A Two Step Approach to Non-Myeloablative Allogeneic Hematopoietic Stem Cell Transplantation for Patients with Hematologic Malignancies

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Abbreviations

AABB	American Associate if Blood Banks
AE	Adverse Event/Experience
ALL	Acute Lymphocytic Leukemia
ALT	Alanine Aminotransferase
AML	Acute Myeloid Leukemia
ANC	Absolute Neutrophil Count
AST	Aspartate Aminotransferase
BID	Twice daily
BMT	Blood and Marrow Transplant
C&S	Culture and Sensitivity
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CLL	Chronic Lymphocytic Leukemia
CML	Chronic Myeloid Leukemia
CMML	Chronic Myelomonocytic Leukemia
CR	Complete remission
CRO	Clinical Research Office
CY/CTX	Cyclophosphamide
CX	Culture
DLCL	Diffuse Large Cell Lymphoma
DLCO	Diffusing Capacity of the Lung for Carbon Monoxide
DLI	Donor Lymphocytes Infusion
DS	Double strength
DSMC	Data and Safety Monitoring Committee
DSMP	Data and Safety Monitoring Plan
DNA	Deoxyribonucleic acid
EF	Ejection Function
FACT	Foundation of Accreditation for Cell Therapy
FDA	Food and Drug Administration
FEV	Forced expiratory volume
G-CSF	Granulocyte colony stimulating factor
GM-CSF	Granulocyte macrophage colony-stimulating factor
GVHD	Graft Versus Host Disease
GVT	Graft versus Tumor
Gy	Gray
HCT-CI	Hematopoietic Cell Transplantation-Comorbidity Index
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen

HPC	Hematopoietic Progenitor Cell
HSCT	Hematopoietic Stem Cell Transplantation
IRB	Institutional Review Board
IV	Intravenous
SKCC	Sidney Kimmel Cancer Center
KM	Kaplan-Meier
KPS	Karnofsky Performance Status
LVEF	Left Ventricular End Diastolic Function
MA	Myeloablative
MDS	Myelodysplastic Syndromes
MDS NOS	Myelodysplastic Syndrome Unclassified
MLE	Maximum Likelihood Estimation
MMF	Mycophenolate mofetil
MPA	Mycophenolic acid
MPD	Myeloproliferative disease
MV	Megavolts
NHL	Non-Hodgkin's Lymphoma
NRM	Non-Relapsed mortality
NM HSCT	Non-Myeloablative hematopoietic stem cell transplantation
OS	Overall Survival
PBSC	Peripheral Blood stem cell
PCP	Pneumocystispneumonia
PCR	Polymerase chain reaction
PI	Principal Investigator
PRC	Protocol Review Committee
RAEB	Refractory Anemia with Excess Blasts
RCMD-RS	Refractory cytopenia with multilineage dysplasia and ringed sideroblasts
RIC	Reduced Intensity
RRM	Relapse related mortality
SAE	Serious Adverse Event/Experience
SIADH	Secretion of anti-diuretic hormone
SPO2	Peripheral Capillary Oxygen saturation
TBI	Total Body Irradiation
TJU	Thomas Jefferson University
TJUH	Thomas Jefferson University Hospital
TMP-SMZ	Trimethoprim/Sulfamethoxazole
TRM	Transplant Related Mortality
WBC	White Blood Count

1.0 Introduction and Background

Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative therapy for many disorders of lymphohematopoiesis. (Armitage, 1994; Blume, 1993; Bortin & Rimm, 1986; Thomas, 1992; Thomas, 1995) While allogeneic transplants are often associated with lowerrates of relapse than autografts or conventional dose treatment, this advantage is partially offset by higher regimen related mortality. (Archimbaud et al., 1994; Attal et al., 1996; Ballester, 1993; Barlogie et al., 1997; Bensinger et al., 1996; Björkstrand et al., 1996; Cassileth et al., 1992; Cavo, Benni, Cirio, Gozzetti, & Tura, 1995; Couban, Stewart, Loach, Panzarella, & Meharchand, 1997; Gahrton, Tura, Ljungman, Blade, Cavo et al., 1995; Gahrton, Tura, Ljungman, Blade, Brandt et al., 1995; Mehta et al., 1998; Reece et al., 1995; Samson, 1992; Samson, 1996; Varterasian et al., 1997; Vesole et al., 1996; Zittoun et al., 1995) 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. (Antin & Ferrara, 1992; Brent, 1995; Champlin, 1991; Ferrara & Deeg, 1991; Irschick et al., 1992; Vogelsang & Hess, 1994) Nowhereis this more apparent than in published outcomes after HSCT for multiple myeloma. In many of these trials, there is a survival benefit with autologous HSCT over allogeneic HSCT, despitea graft versus myeloma effect, on the basis of the toxicity associated with the latter procedure. (Armeson, Hill, & Costa, 2013)

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 inthe absence of overt graft versus host disease (GVHD). (Horowitz et al., 1990) In acute myeloid leukemia (AML) and chronic myeloid leukemia (CML), for example, relapse ratesare higher in recipients of twin transplants than in GVHD-free recipients of matched sibling grafts. (Gale et al., 1994) 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. (Collins Jr. et al., 1997; Drobyski et al., 1993; Kolb et al., 1995; Mackinnon et al., 1995; Porter, Roth, McGarigle, Ferrara, & Antin, 1994)

1.1 Development of Less Intensive Conditioning Regimens

Based on this recognition that long term disease control is mediated through the transplanted immune system, HSCT 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 GVT effects of the donor immune system. They are associated with less treatment-related mortality (Hamadani, Awan, & Copelan, 2008) and have allowed older and heavily pretreated patients who otherwise would not tolerate the rigors of a fully myeloablative HSCT, to undergo transplant successfully. (Kroger & Mesa, 2008) 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. (Giralt et al., 1996; Giralt et al., 1997; Khouri et al., 1998; Slavin et al., 1996)

1.2 Ablative versus Non-Myeloablative HSCT

Despite the demonstration of successful outcomes after NM HSCT, this type of therapy is not universally successful. This is because in order to optimize the graft versus tumor (GVT) effects of the transplanted donor immune system, malignant cells should be controlled to a degree that they do not outgrow the graft. The "permissible" disease burden at the time of HSCT varies by the kinetics of the specific malignancy and to some extent, the dose intensity of the conditioning regimen which reduces the malignant burden. 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. (Aoudjhane et al., 2005; Martino et al., 2006; Vela-Ojeda et al., 2004) This issue becomes particularly important in diseases not known to have a strong GVT effect such as acute lymphocytic leukemia (ALL) (Fielding & Goldstone, 2008; Stein & Forman, 2008) and certain lymphoma subtypes, (Armand et al., 2008; Corradini et al., 2007; Smith, 2006) 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. (Couriel et al., 2004; Martino et al., 2006; Slavin et al., 1998) The latter approach, alternately referred to as "reduced intensity" (RIC) HSCT, has been associated with more TRM but less relapse. (Anderlini et al., 2005; de Lima et al., 2004) A NM HSCT approach that is not an "either/or" proposition has not been clearly identified.

Based on the typically more aggressive diagnoses of the population presenting for treatment to the Thomas Jefferson University (TJU) Blood and Marrow Transplant, RIC as opposed to NM approaches have been developed in recent years at our institution.

1.3 The TJU 2-Step Approach

Since 2006, investigators in the TJU Blood and Marrow Transplant Program have opened three (IRB # 06U.328, 11D.247, and 12D.501) 2-step haploidentical RIC HSCT clinical trials using cyclophosphamide (CY) as a tolerization agent. The initial trial completed accrual in 2010; the later trials are still accruing patients. The basic 2-step approach for both myeloablative (MA) and RIC HSCT is comprised of a chemoradiotherapy conditioning backbone which is followed by an infusion of donor lymphocytes (DLI) containing 2 x 108/kg T cells. The DLI results in a haploimmunostorm characterized by high fevers and in some cases, rash and diarrhea. During this time period, it is theorized that the activation of the donor lymphocytes results in GVT effects. This is based on literature documenting disease responses in the non-HSCT setting using a comparable dose of lymphocytes. (Colvin et al., 2009; Guo et al., 2012) The haploimmunostorm also results in the activation of the most alloreactive donor and host T cells. Two days after the DLI, CY is administered to eradicate these most reactive T cells establishing bidirectional tolerance and leaving behind a subset of less alloreactive T cells to form the basis of post HSCT lymphoid immunity. One day after the completion of CY, a CD 34 selected stem cell product is infused to restore hematopoiesis. In patients with controlled disease at the time of HSCT, overall survival rates (2-8 years of follow-up) have been 75% in the MA setting. (Grosso, Carabasi et al., 2011; Grosso et al., 2014) In terms of the 2 step RIC approaches, OS to date has also been high in fit patients with controlled disease at HSCT. However, in subgroups of patients who are older, are less physically fit, or who have multiple associated comorbidities, rates of

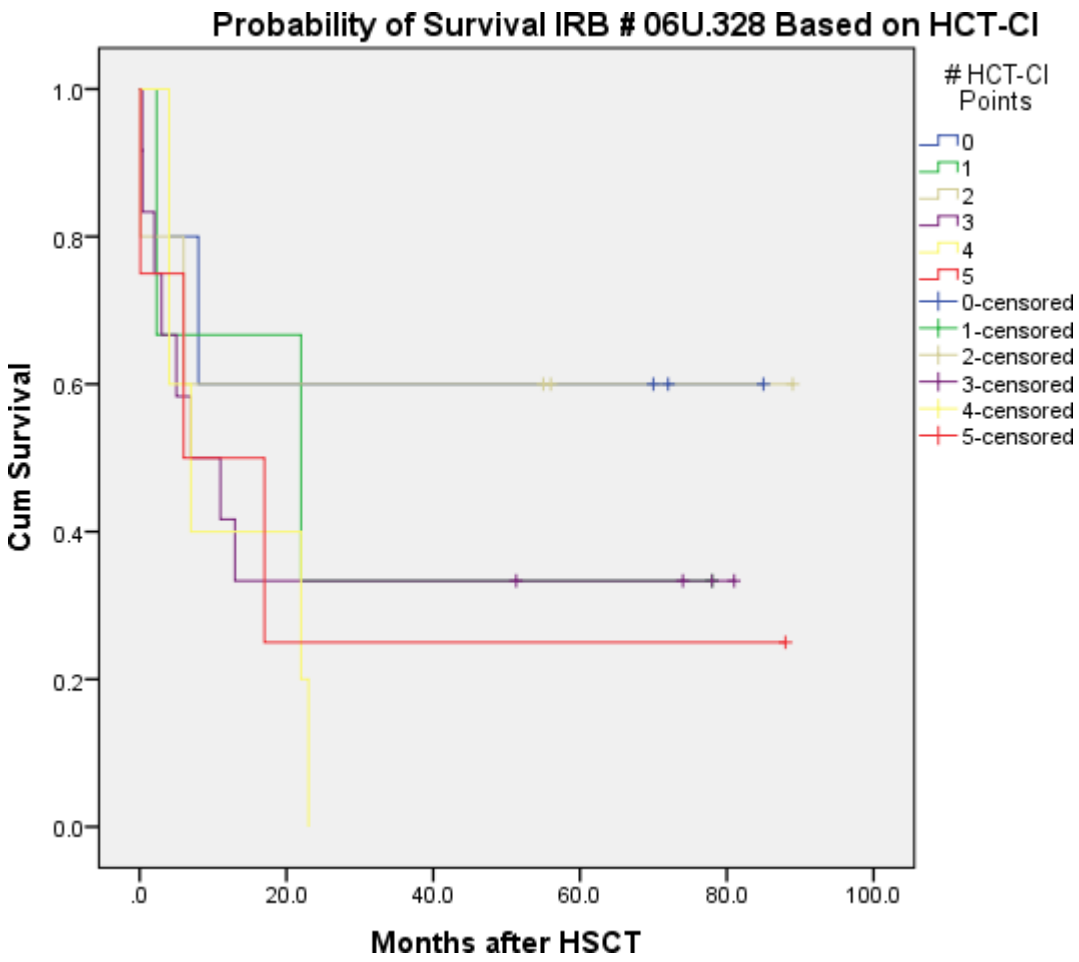
non-relapse mortality (NRM) have been greater than acceptable, resulting in decreased overall survival (OS). (Gaballa, Alpdogan, Carabasi, Filicko-O'Hara, Kasner, Martinez et al., 2014a; Grosso et al., 2010; Palmisiano et al., 2014)

Thirty-four patients were treated on the completed initial 2 step RIC trial (IRB # 06U.328). Diagnoses were AML (17), MPD (1), MDS (3), B cell ALL (3), CLL (3), Myeloma (2), Aplastic Anemia (1), DLCL (1), NHL (2), and CMML (1). Median age was 67 years old at the time of treatment. The patients are now a median of 76 (range 51 to 89 months) post HSCT with an OS of almost 50% at 12 months and 35% at the median follow-up time. Of the patients that died after being treated on the initial RIC trial, 50% died of relapse causes and 50% died of non-relapse causes. This is profoundly different from the MA 2 step trials in which relapsed disease is the primary cause of mortality and death from non-relapse causes is low.

Analysis of this initial trial revealed that multiple comorbidities were associated with decreased OS. While organ function guidelines were part of the eligibility criteria for this trial (as was typical for transplant protocols at the time of the initiation almost 10 years ago), the sum of all of all of the comorbidities that each patient may have possessed were not accounted for. For example, patients could minimally meet organ criteria across 2-3 systems and still be eligible for HSCT despite having cumulative comorbidities which would have predicted increased risk of toxicity. Conversely, gentler NM approaches to haploidentical HSCT have not been associated with high OS rates either. For example, the Hopkins group, with one of the largest experiences with haploidentical HSCT in the United States, reported a 1 year OS in younger patients (median age 46 years) undergoing NM haploidentical HSCT using post transplantation CY of 46% (event free survival at 1 year was only 34%) (Luznik et al., 2008) with mortality from recurrent disease the primary cause of treatment failure. Therefore, OS after RIC and NM haploidentical HSCT has been low, with causes of mortality varying with the intensity of the conditioning regimen.

To help increase the safety of HSCT at TJUH, a widely tested comorbidity index developed by Mohamed Sorror, the HCT-CI, (Sorror et al., 2005) was used in subsequent trials for risk assessment. Outcomes stratified by comorbidity points using this tool were first published in 2005, with further testing and widespread use occurring a few years after the initial publication.

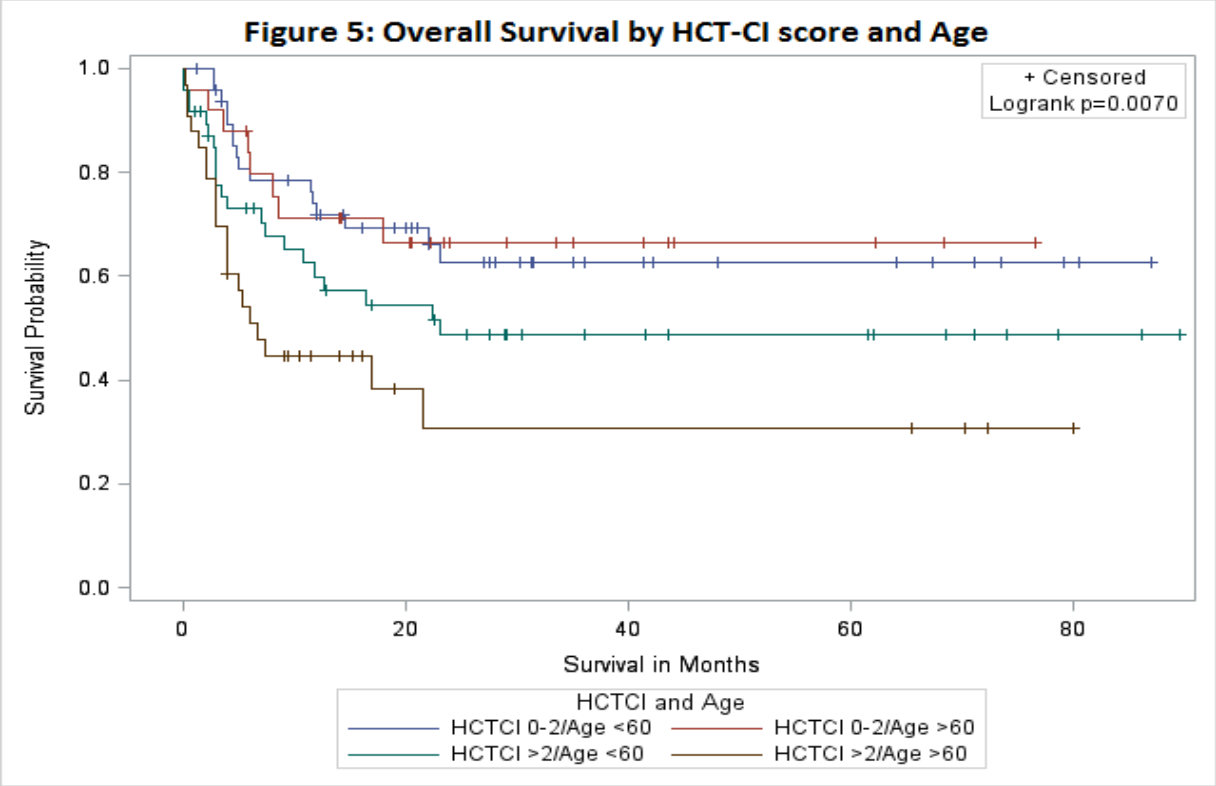
We retrospectively analyzed the outcomes of the 34 patients treated on the initial RIC 2 step trial and as seen below, the number of comorbidity points is also correlated with outcomes in this older population treated at our institution.



The other primary predictor of outcomes after HSCT is age at the time of the procedure. Accounting for age in association with the HCT-CI strengthens the predictability of OS probability of the HCT-CI. This has been demonstrated by Dr. Sorror and colleagues in a multivariate analysis of over 3000 patients undergoing HLA matched donor HSCT. (Sorror et al., 2014) In this analysis, 1 comorbidity point for age ≥ 40 was added to the HCT-CI scores for the patients analyzed. OS at 1 year for patients undergoing intensive regimens with HCT-CI scores of 3 and 4 (2 or 3 points plus age >40 years) resulted in an OS of approximately 60% and 50% at 12 and 24 months respectively, and for scores of 5 (4 points plus age >40 years) of OS of 40% and 30% at 12 and 24 months respectively.

Dr. Neil Palmisiano, as part of his graduate work in the Jefferson School of Pharmacology, performed a survival analysis based on the combination of age and HSCT of patients undergoing HSCT on the TJU 2 step trials to date. All of the trials use intensive conditioning regimens. The patients analyzed included those treated on both the first and 2nd generation 2-step RIC trials. Consistent with the Sorror et al. findings, Dr. Palmisiano found that patients with HCT-CI scores of 0-2 did well in terms of OS regardless of age, but comorbidity scores of >2 were not as well tolerated in older patients (> 40 years-Sorror; > 60 years Palmisiano) with increasing detriment correlating with increasing comorbidity score.

From Palmisiano:



As shown in the table above, patients greater than age 60 years with HCT-CI scores of > 2 have a 1 year OS of approximately 45%, decreasing to about 30% at 2 years. While these outcomes are similar to Dr. Sorror's data based on HCT-CI score alone, we note that 87% of patients in the Sorror et al. analysis were < age 60, while the median age of the population in the initial 2 step RIC trial was 67 years. This highlights the fact that at TJUH, the cut-off age for the same outcomes based on the HCT-CI is higher. Other analyses support the age of 60 years or greater as a point of increasing vulnerability in the 2 step population. Dr. Sameh Gaballa et al.(Gaballa, Alpdogan, Carabasi, Filicko-O'Hara, Kasner, Martinez et al., 2014b) performed a multivariate analysis of factors influencing outcomes after the TJUH 2 step approach in older patients. In addition, an internal analysis of all patients treated to date on the 2 step trials (n=163), show the beginning of a decreasing probability of OS at age 60 years, further supporting that demarcation as significant in the 2 step trials (Grosso, institutional data).

1.4 Addressing the Needs of a Growing Population

In 2011, a second generation 2-step RIC trial for patients with good risk disease was opened at TJUH. Good risk was defined as having leukemia in remission or an indolent lymphoma such as CLL or follicular lymphoma in which GVT effects were stronger and the incidence of post HSCT relapse was lower based on these more indolent diagnoses. The twenty-three patients treated on this trial to date have a median age of 56 (range 20- 74 years) with a median follow-up of 25.5 (range 2-40 months). In part based on the more stringent entry criteria that have been adopted in the TJUH Blood and Marrow Transplant Program for the second generation 2 step trials, all of the patients were discharged from the HSCT stay and lived at least 12

months after HSCT. Furthermore, of the 4 patients that died, 3 succumbed to relapsed disease and only one patient died of complications of GVHD. The KM estimate of OS at 2 years is 81%. These excellent outcomes are based on the types of diseases transplanted (ie more indolent with less aggressive pretreatment history), the lower ages of the cohort, and the more stringent prescreening process based on comorbidity analyses added to the 2 step protocols. The majority of these patients were accrued by 2012 however, and only 1 patient wastreated on this trial in 2013. The primary reason for this precipitous drop in accrual is the development of targeted drugs in B cell malignancies which have dramatically changed the landscape of lymphoma treatment, with less patients requiring allogeneic HSCT to achieve disease control. This change in the field has shifted the cohort of patients requiring HSCT to those with acute leukemia, MDS, or highly resistant lymphoma, all more aggressive diseases requiring aggressive approaches. In addition, the median age of patients with AML and MDS atpresentation is > 60 years, resulting in a growing cohort of patients who are not only older, but often have multiple comorbidities related to their age and aggressive pre-HSCT treatment of their disease. Based on our own in-house analyses and the Sorror et al. data, much of this emerging population of older patient is unlikely to withstand the rigors of an RIC approach. This point was demonstrated above in the KM figure of the initial 2 step RIC trial in which the 34 patients (median age of 67 years), had significant decreases in OS when the analysis was broken down by HCT-CI points.

Of note in the Sorror study, (Sorror et al., 2014) patients undergoing NM HSCT as opposed to RIC and MA HSCT tolerated a HCT-CI/age score (=HCT-CI score plus 1 if > than 40 years), of 3-4 points better in terms of NRM. Patients undergoing NM HSCT with an adjusted HCT-CI/age score of 3-4 had a NRM rate of 17% at 2 years as opposed to 36% and 37% for RIC and MA HSCT respectively. Therefore, it is possible to decrease NRM in vulnerable patients (i.e. advanced age/increased comorbidities), with the use of a NM HSCT regimen. There is currently no 2 step regimen that is NM. Two year OS rates for patients with an HCT-CI/age score of ≥ 5 were less than 35% at 2 years in all categories of conditioning regimens.

1.5 Purpose of the Clinical Trial

This clinical trial is aimed at reducing NRM in the expanding population of high-risk patients with higher comorbidity scores and/or who are older, undergoing HSCT with the use of a gentler NM 2 step approach. To compensate for the higher risk of post HSCT relapse with this type of gentler approach, strategies to immunologically increase GVT effects will be employed. First, patients will undergo HSCT using the TJU 2 step approach, developed at Jefferson in 2006,which has been an associated with low rates of relapse in patients with controlled disease at HSCT.(Grosso et al., 2011; Grosso et al., 2014) The GVT effects associated with the 2 step approach are thought to be due to the use of a haploidentical donor, the administration of high doses of tolerized T cells, the selection of the most predicted alloreactive donor, and the avoidance of T cell polarization to a Th2 type by G-CSF. A second strategy to increase GVT effects will be the infusion of higher doses of donor stem cells, which can now be reliably obtained with the drug plerixifor. The administration of a high dose of CD34 cells has been associated with decreased relapsed rates in Jefferson 2 step patients (Gaballa et al., 2014a)and at other HSCT centers. (Gõmez-Almaguer et al., 2013; Klingebiel et al., 2010; Törlén et al.,2014)

2.0 Hypothesis and Objective

The hypothesis of this research study is that haploidentical HSCT using a NM 2 step approach will decrease NRM as compared to the historical 2 step RIC approaches. Immunological strategies to increase GVT effects will compensate for the gentler conditioning regimen such that relapse related mortality (RRM) will remain acceptable. Therefore, OS rates (impacted by NRM + RRM) will be higher at 1 year using a NM HSCT versus historical TJU 2 step RIC outcomes. Examples of vulnerable patients who will be treated on this protocol include but are not limited to patients with higher comorbidity scores, and/or who are older, or have had previous histories that predispose to higher toxicity. An example of patients who are predisposedto higher toxicities are patients who have received autologous HSCT in the past, who are heavilypretreated, or those who received agents such as bleomycin which can affect the lungs. Patients who do not require intensive conditioning may also be treated on this protocol.

Patients who are younger and fit, will continue to be treated on the 2 step RIC or MAregimens.

2.1 Primary Objective

Our primary objective is to demonstrate efficacy of this approach over the historical 2 step RIC approaches in the "vulnerable" population as defined above. To prove efficacy, 1 year OS of the patient population should be 60%, increased from 45% based on the Palmisiano analysis, and equal to the patients with HSCT-CI scores of >2 in the Sorror et al. analysis (87% of which were < 60 years old) *undergoing HLA matched* HSCT. If an OS of 60% were reached at 1 year, we would conclude that we have been able to improve OS in patients with a higher number of comorbid conditions and/or are older, undergoing HSCT at our institution, and that haploidentical HSCT using the 2 step approach is a safe alternative to HLA matched HSCT in this patient subset.

2.2 Secondary Objective

1. To compare the NRM and RRM rates at 1 year for patients treated on this study to the that of patients undergoing haploidentical RIC HSCT as reported in the literature and as observed in the 2 step RIC trials (06U.327 and 12D.501).
2. To determine the incidence and severity of graft-versus-host disease (GVHD) in patients undergoing treated on the TJU RIC 2 step approach.
3. To evaluate engraftment rates and lymphoid reconstitution in patients treated in the TJU RIC 2 step approach.

3.0 Patient and Donor Selection

3.1 Patient Selection

The majority of patients treated on this protocol will have have a history of heavy pretreatment, or an age or comorbidity score which is higher than allowable on the current TJUH 2 step trials. Because of the NM conditioning regimen, patients must not have rapidly evolving disease as described below:

3.2 Inclusion Criteria

1. Patients treated on this study will have:
    a. Acute myeloid leukemia in morphologic CR not requiring treatment for their disease for 4 weeks

b. A history of AML with ≤10% residual blasts (use highest count on staging studies) after induction therapy and persisting with <10% blasts for at least 8 weeks without reinduction and at the time of HSCT.
c. RA or RARS or isolated 5q-
d. RAEB-1, RCMD+/-RS, or MDS NOS with stable disease for at least 3 months.
e. RAEB-2 must demonstrate chemo-responsiveness. Chemo-responsiveness is defined as a persistent blast percentage decrease by at least 5 percentage points to therapy and there must be ≤ 10% blasts (use highest count on staging studies) after treatment and at the time of transplant.
f. Hodgkin or indolent Non-Hodgkin's lymphoma
g. Myeloma with <5% plasma cells in the marrow
h. Myeloproliferative disorders (excludes CMML)
i. Aplastic Anemia
j. A hematological or oncological disease (not listed) in which allogeneic HSCT is thought to be beneficial, and the disease is chemoresponsive
k. Patients without clear manifestations of their disease status in terms of stage and/or responsiveness should be discussed with the PI and enrollment analysis should be documented in the study records.

2. Patients must have 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 their outcomes will not be part of the statistical aims of the study (see Statistical Section). The HLA matched related category includes patients with a syngeneic donor.
3. **Patients must have had front line therapy for their disease**.
4. Patients must have adequate organ function:
    a. LVEF (Left Ventricular End Diastolic Function) of ≥45%.
    b. DLCO (Diffusing Capacity of the Lung for Carbon Monoxide) ≥45% of predicted corrected for hemoglobin FEV-1 (forced expiratory volume at 1 second ≥50% of predicted
    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. HCT-CI/Age Score ≤5 points (Patients with greater than 5 points will be allowed for trial with approval of the PI and the Co-PI or his designee. This is an adjustment to account for healthy patients who meet the spirit of this protocol but have histories that result in higher than HCT-CI 5 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. KPS ≥90% patients older than 70 years, KPS ≥80% patients younger than 70 years
7. Patients must be willing to use contraception if they have childbearing potential.

3.3 Exclusion Criteria

1. Performance status <90% in patients 70 years or greater, <80% in patients less than age 70 years
2. HCT-CI/age score >5 points (Patients with greater than 5 points will be allowed for trial

with approval of the Principal Investigator and the Co-Principal Investigator or his designee. This is an adjustment to account for healthy patients who meet the spirit of this protocol but have histories that result in higher than HCT-CI 5 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.

3. A diagnosis of CMML, unless in morphologic CR
4. HIV positive
5. Active involvement of the central nervous system with malignancy
6. Inability to obtain informed consent from patient or surrogate
7. Pregnancy
8. Patients with life expectancy of ≤6 months for reasons other than their underlying hematologic/oncologic disorder
9. Patients who have received alemtuzumab or antithymocyte globulin within 8 weeks of the transplant admission. The absence of these therapies in the medical record will serve as documentation that they were not given.
10. Patients with evidence of another malignancy, exclusive of a skin cancer that requires only local treatment, should not be enrolled on this protocol

3.4 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 the 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, anti0HBc IgC 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.

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

Per FACT guidelines, pregnancy will be assessed during the initial donor evaluation and just prior to the start 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. The risks and hazards of the procedure, as well as alternative forms of therapy will be presented to the patient in detail. Patients will receive a signed copy if 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 risk 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:

	-11	**-10**	**-9**	**-8**	**-7**	**-6**	**-5**	**-4**	**-3**	**-2**	**-1**	**0**
AM		Fludara 30 mg/m^2	Fludara 30 mg/m^2	Fludara 30 mg/m^2	TBI 2Gy		Rest	Rest	CY 60 mg/kg	CY 60 mg/kg	Rest Start FK 506 & MMF	CD-34+ PBSC Infusion
PM					TBI 2 Gy	DLI						

May not use voriconazole until Day -1

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

Donor Schedule

	-7	**-6**	**-5**	**-4**	**-3**	**-2**	**-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 Plerixafor	G-CSF Plerixafor	

Table Definitions: G-CSF = granulocyte colony stimulating factor, PBSC = peripheral blood stem cell

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 steroids equivalent to 5 mg of prednisone through day -10. After day -10, a significant portion of the conditioning regimen is completed. 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.

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

The absence of agents that suppress lymphocyte reactivity and voriconazole in the medical record serves as documentation that they were not given.

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

5.1 Administration of Fludarabine

Fludarabine is administered for 3 days on (days -10 through – 8) at a dose of 30 mg/m2 IV. Creatinine should be checked prior to each dose of fludarabine. If renal insufficiency develops, the attending physician must be notified in cases where a dose adjustment needs to be made.

5.2 TBI

4 Gy of TBI will be administered in two divided doses of 2 Gy each on day -7. 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, because of the heterogeneous past histories of the patients to be treated on this protocol, all patients will be evaluated by the radiation oncologist for assessment of radiation toxicity in the context of previous treatment. In addition there may be technical or patient related factors which will require some minor modification in the TBI technique utilized. Selected patients may require local boosting of certain organ sites prior to conditioning therapy. 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. Approximately 18-27 liters will be processed the first day of donor lymphocyte collection. If a second day of collection is needed, the volume processed will be based on the amount of T cells required to meet the T cell target.

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

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

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

Panel:

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

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

All donors will be apheresed for lymphocytes on day -7. If the target number of CD3+ T cell lymphocytes, 2 x 108/recipient kg is not obtained, apheresis will be repeated on day -6.

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

Patients will receive 2 x 10^8/kg T cells on day -6. During the infusion, the patient will be monitored for any untoward reactions. Donor lymphocyte infusions will be administered by nursing staff experienced in the administration of blood products.

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

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. 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 days -5 to -1. They may additionally receive Plerixafor administered at a dose of 0.24 mg/kg/day in the evening on day -3 or -2. The donor will return for a primed progenitor cell collection on days -2 and -1. 18 to 27 liters will be processed per day. Collection days and Plerixafor dosing may be adjusted by one day less or one day more based on initial progenitor cell collection numbers. It is anticipated that with the use of Plerixafor, only one day of PBSC collection may be needed to achieve the desired CD-34 cell/kg dose. CD34+ cell enrichment will be performed via the closed system method using the CliniMACS® CD34 Reagent System (Miltenyi Biotec Inc., Auburn, CA). The CliniMACS system utilizes super-paramagnetic particles composed of iron oxide and dextran conjugated to monoclonal antibodies. These antibodies bind to target cells with the corresponding cell surface antigen (in this case, CD34). After magnetic labeling, the cells are separated using a high-gradient magnetic separation column. The magnetically labeled cells are retained in the column

and separated from the unlabeled cells. Removing the magnetic field from the separation column elutes the retained cells. Eluted cells will be characterized using fluorescent-activated cell sorting (FACS) analysis. All procedures will be performed in a sterile environment with strict adherence to all applicable regulations regarding the processing and use of human stem cells. The use of this device will conform to TJU BMT Laboratory standard operating procedures.

The target dose of donor PBSCs to be infused into the recipient is 15 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 2.0 x 10^7 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.

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

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

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

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

Significant red cell incompatibility between donor and recipient will be managed according to standard operating procedure, CL: Ppp033, of the Thomas Jefferson University Hospital Blood and Marrow Transplant Processing Lab. Pre-medications (if any) prior to PBSC infusion will be at the discretion of the physician.

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

5.6 GVHD Prophylaxis

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

due to interpatient variability.

If grades II-IV GVHD develop at any time after transplant (inpatient or outpatient), any GVHD treatment deemed necessary by the covering attending physician may be utilized.

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**	**Days 180-365**
History and physical with vital signs, including SPO2. Assessment of infectious signs, pregnancy test for females of childbearing potential done on baseline assessment	X	Every 1-2 days	Daily if in hospital weekly until day 28 after discharge	Twice Monthly	Monthly		Every other Month
Laboratory Studies*	X	Every 1-2 days	Daily if in hospital weekly until day 28 after discharge	Twice monthly or as clinically indicated	Twice monthly or as clinically indicated		Every other Month
Quantitative cytomegalovirus by polymerase chain reaction PCR		Weekly or as clinically indicated	Weekly until discharge or as clinically indicated	Twice monthly or as clinically indicated	As clinically indicated		Monthly or as clinically indicated

<u>GVHD Assessment:</u> Presence and degree of skin rash, presence and amount of diarrhea, LFTs	N/A	Daily after engraftment until discharge and then weekly if indicated	X	Twice Monthly	Monthly		Every other Month
<u>Chimerism/ Disease</u> Assessment							
Peripheral blood for CD3+ chimerism & Total chimerism			At d +28	Twice Monthly until >95% donor chimerism	Once d+90	Once d+180	As clinically indicated
Bone marrow exam (morphology, flow cytometry. Cytogenetics, buffy coat chimerism)			At d+28				As clinically indicated
<u>Immune Reconstitution Studies</u>							
Flow cytometry for lymphocyte subsets			At d+28		Once d+90	Once d+180	Once d +365
<u>Radiographic Studies:</u> In applicable situations for disease staging	X				Day +90 or as clinically indicated		Day +365 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 where applicable.**

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

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

6.1 Hematopoietic Engraftment: Defined as

Hematopoietic engraftment will be defined as:

- ANC ≥ 0.5 x10e9/L for at least 3 days
- Platelet engraftment > 20,000 with no transfusion x7 days

6.2 Toxicity Criteria
Regimen-related toxicity will be graded according to the NCI Common Toxicity Criteria version 4.0.

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 non-serious), regardless of relationships to study treatment, from the time of enrollment until d+100 after transplant. The following events are expected side effects of high-dose chemotherapy and transplant and will be recorded but not reported:

- Alopecia, headache, dry skin
- Emesis from chemotherapy or other agents unless refractory to standard supportive care, nausea, anorexia
- Weight loss, cough, dry mouth
- Grades 1-3 fever
- Grades 1-3 infectious sequellae
- Grades 1-3 electrolyte imbalances
- Grades I-III abnormalities in alkaline phosphatase, AST and ALT
- Neutropenia/uncomplicated neutropenic fever
- Thrombocytopenia, petechiae, ecchymoses, minor vaginal bleeding, epistaxis, hemorrhoidal bleeding, or other similar bleeding events will not be reported. (Bleeding events requiring intervention such as endoscopy or radiologic evaluation will be reported)
- Anemia
- Grades 1-3 rash
- Grades 1-3 fatigue
- Grades 1-3 mucositis
- Grades 1-3 diarrhea
- Allergic or other reactions to drugs used for supportive care or GVHD prophylaxis unless grade 4-5

Serious adverse events 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.

6.6 Reporting to SKCC DSMC

All AEs and SAEs except those listed in section 6.5, safety and toxicity data, and any corrective actions will be submitted to the DSMC per the frequency described in the SKCC DSMP. The report to the SKCC DSMC will also include any unanticipated problems that in the opinion of the PI should be reported to the DSMC.

For expedited reporting requirements, see table below:

DSMC AE/SAE Reporting Requirements

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

6.7 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.

6.8 Study Endpoint
The endpoint of this study is OS at 1 year.

7.0 Supportive Care
7.1 Avoidance of Infection

It is recommended that IVIG 0.5 g/kg IV is administered every 4 weeks post-transplant to support immune function, until the IgG level is ≥ 500 mg/dL on 2 consecutive monthly measurements. The first dose will be administered on day +7 if the patient is clinically stable enough to receive it. If not, IVIG should be administered as soon as feasibly possible after d +7. .

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, usually voriconazole 200 mg BID. It is at the discretion of the treating attending physician to change agents as clinically indicated.

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

Patients post partially-matched related donor transplantation will be maintained on PCP prophylaxis, usually TMP-SMZ DS 1 tablet daily weekly. It is at the discretion of the treating attending physician to change agents based on culture results, drug intolerance.

Prophylactic medications may be discontinued at the discretion of the attending physician. Their absence in the medical record serves as documentation that they were discontinued.

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 to target a hemoglobin ≥ 7-8g/L.

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

It is recognized that values for hemoglobin and platelet count may go below targets as these labs are not continuously checked and a time delay need to order blood products.

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. GCSF 5μg/m2 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 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 as a 60 mg/kg/continuous IV infusion over 24 hours starting 30 minutes 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.

8.2 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 cell product to the recipient are provided in the treatment section. All drugs 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.

8.3 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 3 days on (days -10 through – 8) at a dose of 30 mg/m^2 IV. Creatinine should be checked prior to each dose of fludarabine. If renal insufficiency develops, the attending physician must be notified in cases where a dose adjustment needs to be made.

Reference: MicroMedex Health Care Series, Thomson

8.4 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.

8.5 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.

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

8.6 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 other unforeseen circumstances in which the drug is felt to be deleterious to the plan of care.

8.7 Plerixafor
Mechanism: Reversibly inhibits binding of stromal cell-derived factor-1-alpha (SDF-1α), expressed on bone marrow stromal cells, to the CXC chemokine receptor 4 (CXCR4), resulting in mobilization of hematopoietic stem and progenitor cells from bone marrow into peripheral blood. Plerixafor used in combination with filgrastim results in synergistic increase in CD34+ cell mobilization. Mobilized CD34+ cells are capable of engrafting with extended repopulating capacity.

Metabolism: Plerixafor is absorbed rapidly after subcutaneous administration and is distributed primarily in the extravascular fluid space. The terminal half-life elimination is 3-6 hours and the drug is excreted in the urine, about 70% as parent drug.

Incompatibilities: There are no known significant incompatibilities.

Toxicities: Adverse reactions include fatigue, headache, dizziness, local injection site reaction, and arthralgia

Administration: Allogeneic donors may receive a dose of 0.24 mg/kg daily x 2 days in the PM of days -3 and day -2 as needed.

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. The drug dose will be titrated to a goal level of 7 ng/ml +/- 2, although it is recognized that there may be variations beyond the target range due to interpatient variability.

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 t Office (CRO) 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 then submitted to the PRC and IRB annually. 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.

The medical monitor will be a TJU physician who is not a collaborator in this trial. The medical monitor will review all adverse events (in addition to unexpected adverse events), safety data and activity data observed when this trial is ongoing. The medical monitor may recommend reporting

adverse events and relevant safety data not previously reported, and may recommend suspension or termination of the trial. The summary of all discussions of adverse events will be submitted to the DSMC after completion and included in the PI's reports to the PRC and the TJU IRB as part of the study progress report. The PRC, 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.

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.

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 SKCC 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, PRC and/or the Director of Clinical Investigations, SKCC. Studies are re-audited once they have achieved 50% of target accrual. Special audits may be recommended by the IRB, DSMC or PRC 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 CRO randomly audits at least 10 percent of all patients entered into therapeutic SKCC 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 PRC 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.

9.1 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 interventional study in which patients with hematological malignancies are treated with haploidentical HSCT using a NMA conditioning regimen.

10.2 Accrual and Study Duration

The total of 35 patients will be accrued in about 6 years and 6 months and then followed for at least 1 more year. The total study duration is approximately 7 years 6 months.

10.3 Analysis of the Primary Endpoint

The primary endpoint for this study is OS at 1 year post HSCT.

The primary null hypothesis is that 1 year OS rate is at most 45%. 45% is the figure representing the OS at 1 year of patients treated on the initial TJU 2 Step RIC HSCT trial and would represent a failure to improve OS in patients undergoing haploidentical HSCT at our institution and others reported in the literature. Alternative hypothesis is OS at 1 year of 60%. OS will be estimated using Kaplan-Meier curves. The 1-year OS rate and corresponding 95% confidence interval will be estimated from the Kaplan-Meier curve for the OS. The null hypothesis will be rejected if the lower bound of the 95% confidence interval for the 1-year OS rate is above 0.45.

10.4 Sample Size

Assuming that 35 patients will be accrued in 6 years and 6 months and then followed for 1 more year there is 74% power to show that 1-year OS is greater than 45% if the true 1-year survival is 60% or higher (calculations are based on the assumptions of uniform accrual over time, no loss to follow-up, exponentially distributed death times, and use of the exponential MLE one-sided testwith alpha=0.05).

10.5 Assessment of the Secondary Endpoint

The secondary endpoints include (1) relapse-related mortality (RRM) rate at 1 year; (2) NRM at 1 year; (3) the incidence and severity of graft-versus-host disease (GVHD); (4) engraftment rates; and (5) the evaluation of lymphoid reconstitution monthly to every other month during the first year post HSCT. These secondary endpoints will be reported descriptively. RRM and NRM may also be estimated using Kaplan Meier curves and/or cumulative incidence analyses.

10.6 Analysis of Safety

The safety data analysis will be descriptive. The estimates of the incidence 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 20%, 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 5 patients with matched sibling donors will undergo HSCT on this approach during the time that the research study is open. The small number of patients undergoing matched sibling RIC HSCT in our transplant program precludes a separate research protocol for that group. To prevent withholding of transplant therapy, these patients will be treated on this protocol. Only the outcomes of the patient group undergoing HSCT from haploidentical donors (2, 3, or 4 antigen mismatches in the GVH direction) will be used in the analysis of outcomes for the statistical ends of the trial. Outcomes for patients with matched sibling donors will be reported descriptively.

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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 Unit
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 4 Gy delivered administered in two divided doses of 2 Gy each on day -7.

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.

RefWorks: New Mini Haplo File