A Two Step Approach to Reduced Intensity Allogeneic Hematopoietic Stem Cell Transplantation for Patients with Hematologic Malignancies

Principal Investigator
Dolores Grosso, DNP, CRNP

Co-Principal Investigator
Usama Gergis, MD

Co-Investigators
S. Onder Alpdogan, MD
Matthew Carabasi, MD
Joanne Filicko-OHara, MD
Margaret Kasner, MD
William O'Hara, PharmD
Ubaldo Outschoorn Martinez, MD
John Wagner, MD
Neil Palmisiano, MD
Thomas Klumpp, MD

Pierluigi Porcu, MD
Lindsay Wilde, MD

Biostatistics
Inna Chervoneva, PhD

Radiation Oncologists
Wenyin Shi, MD, PhD
Maria Werner-Wasik, MD

Thomas Jefferson University (TJU)
Blood and Marrow Transplantation Program
Department of Medical Oncology
Suite 320, 834 Chestnut Street
Philadelphia, PA 19107

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1.0 Purpose, Hypothesis, and Objectives

Haploidentical hematopoietic stem cell transplantation (HSCT) is a life-saving therapy for patients with hematological malignancies who do not have a fully HLA (Human Leukocyte Antigen)-matched donor. This type of approach has been characterized by lower survival rates as compared to matched related HSCT. These poorer outcomes are at least in part due to the attenuation of the donor graft necessary to avoid catastrophic graft versus host effects and the late stage disease status of the patients undergoing haploidentical HSCT.

Recently, approaches to haploidentical HSCT utilizing reduced intensity conditioning (RIC) and the drug cyclophosphamide (CY) to establish bidirectional tolerization of donor/recipient T cells [1, 1-3] have resulted in less graft versus host disease (GVHD) and treatment-related mortality (TRM) as compared to historical approaches using haploidentical donors. At our institution, the RIC HSCT regimen utilizing CY to tolerize T cells is called the TJU 2 Step approach because patients receive the lymphoid and myeloid portions of their graft in two separate steps. This transplant methodology has greatly improved overall survival rates for patients undergoing haploidentical HSCT at Jefferson.

While these newer approaches have made this type of transplant safer, relapsed disease is a major source of mortality in these trials, specifically in patients with evidence of their disease at HSCT. [1, 4] Although increasing HLA disparity is known to be associated with a greater risk of significant graft versus host disease (GVHD), there is less data as to whether increasing HLA disparity is correlated with stronger, clinically significant graft versus tumor (GVT) effects. Based on the high degree of major histocompatibility complex (MHC) mismatch in haploidentical HSCT, one would expect relapse to occur less frequently than in matched sibling HSCT, yet in both myeloablative and RIC haploidentical HSCT, mortality from relapse in patients with evidence of their disease at HSCT is very common especially in the acute leukemias. It is unclear whether GVT effects are not discerned because the patient has resistant disease at the time of HSCT or because the attenuation of the haploidentical graft to avoid catastrophic GVHD also circumvents clinically meaningful GVT effects. Of interest, there is some data suggesting a superior GVT effect in haploidentical HSCT for the lymphomas as compared with other diagnoses. [2, 5]

The purpose of this research study is to examine GVT effects in RIC haploidentical HSCT utilizing CY tolerization of T cells within the context of lymphoid and myeloid malignancies. Because post HSCT relapse may be associated with disease resistance to the conditioning regimen or the provision of an RIC approach in patients with active or resistant disease at HSCT, patients in remission or with chemosensitive or indolent diseases will be the study group in this trial. Durable disease control in this lower risk patient population would be more specifically related to GVT effects of the haploidentical donor immune system as opposed to an up-front failure to control the disease with chemoradiotherapy.

The hypothesis of this research study is that haploidentical RIC HSCT using the TJU 2 Step approach will result in equal or superior overall survival (OS) in patients with better risk disease as compared to patients undergoing matched sibling or unrelated donor (URD) RIC HSCT despite the fact that a haploidentical donor is used as the hematopoietic cell source. This hypothesis is based on the assumption that the Jefferson 2 Step approach to haploidentical HSCT

has an equivalent rate of treatment related mortality as compared to matched sibling RIC HSCT, and that whatever diminution in GVT effects that occur as a result of CY tolerization are compensated for by increased alloreactivity from the mismatched graft.

Specific Objectives are:

Primary

- To compare the OS rate at 2 years post treatment using the Jefferson 2 Step RIC approach in patients with haploidentical family donors with hematological malignancies in morphological or radiographic remission or with chemosensitive, indolent diseases to historical OS rates in similar populations after RIC matched donor HSCT as reported in the literature. Based on the historical literature, reviewed in the background section of this protocol, we believe that:

    - An OS of < 55% at 2 years would demonstrate poor efficacy for the TJU 2 Step haploidentical regimen as compared to regimens using matched related and unrelated donors, and

    - An OS of ≥75% at 2 years would demonstrate that the TJU 2 Step haploidentical RIC regimen is equal or superior to those using matched related or unrelated donors.

Although there has been approximately 4 years of experience with the TJU 2 Step approach in the Jefferson Blood and Marrow Transplant Program, continuing assessment of this transplant methodology will be performed on the outcomes of GVHD, engraftment, immune reconstitution, TRM, and relapse-related mortality in specific disease groups. Therefore, secondary objectives are:

Secondary

- To compare the TRM rate at 2 years for patients treated on this study to the historical TRM rates of patients undergoing RIC matched-sibling HSCT as reported in the literature.

- To compare the 2 year relapse rates and relapse related mortality of patients with myeloid diseases to that of patients with lymphoid diseases who are treated on this TJU RIC 2 step approach.

- To determine the incidence and severity of graft-versus-host disease (GVHD) in patients undergoing treated on the TJU RIC 2 step approach.

- To evaluate engraftment rates and lymphoid reconstitution in patients treated on the TJU RIC 2 step approach.

- To evaluate the incidence of TRM at 100 days in patients treated on the TJU RIC 2 step approach.

2.0 Introduction and Background

Allogeneic HSCT is a curative therapy for many disorders of lymphohematopoiesis.[6-10] 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. [11-28] 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. [29-34] For more than a decade, it has been recognized that long-term disease control after allogeneic HSCT is mediated through the anti-tumor effects of the transplanted immune system and less so by the intensity of the conditioning regimen. This GVT effect can occur even in the absence of overt GVHD. [35] In acute myeloid leukemia (AML) and chronic myeloid leukemia (CML), for example, relapse rates are higher in recipients of twin transplants than in GVH free recipients of matched sibling grafts. [36] 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. [37-41]

Development of less intensive conditioning regimens

Transplant regimens that are not lethally myeloablative (NM HSCT) have been developed over the last decade. These approaches do not use 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 [42] and have allowed older and heavily pretreated patients who otherwise would not tolerate the rigors of a fully myeloablative HSCT, to undergo transplant successfully.[43] Nonmyeloablative HSCT has been dramatically effective in CML, chronic lymphocytic leukemia (CLL), and follicular lymphoma in its original application and may have utility in other diseases as well. [44-47]

Ablative Versus Nonmyeloablative HSCT

Despite the demonstration of successful outcomes after NM HSCT, this type of therapy cannot be universally applied. Many studies contrasting the outcomes between ablative and non-ablative conditioning show that the superior results for NM HSCT in terms of treatment related mortality are offset by higher rates of relapse.[48-50] This issue becomes particularly important in diseases not known to have a strong GVT effect such as acute lymphocytic leukemia (ALL)[51, 52] and certain lymphoma subtypes,[53-55] where dose intensity may be just as important as a GVT effect in terms of overall survival. 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.[48, 56, 57] The latter approach, alternately referred to as “reduced intensity” HSCT, has been associated with more TRM but less relapse.[58, 59] A NM HSCT approach that is not an “either/or” proposition has not been clearly identified.

Because many patients, due to age, previous treatment, or comorbid conditions, cannot tolerate the rigors of a fully ablative regimen, decreasing the incidence of relapse after this procedure

could result in significant gains in overall survival. Thus, the use of a haploidentical donor as opposed to a fully matched related or unrelated donor in NM HSCT may represent a way to increase graft versus tumor (GVT) effects resulting in an increase in OS in the setting of RIC HSCT. At our institution, the use of haploidentical donors in RIC HSCT has resulted in durable disease control with minimal toxicity especially in fit patients without evidence of their disease at HSCT. It is this subgroup of patients that may benefit from the GVT effects of a haploidentical donor as compared to a matched donor, provided the toxic effects of the regimen are commensurate with matched sibling HSCT. In our experience, the 2 Step TJU RIC HSCT approach meets this criteria.

TJU 2 Step RIC HSCT

A Two Step Approach to Reduced Intensity Allogeneic Hematopoietic Stem Cell Transplantation for Hematologic Malignancies from HLA Partially-Matched Related Donors 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 receive fludarabine and either ARA-C or Thiotepa for 4 days. The patients then receive one day of TBI (2Gy), followed by an exact dose of 2.0 x 10e8 of their donor's T cells (step 1 of the transplant). After the donor T cell infusion (DLI) the patients consistently develop fevers and in many cases a skin rash and diarrhea, consistent with an immune reaction. On the 3rd day after the DLI the patients receive 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 receive 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	**CTX 60 mg/kg**	**CTX 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 receive Thiotepa if there is evidence of an active malignancy where therapy with an alkylating agent is thought to be more beneficial than therapy with an anti-metabolite. This is 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 is given to patients who do not have evidence of disease at the time of HSCT or have diseases in which ARA-C is thought to be beneficial such as CLL or follicular NHL. The conditioning regimen has been developed in this manner to allow for patient-specific adjustments within a consistent

regimen. Patients are treated with ARA-C versus Thiotepa based on defined programmatic guidelines which are part of a greater policy governing patient placement on the various TJU 2 Step protocols.

Review of the TJU 2 Step RIC HSCT

Figure 1

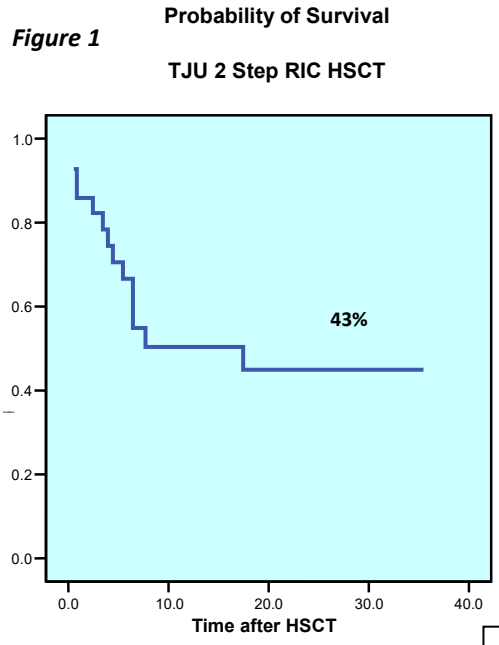

Figure 2

Thirty-four patients were treated on the TJU 2 Step RIC approach from 2006-2010, which is now closed to accrual. The probability of overall survival at 1 year is 50% and 43% at 2 years, with a follow-up of 5 to 42 months (Figure 1). The median age of this group of patients is 67 years.

To our knowledge, there are no haploidentical reduced intensity trials with a patient population as old as the group being treated on our study. Therefore, we do not have comparison survival data for which to assess our current results. However, based on CIBMTR data for patients over the age of 50, the 1 year survival rate advanced AML is close to 50% (Figure 2), which is comparable to our haploidentical results at the 1 year mark, despite a significantly older population.

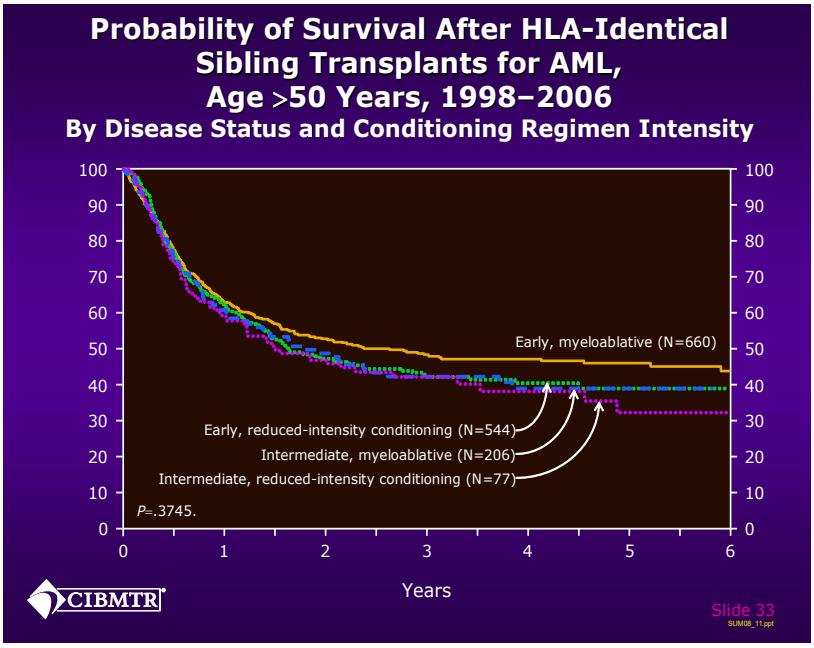

Table 2 below contains patient information and outcomes to date for this trial.

Table 2 **Interim Summary (Follow Up 1-39 Months) TJUH 2 Step Approach to Reduced Intensity Haploidentical HSCT 3/10/2010**	
Sample Size	**34**
Median Age	**67 years**
Diagnoses	**MDS-2 AML-18 ALL-3 CLL-3 NHL-3 MPD-1 Myeloma-2 Biphen Leuk-1 Aplastic Anemia-1 5 patients are S/P previous transplants**
Disease at the time of HSCT	**24/34 (71%)**
Overall Survival **Patients in Remission or with Indolent Disease** **Patients Not in Remission**	**16/34 (47%)** **10/14 (71%)** **6/20 (30%)**
Overall Treatment-Related Mortality **Treatment Related Mortality: Patients in Remission or with Indolent Disease**	**9/34 (26%)** **2/14 (14%)**
Relapse-Related Mortality	**9/34 (26%)**
Mortality Related to GVHD	**2/34 (6%)**
Rejection	**1/34 (3%)**

MDS=myelodysplastic syndrome, AML=acute myeloid leukemia, CLL=chronic lymphocytic leukemia, NHL=non-Hodgkin lymphoma, MPD=myeloproliferative disorder, Myeloma=multiple myeloma, Biphen Leuk=Biphenotypic leukemia

As shown in the table above, the TRM of all of the patients treated on the 2 Step RIC regimen is 26%, and only 14% in the patient group with better risk disease. We believe this rate is acceptable considering the older age of the patient population, although efforts to improve pre-transplant screening and optimization of the clinical condition of patients continue in the

Jefferson Transplant program in order to decrease TRM rates further. Despite this, we believe the TJU 2 Step RIC regimen is commensurate in terms of TRM to historical RIC regimens using fully matched and unrelated donors especially for patients with better risk disease. Patients treated on the TJU 2 Step RIC approach who were in remission at the time of the transplant have an 80% probability of OS at 1 year post HSCT. Therefore, we assume that with similar patient populations and treatment toxicity, comparisons can be made regarding GVT effects. The hypothesis of this current research study is to ascertain if haploidentical HSCT is associated with a greater survival benefit as compared to matched donor RIC HSCT in a larger group of patients than studied in the initial protocol.

Relapse and Overall Survival in Matched Related and Unrelated RIC HSCT

In order to demonstrate that RIC HSCT using haploidentical donors may be comparable or even superior in terms of GVT effects and OS to regimens utilizing completely matched donors, an estimate of OS after related donor RIC HSCT is required for comparison purposes. Because the patient population that is served in our institution is older with a preponderance of AML/MDS, the comparator studies will primarily reflect this type of population, although other studies will be reviewed in order to capture data for patients with other diagnoses. Patients with all of the diagnoses discussed below are treated in the Jefferson transplant program and may be treated according to this protocol. All of the studies reviewed below are summarized in table 3 which follows the discussion section.

AML/MDS

Several larger studies of RIC HSCT are available for patients with better risk AML. A European Group for Blood and Marrow Transplantation (EBMT) trial reported by Aoudjhane and colleagues[49] reviewed the results of 315 patients, median age of 57 years, with intermediate or good risk AML (71% in first complete remission (CR1) or second complete remisison CR2) undergoing transplant with matched related donors. Probability of OS at 1 year in this group was 55% which dropped to 44% at 2 years. Relapse related mortality was 18% at 2 years, and relapsed disease was the major cause of mortality with 27% of patients dying from relapse at 2 years most significant in the group with more advanced disease. A second EBMT study utilizing the same database and reported by Herr et al.,[60] reviewed the outcomes of 361 patients greater than 50 years old with AML, 76% in CR1 or CR2, and compared them to a similar group of patients undergoing autologous HSCT. In this analysis, the probability of OS in patients undergoing RIC HSCT was 54% at 2 years with 29% of patients dying of their disease at 2 years. Consistent with the Aoudjhane et al. analysis, patients with advanced disease had a higher relapse rate than patients in CR1 or CR2. TRM in this study was 16% at 2 years.

In contrast to the above studies, Kurosawa et al.[61] reported on the outcomes of 93 patients (median age 55 years) with AML who were all in CR1 undergoing matched related RIC HSCT. This type of patient population, i.e. having early stage disease with a matched related donor, would be predicted to have the best outcomes. Not surprisingly, the OS rate of 61% at 3 years reported for this trial is higher than most survival rates reported for any RIC HSCT trial in AML. CIBMTR data (available on the CIBMTR website at www.cibmtr.org/) for 1,198 patients with early stage AML undergoing matched related non-myeloablative HSCT showed a 2 years probability of OS of 55%, consistent with higher survival rates for good risk disease. The EBMTR results reported by Aoudjhane and colleagues contain outcomes of patients with

intermediate risk disease and consequently the 2 years probability of OS was lower at 44% which is also consistent with the lower 2 year probability of OS reported by the CIBMTR for patients (N=377) with intermediate-risk AML of 50% as compared to the 55% rate of earlier stage patients.

In trials in which outcomes for patients treated with AML are combined with outcomes of patients with MDS, OS rates are lower for patients with MDS than patients with early stage AML. The probability of OS for patients over the age of 40 reported in a CIBMTR study [62] involving 545 patients with AML in CR1 and 535 patients with MDS was 34-50% for the patients with AML and 35-45% for patients with MDS. Outcomes were worse for older patients with a TRM of 33-35% at 2 years in the MDS group. Surprisingly, the relapse rate for the AML patients was higher at 33-36% than in the MDS group (25-29%), but this may have been due early censoring of the patients in the MDS arm that died of toxicity before they were able to relapse. Two additional studies, Lim et al.[63] which looked at 833 patients with MDS or secondary AML (median age of 59) undergoing HSCT with fully matched donors and Martino et al.[48] reporting on 215 patients with MDS (median age of 56) undergoing matched related RIC HSCT reported OS rates of only 32% at 4 years and 41% at 3 years respectively for these groups of patients. In both of these studies relapsed disease was the major cause of death with relapse rates of 41% at 4 years in the Lim et al. study, and 45% at 3 years in the Martino et al. study. TRM for patients in the LIM et al. study was also a significant cause of mortality at 32% at 4 years and may have been due to the slightly older patient population in the study.

Based on this data, patients with early stage AML and matched sibling donors can achieve OS rates as high as 55-60% after RIC HSCT. Relapse rates are higher and OS is lower in older patients and/or those with intermediate stage AML or MDS, with MDS patients also succumbing to treatment effects in higher numbers. In this group, OS rates range from 30 to 45% depending on the length of follow-up but are certainly lower than patients with better risk disease.

Myeloproliferative Disorders

There is less data regarding patients with CML undergoing RIC HSCT with the advent of transcription kinase inhibiter therapy. One study by Das et al. [64] analyzed the outcomes of 17 young adults with chronic CML undergoing RIC HSCT. In this small series, the OS rate was only 35.5% at a median of 30 months with only 4 patients developing relapsed disease and most deaths attributed to toxicity. Because the GVL effect of HSCT in CML is significant, these results are not surprising and patients with this disease may be the least likely to require a haploidentical donor as opposed to a matched related donor to achieve remission. Stewart and colleagues [65] looked at the outcomes of 24 patients with primary myelofibrosis undergoing RIC HSCT and reported non-relapse mortality (NRM) rate of 32% at 3 years for the group and an OS of only 31%. Relapsed disease was the cause of death for about 1/3 of the patients in this study with regimen toxicity accounting for most of the other deaths. Lissandre and colleagues [66] reported the outcomes of 39 patients with myelofibrosis (1° MF=27, PV=7, ET=5) undergoing RIC HSCT from 1994-2008. The patient numbers were equally distributed between low, intermediate and high Lille prognosis scores. The 3 year OS, relapse-free survival, and regimen related mortality rates were 60%, 54%, and 30%. These figures are consistent with the outcomes reported in the other trials for this disease category in that regimen related mortality, not relapsed disease is the primary cause of mortality for this subgroup of patients.

Lymphoid Disorders

The outcomes of adults with Hodgkin disease undergoing RIC HSCT from matched related donors (N=12) versus umbilical cord blood (N=9) reported by Majhail et al.[67], revealed no difference in OS between the two groups, and a 2 year progression-free survival (PFS) rate of only 20% for the patients undergoing matched related donor RIC HSCT. Progressive disease and relapse related mortality were equal in terms of causes of mortality. Patients with refractory disease at HSCT had a 0% OS rate. Relapsed disease was the primary cause of mortality in a Swedish study reported by Johansson and colleagues [68]of 23 adults with advanced stage Hodgkin disease. By 3 years, 57% of the patients had relapsed and only 20% of patients were alive at 7 years. Conversely, Burroughs et al.[5] reported a significant decrease in relapse rates for patients with Hodgkin disease undergoing RIC HSCT from haploidentical donors. An Italian Group study [69]examined the outcomes of 104 patients with relapsed Hodgkin disease after autologous HSCT undergoing RIC HSCT primarily from matched donors. Relapsed disease was the primary source of mortality in this group with advanced disease, and the PFS and OS rates at 2 years were only 39.3% and 66% respectively. In summary, patients with Hodgkin disease who are treated with RIC HSCT are primarily heavily pretreated with many relapsing after autologous HSCT. For this group, OS at 2 years would be expected to be about 50% with a much lower PFS rate at that time point.

Patients with low grade non-Hodgkin lymphomas have amongst the highest rates of survival after RIC HSCT, with low relapse rates and even higher PFS for patients with chemosensistive disease. In trials of patients with follicular lymphoma undergoing RIC HSCT from matched donors, OS has been reported to be as high as 62%-85% at 3 to 5 years with a PFS of 55%-83%.[53, 53, 55, 70-72] The majority of patients with CLL will undergo HSCT with refractory disease and consequently OS rates are lower than those reported for follicular NHL. Sorror et al.[73] and Peres et al. [74]reported 5 year OS rates of 50-63% for a group of patients with CLL undergoing NM/RIC HSCT with 45%-81% of the population having refractory disease. Dreger et al.[75] reported a 72% OS rate at 2 years in patients with CLL, only one-third of whom had refractory disease. Whether the higher OS rate in this study is due to shorter follow-up time versus higher numbers of better-risk patients is not known. In the Sorror et al. study, patients without refractory disease or comorbidities as scored on the hematopoietic cell transplant comorbidity index (HCT-CI) had an OS rate of 80% at 2 years which decreased to only about 75% at 5 years and is comparable to the Dreger et al. study. However for patients with ≥ 1 point on the HCT CI or ≥ 5cm adenopathy at HSCT, OS rates were lower: 45% to 50% at 2 years and 20% to 40% at 5 years. Therefore, for fit patients with chemosensitive CLL, OS rates at 2 years may be as high as 70%. Conversely, patients with resistant disease who have even a few comorbidities, OS rates may be as low as 45 to 50%.

Two studies by Corradini et al. [55] and Armand et al.[53] reported the outcomes of patients with NHL, mostly with responsive disease in both trials, undergoing RIC HSCT based on NHL subtype. The Corradini et al. trial was the larger study and consistent with the aforementioned CLL and follicular NHL trials, reported a high, 69% OS rate at 3 years for the patients with indolent NHL, with this group of patients having the best PFS. The trial by Armand et al. also included patients with indolent NHL, and these authors also reported a favorable OS rate of 81% at 3 years for patients in this subgroup. Patients with aggressive lymphomas faired well in the

Corradini et al. study as well, with a 3 year OS rate of 69% at 3 years and a PFS rate of 54%. In contrast, the 23 patients with diffuse large cell lymphoma or transformed follicular lymphoma in the Armand et al. trial had a 3 year PFS and OS of only 22% and 42% despite the fact that most of the patients in this study did not have resistant disease. Data specific to patients with this disease subtype was not reported by Armand et al. with enough detail to attempt to identify the reasons for the discrepancy in outcomes for patients with aggressive NHL in the two trials. Patients with mantle cell lymphoma had 3 year survival rates of only 40% to 45% in both trials with a 35% relapse rate in patients in the Corradini et al. trial and a higher risk of relapse as compared to the indolent lymphomas in the Armand et al. trial. Consistent with the previously discussed trials of patients with Hodgkin disease, patients in this subcategory in these two trials fared poorly with high relapse rates and an OS rate of only 32% at 3 years in the Corradini study and a PFS rate of only 22% in the Armand et al. study.

In summary, patients with chemosensitive indolent lymphoid diseases would be predicted to have OS rates of about 70% (60%-80%) at 2 years. Subgroups of patients with more aggressive chemosensitive disease would be predictably lower. Patients in any subgroup with resistant disease would be expected to have OS rates of closer to 40 to 50% at 2 years and those with Hodgkin disease fair the worst with OS rates consistently below 50%.

Multiple Myeloma

The role of RIC HSCT in the treatment of multiple myeloma remains controversial with many studies showing no survival benefit between allogeneic HSCT and autologous HSCT for this patient population.[76, 77] Some trials have shown a benefit in terms of progression-free survival, [78]but the median rate of OS at 2 to 3 years for 12 trials reviewed by Gahrton was only 49% (range 26% to 78%). Four papers, two very recently published, are reviewed here. Patients with early stage myeloma with residual disease after their first autologous HSCT had an OS rate of 61.8% at 5 years after RIC HSCT without a high degree of relapse.[77] In contrast, for patients with later stage disease, Mohty et al.[79] reported only a 41% PFS at 2 years, Shimoni et al. a 34% OS at 7 years[80], and Efebera et al.[81] a 32% OS at 2 years with 43% of patients dying of relapsed disease in this study. In summary, despite a small number of patients who are treated with allogeneic HSCT earlier in their disease course because of identified high-risk features, patients with multiple myeloma undergo RIC HSCT usually later in the disease course when front line therapy including autologous HSCT has failed. Based on the above data for this latter population, DFS rates in the 40 percentage range would not be unexpected with slightly higher OS percentages.

Table 3

Authors	Disease	NM vs. RIC	Probability Overall Survival	Disease Progression/ Relapse Data	Deaths from Toxicity	Median Age	Comments
Myeloproliferative Disorders							
Das et al.[64]	CML **N=17 -matched related donors**	RIC	35.3% at 30 months	Not given	47% at 30 months	34	
Stewart et al. [65]	1º Myelofibrosis **N=24 -matched related and unrelated donors**	RIC	31% at 3 years	25% died from relapsed disease	32% at 3 years	54	
Lissandre et al. [66]	1º Myelofibrosis 2º Myelofibrosis N=39 **- matched related and unrelated donors**	RIC	60% at 3 years	Relapse-free survival 54% at 3 years	30% at 3 years	49	
Hodgkin Disease							
Majhail et al. [67]	Hodgkin **N=12 -matched related donors**	RIC	~65% at 1 year, 50% at 2 years	20% PFS @ 2 years	25% at 6 months	42	
Johansson et al.[68]	Hodgkin **N=39, majority relapsed after auto BMT** -primarily matched unrelated donors with a few matched related donors	RIC	Only 20% alive at 7 years	57% relapsed by 3 years	22% at 100 days	36	
Sarina et al. [69]	Hodgkin **N=104 relapsed after auto BMT** -primarily matched related and unrelated donors	RIC	66% at 2 years	39.3% PFS at 2 years	12% at 2 years years	31	

Authors	Disease	NM vs. RIC	Probability Overall Survival	Disease Progression/ Relapse Data	Deaths from Toxicity	Median Age	Comments
			NHL				
Hari et al. [71]	Follicular NHL Primarily Chemosensitive disease **N=88 -all matched related donors**	RIC	62% at 3 years	55% Progression free survival at 3 years	23% 1 year Cumulative incidence of death from treatment	51	CIBMTR Study
Khouri et al.[72]	Relapsed Chemosensitive Follicular NHL **N=47 -mostly matched related donors**	NM + Rituxan	85% @ 60 months	PFS 83% at 60 months	7/47 (15%) died primarily infection	53	
Dreger et al.[75]	CLL, 35% with refractory disease **N=77 -primarily matched related donors**	RIC	At 2 years: OS 72% DFS 56%	Probability of relapse at 2 years was 31% (Patients with refractory disease fared poorly)	18% at 12 months	54	EBMT Study
Peres et al. [74]	CLL (81% fludarabine refractory) **N=21 -primarily matched related and matched unrelated**	RIC	63% at 5 years	Probability if relapse at 2 years 15.1%	20% Cumulative incidence of TRM at 2 years	54	
Sorror et al. [73]	CLL (45% unresponsive disease) **N=82 - matched related and matched unrelated**	NM	50% 5 year cumulative incidence OS HCT-CI +adenopathy: 0 + <5 cm ~ 70% at 5 y HCT-CI +adenopathy ≥1 + ≥ 5cm 0% at 5 y	38%/39% 5 year cumulative incidence of relapse-PFS	23% 5 year cumulative incidence of non-relapse mortality	56	

Corradini et al.[55]	Relapsed NHL: Indol-NHL(63) Aggres-NHL(61) MCL (14) HD(32) **N=170** **-matched related donors**	RIC	At 3 years: 69% 69% 45% 32%	32/170 died from relapse (19%), highest relapse rate in HD	11% at 1 year	51	
Armand, P. et al. [53]	Advanced NHL: Indol-NHL(13) Aggres-NHL(23) MCL (15) HD(36) **N=87** **-primarily matched related and matched unrelated**	RIC	At 3 years: 81% 42% 40% 56%	PFS 3 years: 59% 22% 30% 22%	38% NHL 15% HD	51 NHL 36 HD	

Authors	**Disease**	**NM vs. RIC**	**Probability Overall Survival**	**Disease Progression/ Relapse Data**	**Deaths from Toxicity**	**Median Age**	**Comments**
			Multiple Myeloma				
Mohty et al.[79]	Multiple Myeloma 70% in partial remission, 27% with progressive disease **N=41** **-matched related donors**	RIC	At 2 years: OS 62%	At 2 years: DFS 41% 51% progressive disease after HSCT	17% (at a median follow-up of 389 days)	52	
Shimoni et al. [80]	Recurrent/ Refractory Multiple Myeloma **N=50** **-matched related and unrelated**	RIC	34% at 7 years	48% had disease progression after HSCT	26% at 5 years	53	
Efebera et al. [81]	Relapsed/refractory Myeloma, 82% stable to responsive disease) **N=51** **-primarily matched related donors**	RIC	32% at 2 years	19% at 2 years 43% died of disease	34% died of non-relapse causes	51	

Rosiñol et al. [77]	Patients with early stage multiple myeloma failing to achieve a CR after 1st auto BMT **N=25 -all matched related donors**	RIC	61.8% at 5 years	61% PFS	16% TRM	52	

Comparison Point for the Trial

The hypothesis of this research study is that haploidentical RIC HSCT using the TJU 2 Step approach will result in equal or superior overall survival (OS) in patients with better risk disease as compared to patients undergoing matched sibling or unrelated donor (URD) RIC HSCT. In the initial TJU 2 Step RIC HSCT trial, patients with better-risk disease included those with acute leukemia in CR1 or CR2 (n=8), indolent NHL (n=2), myelofibrosis (n=1), myeloma with near CR (n=2), and aplastic anemia (n=1). We would expect that patients with the same diagnoses would be treated on this current study. We would also expect that our population would continue to be significantly older than that of the patients cited in the trials cited above. The median age of the patients on the initial RIC haploidentical HSCT trial at Jefferson was 67 years old, which is about 10 to 30 years higher than that reported for the trials reviewed above. Based on these trials, we anticipate that an OS rate of ≥60% at 2 years would prove the superiority of this 2 step approach over matched related or unrelated trials. Conversely, an OS rate of ≤ 35% at 2 years would demonstrate poor efficacy of this trial as compared to historical trials utilizing matched related or unrelated donors. These figures take into account both the older age of the patient population treated at Jefferson as compared to the cited literature, as well as the treatment of a preponderance of patients with myeloid malignancies as opposed to those treated for indolent non-Hodgkin lymphoma.

The Substitution of Alkylating Agents

This protocol opened in 2011 and 23 patients has been treated to date on the current conditioning regimen of Fludarabine, Thiotepa, 2 Gy TBI and CY. Thiotepa has been used in the 2 step RIC approaches since 2008 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, (Adienne Pharma & Biotech, 2013) originating from market forces which have affected many oncology medications.(Chabner, 2011) The drug is no longer manufactured in the United States (American Society of Health System Pharmacists, 2014) and purchasing the drug from overseas has been associated with increasing cost.(Neergaard, 2011) Therefore, the current expense of the drug makes its use in HSCT at TJUH (and most other centers) no longer feasible, and a drug substitution in the regimen is required.

Mortality in patients undergoing allogeneic HSCT comes from non-relapse causes in the forms of GVHD, organ toxicity, or infection versus mortality from disease recurrence. This protocol explores whether a haploidentical donor is superior to a matched related donor in terms of preventing relapse and by extension, increasing OS rates at 2 years. Therefore, regimen toxicity must be minimized so that disease free survival comparisons can be made. To date, none of the 23 patients treated on this protocol have died of toxicity. To keep this non-relapse mortality low, we will substitute Busulfan 3.2 mg/m^2 IV daily x 2 days for Thiotepa in the regimen. Busulfan has been used in HSCT for over 30 years and is commonly used in RIC HSCT.(Devillier et al.,

2014; Gupta et al., 2014; Kanda et al., 2014) A comprehensive review of recent data regarding Busulfan in HSCT was written by Champlin in 2013(R. E. Champlin, 2013) 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 only two doses of Busulfan will be used in this regimen, dosing based pharmacokinetic analysis is not necessary. In October of 2014, patients treated on the TJUH high risk RIC protocol (IRB #12D.501), started to receive Busulfan instead of Thiotepa for the same reason. Three patients have been treated on that study, and there have been no deaths and no toxicity related to Busulfan.

We will use the dose of Busulfan that is most commonly used with Fludarabine in other RIC regimens, including IRB #12D.501. Typically, 3-4 doses of Busulfan are administered in these approaches.(Alatrash et al., 2011; Andersson et al., 2008; De Lima et al., 2004; Parmar et al., 2013) 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.

Summary

The purpose of this clinical trial is to compare overall survival rates of patients with lower risk hematological malignancies who undergo haploidentical HSCT on the TJU 2 Step approach versus patients with similar diseases and disease states who have undergone matched related or unrelated RIC HSCT as reported in the literature. Our hypothesis is that patients with haploidentical donors will have lower relapse rates than patients with matched donors because of the greater degree of HLA mismatch between recipients and donors resulting in greater OS.

Historically, the 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, they will be offered this treatment option in lieu of an RIC trial specifically for matched sibling transplantation. 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 siblings will be reported descriptively.

3.0 Patient and Donor Selection

Patient Selection

Inclusion Criteria

1) Any patient with hematologic or oncologic diagnosis in which allogeneic HSCT is thought to be beneficial, and in whom front-line therapy has already been applied. Patients treated on this protocol will be without morphological evidence of disease, or if the patient has evidence of disease, the patient must have had at least a good partial response (PR) to the most recent therapy and the disease must be chemoresponsive.
2) Patients treated on this study will have:
   a. Acute leukemia in 1st or 2nd CR

b. MDS (myelodysplastic syndrome), specific subtypes of RA (refractory anemia) or RARS (refractory anemia with ringed sideroblasts) subtypes.
c. Hodgkins or Indolent Non-Hodgkin's lymphoma with chemosensitive disease
d. Myeloma without morphological evidence of disease, or a PR to the most recent therapy
e. Myeloproliferative disorders with at least a PR to current therapy
f. Aplastic Anemia
g. A hematological or oncological disease (not listed) that meets the criteria reviewed above (section 3, number 1).

3) Patients must have a related donor who is HLA mismatched at 2, 3, or 4 antigens at the HLA-A; B; C; DR loci in the GVHD direction. (Patients with related donors who are HLA identical or are a 1-antigen mismatch may be treated on this therapeutic approach, but will have their outcomes will not be part of the statistical aims of the study (see Summary section).
4) Patients must have adequate organ function:
   a. LVEF (Left ventricular end diastolic function) of ≥50%
   b. DLCO (Diffusing Capacity of the Lung for Carbon Monoxide) ≥50% of predicted corrected for hemoglobin
   c. Adequate liver function as defined by a serum bilirubin ≤1.8, AST or ALT ≤2.5 x upper limit of normal
   d. Creatinine clearance of ≥60 mL/min
5) 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

6) Patients must be willing to use contraception if they have childbearing potential
7) Able to give informed consent.

Exclusion Criteria

1) HIV positive
2) Active involvement of the central nervous system with malignancy
3) Inability to obtain informed consent
4) Pregnancy
5) Patients with life expectancy of ≤6 months for reasons other than their underlying hematologic/oncologist disorder
6) Patients who have received alemtuzumab or ATG within 8 weeks of the transplant admission. (documented by the absence of these agents in the medical record)
7) 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, evaluation and treatment will be in compliance with 21 CFR 1271 and all TJU BMT program SOPs relating to the use of allogeneic donor for HSCT.

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
Patients referred for the trial will have their eligibility criteria verified. On meeting the eligibility for the trial as outlined, informed consent will be obtained using forms approved by the Thomas Jefferson University Hospital Institutional Review Board and following guidelines related to the use of human subjects in research. This 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 after the consent interview. 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

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
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
		Bu 3.2 mg/kg	Bu 3.2 mg/kg			DLI					Start FK 506 & MMF	→

May not use voriconazole until day -1

Table Definitions: Bu=Busulfan, DLI=Donor lymphocyte Infusion, Fludara=fludarabine, TBI=total body irradiation, Gy-gray, CY=cyclophosphamide, FK 506=tacrolimus, MMF=mycophenolate mofetil, PBSC=peripheral blood stem cell

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 colony stimulating factor

There should be no administration of agents that suppress lymphocyte reactivity from admission until day -1 in this protocol. This includes steroids, calcineurin inhibitors, MMF, or monoclonal antibodies that affect lymphocyte number or function. If patients have previously required steroids as a premedication for transfusion, they may receive a dose of steroid equivalent to 5 mg of prednisone through day -10. After day -10, a significant portion of the conditioning regimen is complete. At this time, the immune system response to alloantigens should be somewhat attenuated. Diphenhydramine and meperidine may be used if necessary. Any use of steroids from day -10 through day 0 should not be administered without approval from the PI.

Patients will not receive azole druges, Acetaminophen, Metronidazole, or any drug inhibiting Busulfan metabolism from day -11 through day -8.

Voriconazole os prohibited until day-1 due to its interaction with cyclophosphamide.

The absence of prohibited medications in the medical record will serve as documentation that they were not given.

All chemotherapy and HPC 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 -11 through – 8) 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 -10 and -9 at a dose of 3.2 mg/kg/day IV. The infusion can be started upon the completion of the fludarabine.

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 -11 through -8.

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 should be reduced by 20-50% (to the nearest 0.5 mg) if the patient experiences excessive sedation

Day -7 is a day of rest

5.2 TBI

2 Gy of TBI will be administered on day -6. . 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.

5.3 Donor Lymphocyte Infusion

Hematopoietic 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 white cell growth factor 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. It is expected that in many cases, a second day of donor lymphocyte collection will be required. 18-27 liters will be processed the first day of donor lymphocyte collection. It is expected that in most cases, a second day of donor lymphocyte collection will not be required. A 3rd day of collection for 2 x 10^8 T cells has never been required in over 200 previous 2 Step donor collections.

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

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 at the American Red Cross, by trained apheresis personnel using standard techniques. No hematopoietic growth factors will be administered to apheresis donors prior to lymphocyte collection. The donor will have venous catheters placed for the purposes of undergoing apheresis. Leukocyte collections will be performed using a standard apheresis machine.

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.

5.4 Cyclophosphamide
CTX 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.

Voriconazole can block the conversion of CTX to its active metabolite, 4-hydroxycyclophosphamide. For this reason, patients may not receive voriconazole until day -1.

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 substitutes for G-CSF. The donor will return for a primed progenitor cell collection of days -2 and -1. 18 to 27 liters will be process per day. CD34+ cell enrichment will be performed via the closed system method using the CliniMACS® CD34 Reagent Syste, (Miltenyi Biotec Inc., Auburn, CA). The CliniMACS system utilitizes 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.

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. 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. The donor will have venous catheters placed for the purposes of undergoing leukopheresis. Leukocyte collections will be performed using a standard apheresis machine.

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 ULFILTERED 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 cells 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 take place at Thomas Jefferson University. PBSC infusions will be administered by nursing staff experienced in the administration of blood products. Patients will have vital signs taken before, during, and after the infusion. The patient will be observed for at least 15 minutes after the infusion is completed. 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: Ppp040.07, 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.

Benadryl, epinephrine, and hydrocortisone should be available at the bedside for emergency use if necessary. Oxygen with nasal cannula should be available.

5.6 GVHD Prophylaxis

Tacrolimus and MMF 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. Only the day +28 studies are mandatory. 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 Conditioning through Day + 28**	**Days 28-90**	**Days 90-180**	**Day 180**	**Day 180-2 years post HSCT**
History and physical with vital signs **including SPOs**. Assessment of infectious signs, pregnancy test for females of childbearing potential done on baseline assessment	X	Every 1-2 days	Every 1-2 days if in hospital, weekly until day 28 after discharge	Monthly	As clinically indicated		As clinically indicated
Laboratory Studies*	X	Every 1-2 days	Every 1-2 days 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		As clinically indicated
Viral resp swab by PCR//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						
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	As clinically indicated	As clinically indicated		As clinically indicated
Chimerism/ Disease Assessment							

Peripheral blood for CD3+ chimerism & Buffy coat chimerism				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 is optional	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	Suggested at days+ 180and +270	At d+365
Radiographic Studies In applicable situations for disease staging	X				Day +90 or as clinically indicated		Day +90 or as clinically indicated

* Study measurements are minimum requirement
**Laboratory studies include a complete blood count with differential comprehensive metabolic panel, and GVHD prophylaxis drug levels when applicable.

It is recognized that due to scheduling issues, some of the required studies cannot be performed on the exact days noted on the table. Day +28 studies can be done 7 days before or 7 days after day 28. Other required studies should be performed within 1 month before or after their due date.

The formal endpoint of this study for efficacy is 2 years post HSCT and patients are required to follow-up with their TJU HSCT team during this time period for assessment of their major outcomes. Testing is performed as clinically indicated during the second year post HSCT. Patients will come off study after 2 years. Outcomes for patients undergoing HSCT at TJUH are followed programmatically indefinitely with results reporting allowable beyond the 2 year follow-up.

6.1 Hematopoietic Engraftment

Hematopoietic engraftment will be defined as:

- ANC ≥ 0.5x10e9/L for at least 3 days
- Platelet engraftment >20,000 with no transfusion 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 at:
https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf

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. Expected non-life-threatening 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, headache
- Grades I-III hypoxia
- Grades I-III fever
- Grades I-III infectious sequellae
- Grades I-III abnormalities in ALP, AST and ALT
- Grade I-III electrolyte disturbance
- Neutropenia/uncomplicated neutropenic fever, grades I-III infectious sequellae
- Thrombocytopenia, Petechiae, ecchymoses, minor vaginal bleeding, epistaxis, hemorrhoidal bleeding, or other similar bleeding events will not be reported.
- Anemia
- Mucositis or esophagitis (non-life-threatening)
- Diarrhea (non-life-threatening)
- Allergic or other common reactions to drugs used for supportive care unless grade 4-5
- Grades I-III Rash
- Grades I-III Fatigue
- Pancytopenia
- 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
-

Serious adverse event reporting to the TJU Institutional Review Board will occur for grade 4 and grade 5 events and/or for an event that results in hospitalization or permanent disability regardless to the relationship to the study treatment.

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

The formal endpoint of this study is 2 years 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.

6.6 Reports to the Federal Drug Administration (FDA)
All grade 3-5 hematopoietic cell infusion reactions and all unexpected SAEs as defined in 21 CFR 312.32 will be reported to the FDA in an expedited fashion.

All Unanticipated Adverse Device Effects will also be reported to the FDA within 10 working days as defined in 21 CFR 812.150.

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.

6.7 Study Endpoints
The endpoint of this study is OS at 2 years.

7.0 Supportive Care
7.1 Avoidance of Infection

Patients who are post HSCT are susceptible to infection. BMT Clinical Program SOPs CP:P050.02 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 every 4 weeks post transplant to support immune function, until IgG level is ≥ 500 mg/dL on 2 consecutive monthly measurements for haploidentical graft recipients. The first dose will be targeted for day +7, but may be timed differently based on patient condition.

The infusion of IVIG to patients undergoing matched sibling transplant is at the discretion of the attending physician.

7.2 Infectious Prophylaxis-General Guidelines
Patients post partially-matched related donor transplantation will be maintained on antifungal prophylaxis, such as voriconazole or noxafil. . It is at the discretion of the treating attending physician to change agents or discontinue agents as clinically indicated.

Patients post partially-matched related donor transplantation will be maintained on HSV prophylaxis such as valacyclovir.. It is at the discretion of the treating physician to change agents or discontinue agents based on culture results and sensitivities as well as patient condition.

Patients post partially-matched related donor transplantation will be maintained on PCP prophylaxis, such as TMP-SMZ. . It is at the discretion of the treating physician to change agents based on culture results, drug tolerance, and patient condition.

Management of prophylactic post HSCT medications is per BMT SOP available on the TJUH Intranet.

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 sequela.

Packed red blood cell transfusions will be given as necessary with the goal of keeping the hemoglobin ≥ 7-8g/L.

Platelet transfusions will be used as needed with the goal of keeping the morning count ≥20x10e9/L, with 10x10e9/L used for situations without an excessive bleeding risk.

GM-CSF (granulocyte-macrophage colony-stimulating factor) 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 ≥ 1000 for all patients post partially-matched related donor transplantation. G-CSF 5µg/m^2 (or other white cell growth factors) 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.

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.

Reference: Skeel R & Lachant N. Handbook of Cancer Chemotherapy, 4th Ed. Little, Brown & Co.: Boston.

8.3 Donor Leukocyte Infusion (DLI)
Administration: All patients will receive a dose of donor CD3+ T cells per kilogram of dosing body weight as outlined in the treatment design. Details of the apheresis procedure to obtain white blood cells, quantification of CD3+ T cells by flow cytometry, and administration of the white cells product to the recipient are provided in the treatment section. All drug that may cause lymphocyte suppression are starting on the day of admission through day 0 as detailed in the treatment section.

Toxicity: Infusion reactions, GVHD.

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 -11 through – 8) at a dose of 30 mg/m^2/day IV 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.

Reference: MicroMedex Health Care Series, Thomson

8.5 G-CSF
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.

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.

Reference: Physician's Desk Reference, Edition 58, 2004.

8.6 GM-CSF

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 in the PM. The drug should continue until the patient has a self-sustaining ANC of 1500.

Reference: Physician's Desk Reference, Edition 58, 2004.

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: MPA¾17.9 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 or intolerance to the drug.

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-γ^{99}. 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.

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. It is suggested that a tacrolimus taper be initiated around d_42 in the absence of GVHD.

9.0 Patient Safety

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

The study will be monitored on an ongoing fashion by the Principal Investigator (PI) and the study medical monitor. Monitoring reports will be submitted to the Clinical Research Management Office (CRMO) for review by the DSMC during their quarterly review. Adverse events and a report summarizing their impact on the conduct of the trial are submitted to the Data Monitoring and Safety Committee (DMSC) quarterly, and the DSMC reports are them submitted to the CCRRC and IRB. The PI will submit serious adverse events (SAE) to the TJU IRB utilizing the electronic Kimmel Cancer Center Clinical Trials Adverse Event Reporting system. 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 DSM 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 related to this protocol will be reported within 24 hours.

In addition to the Cancer Center's DMSC, the TJU BMT program members meet weekly to discuss the status of patients on trial and generate discussion regarding the progress of the patients on the trial.

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 CCRRC and the TJU IRB as part of the study progress report. The CCRRC, DMSC, 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.

Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the funding sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities.

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

In addition to review by the DSMC, all studies initiated by KCC investigators are audited by an independent auditor once they have achieved 10% of target accrual. However, a study can be audited at any time based on recommendations by the IRB, DSMC, CCRRC and/or the Director

of Clinical Investigations, KCC. Studies are re-audited once they have achieved 50% of target accrual. Special audits may be recommended by the IRB, DSMC or CCRRC based on prior findings, allegations of scientific misconduct and where significant irregularities are found through quality control procedures. Any irregularities identified as part of this process would result in a full audit of that study.

In addition to the audits at 10 and 50%, the CRMO randomly audits at least 10 percent of all patients entered into therapeutic KCC trials and other trials as necessary, on at least a bi-annual basis, to verify that there is a signed and dated patient consent form, the patient has met the eligibility criteria, and that SAEs are documented and reported to the TJU IRB.

All audit reports are submitted to the DSMC for review and action (when appropriate). A copy of this report and recommended DSMC action is sent to the CCRRC and TJU IRB. The committee regards the scientific review process as dynamic and constructive rather than punitive. The review process is designed to assist Principal Investigators in ensuring the safety of study subjects and the adequacy and accuracy of any data generated. The TJU IRB may, based on the DSMC and auditor's recommendation, suspend or terminate the trial.

FDA Reports

All grade 3-5 hematopoietic cell infusion reactions and all unexpected SAEs as defined in 21 CFR 312.32 will be reported to the FDA in an expedited fashion
All Unanticipated Adverse Device Effects will also be reported to the FDA within 10 working days as defined in 21 CFR 812.150.

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.

Lastly, a current list of investigators, including the names and addresses of all investigators participating in this trial, will be provided to the FDA every six months.

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. The total of 40 patients will be accrued in 106 months and then followed for at least 1 more year.

10.2 Analysis of the Primary Endpoint

The primary endpoint for this study is overall survival (OS). The primary null hypothesis is that 2 year OS rate is at most 55%. This hypothesis will be rejected if the 95% confidence interval for year OS rate computed from the estimated Kaplan-Meier survival curves will be entirely above 0.55.

10.3 Sample Size

Assuming that 40 patients will be accrued in 4 years and then followed for 1 more year there is 92% power to show that 2-year survival is greater than 55% if the true 2-year survival Is 75% 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-side test with alpha=0.05).

10.4 Assessment of the Secondary Endpoint
The secondary endpoints include (1) relapse-related mortality (RRM) rate at 2 years; (2) 2 year relapse rate; (3) the incidence and severity of graft-versus-host disease (GVHD); (4) engraftment rates; (5) the incidence of treatment related mortality (TRM) at 100 days.

The survival type secondary endpoints (relapsed-related mortality and relapse) will be evaluated similarly to the primary endpoint by estimating Kaplan-Meier survival curves. From these curves, the 95% confidence interval for 2 year rates will be computed. The estimates of incidence rates will be presented with corresponding 95% confidence intervals using the exact method.

10.5 Analysis of Safety
The safety date analysis will be descriptive. The estimates of the incidences rates will be presented with corresponding confidence intervals using the exact method.

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, we anticipate that 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.

In addition, it is estimated that up to 15 patients with matched sibling donors will undergo HSCT on this approach during the time that the research is open. The small number of patients undergoing matched sibling RIC HSCT in out 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.

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12.0 Appendices

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 ≥ 4 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.

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.