high risk features as defined by:

        Age greater than or equal to 60
        Secondary AML (prior therapy or hematologic malignancy)
        Normal cytogenetics but FLT3/ITD positive
        Any relapse or primary refractory disease
        Greater than 3 cytogenetic abnormalities or any one of the following cytogenetic abnormalities:
        -5/del(5q), -7/del(7q), Abn(9q),(11q),(3q),(21q),(17p),t(6;9), t(6;11), t(11;19), +8,del(12p),inv(3),t(10;11),-17, 11q 23

Any single autosomal monosomy[105]

    b. Acute lymphoid leukemia in 1st or 2nd morphological remission. ALL with <u>any</u> morphological evidence of disease will not be eligible.
    c. Myelodysplasia (MDS) other than refractory anemia (RA), refractory anemia with rare sideroblasts (RARS), or isolated 5q-syndrome subtypes.
    d. Hodgkin's or Non-Hodgkin's lymphoma in 2nd or greater remission or with persistent disease.
    e. Myeloma with evidence of persistent disease after front-line therapy.
    f. Chronic myeloid leukemia (CML) resistant to signal transducer inhibitor (STI) therapy
    g. Myelofibrosis and CMML

h. Essential Thrombocytopenia or Polycythemia Vera with current or past evidence of evolution to acute leukemia
i. Patients with CLL, follicular NHL, or other lymphoid malignancies who have highly adverse cytogenetics (such as p53 deletion), are chemo-insensitive, are not responsive to highly effective novel treatments such as CART or Ibrutinib, or who have transformed disease
j. Any hematological malignancy or dyscrasia not cited above which is thought to be high-risk with increased chance of post HSCT relapse.
k. Any patient who has an aggressive disease that would normally be treated on a myeloablative study, but is prevented from doing so by factors in their past medical history. Examples are patients with previous treatment with radiation therapy precluding TBI, or a past history of myeloablative therapy, precluding a 2nd myeloablative regimen.
l. Patients with aplastic anemia may be treated on this protocol, with outcomes reported descriptively.

2) Patients must have a related donor who is at least a 2-4/8 antigen mismatch at the Human Leukocyte Antigen (HLA)-A; B; C; DR loci. Patients with only a 1 out of 8 mismatch in the GVH direction will be classified in the matched related category

3) Patients must have adequate organ function:

a. Left ventricular end diastolic function (LVEF) of ≥50%

b. Diffusion Lung Capacity of Oxygen (DLCO) ≥50% of predicted corrected for hemoglobin

c. Adequate liver function as defined by a serum bilirubin ≤1.8, aspartate aminotransferase (AST) or alanine aminotransferase (ALT) ≤ 2.5X upper limit of normal

d. Creatinine Clearance of ≥ 60 mL/min

4) Patients must have adequate KPS and HCT-CI scores:

a) Patients < age 60 years must have a KPS of ≥80% and an HCT-CI score of 5 or less

b) Patients aged 60 to 65 years must have a KPS of ≥80% and an HCT-CI score of 4 or less

c) Patients aged 66 to 69 years must have a KPS of 90% and an HCT-CI score of 3 or less

d) Patients aged 70 years or more must have a KPS of 90% and an HCT-CI score of 2 or less

(Patients with greater than the allowable HCT-CI points for age can be enrolled for trial with approval of the PI and at least 1 Co-I not on the primary care team of the patient). This is an adjustment to account for healthy patients who meet the spirit of this protocol but have histories that result in higher than guideline HCT-CI points. An example is a patient with a solid tumor malignancy in their remote history (adds 3 points to HCT-CI total) where the treatment for the malignancy occurred years to decades before and there has been complete recovery of toxicities

5) Patients must be willing to use contraception if they have childbearing potential

6) Patient or patient's guardian is able to give informed consent

Exclusion Criteria

1) HIV positive

2) Active involvement of the central nervous system with malignancy. This can be documented as a normal neurological exam and/or a negative CSF analysis

3) Pregnancy

4) Patients with life expectancy of $\leq$ 6 months for reasons other than their underlying hematologic/oncologic disorder

5) Patients who have received alemtuzumab or ATG within 8 weeks of the transplant admission.

6) Patients with evidence of another malignancy, exclusive of a skin cancer that requires only local treatment, should not be enrolled on this protocol

Donor Selection

All donors are selected and screened for their ability to provide adequate infection-free apheresis products for the patient in a manner that does not put the donor at

risk for negative consequences. Donor selection will be in compliance with 21 CFR 1271 and TJU BMT Program SOP CP: P009.03.

Specifically, donors will be tested, using the appropriate FDA-licensed and designated screening tests, for:

1. HIV, type 1
2. HIV, type 2
3. HBV (HBsAg, anti-HBc IgG and IgM)
4. HCV
5. Treponema pallidum
6. Human T-lymphotropic virus, types I and II
7. Cytomegalovirus
8. West Nile Virus
9. Trypanosoma cruzi

As per the Jefferson Blood Donor Center Quality Plan, all allogeneic donor testing samples (including HPC donors) will be sent to a laboratory that is FDA and CLIA licensed. Agreements/contracts for these services will be developed according to TJUH policies and all pertinent regulatory requirements will be retained by the Blood Bank.

Additional donor testing may be performed as required to assess the possibility of transmission of other infectious and non-infectious diseases.

TJUH HPC transplant personnel will discuss the potential for disease transmission from donor to recipient (i.e. the purpose of infectious disease testing) during the donor evaluation.

Infectious disease testing must be completed by the time of the recipient's transplant admission date.

As per FACT guidelines, pregnancy will be assessed during the initial donor evaluation and just prior to the initiation of the recipient's conditioning regimen in female donors of childbearing age.

4.0 Informed Consent

Upon meeting the eligibility criteria for the trial, informed consent will be obtained using forms approved by the TJUH Institutional Review Board and following guidelines related to the use of human subjects in research. The risks and hazards of the procedure, as well as alternative forms of therapy will be presented to the patient in detail. Patients will receive a signed copy of the consent form. In addition, donors will be asked to sign consent after they have been fully informed about the procedures and risks of donating.

5.0 Treatment Plan

While the days of radiation and drug administration are fixed, the exact timing of these treatments on the day they are due is not specified because of expected variations in clinical care.

Treatment Schema

Patient Schedule

	-15	-14	-13	-12	-11	-10	-9	-8	-7	-6	-5 & -4	-2 &-3	-1	0
	Fludar 30 mg/m^2	Fludar 30 mg/m^2	Fludar 30 mg/m^2	Fludar 30 mg/m^2	Rest	**TBI 2 Gy**	Rest	Rest	Rest		Rest	CY 60 mg/kg		Stem Cell Infus
		Bu 3.2 mg/kg	Bu 3.2 mg/kg							**DLI**			Tacro & MMF	→

Bu=Busulfan, Fludar=fludarabine, TBI=total body irradiation, DLI= donor lymphocyte infusion (2 x 10^8 T cells/kg recipient weight), CY=cyclophosphamide, Tacro=tacrolimus, MMF=mycophenolate mofetil

Donor Schedule

	Wed -7	Thur -6	Fri -5	Sat -4	Sun -3	Mon -2	Tues -1
AM	Lymphocyte Collection	Lymphocyte Collection	G-CSF	G-CSF	G-CSF	G-CSF PBSC Collection	G-CSF PBSC Collection
PM			G-CSF	G-CSF	G-CSF	G-CSF	

G-CSF=Granulocyte Stimulating Factor (Neupogen, Filgrastim)

a.There should be no administration of agents that suppress lymphocyte reactivity from day-15 until day -1 in this protocol. This includes steroids, calcineurin inhibitors, MMF, or monoclonal antibodies that affect lymphocyte number or function. Diphenhydramine and meperidine may be used if necessary for transfusion reactions after day -12. Any use of steroids from day -15 through day -1 should not be administered without approval from the PI.

b. Patients will not receive azole drugs, Acetaminophen, Metronidazole, or any drug inhibiting Busulfan metabolism from d-15 through d-12.

c. Voriconazole is prohibited until d-1 due to its interaction with cyclophosphamide.

The absence of prohibited drugs (5.0 a, b, c) in the medical record serves as documentation that they were not given.

All chemotherapy and HPC doses in this protocol are to be based on dosing weight (40% the difference between actual and ideal weight).

5.1 Administration of Fludarabine and Busulfan

Fludarabine is administered for 4 days on (days -15 through – 12) at a dose of 30 mg/m^2 IV daily for 4 days. Creatinine must be checked prior to each dose of fludarabine. If renal insufficiency develops, the attending physician must be notified in cases where a dose adjustment needs to be made.

Busulfan is administered for 2 days on days -14 and -13 at a dose of 3.2 mg/kg/day IV.[99-101, 106] The infusion can be started upon the completion of the fludarabine.[99, 101, 106]

PK levels are not required for Busulfan dosing based on the low dose and low number of administrations of the drug.

Seizure prophylaxis is required with the use of Busulfan. The recommended schedule is:

Clonazepam 0.5 mg and Levetiracetam 500 mg, both drugs administered orally and given BID, beginning the evening prior to the first Busulfan dose and ending the morning after the last dose of Busulfan, days -15 through -12.[107-109]

If patients cannot tolerate Levetiracetam, the suggested alternate regimen is:

Lorazepam 0.02/kg (max 2 mg) orally or intravenously every 6 hours starting 30 minutes prior to the first dose of Busulfan. and continuing for 4 doses after the 2nd dose of Busulfan. The dosage can be reduced by 20-50% (to the nearest 0.5 mg) if the patient experiences excessive sedation.[110]

Day -11 is a rest day.

5.2 TBI

2 Gy of TBI will be administered on day -10. At this low dose, there is almost no clinical scenario in which this small dose of radiation would be associated with added toxicity from prior radiation. However, all patients will be evaluated by a radiation oncologist in preparation for radiation treatment. See Appendix A for radiation guidelines.

Days 9- to -7 are days of rest.

5.3 Donor Lymphocyte Infusion

$CD3^+$ T cell and progenitor cell doses and cyclophosphamide dosing will be based on adjusted dosing weight (40% the difference between actual and ideal body weight + the actual body weight). The dose of the donor lymphocyte infusion (DLI) will be based on $CD3^+$ T cells per kilogram of recipient adjusted body weight. Donor lymphocytes will be collected prior to the use of G-CSF for progenitor cell collection.

The goal of the first day of donor lymphocyte collection is to process a blood volume that is both safe for the donor as well as to obtain the prescribed dose of $CD3^+$ T cells/recipient kg. Approximately 18-27 liters will be processed the first day of donor lymphocyte collection. If a second day of collection is needed, the volume processed will be based on the amount of T cells required to meet the T cell target.

DLI specimen handling and labeling conventions will be performed in accord with the relevant AABB (American Association of Blood Banks) and/or FACT (Foundation for Accreditation for Cell Therapy) regulations and guidelines. All DLI specimens must be appropriately labeled in accord with these standards to be accepted by the Processing Laboratory. A valid prescription and request form must be submitted by the requesting physician.

Determination of the targeted T cell dose from the apheresis product is as follows:

Total T-cells required for the initial infusion = ($2x10^8$ T-cells/kg) * (Weight in kg)

Panel:

	FITC	PE	PE-Cy7	APC	APC-H7
Tube1		CD19	CD16+56	CD3	CD45
Tube2	CD8		CD4	CD3	CD45
Tube3	TCR-ab	TCR-gd		CD3	

CD3 count is calculated directly with single-platform flow cytometry. Reported CD3 absolute count is the mean from 3-tube counts

All donors will be apheresed for lymphocytes on day -7. If the target number of $CD3^+$ T cell lymphocytes, 2 x 10^8/recipient kg is not obtained, apheresis will be repeated on day -6.

Lymphocyte apheresis will be performed at Thomas Jefferson University Hospital or the American Red Cross, by trained apheresis personnel using standard techniques and equipment.

Patients will receive 2 x 10^8/kg T cells on day -6. During the infusion, the patient will be monitored for any untoward reactions. Donor lymphocyte infusions will be

administered by nursing staff experienced in the administration of blood products.

DLI must **NOT** be irradiated. DLI should **NEVER** be administered through a leukocyte depletion (PALL) filter. If blood filtration is necessary, the filter should be a standard blood product filter with pore size of at least 170 microns.

Days -5 and -4 are rest days.

5.4 Cyclophosphamide

CY 60 mg/kg IV will be administered on days –3 and –2 of the conditioning regimen. Mesna 60 mg/kg continuous IV infusion over 24 hours X 2 doses will be infused on days –3 through –2.

Day –1 is a day of rest.

5.5 Collection and Infusion of Progenitor Cells (PBSCs)

Donors will begin G-CSF, 5µg/kg bid, on day -5. Adjunctive or alternate white cell stimulators such as Pegfilgrastim and/or Plerixafor are acceptable for use. Progenitor cell collection will occur on days -2 and -1. Approximately 18 to 27 liters will be processed on the first day of donor collection. The volume processed on the second day of collection will be based on the amount of $CD34^+$ cells required to meet the $CD34^+$ cell target. Specific guidelines regarding the reduction of volume processed the second day based on the collection totals of the first day is contained in BMT SOP CP:P022.08. CD34+ cell enrichment will be performed via the closed system method using the CliniMACS® CD34 Reagent System (Miltenyi Biotec Inc., Auburn, CA). The CliniMACS system utilizes super-paramagnetic particles composed of iron oxide and dextran conjugated to monoclonal antibodies. These antibodies bind to target cells with the corresponding cell surface antigen (in this case, CD34). After magnetic labeling, the cells are separated using a high-gradient magnetic separation column. The magnetically labeled cells are retained in the column and separated from the unlabeled cells. Removing the magnetic field from the separation column elutes the retained cells. Eluted cells will be characterized using fluorescent-activated cell sorting (FACS) analysis. All procedures will be performed in a sterile environment with strict adherence to all applicable regulations regarding the processing and use of human stem cells. The use of this device will conform to TJU BMT Laboratory standard operating procedures.

The target dose of donor PBSCs to be infused into the recipient is between 3 –5 x 10^6 CD34 cells/kg of recipient dosing body weight. The acceptable minimum infusion target of PBSCs will be 1 x 10^6 CD34 cells/kg. Recipients will receive no

more than 10 x 10^6 CD34 cells/kg, the maximum dose. If less than 50% of the minimum acceptable CD34 cells/kg target dose is obtained after the first collection, Plerixafor,12 mg may be administered subcutaneously the evening prior to the second collection. Because the meaningful dose of T cells has already been collected and infused by this time, Plerixafor would have not polarization effects on T helper cells.

In our experience, the ideal amount of T-cells left in the PBSC product is no greater than $5x10^4$/kg, so that every effort will be made to keep T-cell amounts to below this threshold. In over 100 2 Step HSCT procedures, approximately 1% of products contained greater than this amount of T cells. In addition, the amount over the targeted minimal dose was negligible. It is recognized that because of donor heterogeneity, every product will have varying percentages of cells. Thus, patients will be advised during the informed consent process that an excess amount of residual T-lymphocytes in the PBSC product may increase the risk of GVHD.

Progenitor cell apheresis will be performed at Thomas Jefferson University Hospital or the American Red Cross, by trained apheresis personnel using standard techniques and equipment.

Handling and labeling of the progenitor cell product will be performed in accord with the relevant AABB (American Association of Blood Banks) and/or FACT (Foundation for Accreditation for Cell Therapy) regulations and guidelines. All donor specimens must be appropriately labeled in accord with these standards to be accepted by the Processing Laboratory. A valid prescription and request form must be submitted by the requesting physician.

The donor PBSC product is infused UNFILTERED or through a filter of at least 170 micron size intravenously through a central catheter. PBSCs should only be piggybacked through normal saline and not other intravenous solutions. Contingency plans for an inadequate collection of progenitor cells via apheresis or non-viable donor cells will be made according to institutional policies. All donors will be available for a third day of progenitor cell apheresis and will be given extra neupogen in case there is a need for a third collection day.

During the infusion, the patient will be monitored for any untoward reactions. Each infusion will be administered by nursing staff experienced in the administration of blood products. PBSC products must **NOT** be irradiated. PBSC products should **NEVER** be administered through a leukocyte depletion (PALL) filter. If blood filtration is necessary, the filter should be a standard blood product filter with pore size of at least 170 microns.

Significant red cell incompatibility between donor and recipient will be managed according to standard operating procedure, CL: Ppp033, of the Thomas Jefferson

University Hospital Blood and Marrow Transplant Processing Lab. Pre-medications (if any) prior to PBSC infusion will be at the discretion of the physician. Diphenhydramine, epinephrine, and hydrocortisone should be available for emergency use if necessary. Oxygen with nasal cannula should be immediately available.

5.6 GVHD Prophylaxis

Tacrolimus will be started on day -1. Tacrolimus dose titration will occur to target a goal level of 7 ng/ml +/- 2. It is recognized that there may be values beyond this target range due to interpatient variability.

MMF will be dosed at 1 gram IV BID beginning on day -1.

The tacrolimus taper can be initiated by day + 42 in the absence of concern for GVHD or interference with a GVHD plan of care that was developed prior to day +42. Because of the variability in patient outpatient office visit times and the need for GVHD assessment, it is not mandatory that the taper begins exactly day on +42.

MMF will be discontinued beginning at day +28 +/- 3 days in the absence of GVHD.

Tacrolimus and MMF may be discontinued earlier if there is count suppression from the drugs or other unforeseen circumstances in which the drug is felt to be deleterious to the plan of care, such as infection, count suppression, drug side effects, or a need for alternate GVHD treatment.

The BMTU attending physician may change these GVHD prophylaxis guidelines if clinically indicated.

6.0 Study Measurements**

The table below outlines the measurements and time points specific to this study. <u>Only the day +28 studies are mandatory</u>. The other elements are recommended. The attending physician may perform assessments/labs more or less frequently based on the patient's unique course.

	Baseline assessment	**During conditioning**	**After Condition-ing through Day + 28**	**Days 28-90**	**Days 90-180**	**Day 180**	**Days 180-365**
History and physical with vital signs, including SPO_2. Assessment of infectious signs, pregnancy test for females of childbearing potential done on baseline assessment	X	Every 1-2 days	Daily if in hospital weekly until day 28 after discharge	Monthly	As clinically indicated		As clinically indicated
Laboratory Studies*	X	Every 1-2 days	Daily if in hospital weekly until day 28 after discharge	Twice monthly or as clinically indicated	As clinically indicated		As clinically indicated
Quantitative cytomegalovirus CMV by polymerase chain reaction PCR		Weekly or as clinically indicated	Weekly until discharge or as clinically indicated	Twice monthly or as clinically indicated	As clinically indicated		Monthly or as clinically indicated
Viral throat gargle/sputum culture and sensitivity C&S		If respiratory symptoms	If respiratory symptoms	If respiratory symptoms	If respiratory symptoms		If respiratory symptoms
Stool culture (cx), viral screening & cx & fungal cx	If clinically indicated	If clinically indicated	If clinically indicated	If clinically indicated	If clinically indicated		If clinically indicated

	Baseline assessment	During condition-ing	Day + 28	Days 28-90	Days 90-180	Day 180	Days 180-365
GVHD Assessment Presence and degree of skin rash, presence and amount of diarrhea, LFT's	N/A	Daily after engraftment until discharge and then weekly as indicated	X	Twice monthly	As clinically indicated		As clinically indicated
Chimerism/ Disease Assessment							
Peripheral blood for CD3+ chimerism & Buffy coat chimerism			X	Twice monthly until >95% donor chimerism	Once d+90	X	As clinically indicated
Bone marrow exam (morphology, flow cytometry, cytogenetics, buffy coat chimerism)			X	Day +90 Marrow	Day +180 Marrow Is optional	Day +270 Marrow Is optional	Day +365 Marrow Is optional
Immune Reconstitution Studies							
Flow cytometry for lymphocyte subsets			X	Monthly	Monthly	X	Quarterly
Radiographic Studies In applicable situations for disease staging	X				Day +90 or as clinically indicated		Day +365 or as clinically indicated

****Laboratory studies include a complete blood count with differential, comprehensive metabolic panel, lactic and GVHD prophylaxis drug levels when applicable**

The day +28 peripheral blood, marrow studies, and IRP can be obtained within 1 week before day 28 (i.e day +21 through day +28) and within 2 weeks after day +28 (i.e. day 28 through day +42) to account for scheduling factors and failed testing.

The formal endpoint of this study <u>for efficacy</u> is 1 year post HSCT. Therefore patients will not be followed for this study after this time. However, outcomes for patients undergoing HSCT at TJUH are followed programmatically beyond this study indefinitely.

6.1 Hematopoietic engraftment. Will be defined as:

- ANC >/= 0.5x10e9/L for at least 3 days
- Platelet engraftment >20,000 with no transfusions X 7 days.

6.2 Toxicity Criteria.

Regimen-related toxicity will be graded according to the NCI Common Toxicity Criteria, version 4.0. These criteria can be found on the Thomas Jefferson University Hospital BMT WEB site available via the TJUH Intranet.

The NCI Common Toxicity Criteria can also be found at the following WEB address: http://ctep.cancer.gov/reporting/ctc.html

6.3 Disease Response:

Disease response will be measured according to the National Comprehensive Cancer Network Guidelines (NCCN). The guidelines are disease specific and the guidelines for each disease can be found at:

http://www.nccn.org/professionals/physician_gls/f_guidelines.asp#site

6.4 GVHD Scoring

GVHD will be graded according to standard criteria contained in Appendix B.

6.5 Adverse event reporting.

All patients will be followed for adverse experiences (AEs) (serious and nonserious), regardless of relationships to study treatment, from the time of enrollment until d +100 after transplant. The following events are expected side effects of high-dose chemotherapy and transplant and will be recorded but will not be reported except as noted:

- Alopecia, headache, dry skin
- Emesis from chemotherapy or other agents unless refractory to standard supportive care, nausea, anorexia
- Weight loss, cough, dry mouth
- Grades 1-3 fever
- Grades 1-3 infectious sequellae
- Grades 1-3 electrolyte imbalances

- Grades I-III abnormalities in alkaline phosphatase, AST and ALT
- Neutropenia/uncomplicated neutropenic fever, grades 1-3 infectious sequellae

- Thrombocytopenia, petechiae, ecchymoses, minor vaginal bleeding, epistaxis, hemorrhoidal bleeding, or other similar bleeding events will not be reported. (Bleeding events requiring intervention such as endoscopy or radiologic evaluation will be reported.)
- Grades 1-3 Rash
- Grades I-III Fatigue
- Anemia
- Grade I - III Mucositis
- Grade I - III Diarrhea
- Allergic or other reactions to drugs used for supportive care or GVHD prophylaxis unless grade 4-5

After d+100, only AEs that are considered by the investigator to be possibly or probably associated with the treatment regimen will be reported.

6.6 Reports to the Federal Drug Administration (FDA)

All grade 3-5 infusion reactions and all unexpected SAEs as defined in 21 CFR 312.32 will be reported to the FDA in an expedited fashion

An annual report will be sent to the FDA regarding the progress to date of patients on the trial. In the report, a separate listing of infusion toxicities and all biological product deviations will be included in addition to the other required elements.

These requirements will end with cessation of the need for an IDE (ie CliniMacs approval and/or reporting through an alternate mechanism)

6.7 Study Endpoint

The endpoint of this study is DFS at 1 year post HSCT.

7.0 Supportive Care

7.1 Avoidance of Infection

Patients who are post HSCT are susceptible to infection. BMT Clinical Program SOPs CP:P050.01 and CP:P001.04 address infectious prophylaxis and management of suspected infection.
Central venous catheters will be removed as soon as clinically manageable.

It is recommended that IVIG 0.5 g/kg IV will be administered monthly post-transplant to support immune function, until the IgG level is ≥ 500 mg/dL on 2 consecutive monthly measurements. The first dose will be targeted for administration on day +7. It is recognized that fluid overload, changes in renal function, or outpatient lack of coverage for the IVIG may prohibit or delay this therapy.

7.2 Infectious Prophylaxis-General Guidelines

Patients post allogeneic HSCT will be maintained on antifungal prophylaxis, usually voriconazole 200 mg BID. It is at the discretion of the treating attending physician to change agents as clinically indicated.

Patients post allogeneic HSCT will be maintained on HSV prophylaxis, usually valacyclovir 500 mg daily. It is at the discretion of the treating attending physician to change agents based on culture results and sensitivities.

Patients post allogeneic HSCT will be maintained on Pneumocystis jiroveci pneumonia prophylaxis, usually TMP-SMZ DS 1 tablet daily. It is at the discretion of the treating attending physician to change agents based on culture results or drug intolerance.

7.3 Growth Factor and Transfusion Support

To prevent inadvertent lymphoid engraftment, all blood cell products must be irradiated.

All red cell and platelet products will be leukodepleted to prevent alloimmunization and decrease infectious sequellae.

Packed red blood cell transfusions will be given as necessary to keep the hemoglobin >/= 7-8g/L.

Platelet transfusions will be used as needed to keep the morning count >/= 10-20x10e9/L, with 10x10e9/L used for situations without an excessive bleeding risk.

GM-CSF 250μg/m^2 will be administered daily beginning on day +1. GM-CSF will be weaned/discontinued at the discretion of the attending physician. Every effort should be made to keep the ANC $\geq$ 1000 for all patients post allogeneic HSCT. G-CSF 5μg/m^2 can be substituted for GM-CSF in the event of a GM-CSF shortage or if a patient has a deleterious reaction to GM-CSF as determined by the BMTU attending physician.

Red cell growth factors are permissible after transplantation.

8.0 Drug Information and Administration

8.1 Busulfan

Mechanism: Busulfan is an alkylating agent which reacts with the N-7 position of guanosine and interferes with DNA replication and transcription of RNA. Busulfan has a more marked effect on myeloid cells than on lymphoid cells and is also very toxic to hematopoietic stem cells. Busulfan exhibits little immunosuppressive activity, and therefore in this protocol is given with fludarabine and TBI both of which have lymphopenic affects. Busulfan interferes with the normal function of DNA by alkylation and cross-linking the strands of DNA.
Metabolism: Extensively hepatic; glutathione conjugation followed by oxidation
Incompatibilties: Busulfan does not have an extensive list of medications that cause problematic interactions. However, there are a few drugs, commonly used with Busulfan that may affect its metabolism. Phenytoin may decrease the serum concentration of Busulfan and Azoles may decrease the metabolism of Busulfan. Acetominophen and Metronidazole may increase the serum concentration of Busulfan.
Toxicity: Side effects of Busulfan include but are not limited to: tachycardia, hypertension, insomnia, anxiety, headache, fever, vomiting, mucositis, diarrhea, anorexia, myelosupression, hyperbilirubinemia, VOD, weakness, and arthralgias,
Administration: Busulfan is administered for 2 days on days -14 and -13 at a dose of 3.2 mg/kg/day IV.(Alatrash, deLimaAndersson McCune) The infusion can be started upon the completion of the fludarabine.
Reference:
http://online.lexi.com.proxy1.lib.tju.edu/lco/action/doc/retrieve/docid/patch_f/6487#f_adverse-reactions

8.2 Cyclophosphamide

Mechanism: A multistep process activates it by conversion to 4-hydroxycyclophosphamide by the liver microsomal oxidase system and to aldophosohamide by tautomerization in the peripheral tissues. Aldophosphamide spontaneously degrades into acrolein and phosporamide mustard, which cause

cellular glutathione depletion and DNA alkylation. This results in inhibition of DNA replication and transcription. Cells expressing high levels of aldehyde dehydrogenase (e.g. stem cells, L1210 leukemia cells) resist cyclophosphamide-mediated cytotoxicity as aldophosphamide is inactivated by this enzyme. The drug also does not affect quiescent cells and therefore stem cells are generally protected, an important factor if autologous hematopoietic recovery is relied on in the event of graft failure.

Metabolism: Cyclophosphamide is broken down as described above and the break down products are excreted by the kidneys. It is a substrate of CYP2A6 (minor), CYP2B6 (major), CYP2C19 (minor), CYP2C9 (minor), CYP3A4 (minor); **Note:** Assignment of Major/Minor substrate status based on clinically relevant drug interaction potential; **Inhibits** CYP3A4 (weak); **Induces** CYP2B6 (weak/moderate), CYP2C9 (weak/moderate).

Incompatibilties: Phenobarbital or rifampin may increase the toxicity of cyclophosphamide. Concurrent allopurinol or thiazide diuretics may exaggerate bone marrow depression May prolong neuromuscular blockade from succinylcholine Cardiotoxicity may be additive with other cardiotoxic agents (cytarabine, daunorubicin, doxorubicin). May decrease serum digoxin levels. Additive bone marrow depression with other antineoplastics or radiation therapy. May potentiate the effects of warfarin. May decrease antibody response to live-virus vaccines and increase the risk of adverse reactions. Prolongs the effects of cocaine.
Toxicity: Nausea, vomiting, water retention due to inappropriate secretion of anti-diuretic hormone (SIADH), cardiomyopathy with myocardial necrosis and congestive heart failure, hemorrhagic cystitis, alopecia, skin rash, pulmonary fibrosis, sterility and secondary malignancies.
Administration: Patients will receive a dose of cyclophosphamide 60 mg/kg IV, on days –3 and -2. The dose of cyclophosphamide will be calculated according to the dosing body weight. MESNA (sodium-2-mercaptoethane sulfonate) will be administered prior to cyclophosphamide infusion and ending approximately 24 hours after the last dose of cyclophosphamide. The dose of MESNA will also be calculated based on dosing body weight.

References: Skeel R & Lachant N. Handbook of Cancer Chemotherapy, 4th Ed. Little, Brown & Co.: Boston.
Information from LexiComp on line reviewed on 7/4/12 at http://online.lexi.com.proxy1.lib.tju.edu/lco/action/doc/retrieve/docid/patch_f/6674#f_interactions

8.3 Donor Leukocyte Infusion (DLI)

Administration: All patients will receive a dose of $CD3^+$ T cells per kilogram of dosing body weight as outlined in the treatment design. Although unlikely, if more

than a single apheresis of the donor is required to obtain the target cell dose, the white cells that are obtained from each procedure will be given to the patient on the same day. Details of the apheresis procedure to obtain white blood cells, quantification of $CD3^+$ T cells by flow cytometry, and administration of the white cell product to the recipient are provided in the treatment section. All drugs that may cause lymphocyte suppression are held prior to lymphocyte infusion (day -6), through day 0 as detailed in the treatment section. Every effort will be made to administer the donor lymphocytes around or as close to the designated day of lymphocyte infusion. Moreover the viability of the lymphocytes will be tested by flow cytometry and the number of viable $CD3^+$ T cells will be used to dose the DLI.
Toxicity: GVHD, delayed myelosuppression, infusion reactions.

8.4 Fludarabine

Mechanism: Fludarabine phosphate is fluorinated nucleotide and analog of antiviral agent vidarabine, that is relatively resistant to adenosine deaminase deamination. It is actively dephosphorylated to 2-fluoro-ara-A and phosphorylated further by deoxycytidine kinase to 2-fluoro-ara-ATP, then acts by inhibiting DNA polymerase alpha, ribonucleotide reductase and DNA primase resulting in DNA synthesis inhibition.
Metabolism: Renal Excretion
In a pharmacokinetic study of patients treated with fludarabine for rheumatoid arthritis, the mean total clearance was 14.01 L/hr following a dose of 20 mg/m(2)/day, and 13.4 L following a dose of 30 mg/m(2)/day (Knebel et al, 1998). The median total body clearance was 9.6 L/hr after intravenous or subcutaneous fludarabine 30 mg/m(2) for 3 days in 5 patients with lupus nephritis (Kuo et al, 2001).
Incompatibilities: Fludarabine has drug interactions with several vaccines and its simultaneous use with Rotavirus vaccine is contraindicated.
Toxicities: Common: Endocrine/Metabolic: Shivering, Gastrointestinal: Loss of Appetite, Nausea, Vomiting, Neurologic: Asthenia, Other: Fatigue, Malaise**,** Serious: Cardiovascular: Edema (frequent), Dermatologic: Aplasia of skin (rare), Hematologic: Autoimmune Hemolytic Anemia, Graft versus host disease, Transfusion-associated, with non-irradiated blood (rare), Myelosuppression (frequent), Neurologic: Neurotoxicity, Respiratory: Pneumonia (frequent), Other: Fever (frequent), Infectious disease.
Administration: In this protocol, Fludarabine is administered for 4 days on (days -15 through – 12) at a dose of 30 mg/m^2 IV daily for 4 days. Creatinine should be checked prior to each dose of fludarabine. If renal insufficiency develops, the attending physician must be notified in cases where a dose adjustment needs to be made.

References: MicroMedex Health Care Series, Thomson. In addition, information from LexiComp on line reviewed on 7/4/12 at http://online.lexi.com.proxy1.lib.tju.edu/lco/action/doc/retrieve/docid/patch_f/6674#f_interactions

8.5 G-CSF (Figrastim, Neupogen)

Mechanism: G-CSF is a human granulocyte colony-stimulating factor produced by recombinant DNA technology. It is a glycoprotein which acts on hematopoietic cells by binding to specific cell surface receptors and stimulating proliferation, differentiation, commitment, and some end-cell functions. Activates neutrophils to increase migration andtoxicity.
Metabolism: Absorption and clearance of G-CSF follows first-order pharmacokinetic modeling without apparent concentration dependence. The elimination half-life in both normal and cancer patients is 3.5 hours.
Incompatibilties: Safety and efficacy of G-CSF when used simultaneously with chemotherapy or radiotherapy has not been evaluated. Donors receiving either of these 2 modalities will not be permitted on study.
Toxicities: Allergic reactions consisting of rash, wheezing and tachycardia. Splenic rupture, ARDS, and exacerbation of sickle cell disease have been reported rarely.
Administration: In this protocol, G-CSF will be administered to healthy donors at a dose of 10 µg/kg (actual weight) subcutaneously on days -5 through day -1.

References: Physician's Desk Reference, Edition 58, 2004. In addition, information from LexiComp on line reviewed on 7/4/12 at http://online.lexi.com.proxy1.lib.tju.edu/lco/action/doc/retrieve/docid/patch_f/6674#f_interactions

8.6 GM-CSF (Sargramostim, Leukine)

Mechanism: GM-CSF is a recombinant human granulocyte-colony stimulating factor produced by recombinant DNA technology in a yeast expression system. It supports survival, clonal expansion, and differentiation of hematopoietic cells. GM-CSF is also capable of activating mature granulocytes and macrophages, and is a multilineage factor with effects on the myelomonocytic, erythroid, and megarkaryocytic lines.
Metabolism: GM-CSF is detected in the serum at 15 minutes after injection. Peak levels occur about 1 to 3 hours after injection, and it is detectable in the serum for up to 6 hours after injection.
Incompatibilities: Interactions between GM-CSF and other drugs have not been fully evaluated. Drugs which may potentiate the myeloproliferative effects of GM-CSF, such as lithium and corticosteroids, should be used with caution.
Toxicities: Allergic and anaphylactic reactions have been reported. A syndrome characterized by respiratory distress, hypoxia, flushing, hypotension, syncope and or tachycardia has been associated with the first administration of GM-CSF in a cycle. These signs have resolved with treatment.

Administration: In this protocol, GM-CSF will be given to the patients beginning on Day +1. The drug should continue until the patient has a self-sustaining ANC of ≥ 1000.

References: Physician's Desk Reference, Edition 58, 2004. In addition, information from LexiComp on line reviewed on 7/4/12 at http://online.lexi.com.proxy1.lib.tju.edu/lco/action/doc/retrieve/docid/patch_f/6674#f_interactions

8.7 Mycophenolate Mofetil (MMF)

Mechanism: Inhibits the enzyme inosine monophosphate dehydrogenase, which is involved in purine synthesis. This inhibition results in suppression of T- and B-lymphocyte proliferation.
Metabolism: Following oral and IV administration, mycophenolate is rapidly hydrolyzed to mycophenolic acid (MPA), its active metabolite. Distribution is unknown. MPA is extensively metabolized; <1% excreted unchanged in urine. Some enterohepatic recirculation of MPA occurs. Half Life: *MPA*¾17.9 hr.
Incompatibilities: Combined use with azathioprine is not recommended (effects unknown) · Acyclovir and ganciclovir compete with MPA for renal excretion and, in patients with renal failure, may increase each other's toxicity. · Magnesium and aluminum hydroxide antacids decrease the absorption of MPA (avoid simultaneous administration). Cholestyramine and colestipol decrease the absorption of MPA (avoid concurrent use). Toxicity may be increased by salicylates. · May interfere with the action of oral contraceptives (additional contraceptive method should be used). · May decrease the antibody response to and increase risk of adverse reactions from live-virus vaccines, although influenza vaccine may be useful. ·When administered with food, peak blood levels of MPA are significantly decreased.
Toxicities: GI: Bleeding, Diarrhea, Vomiting, Hematopoietic: Leukopenia Miscellaneous: Sepsis, Increased Risk of Malignancy
Administration: In this protocol, MMF will be administered at a dose of 1 gram IV BID beginning on day -1. MMF will be discontinued on day +28 in the absence of GVHD. MMF may be stopped earlier if there is count suppression from the drug.

Reference: Information from LexiComp on line reviewed on 7/4/12 at http://online.lexi.com.proxy1.lib.tju.edu/lco/action/doc/retrieve/docid/patch_f/6674#f_interactions

8.8 Tacrolimus

Mechanism: Tacrolimus, it is a macrolide immunosuppressant. It inhibits lymphocytes by forming a complex with FKBP-12, calcium, calmodulin leading to the decrease in the phosphatase activity of calcineurin. This in turn prevents generation of NF-AT, a nuclear factor for initiating gene transcription for

lymphokines like interleukin-2 and interferon-□□□. This drug is used with corticosteroids for prophylaxis of organ rejection in patients receiving allogeneic liver transplants. Its use is also currently being investigated in kidney, bone marrow, cardiac, pancreas, pancreatic island cell and small bowel transplantation.
Metabolism: This drug is well absorbed orally. It is metabolized in the liver by unknown mechanisms and demethylation and hydroxylation has been proposed based on *in vitro* studies. The metabolized products are excreted in the urine. Tacrolimus is a substrate of CYP3A4 (major), P-glycoprotein; Note: Assignment of Major/Minor substrate status based on clinically relevant drug interaction potential; Inhibits CYP3A4 (weak), P-glycoprotein.
Incompatibilities: Nephrotoxic drugs, antifungals (azoles), calcium-channel blockers, cimetidine, danazol, erythromycin, methylprednisone and metoclopramide increase the bioavailabilty of tacrolimus. On the other hand phenobarbital, phenytoin, rifamycins and carbamazepine decrease tacrolimus levels.
Toxicities: Adverse reactions include: tremor, headache, neurotoxicity; diarrhea, nausea; hypertension; TTP and renal dysfunction.
Administration: Tacrolimus will be started on day -1. with a goal target level of 7ng/ml +/- 2 as noted in section 5.

Reference: Information from LexiComp on line reviewed on 7/4/12 at http://online.lexi.com.proxy1.lib.tju.edu/lco/action/doc/retrieve/docid/patch_f/6674#f_interactions

9.0 Patient Safety

To ensure patient safety, a number of steps will be taken.

The study will be monitored monthly by the Principal Investigator (PI) and the study medical monitor. Monitoring reports will be submitted to the Clinical Research Organization (CRO), Protocol Review Committee (PRC), and the Data Monitoring and Safety Committee (DMSC). The PI will submit all unexpected serious adverse events (SAE) to the TJU IRB utilizing the electronic Sidney Kimmel Cancer Center Clinical Trials Adverse Event Reporting system, with hard copies also submitted to the Office of Scientific Affairs within 48 hours of occurrence. Due to the nature of the study treatment as outlined in this protocol, expected grade 3 AE/SAEs that occur while receiving standard inpatient protocol treatment may be included on the patient's AE log for quarterly review by the DSMC rather than be reported via the eSAEy System per the DSMC Plan. It is the responsibility of the study Principal Investigator (PI) to report any grade 3 AE/SAE to the DSMC per the DSM Plan should the length of standard protocol treatment hospitalization be extended and/or the grade 3 AE/SAE is more acute than expected as outlined in the informed consent form. Unexpected deaths due related to this protocol will be reported within 24 hours.

The medical monitor will be a TJU physician who is not a collaborator in this trial. The medical monitor will review all adverse events (in addition to unexpected adverse events), safety data and activity data observed when this trial is ongoing. The medical monitor may recommend reporting adverse events and relevant safety data not previously reported, and may recommend suspension or termination of the trial. The summary of all discussions of adverse events will be submitted to the DSMC after completion and included in the PI's reports to the PRC and the TJU IRB as part of the study progress report. The PRC, DMSB, and/or the TJU IRB may, based on the monitor's recommendation suspend or terminate of the trial. The quarterly safety and monitoring reports will include a statement as to whether this data has invoked any stopping criteria (dose-limiting toxicities) in the clinical protocol.

9.1 Safety and Adverse Event Reporting

Unanticipated Problems

Unanticipated problems (UAPs) include, in general, any incident, experience, or outcome that meets the following criteria:

- unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;

UAPs are considered to pose risk to participants or others when they suggest that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Adverse Events

An adverse event is any untoward or unfavorable medical occurrence in a human participant, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the participant's participation in the research, whether or not considered related to the participant's participation in the research.

Serious Adverse Events

A serious adverse event (SAE) is one that meets one or more of the following criteria:

- Results in death
- Is life-threatening (places the participant at immediate risk of death from the event as it occurred)
- Is disabling or incapacitating
- Results in inpatient hospitalization or prolongation of existing hospitalization

- Results in a persistent or significant disability or incapacity
- Results in a congenital anomaly or birth defect
- An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the participant or may require intervention to prevent one of the outcomes listed in this definition. Potential drug induced liver injury (DILI) is also considered an important medical event
- Suspected transmission of an infectious agent (e.g. pathogenic or nonpathogenic) via the study drug is an SAE
- Although pregnancy, overdose, cancer and potential DILI are not always serious by regulatory definition, these events must be handled as SAEs

Following the subject's start of treatment, all SAEs, whether related or not related to study drug, must be collected, including those thought to be associated with protocol-specified procedures. All SAEs must be collected, that occur within 100 days of discontinuation of dosing.

A ***nonserious adverse event*** is an AE not classified as serious.

Nonserious Adverse Event Collection and Reporting

The collection of nonserious AE information should begin at initiation of study drug. All nonserious adverse events (not only those deemed to be treatment-related) should be collected continuously during the treatment period and for a minimum of 100 days following the last dose of study treatment.

Safety Assessment and Follow-Up

See Section 6.6 for information on protocol specific adverse event reporting.

Recording Adverse Events

The following subsections detail what information must be documented for each adverse event occurring during the time period specified in Section

Relationship to Study Intervention

The relationship to study intervention or study participation must be assessed and documented for all adverse events. Evaluation of relatedness must consider etiologies such as natural history of the underlying disease, concurrent illness, concomitant therapy, study-related procedures, accidents, and other external factors.

The following guidelines are used to assess relationship of an event to study intervention:

1. Related (Possible, Probable, Definite)
    a. The event is known to occur with the study intervention.
    b. There is a temporal relationship between the intervention and event onset.
    c. The event abates when the intervention is discontinued.
    d. The event reappears upon a re-challenge with the intervention.

2. Not Related (Unlikely, Not Related)
    a. There is no temporal relationship between the intervention and event onset.
    b. An alternate etiology has been established.

Expectedness
The PI is responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the intervention. Risk information to assess expectedness can be obtained from preclinical studies, the investigator's brochure, published medical literature, the protocol, or the informed consent document.

Severity of Event
Adverse events will be graded for severity according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03.

Intervention
Any intervention implemented to treat the adverse event must be documented for all adverse events.

Safety Reporting to IRB *Unanticipated Problems*

All incidents or events that meet criteria for unanticipated problems (UAPs) as defined in Section 0
Unanticipated ***Problems*** require the creation and completion of an unanticipated problem report form (OHR-20).
UAPs that pose risk to participants or others, and that are not AEs, will be submitted to the IRB on an OHR-20 form via the eazUP system within 10 working days of the investigator becoming aware of the event.
UAPs that do not pose risk to participants or others will be submitted to the IRB at the next continuing review.

9.2 Reporting to SKCC DSMC

All AEs and SAEs, safety and toxicity data, and any corrective actions will be submitted to the DSMC per the frequency described in the SKCC DSMP. The report to the SKCC DSMC will also include any unanticipated problems that in the opinion of the PI should be reported to the DSMC.
For expedited reporting requirements, see table below:

DSMC AE/SAE Reporting Requirements

	Grade 1	Grade 2		Grade 3				Grades 4 and 5
				Unexpected		Expected		
	Unexpected and Expected	Unexpected	Expected	With Hospitalization	Without Hospitalization	With Hospitalization	Without Hospitalization	Unexpected and Expected
Unrelated Unlikely	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	5 Working Days	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	5 Working Days	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	Phase I - 48 Hours (Death: 24 Hours) Phase II - 5 working days
Possible Probably Definite	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	48 Hours (Death: 24 Hours)	Phase I - 48 Hours Phase II - 5 working days	48 Hours (Death: 24 Hours)	Reviewed at Quarterly DSMC Meeting and IRB Annual Review	Phase I and Phase II - 48 Hours (Death: 24 Hours)

10.0 Statistical Analysis

10.1 Study Design

This is a one-arm study in patients with hematological malignancies with haploidentical family donors and treated with haploidentical transplant.

10.2 Analysis of the primary endpoint

The primary endpoint for this study is DFS at 1 year post HSCT. The primary null hypothesis is that 1 year DFS rate is at most 35%. 35% is the rounded number (actual 36%) representing the DFS at 1 year of patients treated on the initial TJU 2 Step RIC HSCT trial and consistent with the outcome of patients treated on similar protocols outside of our institution (reviewed in background section). This

hypothesis will be rejected if the 95% confidence interval for year DFS rate computed from the estimated Kaplan-Meier survival curves will be entirely above 0.35.

10.3 Sample Size

This is a one arm study utilizing new methods to improve DFS. Assuming that 62 patients will be accrued in 8 years and then followed for 1 more year there is an 80% power to show that 1-year DFS is greater than 35% if the true 1-year DFS is 50% or higher (calculations are based on the assumptions of uniform accrual over time, no loss to follow-up, exponentially distributed death times, and use of the exponential MLE one-sided test with alpha=0.05).

10.4 Assessment of Other Secondary Objectives

The secondary objectives of overall survival, regimen related toxicity, immune reconstitution, incidence and degree of GVHD, and engraftment rates will be analyzed and reported descriptively.

10.5 Analysis for Safety

Patient outcomes are routinely monitored in an ongoing fashion for all patients on investigational trials, beyond their formal endpoints. Based on prior experience using a two-step approach similar to that described in this trial, the incidence of graft failure should be less than 10%, the incidence of severe GVHD should be less than 20%, and the non-relapse mortality should be less than 30% at 100 days. If at any point incidences higher than these thresholds are seen, that would trigger a protocol review to assess whether there are any obvious reasons for the inferior outcomes observed. Depending on the results of the review, enrollment may continue on a limited basis with careful further observation, the protocol may be revised, or the protocol may be terminated. Incidences will be calculated after 10 patients are treated on this trial in order to have a sufficient denominator in which to examine outcomes based on percentages.

10.6 Targeted Accrual Number

The small number of patients undergoing matched sibling RIC HSCT in our transplant program precludes a separate research protocol for that group. To prevent withholding of transplant therapy, these patients will be treated on this protocol. Only the outcomes of the patient group undergoing HSCT from haploidentical donors (2, 3, or 4 antigen mismatches in the GVH direction) will be used in the analysis of outcomes for the statistical ends of the trial. Outcomes for patients with matched sibling donors will be reported descriptively.

"Therefore, when 62 evaluable patients undergoing haploidentical HSCT are treated on this protocol, the study will close to accrual. Up to 10 patients may undergo matched related donor HSCT on this protocol, but will not count toward the statistical ends of the study. These patients will be reported descriptively. Highest possible accrual number is **72** patients."

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11.0 References

1. Thomas ED. History, current results, and research in marrow transplantation. Perspect Biol Med. 1995;38(2):230-237.

2. Thomas ED. Bone marrow transplantation: Past experiences and future prospects. Semin Oncol. 1992;19(3 SUPPL. 7):3-6.

3. Bortin MM and Rimm AA. Increasing utilization of bone marrow transplantation. Transplantation. 1986;42(3):229-234.

4. Blume KG. A general overview of the status of bone marrow transplantation for hematologic diseases. Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K. 1993;7(7):1078-1079.

5. Armitage JO. Medical progress: Bone marrow transplantation. N Engl J Med. 1994;330(12):827-838.

6. Ballester OF. Allogeneic bone marrow transplantation for multiple myeloma. Semin Oncol. 1993;20(SUPPL. 6):67-71.

7. Bensinger WI, Buckner CD, Anasetti C, et al. Allogeneic marrow transplantation for multiple myeloma: An analysis of risk factors on outcome. Blood. 1996;88(7):2787-2793.

8. Björkstrand B, Ljungman P, Svensson H, et al. Allogeneic bone marrow transplantation versus autologous stem cell transplantation in multiple myeloma: A retrospective case-matched study from the European Group for Blood and Marrow Transplantation. Blood. 1996;88(12):4711-4718.

9. Attal M, Harousseau J-, Stoppa A-, et al. A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. N Engl J Med. 1996;335(2):91-97.

10. Barlogie B, Jagannath S, Vesole DH, et al. Superiority of tandem autologous transplantation over standard therapy for previously untreated multiple myeloma. Blood. 1997;89(3):789-793.

11. Archimbaud E, Thomas X, Michallet M, et al. Prospective genetically randomized comparison between intensive postinduction chemotherapy and bone marrow transplantation in adults with newly diagnosed acute myeloid leukemia. Journal of Clinical Oncology. 1994;12(2):262-267.

12. Cassileth PA, Lynch E, Hines JD, et al. Varying intensity of postremission therapy in acute myeloid leukemia. Blood. 1992;79(8):1924-1930.

13. Cavo M, Benni M, Cirio TM, Gozzetti A, Tura S. Allogeneic bone marrow transplantation for the treatment of multiple myeloma: An overview of published reports. Stem Cells. 1995;13(SUPPL. 2):126-131.

14. Couban S, Stewart AK, Loach D, Panzarella T, Meharchand J. Autologous and allogeneic transplantation for multiple myeloma at a single centre. Bone Marrow Transplant. 1997;19(8):783-789.

15. Goldschmidt H, Hegenbart U, Wallmeier M, Moos M, Haas R. High-dose chemotherapy in multiple myeloma. Leukemia. 1997;11(SUPPL. 5):S27-S31.

16. Gahrton G, Tura S, Ljungman P, et al. Prognostic factors in allogeneic bone marrow transplantation for multiple myeloma. Journal of Clinical Oncology. 1995;13(6):1312-1322.

17. Gahrton G, Tura S, Ljungman P, et al. An update of prognostic factors for allogeneic bone marrow transplantation in multiple myeloma using matched sibling donors. Stem Cells. 1995;13(SUPPL. 2):122-125.

18. Mehta J, Tricot G, Jagannath S, et al. Salvage autologous or allogeneic transplantation for multiple myeloma refractory to or relapsing after a first-line autograft? Bone Marrow Transplant. 1998;21(9):887-892.

19. Reece DE, Shepherd JD, Klingemann H-, et al. Treatment of myeloma using intensive therapy and allogeneic bone marrow transplantation. Bone Marrow Transplant. 1995;15(1):117-123.

20. Samson D. High-dose therapy in multiple myeloma. Curr Opin Hematol. 1996;3(6):446-452.

21. Samson D. The current position of allogeneic and autologous BMT in multiple myeloma. Leukemia and Lymphoma. 1992;7(SUPPL. 1):33-38.

22. Varterasian M, Janakiraman N, Karanes C, et al. Transplantation in patients with multiple myeloma: A multicenter comparative analysis of peripheral blood stem cell and allogeneic transplant. American Journal of Clinical Oncology: Cancer Clinical Trials. 1997;20(5):462-466.

23. Vesole DH, Tricot G, Jagannath S, et al. Autotransplants in multiple myeloma: What have we learned? Blood. 1996;88(3):838-847.

24. Zittoun RA, Mandelli F, Willemze R, et al. Autologous or allogeneic bone marrow transplantation compared with intensive chemotherapy in acute myelogenous leukemia. N Engl J Med. 1995;332(4):217-223.

25. Brent L. Medawar prize lecture: Tolerance and graft-vs-host disease: Two sides of the same coin. Transplant Proc. 1995;27(1):12-14.

26. Antin JH and Ferrara JLM. Cytokine dysregulation and acute graft-versus-host disease. Blood. 1992;80(12):2964-2968.

27. Champlin R. Immunobiology of bone marrow transplantation as treatment for hematologic malignancies. Transplant Proc. 1991;23(4):2123-2127.

28. Ferrara JLM and Deeg HJ. Graft-versus-Host disease. N Engl J Med. 1991;324(10):667-674.

29. Irschick EU, Hladik F, Niederwieser D, et al. Studies on the mechanism of tolerance or graft-versus-host disease in allogeneic bone marrow recipients at the level of cytotoxic T-cell precursor frequencies. Blood. 1992;79(6):1622-1628.

30. Vogelsang GB and Hess AD. Graft-versus-host disease: New directions for a persistent problem. Blood. 1994;84(7):2061-2067.

31. Horowitz MM, Gale RP, Sondel PM, et al. Graft-versus-leukemia reactions after bone marrow transplantation. Blood. 1990;75(3):555-562.

32. Gale RP, Horowitz MM, Ash RC, et al. Identical-twin bone marrow transplants for leukemia. Ann Intern Med. 1994;120(8):646-652.

33. Drobyski WR, Keever CA, Roth MS, et al. Salvage immunotherapy using donor leukocyte infusions as treatment for relapsed chronic myelogenous leukemia after allogeneic bone marrow transplantation: Efficacy and toxicity of a defined T-cell dose. Blood. 1993;82(8):2310-2318.

34. Mackinnon S, Papadopoulos EB, Carabasi MH, et al. Adoptive immunotherapy evaluating escalating doses of donor leukocytes for relapse of chronic myeloid leukemia after bone marrow transplantation: Separation of graft-versus-leukemia responses from graft-versus-host disease. Blood. 1995;86(4):1261-1268.

35. Porter DL, Roth MS, McGarigle C, Ferrara JLM, Antin JH. Induction of graft-versus-host disease as immunotherapy for relapsed chronic myeloid leukemia. N Engl J Med. 1994;330(2):100-106.

36. Kolb H-, Schattenberg A, Goldman JM, et al. Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. Blood. 1995;86(5):2041-2050.

37. Collins Jr. RH, Shpilberg O, Drobyski WR, et al. Donor leukocyte infusions in 140 patients with relapsed malignancy after allogeneic bone marrow transplantation. Journal of Clinical Oncology. 1997;15(2):433-444.

38. Hamadani M, Awan FT, Copelan EA. Hematopoietic Stem Cell Transplantation in Adults with Acute Myeloid Leukemia. Biology of Blood and Marrow Transplantation. 2008;14(5):556-567.

39. Kroger N and Mesa RA. Choosing between stem cell therapy and drugs in myelofibrosis. Leukemia. 2008;22(3):474-486.

40. Giralt S, Estey E, van Besien K, et al. Induction of graft-versus-leukemia without myeloablative therapy using allogeneic PBSC after purine analog containing regimens.[abstract]. Blood. 1996;88, Suppl 1:614a.

41. Slavin S, Nagler A, Naparstek E, et al. Immunotherapy of leukemia in conjunction with reduced intensity conditioning: Engraftment of blood stem cells and eradication of host leukemia with reduced intensity conditioning based on fludarabine and anti-thymocyte globulin (ATG).[abstract]. Blood. 1996;88 Suppl 1:614a.

42. Giralt S, Estey E, Albitar M, et al. Engraftment of allogeneic hematopoietic progenitor cells with purine analog-containing chemotherapy: harnessing graft-versus-leukemia without myeloablative therapy. Blood. 1997;89(12):4531-4536.

43. Khouri IF, Keating M, Korbling M, et al. Transplant-lite: induction of graft-versus-malignancy using fludarabine-based nonablative chemotherapy and allogeneic blood progenitor-cell transplantation as treatment for lymphoid malignancies. Journal of Clinical Oncology. 1998;16(8):2817-2824.

44. Martino R, Iacobelli S, Brand R, et al. Retrospective comparison of reduced-intensity conditioning and conventional high-dose conditioning for allogeneic hematopoietic stem cell transplantation using HLA-identical sibling donors in myelodysplastic syndromes. Blood. 2006;108(3):836-846.

45. Couriel DR, Saliba RM, Giralt S, et al. Acute and chronic graft-versus-host disease after ablative and nonmyeloablative conditioning for allogeneic

hematopoietic transplantation. Biology of Blood & Marrow Transplantation. 2004;10(3):178-185.

46. Slavin S, Nagler A, Naparstek E, et al. Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. Blood. 1998;91(3):756-763.

47. Anderlini P, Saliba R, Acholonu S, et al. Reduced-intensity allogeneic stem cell transplantation in relapsed and refractory Hodgkin's disease: Low transplant-related mortality and impact of intensity of conditioning regimen. Bone Marrow Transplantation. 2005;35(10):943-951.

48. de Lima M, Anagnostopoulos A, Munsell M, et al. Nonablative versus reduced-intensity conditioning regimens in the treatment of acute myeloid leukemia and high-risk myelodysplastic syndrome: dose is relevant for long-term disease control after allogeneic hematopoietic stem cell transplantation. Blood. 2004;104(3):865-872.

49. Ciurea SO, Saliba R, Rondon G, et al. Reduced-intensity conditioning using fludarabine, melphalan and thiotepa for adult patients undergoing haploidentical SCT. Bone Marrow Transplant. 2010;45(3):429-436.

50. Bethge WA, Faul C, Bornhauser M, et al. Haploidentical allogeneic hematopoietic cell transplantation in adults using CD3/CD19 depletion and reduced intensity conditioning: an update. Blood Cells Molecules & Diseases. 2008;40(1):13-19.

51. Dodero A, Carniti C, Raganato A, et al. Haploidentical stem cell transplantation after a reduced-intensity conditioning regimen for the treatment of advanced hematologic malignancies: posttransplantation CD8-depleted donor lymphocyte infusions contribute to improve T-cell recovery. Blood. 2009;113(19):4771-4779.

52. Keever CA, Small TN, Flomenberg N, et al. Immune reconstitution following bone marrow transplantation: comparison of recipients of T-cell depleted marrow with recipients of conventional marrow grafts. Blood. 1989;73(5):1340-1350.

53. Buckley RH, Schiff SE, Schiff RI, et al. Hematopoietic stem-cell transplantation for the treatment of severe combined immunodeficiency. N Engl J Med. 1999;340(7):508-516.

54. Haddad E, Landais P, Friedrich W, et al. Long-term immune reconstitution and outcome after HLA-nonidentical T-cell-depleted bone marrow transplantation

for severe combined immunodeficiency: a European retrospective study of 116 patients. Blood. 1998;91(10):3646-3653.

55. Williams KM, Hakim FT, Gress RE. T cell immune reconstitution following lymphodepletion. Semin Immunol. 2007;19(5):318-330.

56. Ciceri F, Labopin M, Aversa F, et al. A survey of fully haploidentical hematopoietic stem cell transplantation in adults with high-risk acute leukemia: a risk factor analysis of outcomes for patients in remission at transplantation. Blood. 2008;112(9):3574-3581.

57. Henslee-Downey PJ, Abhyankar SH, Parrish RS, et al. Use of partially mismatched related donors extends access to allogeneic marrow transplant. Blood. 1997;89(10):3864-3872.

58. Aversa F. Haploidentical haematopoietic stem cell transplantation for acute leukaemia in adults: experience in Europe and the United States. Bone Marrow Transplant. 2008;41(5):473-481.

59. Lewalle P, Delforge A, Aoun M, et al. Growth factors and DLI in adult haploidentical transplant: a three-step pilot study towards patient and disease status adjusted management. Blood Cells Molecules & Diseases. 2004;33(3):256-260.

60. Walker I, Shehata N, Cantin G, et al. Canadian multicenter pilot trial of haploidentical donor transplantation. Blood Cells Molecules & Diseases. 2004;33(3):222-226.

61. Ringhoffer M, Wiesneth M, Harsdorf S, et al. CD34 cell selection of peripheral blood progenitor cells using the CliniMACS device for allogeneic transplantation: clinical results in 102 patients. Br J Haematol. 2004;126(4):527-535.

62. Drobyski WR, Klein J, Flomenberg N, et al. Superior survival associated with transplantation of matched unrelated versus one-antigen-mismatched unrelated or highly human leukocyte antigen-disparate haploidentical family donor marrow grafts for the treatment of hematologic malignancies: establishing a treatment algorithm for recipients of alternative donor grafts. Blood. 2002;99(3):806-814.

63. Lacerda JF, Martins C, Carmo JA, et al. Haploidentical stem cell transplantation with purified CD34 cells after a chemotherapy-alone conditioning regimen. Biology of Blood & Marrow Transplantation. 2003;9(10):633-642.

64. O'Donnell PV, Luznik L, Jones RJ, et al. Nonmyeloablative bone marrow transplantation from partially HLA-mismatched related donors using

posttransplantation cyclophosphamide. Biology of Blood & Marrow Transplantation. 2002;8(7):377-386.

65. Luznik L, O'Donnell PV, Symons HJ, et al. HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, posttransplantation cyclophosphamide. Biology of Blood & Marrow Transplantation. 2008;14(6):641-650.

66. Burroughs LM, O'Donnell PV, Sandmaier BM, et al. Comparison of outcomes of HLA-matched related, unrelated, or HLA-haploidentical related hematopoietic cell transplantation following nonmyeloablative conditioning for relapsed or refractory Hodgkin lymphoma. Biology of Blood & Marrow Transplantation. 2008;14(11):1279-1287.

67. Ringden O, Horowitz MM, Gale RP, et al. Outcome After Allogeneic Bone Marrow Transplant for Leukemia in Older Adults. JAMA. 1993;270(1):57-60.

68. Grosso D, Alpdogan O, Besa EC, et al. Haploidentical Transplantation In Adults >= 66 Years of Age. ASH Annual Meeting Abstracts. 2010;116(21):3529.

69. Sorror ML, Maris MB, Storb R, et al. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. Blood. 2005;106(8):2912-2919.

70. Kiss TL, Sabry W, Lazarus HM, Lipton JH. Blood and marrow transplantation in elderly acute myeloid leukaemia patients - Older certainly is not better. Bone Marrow Transplant. 2007;40(5):405-416.

71. Lemoli RM, D'addio A, Marotta G, et al. BU/melphalan and auto-SCT in AML patients in first CR: a Gruppo Italiano Trapianto di Midollo Osseo (GITMO) retrospective study. Bone Marrow Transplant. 2010;45(4):640-646.

72. Deschler B, Binek K, Ihorst G, et al. Prognostic Factor and Quality of Life Analysis in 160 Patients Aged ≥60 Years with Hematologic Neoplasias Treated with Allogeneic Hematopoietic Cell Transplantation. Biology of Blood and Marrow Transplantation. 2010;16(7):967-975.

73. Lim Z, Brand R, Martino R, et al. Allogeneic Hematopoietic Stem-Cell Transplantation for Patients 50 Years or Older With Myelodysplastic Syndromes or Secondary Acute Myeloid Leukemia. J Clin Oncol. 2010;28(3):405-411.

74. van Besien K, Devine S, Wickrema A, et al. Regimen-related toxicity after fludarabine-melphalan conditioning: A prospective study of 31 patients with hematologic malignancies. Bone Marrow Transplant. 2003;32(5):471-476.

75. Nakamura R, Rodriguez R, Palmer J, et al. Reduced-intensity conditioning for allogeneic hematopoietic stem cell transplantation with fludarabine and melphalan is associated with durable disease control in myelodysplastic syndrome. Bone Marrow Transplant. 2007;40(9):843-850.

76. Malladi RK, Peniket AJ, Norton AE, et al. Favourable outcome for patients with myeloid disorders treated with fludarabine-melphalan reduced-intensity conditioning and allogeneic bone marrow stem cell transplantation without the use of T-lymphocyte-depleting antibodies. Eur J Haematol. 2004;73(2):85-92.

77. Pollyea DA, Artz AS, Stock W, et al. Outcomes of patients with AML and MDS who relapse or progress after reduced intensity allogeneic hematopoietic cell transplantation. Bone Marrow Transplant. 2007;40(11):1027-1032.

78. Kebriaei P, Detry MA, Giralt S, et al. Long-term follow-up of allogeneic hematopoietic stem-cell transplantation with reduced-intensity conditioning for patients with chronic myeloid leukemia. Blood. 2007;110(9):3456-3462.

79. Moorman AV, Chilton L, Wilkinson J, Ensor HM, Bown N, Proctor SJ. A population-based cytogenetic study of adults with acute lymphoblastic leukemia. Blood. 2010;115(2):206-214.

80. Stein AS, Palmer JM, O'Donnell MR, et al. Reduced-intensity conditioning followed by peripheral blood stem cell transplantation for adult patients with high-risk acute lymphoblastic leukemia. Biology of Blood and Marrow Transplantation. 2009;15(11):1407-1414.

81. Cho B-, Lee S, Kim Y-, et al. Reduced-intensity conditioning allogeneic stem cell transplantation is a potential therapeutic approach for adults with high-risk acute lymphoblastic leukemia in remission: Results of a prospective phase 2 study. Leukemia. 2009;23(10):1763-1770.

82. Delgado J, Pillai S, Phillips N, et al. Does reduced-intensity allogeneic transplantation confer a survival advantage to patients with poor prognosis chronic lymphocytic leukaemia? A case-control retrospective analysis. Annals of Oncology. 2009;20(12):2007-2012.

83. Anderlini P, Saliba R, Acholonu S, et al. Fludarabine-melphalan as a preparative regimen for reduced-intensity conditioning allogeneic stem cell transplantation in relapsed and refractory Hodgkin's lymphoma: The updated M.D. Anderson Cancer Center experience. Haematologica. 2008;93(2):257-264.

84. Piñana JL, Martino R, Gayoso J, et al. Reduced intensity conditioning HLA identical sibling donor allogeneic stem cell transplantation for patients with

follicular lymphoma: Long-term follow-up from two prospective multicenter trials. Haematologica. 2010;95(7):1176-1182.

85. Bertz H, Illerhaus G, Veelken H, Finke J. Allogeneic hematopoetic stem-cell transplantation for patients with relapsed or refractory lymphomas: Comparison of high-dose conventional conditioning versus fludarabine-based reduced-intensity regimens. Annals of Oncology. 2002;13(1):135-139.

86. Robinson SP, Goldstone AH, Mackinnon S, et al. Chemoresistant or aggressive lymphoma predicts for a poor outcome following reduced-intensity allogeneic progenitor cell transplantation: an analysis from the Lymphoma Working Party of the European Group for Blood and Bone Marrow Transplantation. Blood. 2002;100(13):4310-4316.

87. Rodriguez R, Nademanee A, Ruel N, et al. Comparison of Reduced-Intensity and Conventional Myeloablative Regimens for Allogeneic Transplantation in Non-Hodgkin's Lymphoma. Biology of Blood and Marrow Transplantation. 2006;12(12):1326-1334.

88. Colvin GA, Berz D, Ramanathan M, et al. Nonengraftment Haploidentical Cellular Immunotherapy for Refractory Malignancies: Tumor Responses without Chimerism. Biology of Blood and Marrow Transplantation. 2009;15(4):421-431.

89. Adienne Pharma & Biotech. Urgent Thiotepa Update. 2013.

90. Chabner BA. Drug shortages - A critical challenge for the generic-drug market. N Engl J Med. 2011;365(23):2147-2149.

91. American Society of Health System Pharmacists. Thiotepa for Injection. 2014.

92. Neergaard L. Hospitals hunt substitutes as drug shortages rise. 2011.

93. Devillier R, Fürst S, El-Cheikh J, et al. Antithymocyte globulin in reduced-intensity conditioning regimen allows a high disease-free survival exempt of long-term chronic graft-versus-host disease. Biology of Blood and Marrow Transplantation. 2014;20(3):370-374.

94. Kanda J, Long GD, Gasparetto C, et al. Reduced-intensity allogeneic transplantation using alemtuzumab from HLA-Matched related, unrelated, or haploidentical related donors for patients with hematologic malignancies. Biology of Blood and Marrow Transplantation. 2014;20(2):257-263.

95. Gupta V, Malone AK, Hari PN, et al. Reduced-Intensity Hematopoietic Cell Transplantation for Patients with Primary Myelofibrosis: A Cohort Analysis from

the Center for International Blood and Marrow Transplant Research. Biology of Blood and Marrow Transplantation. 2014;20(1):89-97.

96. Bredeson C, LeRademacher J, Kato K, et al. Prospective cohort study comparing intravenous busulfan to total body irradiation in hematopoietic cell transplantation. Blood. 2013;122(24):3871-3878.

97. Champlin RE. Busulfan or tbi: Answer to an age-old question. Blood. 2013;122(24):3856-3857.

98. Parmar S, Rondon G, de Lima M, et al. Dose Intensification of Busulfan in the Preparative Regimen is Associated with Improved Survival: A Phase I/II Controlled, Randomized Study. Biology of Blood and Marrow Transplantation. 2013;19(3):474-480.

99. Andersson BS, de Lima M, Thall PF, et al. Once Daily i.v. Busulfan and Fludarabine (i.v. Bu-Flu) Compares Favorably with i.v. Busulfan and Cyclophosphamide (i.v. BuCy2) as Pretransplant Conditioning Therapy in AML/MDS. Biology of Blood and Marrow Transplantation. 2008;14(6):672-684.

100. Alatrash G, De Lima M, Hamerschlak N, et al. Myeloablative Reduced-Toxicity i.v. Busulfan-Fludarabine and Allogeneic Hematopoietic Stem Cell Transplant for Patients with Acute Myeloid Leukemia or Myelodysplastic Syndrome in the Sixth through Eighth Decades of Life. Biology of Blood and Marrow Transplantation. 2011;17(10):1490-1496.

101. De Lima M, Couriel D, Thall PF, et al. Once-daily intravenous busulfan and fludarabine: Clinical and pharmacokinetic results of a myeloablative, reduced-toxicity conditioning regimen for allogeneic stem cell transplantation in AML and MDS. Blood. 2004;104(3):857-864.

102. Mayumi H and Good RA. Long-lasting skin allograft tolerance in adult mice induced across fully allogeneic (multimajor H-2 plus multiminor histocompatibility) antigen barriers by a tolerance-inducing method using cyclophosphamide. J Exp Med. 1989;169(1):213-238.

103. Mayumi H, Umesue M, Nomoto K. Cyclophosphamide-induced immunological tolerance: An overview. Immunobiology. 1996;195(2):129-139.

104. Fontalin LN, Chernyakhovskaya IY, Mayumi H, Good RA. Recent state of cyclophosphamide-induced skin allograft tolerance in mice. Immunobiology. 1991;182(3-4):323-333.

105. Breems DA, Van Putten WLJ, De Greef GE, et al. Monosomal karyotype in acute myeloid leukemia: A better indicator of poor prognosis than a complex karyotype. Journal of Clinical Oncology. 2008;26(29):4791-4797.

106. McCune JS, Woodahl EL, Furlong T, et al. A pilot pharmacologic biomarker study of busulfan and fludarabine in hematopoietic cell transplant recipients. Cancer Chemother Pharmacol. 2012;69(1):263-272.

107. Bubalo JS, Kovascovics TJ, Meyers G, et al. 467: Clonazepam Plus Levetiracetam (CL) for the Prevention of Busulfan-Induced Seizures: A Single Center Experience. Biology of Blood and Marrow Transplantation. 2008;14(2, Supplement):165.

108. Soni S, Skeens M, Termuhlen AM, Bajwa RPS, Gross TG, Pai V. Levetiracetam for busulfan-induced seizure prophylaxis in children undergoing hematopoietic stem cell transplantation. Pediatric Blood & Cancer. 2012;59(4):762-764.

109. Caselli D, Rosati A, Faraci M, et al. Risk of Seizures in Children Receiving Busulphan-Containing Regimens for Stem Cell Transplantation. Biology of Blood and Marrow Transplantation. 2014;20(2):282-285.

110. Eberly AL, Anderson GD, Bubalo JS, McCune JS. Optimal prevention of seizures induced by high-dose busulfan. Pharmacotherapy. 2008;28(12):1502-1510.

12.0 Appendices

12.1 **Appendix A** Guidelines for Total Body Irradiation

Modality

Photon irradiation is to be used for the TBI in all patients.

Energy

A linear accelerator with energy ≥ 6 MV may be used. Dose to superficial tissues near skin surface will be increased by using a beam “spoiler” lucite plate close to the patient. Since neoplastic infiltrates may be found in the skin, it is necessary for the superficial dose to satisfy the same total dose requirements as other locations.

Geometry

The treatment configuration shall be such that the patient is entirely included within the treatment beam. It is essential that the correlation between the light field and the radiation field be established and verified for extended TBI distances.

Dose Rate

A dose rate of 0.05 to 0.25 Gy/minute at the prescription point shall be utilized. The physicist of record, involved with TBI treatments, shall be consulted to achieve correct range of treatment dose rate.

Calibration & Beam Data Verification

The calibration of the output of the machine, used for this protocol, shall be verified on a daily basis prior to start TBI treatments. All dosimetric parameters, necessary for the calculation of dose delivered during TBI treatments, shall be measured at the appropriate treatment distance. They shall be documented and made available for calculation of every patient treatment.

Treatment Volume

The patient shall be entirely included within the treatment beam. Care should be taken to guarantee that all of the patient is within the 90% decrement line at each depth. The 90% decrement line is defined as the line in each plane perpendicular to the central axis connecting the points which are 90% of the central axis dose, in that plane.

Treatment Dose

Prescription Point

The prescription point is defined as the midplane point along the longitudinal axis at the level of the umbilicus.

Dose Units

All doses shall be specified in Gray (Gy) to muscle tissue.

Tissue Inhomogeneity Considerations

No inhomogeneity corrections shall be made in the calculation of the dose to the prescription point.

Prescription Point Dose

The total dose shall be 2 Gy.

Time-Dose Considerations

Dose Homogeneity

The total absorbed dose along the patient's head to toe axis (in the midplane of the patient) shall not deviate more than 10% from the prescribed dose.

Treatment Technique

Treatment Fields

Equally weighted parallel opposed portals shall be used. AP/PA fields shall be used.

Field Size

The collimation and treatment distance shall be such that the patient will be entirely included within the treatment beam and that no part of the patient extends beyond that region. The agreement of the light field and the radiation field should be checked periodically for the extended TBI treatment distance.

Treatment Position

The patient shall be treated in any position that is compatible with the homogeneity requirement, allowing for the reproducibility of the patient setup and dosimetry.

Field Shaping

Patients will be treated with open fields.

Calculations

Central Axis Dose

It is recommended that the dose calculation method be based upon measurements that are made in a unit density phantom with the following minimum dimensions:

Length equal to top of shoulder to the bottom of the pelvis.

Width equal to the patient width at the level of the umbilicus.

Thickness equal to the typical patient thickness at the umbilicus.

All measurements should be made at the appropriate extended SSD.

Superficial Dose

For the radiation beam with the Plexiglas plate in place, data should be available demonstrating that the skin dose is within 5% of the prescribed dose.

Quality Assurance Documentation

For purposes of quality assurance the following must be performed on every patient undergoing TBI:

A check of the monitor unit calculation by a second physicist and a radiation oncologist prior to first treatment.

12.2 **Appendix B** GVHD Grading System Grade

Clinical Staging of Acute Graft-Versus-Host Disease

Stage	Skin	Liver	Gut
+	Maculopapular rash < 25% body surface	Bilirubin, 2-3 mg/dl	Diarrhea, 500-1,000 ml/day or persistent nausea
++	Maculopapular rash 25-50% body surface	Bilirubin, 3-6 mg/dl	Diarrhea, 1,000-1,500 ml/day
+++	Generalized erythroderma	Bilirubin, 6-15 mg/dl	Diarrhea, > 1,500 ml/day
++++	Desquamation and bullae	Bilirubin, > 15 mg/dl	Pain +/- ileus

Clinical Grading of Acute Graft-Versus-Host DiseaseStage				
Overall Grade	Skin	Liver	Gut	Functional Impairment
0 (none)	0	0	0	0
I (mild)	+ to ++	0	0	0
II (moderate)	+ to +++	+	+	+
III (severe)	++ to +++	++ to +++	++ to +++	++
IV (life-threatening)	++ to ++++	++ to ++++	++ to ++++	+++

Tables from Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation, 18: 295-304, 1974.