# THOMAS JEFFERSON UNIVERSITY
# Sidney Kimmel Cancer Center

## A 2 Step Approach to Matched Related Hematopoietic Stem Cell Transplantation for Patients with Hematological Malignancies-5+5 Dosing

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## Signature Page

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Principal Investigator:

Signed: _____ Date: _____

Name: Usama Gergis, MD

Title: Chairman, Medical Oncology

## Statement of Compliance

This study will be conducted in accordance with the International Conference on Harmonisation guidelines for Good Clinical Practice (ICH E6), the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46), and Thomas Jefferson University research policies

## List of Abbreviations

| | |
|---|---|
| ALL | Acute Lymphocytic Leukemia |
| AML | Acute Myeloid Leukemia |
| AE | Adverse Event/Adverse Experience |
| CFR | Code of Federal Regulations |
| CIOMS | Council for International Organizations of Medical Sciences |
| CONSORT | Consolidated Standards of Reporting Trials |
| CRF | Case Report Form |
| CRO | Clinical Research Organization |
| CTCAE | Common Terminology Criteria for Adverse Events |
| CY | Cyclophosphamide |
| DSMC | Data and Safety Monitoring Committee |
| DSMP | Data and Safety Monitoring Plan |
| DFS | Disease-Free Survival |
| DLI | Donor Lymphocyte Infusion |
| FDA | Food and Drug Administration |
| GCP | Good Clinical Practice |
| GVHD | Graft versus Host Disease |
| GVT | Graft versus Tumor |
| HSCT | Hematopoietic Stem Cell Transplantation |
| HLA | Human Leukocyte Antigen |
| ICF | Informed Consent Form |
| IDE | Investigational Device Exemption |
| IND | Investigational New Drug Application |
| IRB | Institutional Review Board |
| MHC | Major Histocompatibility Complex |
| mHag | Minor Histocompatibility Antigen |
| MDS | Myelodysplastic Syndrome |
| MPN | Myeloproliferative Disease |
| MRD | Minimal Residual Disease |
| NHL | Non-Hodgkin Lymphoma |
| OHRP | Office for Human Research Protections |
| OS | Overall Survival |
| PHI | Protected Health Information |
| PI | Principal Investigator |

| | |
|---|---|
| PRC | Protocol Review Committee |
| QA | Quality Assurance |
| QC | Quality Control |
| Non-tolerized T Cells | Refers to the Residual T Cells in the CD34 Stem Cell Graft that have not be Exposed to Cyclophophosphamide |
| RRM | Relapse-Related Mortality |
| SAE | Serious Adverse Event/Serious Adverse Experience |
| SKCC | Sidney Kimmel Cancer Center |
| SOP | Standard Operating Procedure |
| TJU | Thomas Jefferson University |
| TJU 08D.85 | 1st Generation 2-Step Matched Related Trial |
| TRM | Treatment-Related Mortality |
| UAP | Unanticipated Problem |
| “5+5” | The proposed minimal amount of non-tolerized T cells (5.0 x $10^3$/kg) and CD34 stem cells (5 x $10^6$/kg) administered to patients undergoing HSCT on this clinical trial |

# Study Summary

**Title:** A 2 Step Approach to Matched Related Hematopoietic Stem Cell Transplantation (HSCT) for Patients with Hematological Malignancies-5+5 Dosing

**Précis:** Based on an analysis of outcomes of historical patients treated from 2007 to 2017 with a 2 step HSCT using matched related donors (TJU-08D.85), it was determined that post HSCT disease relapse represented the primary reason for treatment failure in patients not achieving long term survival. Relapse was associated with a failure to achieve 98% or better donor T cell chimerism in early post HSCT studies and its occurrence correlated with lower doses of donor CD3+ T and CD34+ stem cells. Incomplete, early chimerism in many cases represents a failure to control malignancy resulting in higher relapse rates. In this successor study, a minimal dose of donor CD3+ T cells (5 x $10^5$/kg) and CD34 stem cells (5 x $10^6$/kg), **"5+5"**, will be administered in an attempt to increase early donor chimerism to 98% or more for a higher percentage of patients. It is anticipated that higher earlier chimerism percentages will result in less relapse of the study population as compared to the historical patient group treated on TJU 08D.85.

**Objectives:** Primary: The primary objective of this study is to assess whether providing a patient with "5+5" dosing in a 2 step matched related donor HSCT increases the percentage of patients who achieve full donor T cell chimerism (defined by 98% or greater donor T cell chimerism) by 28 days post HSCT (d+28).

This objective will be measured by the peripheral blood T cell chimerism value at d+28.

Secondary: The secondary objectives are as follows:

-To assess d+90 chimerism in patients receiving "5+5" dosing

-To assess post HSCT relapse incidence in patients receiving "5+5" dosing

-To assess incidence of grade II-IV GVHD in patients receiving "5+5" dosing

-To assess TRM in patients receiving"5+5" dosing

These objectives will be measured by:

-Peripheral blood T cell chimerism at d+90

-Relapse rate at 1 year post HSCT

| | |
|---|---|
| | -The incidence of grades II-IV GVHD occurring within 1 year of HSCT<br>-Rate of TRM at 1 year post HSCT |
| **Population:** | Patients treated on this study will have a hematological malignancy in which treatment with HSCT is thought to be beneficial. The population will reflect the catchment area of Thomas Jefferson University and its referral centers. This area primarily includes Pennsylvania, New Jersey, and Delaware. Patients typically will have undergone primary treatment for their diagnosis and will be fit for the rigors of HSCT. Eligibility criteria in the protocol encompass evaluation of fitness. |
| **Phase:** | II |
| **Number of Sites:** | 1 |
| **Description of Intervention:** | After conditioning, patients will receive 5 x $10^6$/kg CD34 stem cells from their donor. The amount of T cells in the CD34 stem cell product will be at least 5.0 x $10^3$/kg. An amount of 5 x $10^3$/kg CD3+ T cells will be added back to any CD34 stem cell product with < 5 x $10^3$/kg donor T cells. No add back will occur in patients whose CD34 stem cell product already contains 5 x $10^3$/kg donor T cells after processing. With this intervention, patients will receive “5+5”: 5 x $10^6$/kg CD34 cells and 5 x $10^3$/kg donor T cells in the CD34 stem cell product. |
| **Study Duration:** | 6 years |
| **Participant Participation Duration:** | 1 year |
| **Estimated Time to Complete Enrollment:** | 5 years |

# 1 Introduction

## 1.1 Background Information

Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative therapy for many disorders of lymphohematopoiesis. For almost two decades, 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.[1, 2] This "graft versus tumor" (GVT) effect can occur even in the absence of overt graft versus host disease (GVHD),[3] and is the foundation for success in less intensive HSCT approaches.[4]

In human leukocyte antigen (HLA) matched HSCT, the basis for beneficial GVT effects arises from the recognition of minor histocompatibility antigens (mHags) on recipient tumors by the transplanted donor immune system.[5] This recognition can also occur with normal recipient tissues contributing to deleterious GVHD.[6] Nevertheless, HSCT using matched related donors is associated with less GVHD and treatment-related mortality (TRM) than HSCT using other graft sources such as family HLA haploidentical or matched unrelated donors.[7] In haploidentical HSCT, techniques used to counterbalance the effects of crossing the major histocompatibility (MHC) barrier such as T cell depletion or cyclophosphamide (CY) tolerization can result in high rates of TRM due to infection.[8] In unrelated donor HSCT, the risk of TRM is more specific to GVHD which is predominantly due to HLA mismatching, especially at the HLA DP locus.[9] Consequently, an HLA matched family member is still considered the "gold standard" HSCT donor source for most transplant centers.

## 1.2 Correlative Studies

### Matched Related Transplant Outcomes in all Diagnoses According to Center for International Blood and Marrow Transplant Research (CIBMTR)

Despite the well-known benefits of matched related HSCT, long-term overall survival (OS) after this procedure is less than 50% in most contexts requiring optimization of current techniques and application of novel approaches to improve outcomes. CIBMTR data reveals that for patients undergoing matched related transplant (all diagnoses), TRM in the forms of infection, GVHD, and organ failure are the primary causes of death within the first 100 days of matched sibling transplant. After 100 days, relapsed disease emerges as the primary barrier to success, accounting for 57% of all deaths.[10] Acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), myeloproliferative disease (MPD) and B cell acute lymphocytic leukemia (ALL) are the primary disease indications for allogeneic HSCT. The three year OS for patients with early stages of these diseases is 59%, 53%, 53%, and 59% respectively. OS falls below these percentages in patients with intermediate and advanced disease. For example in AML, the most common diagnosis in patients undergoing matched related HSCT nationally and in the Thomas Jefferson University Hospital (TJUH) transplant program, OS at 3 years is 51% and 27% respectively.[10]

### Disease Specific Data-Clinical Trials

#### Matched Related Transplant Outcomes in AML

One of the largest studies examining outcomes after matched related transplant was performed by The Dutch-Belgian Hemato-Oncology Cooperative Group and the Swiss Group for Clinical Cancer Research (HOVON-SAKK). In this analysis of 1032 patients with AML, 326 (median age 39 years) patients underwent a matched related HSCT. Cumulative incidence of relapse in this group was 32% at 4 years and TRM was 21%. DFS was 48% at 4 years[11] In the European Organization for Research and Treatment of Cancer Leukemia Group and Gruppo Italiano Malattie Ematologiche dell' Adulto (EORTC-LG/GIMEMA) acute myeloid leukemia (AML)-10 trial,[12] relapse rate for 293 younger patients (median age 35 years) who underwent matched related HSCT for AML was 30.4%. Disease-free survival (DFS) at 4 years was 52% for the whole group. This study also highlighted the importance of cytogenetics and age on outcomes. DFS for patients with intermediate and poor risk cytogenetics dropped to 48.5% and 43.4% respectively. Older patients (ages 36-45 years) experienced 21.1% rate of non-relapse death, double that of the youngest subgroup of patients ages 15 to 25 years. In a prospective, multicenter analysis of 155 early-stage patients with AML (66.3%) or ALL (33.8%) examining the effect of antithymocyte globulin on HSCT outcomes, Kroger et al. reported a 2-year DFS rate of 59.4% and 64.6% in the ATG versus non-ATG groups, with 2 year relapse rate of 32.2% versus 25.5% respectively. Median age of the patients was 39 versus 43.5 years respectively.[13]

**Matched Related HSCT Outcomes in MDS**

Patients with MDS comprise an older subset of the HSCT population and as such, long-term survival in older patients is approximately 30%.[14, 15] OS estimates vary based on age and stage of disease, but in this category tend to be lower than 50% even in good risk younger patients. Spitzer et al. reported on the outcomes of 102 younger, good risk, patients (median age 55 years) with myeloid malignancies undergoing an all chemotherapy approach to T-cell depleted HSCT. 44% of patients had a diagnosis of MDS, the majority of whom were in complete remission at the time of HSCT. Five-year DFS in the MDS group was 46.7% and cumulative incidence of relapse was 26.5%. The major cause of TRM in this study was infection, and cumulative incidence of TRM was 28.4% at 5 years.[16] Patients with early stage and very late stage disease do not benefit from HSCT due to early introduction of toxic treatment in the former and disease resistance and poor performance status in the latter. Della Porta et al.[17] stratified 1728 patients (median age 66 years) with MDS based on their International Prognostic Score-R (IPSS-R) and examined outcomes after HSCT. Patients with high or very high IPSS-R risk scores had an OS of 40% and 20% respectively at 5 years. Patients with intermediate risk disease fared better with an OS rate of 50% at 5 years. Probability of OS decreased with age across these categories. Deeg et al. examined the outcomes of 1007 patients with MDS undergoing HLA matched HSCT (a majority of whom had matched related donors) based on karyotype and International Prognostic Score (IPSS). Approximate relapse rates at 2 years were 15-45% for good versus poor risk karyotype and 20 to 40% based on IPSS score. The median age of the patients on the trial was only 45 years old.[18] Two-year post HSCT relapse rates varied with stage of disease-early stage-(15% to 31%, increasing with age) and advanced stage 30%-35% in a cohort of 535 patients with MDS in a large CIBMTR study specific older patients. OS in the MDS cohort varied by age and was 35% to 45% at 2 years.[19]

**Matched Related HSCT Outcomes in B- ALL**

The International ALL Trial (MRC UKALL XII/ECOG E2993) analyzed the outcomes of 310 patients with non-Philadelphia chromosome acute lymphocytic leukemia ALL undergoing matched related transplant in first remission. In this study, patients older than 35 years were considered high risk. Relapse incidence at 10 years was 24% and 37% for standard versus high risk patients respectively. Non-relapse mortality at 2 years for both groups was high: 19.5% and 35.8% for standard and high risk patients respectively. The relapse rate and TRM contributed to OS rates of 62% and 41% for patients with standard and high risk ALL respectively at 5 years.[20] In a comparison of 1458 patients with B-cell ALL undergoing HSCT from matched related (n=440) versus matched unrelated donors, Segal et al. reported 5 year relapse, TRM, DFS, and OS rates of 43%, 27%, 32% and 35% respectively in the matched related HSCT group. Median age was 41 years old with 62% in first remission.[21] A multicenter study[22] comparing HSCT outcomes of 221 patients with ALL, 103 of whom received grafts from a matched related donor. Median age of the matched related group was 32 years, 62% were in first complete remission. At 5 years, TRM was 43% and disease-free survival was 40% in this group. Sixty-five of the 221 patients died due to relapsed disease (29%) at a median follow-up time of 7.1 (range 0.03-103) months.

**Matched Related HSCT Outcomes in NHL**

Non-Hodgkin lymphoma is a heterogeneous group of diseases and therefore data specific to a disease subcategory is limited. Patients with two of the most common diagnoses in the NHL umbrella, chronic lymphocytic leukemia and follicular lymphoma, are primarily treated with reduced intensity or non-myeloablative regimens which in general are not applicable to the current study. Many patients with NHL undergoing allogeneic HSCT have a history of a failed autologous HSCT and therefore TRM in category is often high. A multi-center study from Japan analyzed the outcomes of 233 patients, median age 33 years, undergoing myeloablative HSCT from a matched or 1-antigen mismatched related (74%) or unrelated (26%) donor. The diagnosis of the group was primarily aggressive lymphoma of both B and T cell histologies. While outcome reporting was not separated based on donor source, long-term lymphoma-free survival was achieved in approximately 40% of patients and varied based on lymphoma subtype and chemo- sensitivity of the disease. TRM was the primary cause of death in this analysis and was 42% at 4 years.[23] The outcomes of a multicenter European Group for Blood and Marrow Transplantation Registry analysis of 101 patients with diffuse large cell NHL who relapsed after autologous HSCT and subsequently underwent allogeneic HSCT was reported by van Kampen and colleagues in 2011.[24] Median age of the study group was 46 years and the majority of patients (76%) had a matched related donor. Conditioning regimen was myeloablative or reduced intensity. Cumulative incidence of TRM and relapse at 3 years was 29% and 41% respectively. Probability of OS was 52.2% at 3 years, but by 5 years post HSCT, progression-free survival was only approximately 40%.

**Allogeneic HSCT in Patient in Older Patients**

The median ages of most of the patients in the studies cited above are far lower than the median age of the matched related population treated on the current TJUH matched related myeloablative

study of 50 years (TJU-08D.85). Age and OS are negatively correlated in HSCT and therefore DFS and OS will be lower in an older age patient population. Jakubowski et al. published the outcomes of older patients (median age 61 years) with good risk acute leukemias (mostly myeloid disease) undergoing HSCT with related donors. Despite early stage disease, the 4 year OS of this group was 44%, 15% lower than that reported by the CIBMTR for good risk AML.[25] Multivariate analysis by Tan et al. of 132 adult patients primarily with AML and ALL revealed that recipient age of > 35 years was associated with a 1.676 increase in hazard of death as compared to patients younger than 35 years. (P=0.006).[26]

**Literature Summary**

The literature regarding matched related HSCT, reviewed above, is also summarized below in Table 1. Interestingly despite large variations in diagnoses and approach, the studies show relatively consistently, that younger patients transplanted with early stage disease have relapse rates at 2 years between approximately 20-30%. In patients with later stage disease, or with longer follow-up, relapse rates can reach 40%. In the current TJUH matched related myeloablative study (08D.85), the patients were older with higher disease risk than many of the cited reports. Despite that, the 2 year cumulative incidence of relapse is consistent with the good risk reports at 24% at 2 years. However, as seen in Figure 1 (adjacent), with longer follow-up time, relapse after treatment on TJU 08D.85 reached 44% representing the major area for improvement in any second generation trial.

Figure 1. Cumulative Incidence Relapse TJU 08D.85

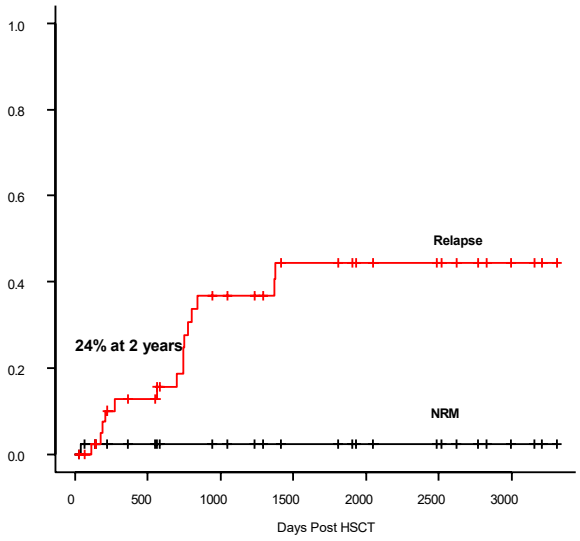


Table 1 Literature Review-HLA Matched HSCT Outcomes and Relapse

| Study | Disease | Number of Patients | Median Age | Disease Risk at HSCT* | Relapse Rate** | Year of Relapse Estimate |
|---|---|---|---|---|---|---|
| **Cornelissen et al.[11]** | AML | 326 | 39 | All CR-1 (good) | **32%** | @4 yrs |
| **Sucin et al.[12]** | AML | 293 | 35 | All CR-1 (good) | **Approx 25%** | @2 yrs |
| **Kroger et al.[13]** | AML | 155 | 39-43 | Good | **25.5-32.2%** | @ 2 yrs |
| **Spitzer et al.[16]** | Myeloid Malignan-cies | 102 | 55 | Variable | **26.7** | @ 5 years |
| **Deeg et al.[18]** | **MDS** | **1007** | **45** | **Variable** | **15-45% based on karyotype** | **@ 2 years** |
| | | | | | **20-40% based on IPSS** | |
| **McClune et al.[19]** | MDS | 535 | 55-59 | Variable | **Appr 30%** | @ 2 years |
| **Goldstone et al.[20]** | **ALL** | **310** | | **Varied (on risk and age)** | **20-25%** | **@ 2 years** |
| **Segal et al.[21]** | ALL | 440 | 43 | Majority good risk | **43%** | @ 5 years |
| **Kiehl et al.[22]** | **ALL** | **264** | **> 17 years** | **Majority good risk** | **25%** | **@ 5 years** |
| **Kim et al.[23]** | Aggress lymphoma | 233 | 33 | Various | **21%** | @ 2 years |
| **Van Kampen et al.[24]** | **Large Cell Lymphom a** | **101** | **46** | **Hi risk** | **41%** | **@ 3 years** |
| **Jakubowski et al.[25]** | Older Age Various | 214 (86 matched related) | 61 | Various | **23%** | @ 2 years |
| **TJUH-08D.85** | Various | 43 (evalua-ble-2 in treat-ment) | 50 | Primarily intermediat e | **24%** | @ 2 years |

*Risk based on age and remission status at HSCT

**Some relapse rates reported from relapse curves in the publication

**The Jefferson 2 Step Approach (TJU 08D.85)**

In the following section, the results of the current matched related HSCT approach at our institution (TJU 08D.85) will be reviewed and areas that require improvement based on results to date will be identified. These areas of needed improvement represent the rationale for the current study.

At Jefferson, a 2 Step approach to myeloablative HSCT was opened in 2008-TJU 08D.85. To date, 45 patients have been treated (43 evaluable). The regimen is depicted below:

| | -9 | -8 | -7 | -6 | -5 | -4 | -3 | -2 | -1 | 0 |
|---|---|---|---|---|---|---|---|---|---|---|
| AM | TBI | TBI | TBI | TBI | Rest | Rest | CY 60 mg/kg | CY 60 mg/kg | Tacrolimus &MMF* | CD 34+ selected HSCT |
| PM | TBI | TBI | TBI | TBI | | | | | | |
| | | | | DLI HSCT Step 1 | | | | | | HSCY Step 2 |

CY=cyclophosphamide; DLI=donor lymphocyte infusion; MMF=mycophenolate mofetil;
TBI=total body irradiation (total dose 12Gy)

The 2 Step approach was initially developed in 2005 and at that time, was used in patients undergoing haploidentical HSCT. In 2008, TJU-08D.85 was opened for patients with matched related donors. In the approach, a high, fixed dose of donor lymphocytes (DLI -2 x$10^8$/kg CD3+ T cells) are administered (Step #1 of HSCT) after a conditioning regimen of eight-1.5 Gy fractions of total body irradiation (TBI). After two days, CY is administered for bidirectional tolerization of the CD3+ cells with the goal of decreasing graft versus host disease incidence and severity. A day later, a CD34-selected stem cell product is administered (Step #2 of HSCT) to repopulate the marrow. The 2 Step approach 1) allows a fixed dose of T cells to be given providing a platform from which to compare and contrast outcomes, 2) avoids exposure of stem cells to CY, 3) allows increases in CD34 cell dosing without large increases in T cell content of the graft, and 4) circumvents exposure of the stem cell product to growth factors avoiding polarization of T cells to a TH2 cytokine type. Current outcomes of the patients treated on this protocol follows.

**2 Step Matched Related Myeloablative HSCT Outcomes-Initial Trial (TJU #08D.85) 2008 to 2017 (43 Evaluable Patients Based on Time Post HSCT)**

Patient data and major outcomes listed in Table 2 and Figures 2&3

Table 2 Patient Data TJU 08D.85

| ***Median Age (Years)*** | ***Diagnoses*** | ***Evidence of Disease at HSCT*** | ***Revised Disease Risk Index (RDRI)**** | ***Karnofsky Performance Score (KPS)*** | ***Hematopoietic Transplant Comorbidity Index Score (HCT-CI)*** |
|---|---|---|---|---|---|
| **50 Range (25-65)** | AML (41.9%)<br>MDS (14%)<br>PH+ ALL (11.6%)<br>B ALL (9.3%)<br>NHL (9.3%)<br>DLCL (4.7%)<br>CMML (4.7%)<br>CML (2.3%)<br>MPD (2.3%) | Yes 25.6%<br>No 74.4% | Intermediate Risk 67.4%<br><br>High Risk 30.2%<br><br>Very High Risk 2.3% | Median 90%<br>Range: (80-100%) | Median 3 points<br>Range: (0-8) points |

*Revised Disease Risk Index (Includes cytogenetic stratification)[27]

Figure 2 Relapse Related Mortality (RRM) and TRM-Censor Date 11-1-17 TJU-08D.85

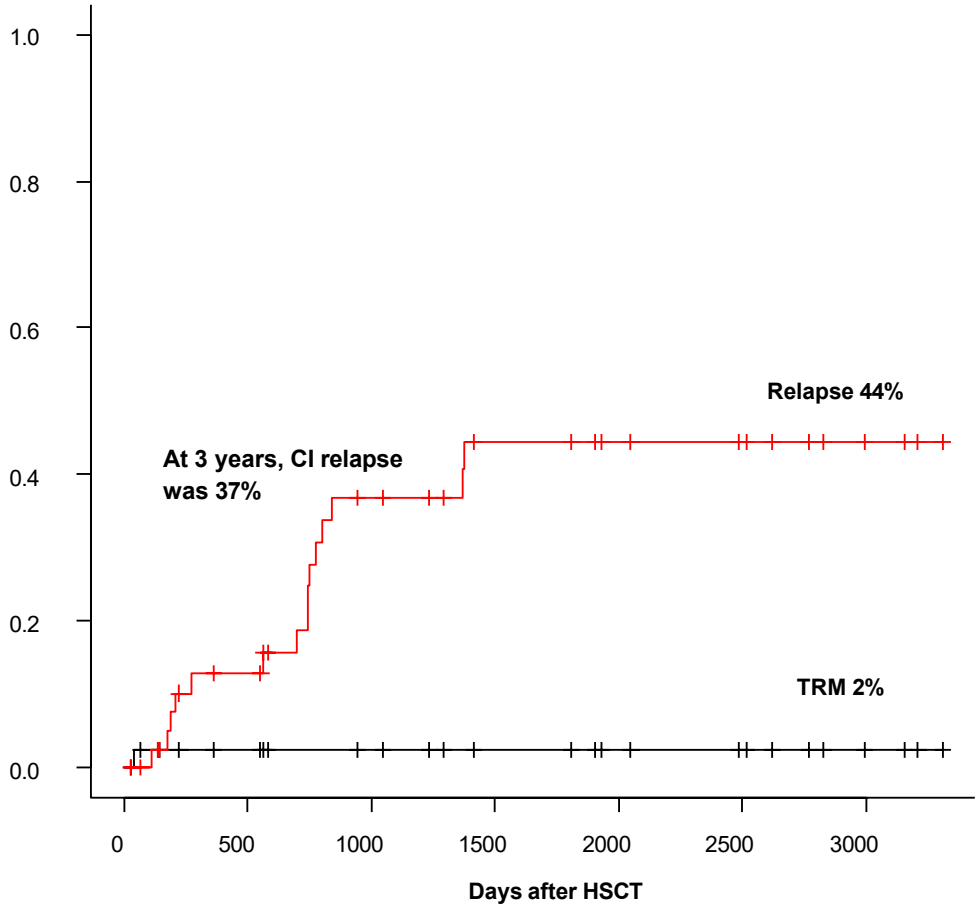
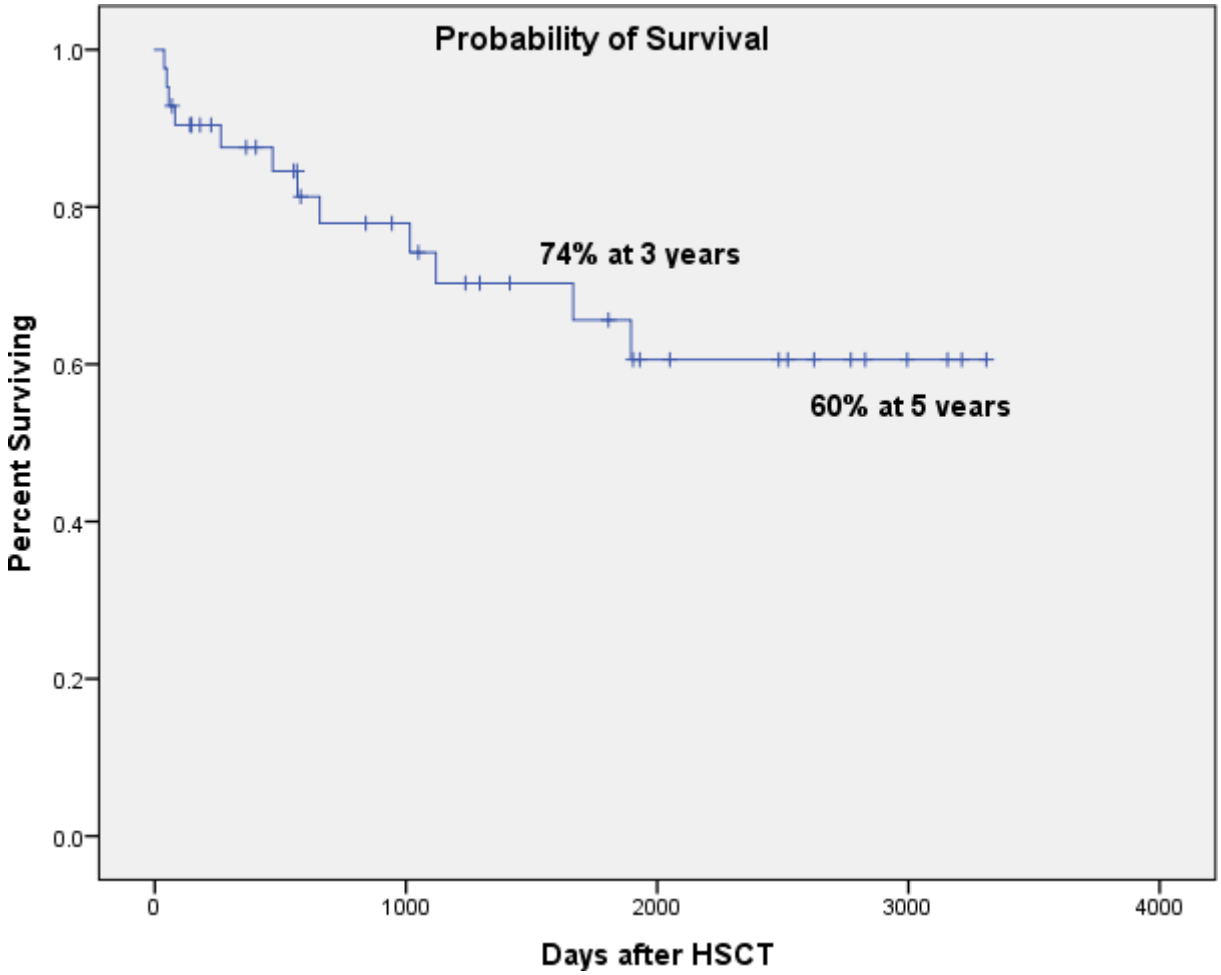


Figure 3 Overall Survival TJU-08D.85




The majority of patients on this initial trial had intermediate risk disease (no good risk patients), and were older with a median age of 50 years. In comparing the outcomes of the trial to those in the literature, the relapse incidence at 3 years of 37% is comparable to that in the literature for older higher risk patients, but is not significantly better. The TRM is far better than what is reported in the literature, resulting in a favorable OS rate as compared to other published trials. *The goal of this current successor trial is then, to decrease relapse incidence while not increasing TRM.*

## 1.3 Rationale for the Proposed Study

### Strategies to Decrease Relapse Rates in the Successor Trial to TJU-08D.85

Because relapse has been the primary barrier to success in matched related recipients of the 2 Step approach, strategies to decrease relapse rates center on increasing the intensity of the approach either through the conditioning regimen or immunologically. The development of relapsed disease early after HSCT is associated with either poor control of the disease at the

time of HSCT, or a failure of the conditiong regimen to sufficiently control disease long enough for the donor immune system to mature and eradicate disease immunologically. This point was highlighlighted by the recent Blood and Marrow Transplant Clinical Trials Network (BMT CTN) trial which showed that relapse-free survival at 18 months for patients with AML or MDS was significantly improved after undergoing a myeloablative (67.7%) as compared to a reduced intensity (47.3%) regimen (p=<0.001).[28] Patients treated on the TJU-08D.85 had fully myeloablative conditioning, and for the most part controlled disease at HSCT. The cumulative incidence of relapse figure above (Figure 2) is most consistent with late relapse of disease. Taken together, this data does not support conditioning regimen failure or status of disease at HSCT as the primary cause of relapse in the trial, suggesting that treatment failure in most cases was an immunological event. Therefore, the incidence of relapsed disease in patients treated on a 2 Step matched related HSCT is potentially reduced by intensifying the immunological effects of the regimen. Because the 2 Step approach is divided into myeloid and lymphoid portions, each immunological arm of the regimen can be intensified without direct impact on the other.

**Early Post HSCT Chimerism and Minimal Residual Disease (MRD) as Predictors of Relapse Following HSCT**

Because recurrent disease is the primary barrier to long term OS for the majority of patients undergoing matched related HSCT, efforts to identify the mechanisms of relapse and to develop preventative strategies have been ongoing for years. The establishment of early, complete donor chimerism is associated with decreased relapse rates, [29-32] even in indolent disease[33] with data supporting the early elimination of MRD [34-38] as the basis for this finding.

A greater percentage of patients undergoing HSCT on TJU 08D.85 experienced early mixed chimerism when compared to TJUH 2 Step haploidentical recipients. As of 11/1/17 and excluding patients who have had graft rejection or relapsed disease, differences in early T cell chimerism (d+28 and d+90) are shown in Table 3.

Table 3 Comparison of Chimerism Based on Donor Source in the 2 Step Approach

| Donor Source | Median day +28 T cell Chimerism (Range) | Median day +90 T cell Chimerism (Range) |
|---|---|---|
| **Matched Related** | 98% (64%-100%) | 99.5% (57%-100%) |
| **Haploidentical** | 100% (86%-100%) | 100% (97%-100%) |

This finding is possibly a reflection of the ability of haploidentical T cells to recognize and eradicate host immune cells more effectively than HLA matched T cells, especially in the context of CY tolerization used in both the matched and haploidentical 2 Step approaches. Interestingly, of 205 evaluable haploidentical versus 42 evaluable matched related recipients (247 total group HSCT

recipients), haploidentical recipients contributed 25.9% versus 35.7% for matched related recipients to the overall group relapse percentage (Chi-Square, p= 0.033).

**Does Failure to Achieve Early Complete Chimerism Correlate with Relapse in TJU 08D.85?**

As observed in Tables 4a and 4b below, the avoidance of relapse is significantly correlated with higher T cell chimerism 28 days after HSCT in 43 evaluable patients and 90 days after HSCT in 40 evaluable patients treated on TJU 08D.85.

**Table 4a Cox Regression d+28:**

**Variables in the Equation**

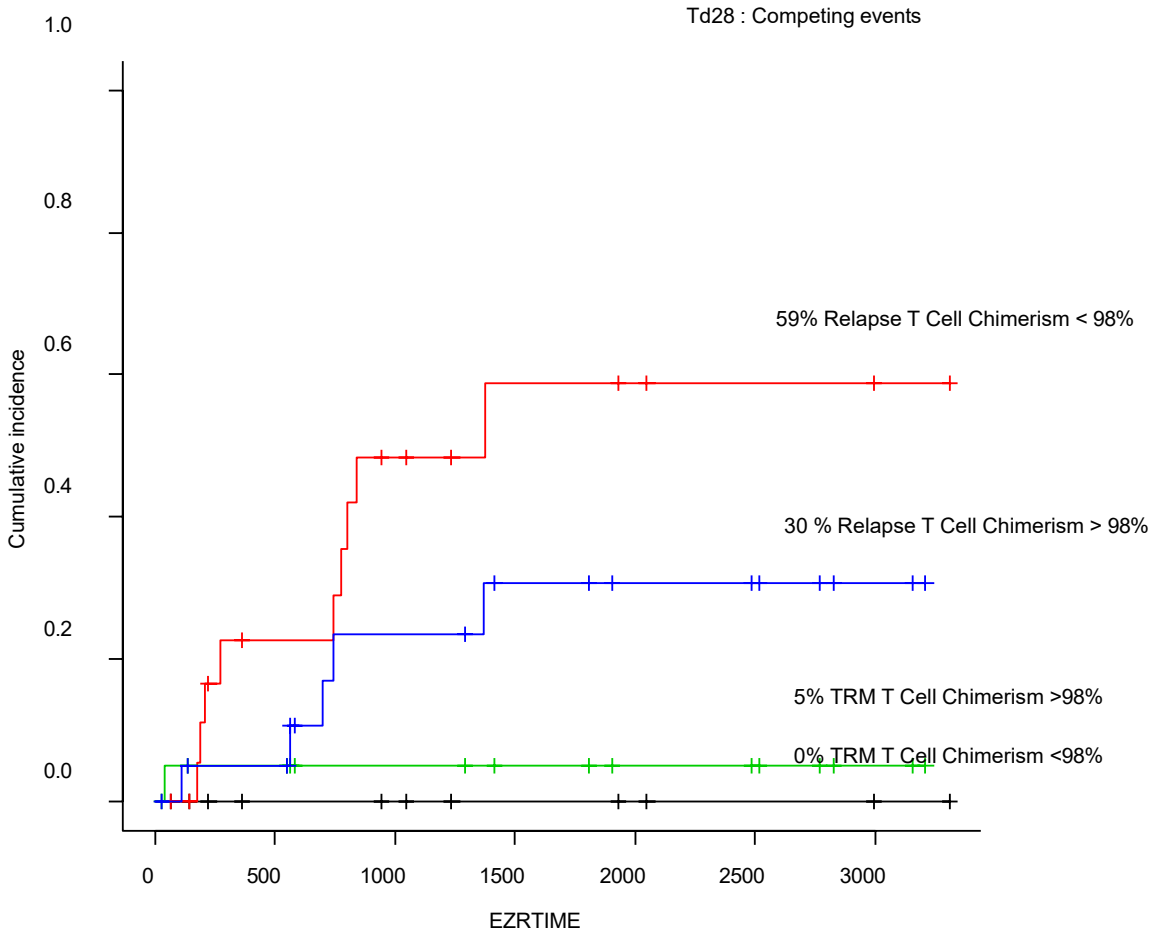
| | B | SE | Wald | df | Sig. | Exp(B) |
|---|---|---|---|---|---|---|
| **TChimerismD28** | -.078 | .040 | 3.842 | 1 | **.050** | .925 |
| Dz at HSCT | -.904 | .698 | 1.680 | 1 | .195 | .405 |
| Age | .004 | .022 | .035 | 1 | .853 | 1.004 |

**Table 4b Cox Regression d+90:**

**Variables in the Equation**

| | B | SE | Wald | df | Sig. | Exp(B) |
|---|---|---|---|---|---|---|
| Dz at HSCT | -.950 | .836 | 1.293 | 1 | .255 | .387 |
| Age | .004 | .027 | .024 | 1 | .876 | 1.004 |
| **TChimerismD90** | -.059 | .034 | 3.006 | 1 | **.083** | .943 |

Cumulative incidence testing also supports a relationship between relapse and incomplete early chimerism (Figure 4)




P=NS

## Can Early Chimerism be Increased in 2 Step Matched Related Recipients to Avoid Relapse?

All allogeneic CD34 stem cell products contain T cells, although the dose of T cells vary based on the specific approach to in-vivo and ex-vivo T cell depletion or the avoidance of T cell depletion altogether. In T cell depleted HSCT, residual T cell doses of < 5 x $10^4$/kg are associated with the circumvention of significant GVHD.[39] For this reason, while patients treated on a 2 Step approach receive a high dose of CY-tolerized T cells (2 x $10^8$/kg), the non-tolerized, residual dose of T cells in the CD34 stem cell product is kept below 5 x $10^4$/kg. The median residual T cell content in the CD34 stem cell products of recipients of TJU 08D.85 was only 2.95 x $10^3$/kg (range 0.96-17), far below this set limit. Therefore, the provision of a higher dose on non-tolerized T cells would

potentially increase T cell chimerism in the recipient, especially very early after HSCT (d+28) as

it is the expansion of this population which would make engraftment more robust as well as mediating GVT effects.[40] CY tolerization preferentially deletes alloreactive T cells and so it may be that a greater dose of non-tolerized T cells, still below the GVHD limit of 5 x $10^4$/kg, would increase engraftment without increasing toxicity. In addition, the provision of a higher CD34 stem cell dose may also increase T cell chimerism, likely more important at 90 days after HSCT, when lymphocytes arising from the engrafted stem cells appear in greater numbers and potentially sustain a GVT effect. The 2 Step approach is uniquely suitable to manipulate numbers of both untolerized T cells and CD34 stem cells.

All patients receive an identical fixed dose of *tolerized* T cells in step 1 of the treatment regimen, providing an ideal platform to contrast and compare outcomes. However, both the residual non-tolerized T cells component in the CD34 stem cell product as well as the dose of CD34 stem cells vary between patients (non-fixed). The fixed and non-fixed portions of the infused cells are shown in Table 5:

Table 5 Similarities and Differences in Allogeneic Cell Doses in 2 Step Recipients

| | **HSCT Step 1** | **CY Tolerization/ GVHD Prophylaxis** | **HSCT Step 2** |
|---|---|---|---|
| **Conditioning** | 2x$10^8$CD3+ cells/kg | CY 60 mg/kg/d x 2 | **Median**/(range) CD 34 cells/kg: **4.9** x $10^6$/kg (1.64-10.0) **Median**/(range) non-tolerized T cells in the CD34 product cells/kg: **2.9** x $10^3$/kg (0.96-17.0) |
| | All Patients | All Patients | Varied Between Patients |

Therefore, the non-fixed portions of the approach, the residual T cell content in the CD34 stem cell graft (non-tolerized T cells) and CD34 stem cell dose itself, can be increased in the regimen. We examined the outcomes of patients treated on TJU 08D.85 to determine whether with higher residual T cell and CD 34 cell doses in their allogeneic graft resulted in 1) increased early chimerism and 2) decreased rates of relapse

### The Effect of a Higher Graft Content of Non-Tolerized T Cells and CD34 Positive Stem Cells on d+28 Chimerism

The median non-tolerized T cell content of the CD34 stem cell graft of patients treated on TJU 08D.85 was 2.95 x $10^3$/kg. We found that 71.5% of patients receiving higher versus 28.6% of patients reciving lower than the median number of non-tolerized T cells in the CD34 graft achieved 98% or greated donor T cell engraftment at day +28, Chi Square, p=0.043. Similarly, 57.1% of

patients receiving higher than the median dose of CD34 stem cells, 4.90/$10^6$/kg, achieved 98% or greater donor chimerism at d+28 as compared to those patients receiving less than this median dose in which only 42.9% achieved achieved 98% or greater donor chimerism at d+28. This result was just short of statistical significance (Chi Square 0.080). At d+90, the effects of higher non-tolerized T cell and CD34 cell doses in the graft were still apparent. 52.4% versus 47.6% recipients receiving a higher versus lower median non-tolerized T cell dose and 52.4% versus 47.6% of recipients receiving a higher versus lower median CD34 stem cell dose achieved 98% or higher T cell chimerism. The d+90 results are less evident because 3 patients had already relapsed by d+90 and were taken out of the analysis. Two of the 3 had lower CD34 stem cell and T cell doses. These results are consistent with higher non-tolerized T cell and CD34 stem cell doses being associated with the achievement of higher percentages of earlier (days 28 and 90), complete donor chimerism post HSCT.

## The Effect of Higher Non-Tolerized T Cell Graft Content on Relapse and TRM

The above data supports the notions that 1) the achievement of early complete donor chimerism is associated with lower relapse rates, and 2) higher doses of non-tolerized T cells and CD34 stem cells in the graft are associated with the attainment of earlier complete donor chimerism. This data suggests an indirect, positive correlation between higher doses of graft non-tolerized T cells and CD34 stem cells and a reduction in relapse. If so, consistently higher doses would address the problem of relapse in the successor trial to TJU 08D.85. To assess this potential relationship further, TRM and RRM were examined based on the receipt of higher or lower than the median non-tolerized T cell doses and CD34 stem cell doses of the patients treated on TJU 08D.85. The impact of non-tolerized T cell doses on these outcomes are presented first, followed by the CD34 stem cell data.

## The Impact of Higher Doses of Non-Tolerized T Cells on TRM and RRM in Patients Treated on 08D.85.

Forty-two patients were evaluable for this analysis. We divided the group into those receiving higher versis lower than the median non-tolerized T cell dose of <2.985x$10^3$/kg. Twenty patients received the median non-tolerized T cell dose or lower, 22 were above the median. In cumulative incidence analysis**,** RRM was higher in patients receiving less than the median number of CD3+ T cells, while OS was higher in patients receiving more than the median number of residual T cells (Figures 5&6):

Figure 5

**Cumulative Incidence of TRM and RRM Based on the Dose of non-Tolerized T cells in the CD34 Product 42 Recipients Initial TJUH 2 Step Matched Sib Trial: Divide Point is Based on Median**

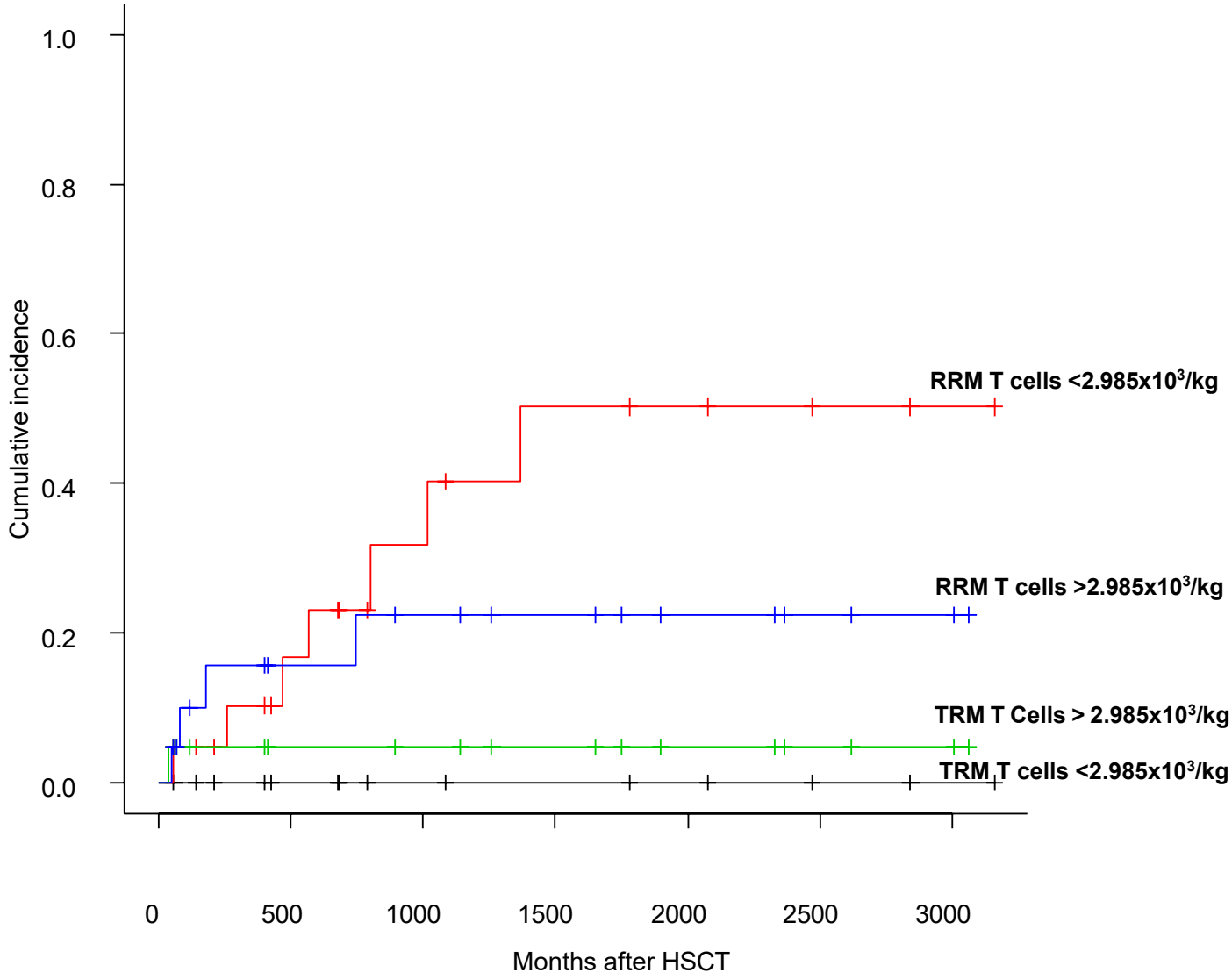
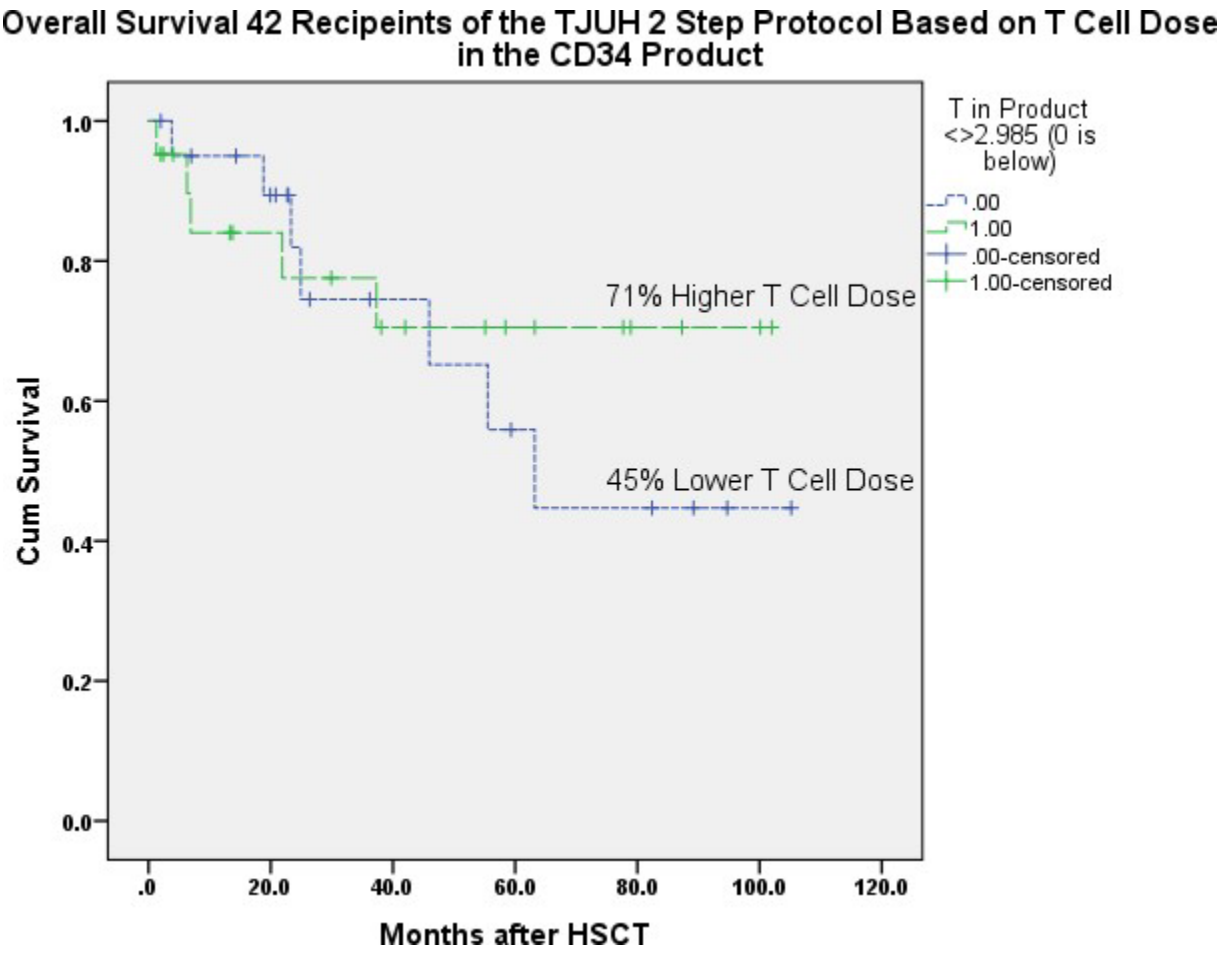


Figure 6




An independent sample T test also showed that the dose of the residual, non-tolerized T cells in the CD34 product appeared to have influenced RRM, TRM, and OS without adversely affecting GVHD incidence (Table 6a-c):

Table 6 a, b, c Independent Samples T Tests Survival, Relapse, GVHD

**a. Group Statistics**

| | Status | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| T Cells in Product (10e3) | Dead | 12 | 3.5625 | 2.83154 | .81740 |
| | Alive | 30 | 4.4120 | 3.96811 | .72447 |

**b. Group Statistics**

| | Relapse Status | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| T Cells in Product (10e3) | Relapse | 14 | 2.9986 | 2.76266 | .73835 |
| | No Relapse | 26 | 4.9377 | 4.05191 | .79465 |

**c. Group Statistics**

| | GVHD Categorical | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| T Cells in Product (10e3) | Grades 2-4 | 6 | 2.2167 | 1.66178 | .67842 |
| | None/Grade 1 | 34 | 4.5326 | 3.92130 | .67250 |

To further identify an optimal threshold for the dose of non-tolerized T cells in the CD34 stem cell product (ie what dose above 2.985 x $10^3$/kg is optimal), we performed both Kaplan Meier and cumulative incidence analyses (Figures 7 and 8) and found that *patients receiving > 5.40 x $10^3$/kg residual non-tolerized T cells had improved rates of RRM and OS without an increase in TRM* as compared to those patients who received a lower dose of T cells. We note that this dose is significantly below the dose of 5 x$10^4$/kg that has previously been identified as a practical threshold to avoid GVHD in haploidentical HSCT.[39]

Figure 7 What Is the Optimal Amount of T cells to be Left in CD 34 Product?-Survival

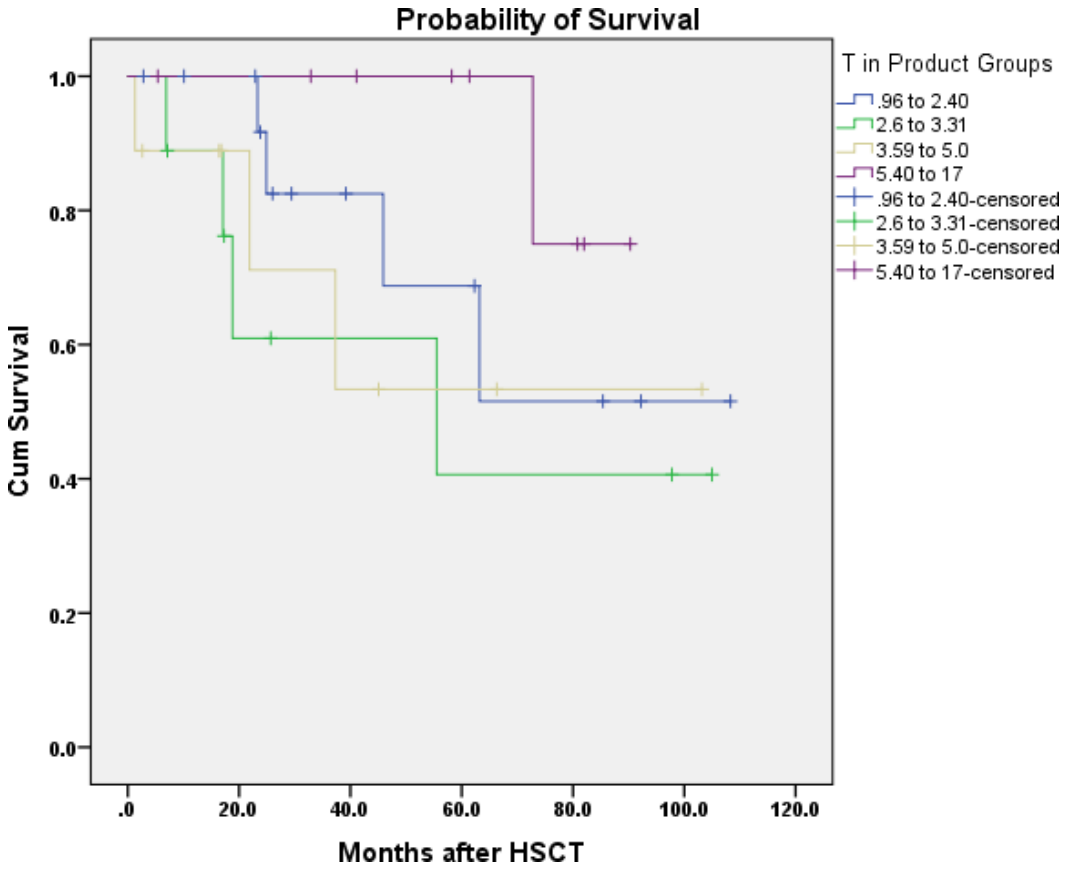
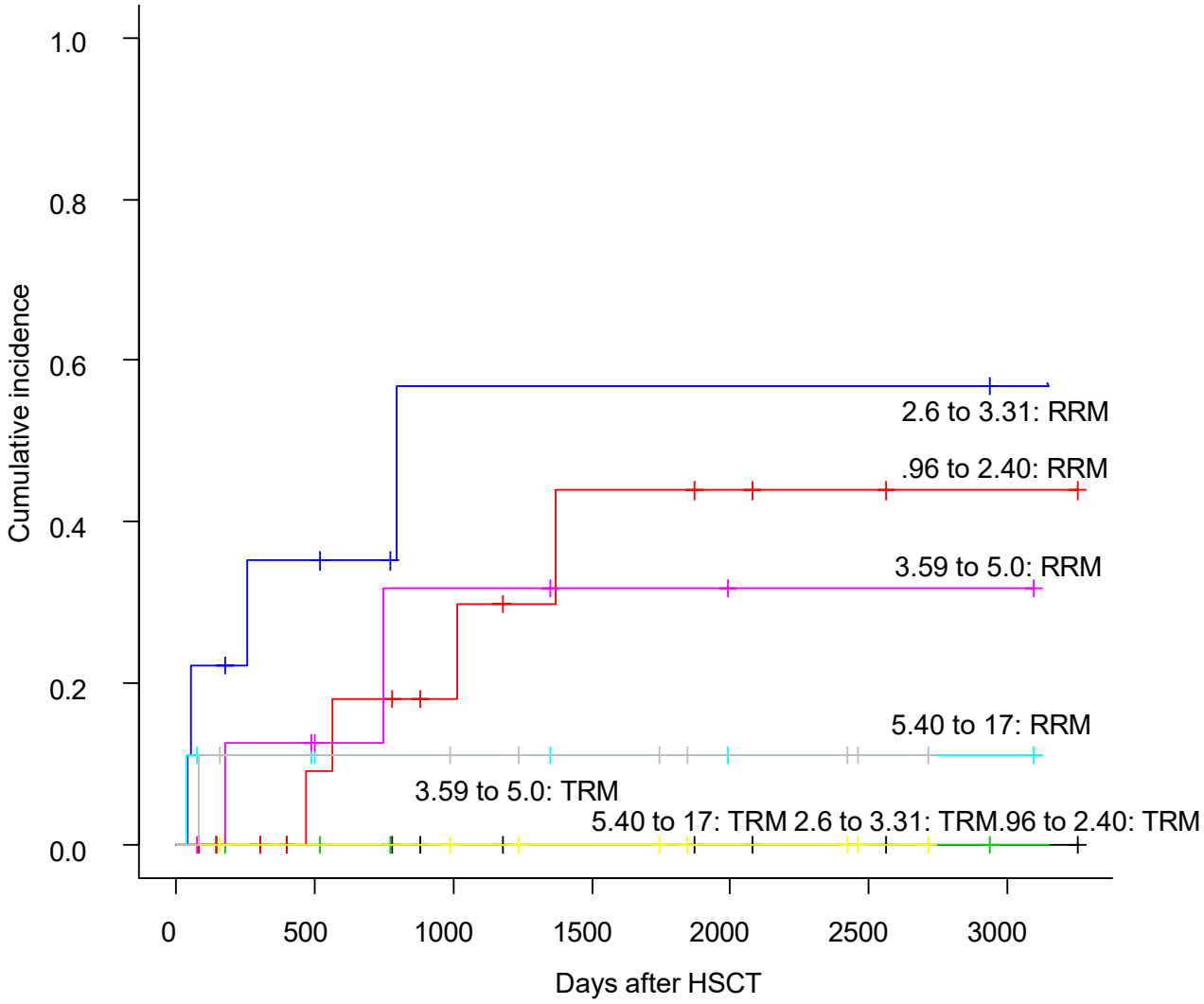


Figure 8 What Is the Optimal Amount of T cells to be Left in CD 34 Product?-RRM and NRM



## Differences in Outcomes TJU-08D.85 Based on CD34 Stem Cell Product Dose

Next, we examined patient outcomes based on CD34 stem cell dose. Again, 42 patients were evaluable, 20 received an equal or lower versus 22 who received a higher than the median dose of CD34 stem cells of 4.90 x $10^6$/kg. Cumulative incidence and Kaplan Meier analyses (Figures 9 & 10) revealed that relapse incidence and OS was improved in patients receiving higher than the median dose of 4.90 x $10^6$/kg donor stem cells without concomitant increase in TRM.

Figure 9 Cumulative Incidence of Relapse and TRM Based on CD34 Stem Cell Dose of >4.90 vs 4.90 or Lower

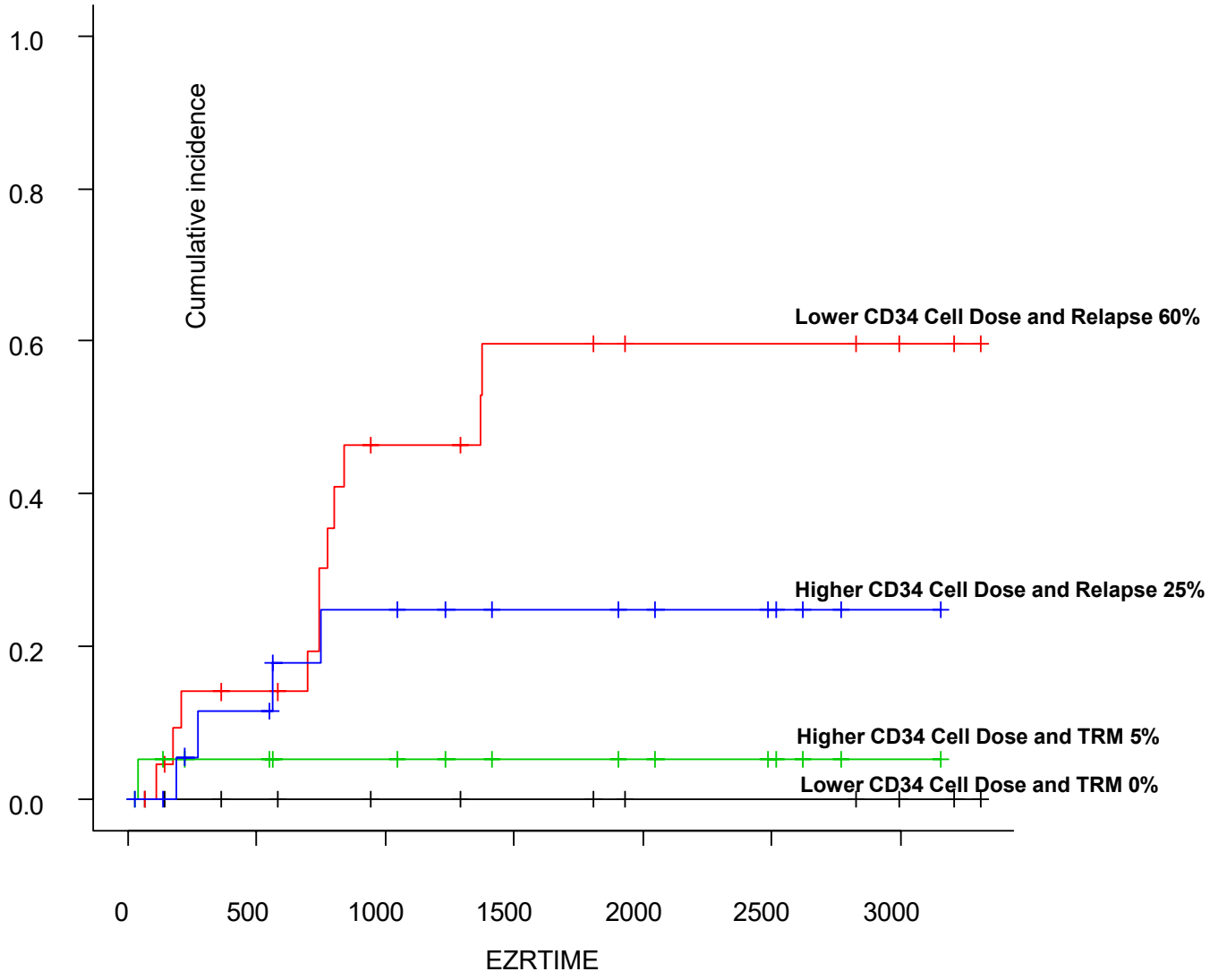
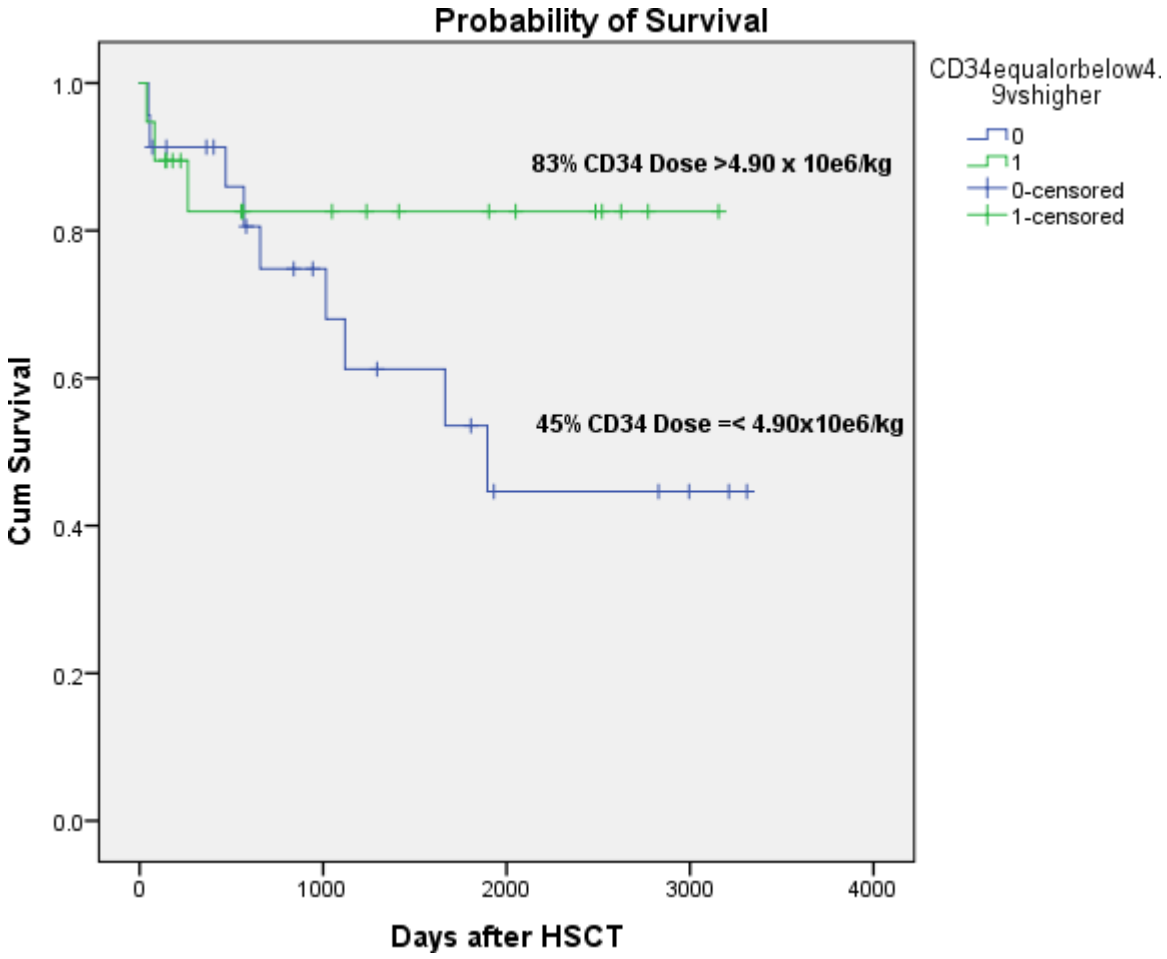


Figure 10 Probability of Survival Based on CD34 Stem Cell Dose of >4.90 vs 4.90 or Lower




To further identify an optimal threshold for the dose of CD34 cells, we analyzed OS, relapse and NRM based on a projected CD34+ stem cell dose above the median, as well as one that could be obtained from most donors without additional growth factor usage. At a CD34 stem cell dose of 6.0 x $10^6$/kg, cumulative incidence of relapse and OS remained higher in the higher CD34 dose group without increasing TRM (Figures 11-12):

Figure 11 Cumulative Incidence of Relapse and Treatment Related Mortality Based on CD34 Stem Cell Dose in Patients Undergoing TJU-08D.85

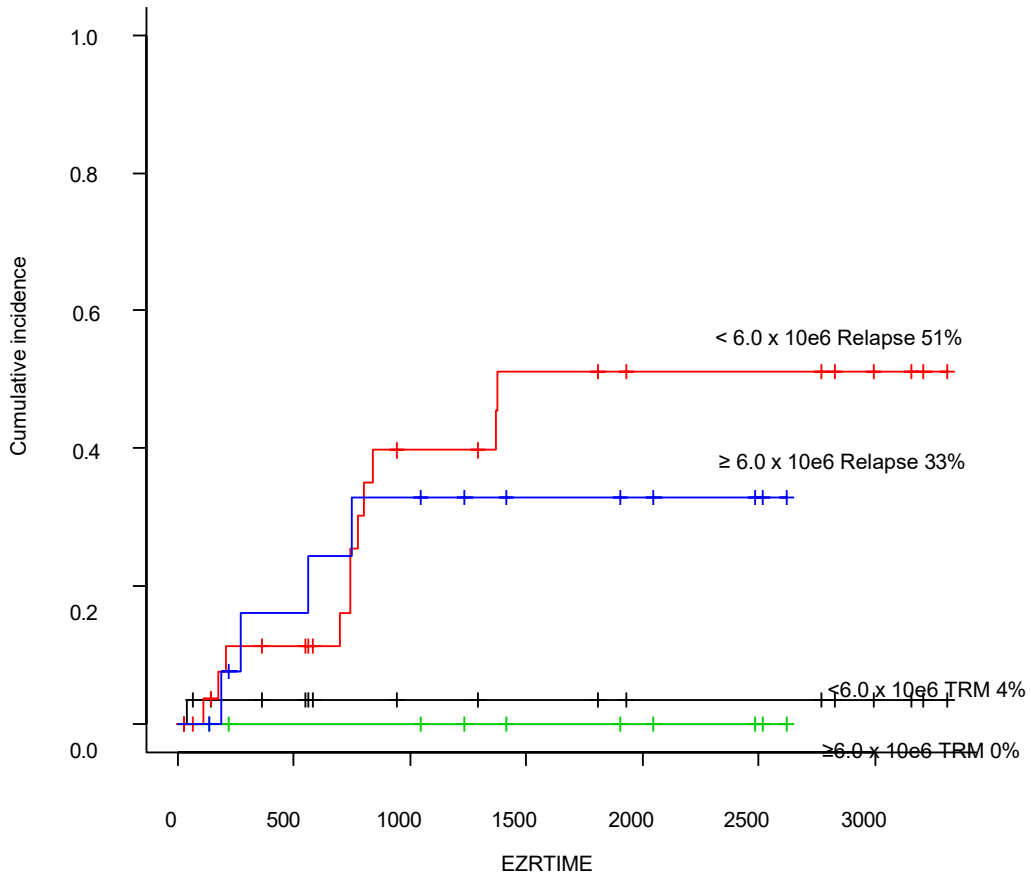
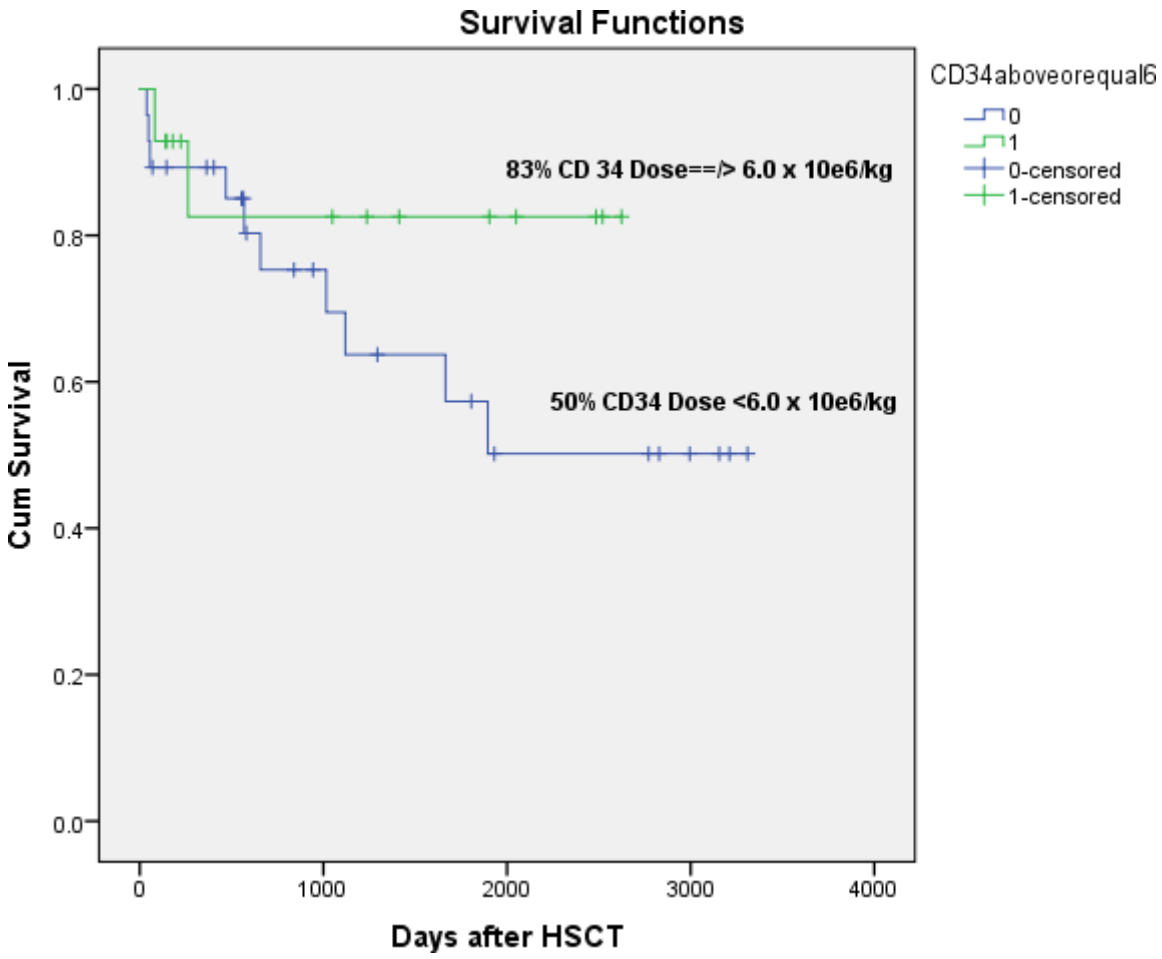


Figure 12 Probability of Survival Based on CD34+ Stem Cell Dose in Patients Undergoing Matched Related 2 Step HSCT (08D.85)



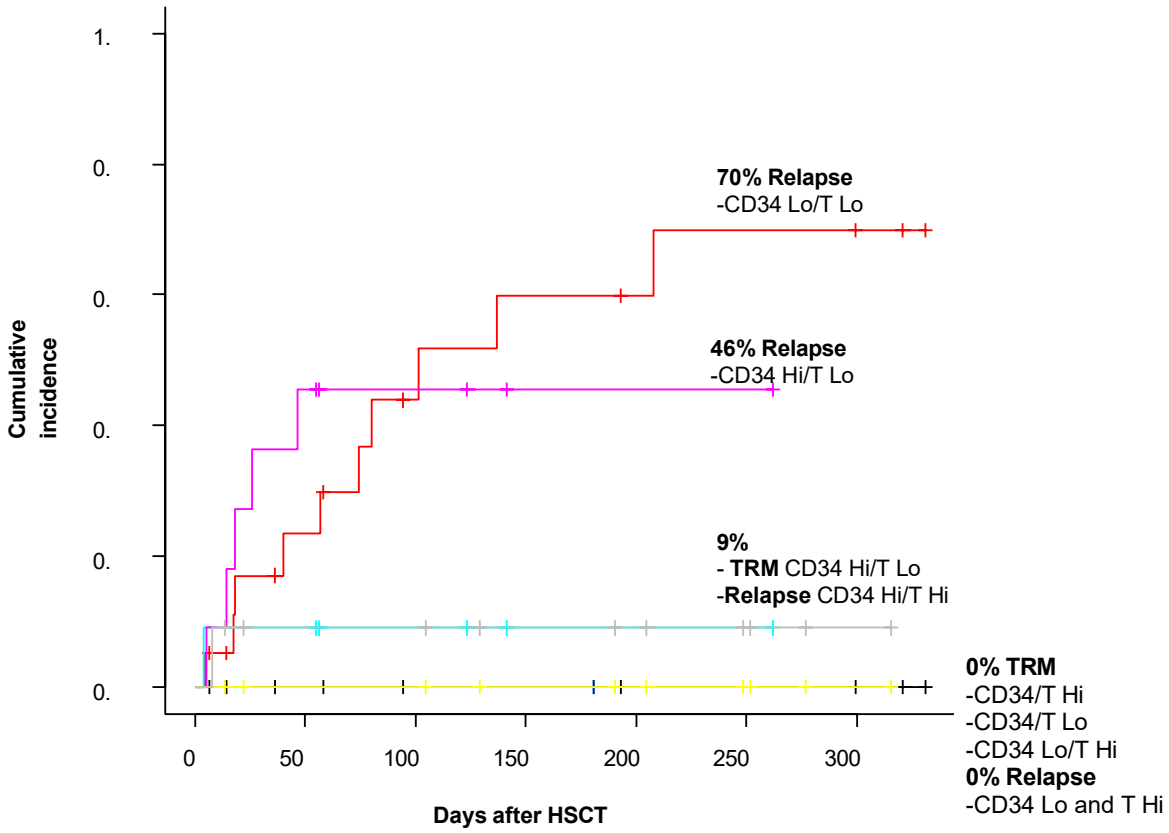

In comparing outcomes between a CD34 stem cell dose of >4.90 versus 6.0 x $10^6$/kg, (Figure 9 vs 11 and Figure 10 vs 12), we note no changes in TRM with the higher CD34 stem cell dose. However, the higher 6.0 CD34+ stem cell does not greatly impact RRM and OS as compared to the lower, 4.90 dose. Taken together, the data suggests that a target CD34 stem cell dose of 6.0 x $10^6$/kg would not increase TRM, but that there does not appear to be a large benefit in increasing CD34 stem cell dose above the median of 4.90 x $10^6$/kg.

**Outcomes Based on Optimized Non-Tolerized T Cell Dose AND CD34 Stem Cell Dose-"5+5"**

Having identified the potentially optimal doses of non-tolerized T cells and CD34 stem cells in the graft, we propose to administer these doses to patients on this successor trial to TJU 08D.85. Based on minor rounding of the above data, a non-tolerized T cell dose of 5.0 x $10^3$/kg and a CD34 stem cell dose of 5.0 x $10^6$/kg (5+5) represent optimal doses for patients treated on the successor trial. We examined the combinatorial effects on relapse (including molecular), TRM and acute GVHD of these optimal doses in the 42 evaluable patients treated on 08D.85. See Figure 13.

## Figure 13 Patients Treated on TJU 08D.85-Analysis Based on the Combination of CD34 Stem Cell and Non-Tolerized T Cell Doses Received

Both Lo=CD34 Stem Cell Dose < 5 x $10^6$/Kg and Non-Tolerized T Cell Dose is <5 (n=19)
Both Hi=CD34 Stem Cell Dose ≥5 x $10^6$/Kg and Non-Tolerized T Cell Dose is ≥5 (n=11)
CD34 Lo/T Cell Hi=CD34 Stem Cell Dose < 5 x $10^6$/Kg and Non-Tolerized T Cell Dose is ≥5 (n=1)
CD34 Hi/T Lo=CD34 Stem Cell Dose ≥5 5 x $10^6$/Kg and Non-Tolerized T Cell Dose is <5 (n=11)



Remarkably, for patients who received the combination of higher CD34 stem cell doses and non-tolerized T cell doses, relapse rates were only 9%, with a concommitant TRM of 0%. This result supports the safety and efficacy of higher doses of these cells in the 2 Step approach.

## Summary of Background Section, Correlative Studies, and Rational for Proposed Trial

A comparison to pertinent literature revealed that relapse rates in patients transplant on TJU 08D.85 (the initial 2 Step matched related trial upon which this proposed trial is the successor) are comparable to other HSCT treatment approaches using HLA matched donor, but the rate of relapse represents a major barrier to long-term survival. The low TRM associated with the regimen contributes to an OS that compares favorably to other published results in this area. In this successor study, reducing relapse would increase OS further. Multiple published reports support the notion that full donor chimerism (within the first 3 months after HSCT) is associated with decreased in relapse theoretically due to early, successful eradication of disease. In recipients of TJU-08D.85, median day+ 28 and d+90 donor chimerism was lower than that observed in 2 Step haploidentical recipients, a group that has lower relapse rates. Examination of outcomes of patients treated on TJU 08D.85 revealed that full donor chimerism was achieved more rapidly in patients who received higher doses of CD34 stem cells and non-tolerized T cells in Step 2 of the HSCT. Further analysis revealed that early chimerism was associated with lower relapse rates without increased TRM, supporting the notion that increasing doses of these cells in this successor protocol would reduce the relapse incidence. The outcomes of patients treated on TJU 08D.85 were further analyzed to identify optimal cell doses associated with lower relapse. Cumulative incidence analysis revealed that > **5** x $10^6$/kg of CD 34 stem cells and > **5** x $10^3$/kg of non-tolerized T cells were associated with improved relapse rates without an increase in TRM. The rationale for the proposed trial is to administer this “5+5” combination to reduce relapse rates after a 2 Step matched related HSCT.

## 1.4 Potential Risks and Benefits

### 1.4.1 Potential Risks

Risks associated with consistently treating with ≥ 5x $10^3$/kg non-tolerized T and ≥ 5 x $10^6$/kg CD 34 stem cells (“5+5”) are expected to be minimal. As reviewed above, 50% of patients treated on TJU 08D.85 were treated with above the median doses of non-tolerized T cells and CD34 cells and there was no correlation with TRM or GVHD. In fact, patients who received a higher dose of non-tolerized T cells had lower rates of grade II-IV GVHD. Nevertheless, dosing of these cells will be higher for the whole population treated on this successor protocol and therefore, unforeseen risk may be present. **We note here that all 2 Step regimens contain upper limits of cell doses allowable on protocol. These upper limits will be in place in this protocol.** Therefore, it is consistency of cell dosing to or above the median of what has been given in historical patients as opposed to increasing cell doses to amounts not used before.

### 1.4.2 Potential Benefits

The outlook for patients relapsing after allogeneic HSCT is dismal[41] creating an urgent need to develop strategies to avoid recurrent disease post HSCT. For patients, the benefit from participating in this clinical trial is the potential for avoidance of post HSCT relapse. The information would also contribute valuable scientific data to this area of HSCT.

# 2 Study Objectives

## 2.1 Objectives

### 2.1.1 Primary

The primary objective of this study is to assess whether providing a patient with“5+5” dosing in a 2 Step matched related donor HSCT increases the percentage of patients who achieve full donor chimerism earlier as defined by 98% or greater donor T cell chimerism at 28 days post HSCT (d+28).

### 2.1.2 Secondary

The secondary objectives are as follows:

-To assess d+90 chimerism in patients receiving “5+5” dosing
-To assess post HSCT relapse rates in patients receiving “5+5” dosing
-To assess rates of grade II-IV GVHD in patients receiving “5+5” dosing
-To assess TRM in patients receiving“5+5” dosing

### 2.1.3 Exploratory

-To assess whether patients receiving “5+5” dosing have lower rates of CMV reactivation as compared to patients treated on TJU 08D.85

## 2.2 Endpoints/Outcome Measures

### 2.2.1 Primary

The primary outcome measure is donor T cell chimerism at d+28 (Yes or No) defined as "Yes" if peripheral blood T cells are 98% or more donor origin at 28 days post HSCT (d+28), and "No" if otherwise (<98% donor origin).

### 2.2.2 Secondary

The secondary outcomes measures are:

-T cell chimerism at d+90
-Relapse rate at 1 year post HSCT
-The incidence of grades II-IV GVHD occurring within 1 year of HSCT
-Rate of TRM at 1 year post HSCT

### 2.2.3 Exploratory

The exploratory outcome measure is the rate of CMV reactivation in the first 100 days after HSCT

## 3 Study Design

This is a phase II, single institution study in which consistent treatment of patients with ≥ 5x $10^3$/kg non-tolerized T and ≥ 5 x $10^6$/kg CD 34 stem cells ("5+5") within the framework of the matched related 2 Step approach is being tested to assess whether more patients undergoing HSCT are able to achieve 98% or greater donor T cell chimerism at d+28. More rapid attainment of donor chimerism has been associated with decreased rates of relapse. The 2 Step approach to HSCT has been used for over 11 years at Jefferson at has been shown to be a safe and effective regimen.[42-44] Therefore, the use of the 2 Step approach itself is not being tested-more consistent dosing of non-tolerized T cells and CD34 stem cells within the 2 Step approach is the focal point of this clinical trial. We adapt Table 5 from Section 1.3 highlighting cell dosing strategies:

| | **HSCT Step 1** | **CY Tolerization/ GVHD Prophylaxis** | **Historical HSCT Step 2 TJU 08D.85** | **Proposed HSCTStep 2 Changes** |
|---|---|---|---|---|
| **Conditioning**<br><br>**TBI (12Gy)**<br><br>**FIXED** | 2x$10^8$CD3/kg<br><br>FIXED | CY 60 mg/kg x 2<br><br>FIXED | **Median**/(range) CD 34 cells/kg:<br>**4.9** x $10^6$/kg (1.64-10.0)<br><br>**Median**/(range) non-tolerized T cells in the CD34 product cells/kg:<br>**2.9** x $10^3$/kg (0.96-17.0) | Patients to receive at least **5 x $10^6$/kg** (limit 10 x $10^6$/kg as per 2 Step routine<br><br>Patients to receive at least **5 x $10^3$/kg** (limit 5 x $10^4$/kg as per 2 Step routine |

As seen in the table, the proposed dosing is well within the range of doses that historical patients treated on TJU 08D.85 have already safely received. This successor protocol will add consistency to dosing by adding back to the allogeneic product an amount of non-tolerized T cells to bring all patients to a minimum dose of 5 x$10^3$/kg. We anticipate that every patient will be able to receive at least 5 x $10^6$/kg CD34 stem cells without specific intervention based on the recent introduction

of more efficient apheresis technology at TJUH as well as the ability to alter apheresis times within the standard 2 day donor aphereses already built into the 2 step protocols. The rationale for the provision of “5+5” dosing to all patients is that variability in doses of cells in step 2 of the HSCT has resulted in some patients receiving less than a desired amount of donor cells resulting in lower donor chimerism and higher relapse rates. This successor protocol corrects this issue.

All patients will be transplanted in the TJUH HSCT program using the 2 Step approach. All historical 2 Step protocol parameters will be followed with the sole exception being the “5+5” intervention as described above. There is no randomization. Patients will undergo peripheral blood chimerism studies at d+28 and d+90. Evaluations for relapse, GVHD, and TRM will occur at d+28, 3, 6, 9, and 12 months post HSCT.

### 3.1 Characteristics

The target population is unchanged from TJU 08D.85. Specifically, patients with hematological malignancies or other dyscrasias that would benefit from allogeneic HSCT and have an HLA matched related donor

### 3.2 Number of Participants

This is a one arm study enrolling 34 patients.

### 3.3 Duration of Therapy

Patient will be receiving active therapy x 10 days which is the length of the conditioning regimen concluding with the HSCT.

### 3.4 Duration of Follow Up

Patients will be followed for the primary endpoint of d+28 and the secondary endpoint of d+90 chimerism studies. Therefore, the primary follow-up period is 90 days. Patients will be followed for the remaining secondary endpoints for 1 year post HSCT.

### 3.5 Study Timeline

#### 3.5.1 Primary Completion

5 Years to accrue 34 patients, 6 years to complete the trial

#### 3.5.2 Study Completion

While information regarding the primary and secondary endpoints, chimerism at d+28 and d+90, will be known by 90 days after HSCT, the secondary endpoints of assessment for GVHD, relapse, and TRM are performed at the 1 year mark. Therefore, patients will need to be followed for 1 year post HSCT and will complete their study enrollment at one year. If 4 patients are accrued each year, we will need 5 years for accrual and 1 additional year for full analysis, bringing the length of the trial to 6 years. Based on the size of the TJU HSCT program and the limited amount of patients having fully matched relative donors based on societal factors, it would be unlikely that any matched related protocol would accrue more quickly than this target.

## 4 Study Enrollment and Withdrawal

Target sample size is 34. Approximately 200 patients will need to be screened to meet this number.

## 4.1 Eligibility Criteria

### 4.1.1 Inclusion Criteria

Individuals must meet all of the following inclusion criteria in order to be eligible to participate in the study. Patients must:

1. Provide signed and dated informed consent form
2. Have a hematological malignancy or any type of dyscrasia in which allogeneic HSCT is thought to be beneficial
3. Have a related donor who is no more than a 1-antigen mismatch at the human leukocyte antigen (HLA)-A; B; C; DR loci in the GVHD direction with the patient. (Patients with a syngeneic donor may be treated on this therapeutic approach, but their outcomes will not be part of the statistical aims of the study.
4. Have adequate organ function:
    a. LVEF (Left Ventricular End Diastolic Function) of ≥45%.
    b. DLCO (Diffusing Capacity of the Lung for Carbon Monoxide) ≥50% 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. Have a Hematopoietic Cell Transplant Comorbidity Index (HCT-CI)[45] Score ≤5 points (Patients with greater than 5 points will be allowed 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 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. Have a Karnofsky performance score[46] (KPS) ≥80%
7. Be age 18 years old or greater
8. Women of reproductive potential (defined as women under the age of 50 years still menstruating within 2 months of HSCT despite past history of chemotherapy) will be counselled to use highly effective contraception including oral, IM, or patch contraceptives, IUD, diaphragm, cervical cap, or contraceptive implant. Pharmacological avoidance of pregnancy and suppression of menstruation may be instituted during the HSCT inpatient stay.
9. Men will be asked to abstain from sexual relations during the treatment period of the HSCT stay

### 4.1.2 Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study. Patients must not:

1. Be human immunodeficiency virus (HIV) positive
2. Be pregnant or breastfeeding
3. Have received alemtuzumab or rabbit antithymocyte globulin (ATG) within 8 weeks or horse ATG within 6 weeks of the transplant admission. This exclusion criterion will be documented by the absence of these drugs in the medical record
4. Patients with evidence of another malignancy, exclusive of a skin cancer that requires only local treatment, should not be enrolled on this protocol without the specific approval of the PI. If the PI disregards this criterion (example of this is localized prostate cancer not yet requiring treatment), the rationale must be documented in the study binder). This exclusion criterion will be documented by the absence of these drugs in the medical record

## 4.2 Gender/Minority/Pediatric Inclusion for Research

Eligibility for this trial is not based on gender, race or ethnicity. All patients in the Jefferson catchment area meeting criteria for this protocol will be eligible for enrollment. Percentages of women and minorities should be reflective of the Jefferson catchment area. Patients under the age of 18 years are not eligible for this study. The protocol and the training of caregivers who are conducting this study is specific to adults and therefore for safety reasons, pediatric patients (age < 18 years) are referred to pediatric institutions.

Prisoners are eligible for this study if they meet inclusion criteria and if they are able to complete the post HSCT study assessments. For safety reasons, prisoners must also be able to meet TJUH HSCT follow-up criteria as described by TJUH standard operating procedure (SOP) CP:P035.02, TJUH BMT Guidelines for Post-Transplant (HSCT) Allogeneic Assessments.

## 4.3 Strategies for Recruitment and Retention

There are no specific strategies for recruitment. Patients treated on this study will be patients presenting to TJUH for treatment or to physicians in the TJUH geographical area who then refer to TJUH for HSCT. In terms of retention, patients undergoing HSCT at TJUH are counseled to be compliant with post treatment care as failure to do so may result in death. Towards this end, patients are interviewed and approved for HSCT by a TJUH social worker. There is no compensation for participation in this study.

## 4.4 Participant Withdrawal

### 4.4.1 Reasons for Withdrawal

Participants are free to withdraw from participation in the study at any time upon request and without affecting the ability to receive medical care at Thomas Jefferson University.

An investigator may terminate a study participant’s participation in the study if:

- Any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant.
- The participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.

#### 4.4.2 Handling of Participant Withdrawals and Participant Discontinuation of Study Intervention

The study specific part of the current protocol is the receipt of the “5+5” cells during HSCT. Prior to receiving them, the patient may withdrawal from the study and proceed with a 2 Step HSCT using historical trial minimal targets or a different type of HSCT approach offered at TJUH. Withdrawal from the study at any time after the conditioning regimen or the receipt of allogeneic cells will result in the encouragement by the patient’s care team to continue to receive post HSCT follow-up care at TJUH or at another HSCT center whether the participant is on study or not. Failure to do so could result in death due to the immunologic instability of the new donor immune system. For this reason, patients are assessed for willingness, ability, and compliance with study procedures as well as the HSCT prior to HSCT. The assessment includes a social work evaluation.

The primary measure of the study is donor T cell chimerism at d+28 and the first secondary measure is donor T cell chimerism at d+90. Therefore, adverse events serious or otherwise will be monitored and recorded/reported through d+90. To increase patient comfort and avoid withdrawals, the outcome measures can be analyzed through blood draws as opposed to marrow exams. Patient refusing to follow-up at TJUH for assessment of chimerism, relapse, GVHD, and treatment related conditions will be encouraged to follow-up at another institution whose care team is familiar with HSCT. The TJUH team would then work with that team, if the patient consents, to provide safety information and obtain follow-up data.

Patients withdrawing from study prior to the 1 year follow-up time may be replaced.

### 4.5 Premature Termination or Suspension of Study

This study may be suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to the investigator, Investigational Device Exemption (IDE) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the principal investigator will promptly inform the IRB and will provide the reason(s) for the termination or suspension.

Circumstances that may warrant termination include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants.
- Insufficient adherence to protocol requirements.
- Data that is not sufficiently complete and/or evaluable.
- Determination of futility.

## 4.6 Donor Eligibility

All donors are selected and screened for their ability to provide adequate infection-free apheresis products for the patient in a manner that does not put the donor at risk for negative consequences. Donor selection will be in compliance with 21 CFR 1271 and TJU BMT Program SOP CP: P009.03.

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

- HIV, type 1
- HIV, type 2
- HBV
- HCV
- Treponema pallidum
- Human T-lymphotropic virus, types I and II
- Cytomegalovirus
- West Nile Virus
- 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.

The timing of infectious disease testing will be based on Foundation for the Accreditation of Cellular Therapy (FACT) guidelines

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.

# 5 Study Intervention

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.

Proposed Schema **- Patient**

| | -9 | -8 | -7 | -6 | -5 | -4 | -3 | -2 | -1 | 0 |
|---|---|---|---|---|---|---|---|---|---|---|
| AM | TBI | TBI | TBI | TBI | Rest | Rest | Cy 60 mg/kg | Cy 60 mg/kg | Tacrolimus & MMF | CD $34^+$ selected HSCT |
| PM | TBI | TBI | TBI | TBI DLI | | | | | | |

Proposed Schema **- Donor**

| | -7 | -6 | -5 | -4 | -3 | -2 | -1 |
|---|---|---|---|---|---|---|---|
| AM | Lymphocyte Collection | Lymphocyte Collection *(If Needed)* | G-CSF | G-CSF | G-CSF | G-CSF PBSC Collection | G-CSF PBSC Collection |
| PM | | | G-CSF | G-CSF | G-CSF | G-CSF | |

DLI= Donor Lymphocyte Infusion; G-CSF=Granulocyte colony stimulating factor; MMF= Mycophenolate Mofetil; PBSC=Peripheral Blood Stem Cell Collection; TBI=Total Body Irradiation

## 5.1 Administration of Immunosuppressive Agents during Conditioning

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. Patients must be off steroids by the time of admission. The only exception is in patients receiving 5 mg prednisone (or equivalent amount of an alternate steroid) for adrenal insufficiency. Patients in this category may be medically required to stay on this dose of steroids for the entire HSCT stay. If patients have previously required steroids as a premedication for transfusion, they may receive a dose of steroid equivalent to 5 mg of prednisone on the first day of TBI. Diphenhydramine and meperidine may be used if necessary. Any use of steroids after the first day of TBI through day -1 should not be administered without approval from the PI. **The absence of prohibited drugs in the medical record serves as documentation that they were not given.**

## 5.2 Total Body Irradiation (TBI)

1.5 Gy TBI will be administered twice daily for 4 days (8 fractions) on days -9 through -6. TBI will be utilized for all patients eligible for this protocol. All patients will be evaluated by a radiation oncologist in preparation for radiation treatment. See Appendix A for radiation guidelines.

## 5.3 Donor Lymphocyte Infusions (DLI)

The target CD3+ T cell dose in the DLI to be administered to the recipient is 2 x $10^8$/kg.

All donors will report for apheresis of lymphocytes on day -7. If the targeted amount of lymphocytes is not collected on the first day, the donor will return for a second day of lymphocyte collection 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. No hematopoietic growth factors will be administered to apheresis donors prior to lymphocyte collection. Leukocyte collections will be performed using a standard apheresis machine.

For the donor lymphocyte apheresis, 24-26 liters will be processed on the first day of collection. In our experience, most donors reach the target of 2 x $10^8$/kg T cells on the first day. 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.

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

DLI specimen handling and labeling conventions will be performed in accord with the relevant AABB (American Association of Blood Banks) and/or FACT 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.

The following guidelines should be used to calculate the correct volume of blood to be obtained from the donor product to achieve the target T-cell dose.

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.

During the infusion of the DLI, the patient will be monitored for any untoward reactions. DLI will be administered by nursing staff experienced in the administration of allogeneic products.

Based on minor variations in donor collection and laboratory processing times, an exact time for the DLI infusion will not be prescribed by this protocol. The DLI will be infused in the afternoon on day -6 after the last dose of TBI. The DLI should be infused before day -5.

DLI must **NOT** be irradiated. DLI should **NEVER** be administered through a leukocyte depletion 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

CY 60 mg/kg IV will be administered on days –3 and –2 of the conditioning regimen. Mesna 60 mg/kg continuous IV infusion over 24 hours X 2 doses will be administered on days –3 and -2. Day –1 is a day of rest.

Voriconazole can block the conversion of CY to its active metabolite, 4-hydroxycyclophosphamide. Isavuconazole is an inducer of CYP2B6 and thus may affect CY metabolism, as CY is a CYP2B6 substrate. Posaconazole may increase plasma concentrations of CY via inhibition of cytochrome P450 3A4-mediated biotransformation. For this reason, these drugs will not be administered to any patient from admission (or the beginning of conditioning) until day -1. **The absence of prohibited drugs in the medical record serves as documentation that they were not given.**

There are no restrictions on the use of liposomal amphotericin.

## 5.5 Collection and Infusion of Progenitor Cells

Donors will begin G-CSF, 5µg/kg bid on days -5 to -1. Pegfilgrastim 12 mg x 1 dose subcutaneously on d -5 may be substituted for G-CSF.

The donor will return for a primed progenitor cell collection on days -2 and -1. 24 to 26 liters will be processed each day. Liters processed during the second day of collection may be reduced based on the amount of CD34 stem cells required to meet the CD34 stem cell target, but will not exceed 26 liters.

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.

## 5.6 Peripheral Blood Stem Cell (PBSC) Dose

The target dose of donor PBSCs to be infused into the recipient is **5.0-10.0 x $10^6$ CD34** cells/kg of recipient dosing body weight. No patient will receive more than 10.0 x $10^6$/kg donor CD34 cells/kg. Every effort will be made to infuse at least **5.0 x $10^6$ CD34** cells/kg in patients to support the scientific aims of the protocol. Donors may be apheresed a 3rd day if the first two collection cell doses do not meet a CD34 cell dose of 2.0 x $10^6$/kg which has been the minimal target in the 2 Step approach for rapid engraftment.

In our experience, the ideal amount of T-cells left in the PBSC product is no greater than 5x$10^4$/kg. In over 250 2 step patients treated on protocol, no patient receiving a CliniMACS® product received more than this allowable dose of residual T cells in the stem cell product, including the 4 patients who received double donor transplants treated on IRB #11D.570. This maximum residual T cell target also applies to this protocol. However, in this protocol, there will be a minimal residual CD3+ T cell dose target of 5 x $10^3$/kg in the CD34 cell product. If the CD34 product contains less than this amount of T cells, an aliquot of donor cells will be added back to the CD34 product to meet this dose.

Specifically, 3 cc will be removed from the donor CD34 stem cell product prior to processing with the CliniMacs® machine. After CD34 cell enrichment, if the residual CD3+ T cell content is ≥5.0 x $10^3$/kg, no add back of T cells is needed. However, an aliquot of the reserved 3 cc of pre CliniMacs® product containing a dose of 5.0 x $10^3$/kg will be added back to any post CliniMacs® CD34 stem cell product containing < 5 x $10^3$/kg CD3+ T cells. The addition of these cells would bring the CD34 stem cell product residual CD3+ T cell dose to no greater than 1 x $10^4$/kg CD3+ T cells, far below the 5x$10^4$/kg maximum dose required in this and every 2 step protocol. BMT Processing Laboratory SOP is specific to this point in the protocol.

The scientific aim of this protocol does not address the phenotype of the residual CD3+ T cells in the CD34 stem cell product. Analysis for the “5+5” target doses was based on the quantity of the residual CD3+ T cells in the CD34 stem cell products of patients treated on TJU-08D.85 and not phenotypical differences between donors.

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. Leukocyte collections will be performed using a standard apheresis machine such as the Cobe Spectra apheresis instrument (Cobe Laboratories Inc., Lakewood, CO).

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.

## 5.7 PBSC Infusion

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.

Significant red cell incompatibility between donor and recipient will be managed according to standard operating procedure, CL:P060, of the Thomas Jefferson University Hospital Blood and Marrow Transplant Processing Lab.

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

## 5.8 Failure to Meet the Targeted Cell Totals

Because of biological differences in the population or tolerability of apheresis, there may be donors who cannot produce the targeted cell amounts specified in this protocol. Patients who do not receive CD34 stem cell or residual CD3+ T cell doses in the targeted range will not be included in this analysis for scientific endpoints and their outcomes will be reported descriptively.

## 5.9 GVHD Prophylaxis

Tacrolimus will be started on day -1.

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

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

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

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

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

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

# 6 Study Schedule

## 6.1 Enrollment/Baseline

All studies and assessments required to assess eligibility criteria will be performed within 3 months of admission for HSCT and will be documented on a case report form (CRF) by the study coordinators. The PI or Co-I will approve and sign the CRF, if eligibility criteria is met, prior to admission for HSCT.

## 6.2 Laboratory Studies/Outcome Assessment

Table 7 outlines the mandatory measurements and time points specific to this study. Only the day +28 and +90 studies are mandatory.

| ***Table 7*** | **Day + 28** | **Days 28-90** | **Days 91 -180** | **Days 180-365** | **Day 365** |
|---|---|---|---|---|---|
| LAB (CBC, CMP) patient status, & GVHD Assessment: Presence and degree of skin rash, presence and amount of diarrhea, liver function | X | Twice Monthly | Monthly | Every other month | X |
| CMV by PCR only in CMV seropositive patients at least one time by day +100 | | | | | |
| Chimerism/ Disease Assessment | | | | | |
| Peripheral blood for Total, mononuclear cell, & CD3+ chimerism | With d+28 studies | Twice monthly until ≥98% donor T cell chimerism and at d+90 studies | At d+180 | At d+270 | With 1 year studies |
| Bone marrow exam (morphology, flow cytometry,cytogenetics, chimerism) | With d+28 studies | As clinically indicated | As clinically indicated | As clinically indicated | As clinically indicated |
| Flow cytometry for lymphocyte subsets (IRP) | With d+28 studies | With d+90 studies | With d+180 studies | At d+270 | With 1 year studies |

The day +28 peripheral blood, marrow studies and the day 28 assessment can be obtained within 1 week of day 28 (i.e. ± 7 days) to account for scheduling factors. The absence of notation of S/S GVHD will serve to document the absence of GVHD. All non-d+28 parameters should be obtained within 1 month before or 1 month after the suggested time frame. This table represents a minimum recommended sampling and visit strategy.

## 6.3 Hematopoietic Engraftment

Will be defined as:

- ANC ≥ 0.5 x $10^9$/L for at least 3 days
- Platelet engraftment >20,000 with no transfusions X 7 days.

## 6.4 Toxicity Criteria

Regimen-related toxicity will be graded according to the NCI Common Toxicity Criteria, version 5.0.

The NCI Common Toxicity Criteria can also be found at the following WEB address: https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_5.0/

## 6.5 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:

https://www.nccn.org/evidenceblocks/default.aspx

## 6.6 GVHD Scoring

GVHD will be graded according to Glucksberg et al.[47]

## 6.7 Study Endpoint

Adverse events and significant adverse events are monitored until day +100. After that, all patients will be monitored for 1 year for major HSCT outcomes of OS, GVHD, and relapse to answer the secondary objectives.

## 6.8 Supportive Care

**Infectious Prophylaxis: General Guidelines**

Patients post matched related donor transplantation will be maintained on antifungal prophylaxis, usually voriconazole 200 mg BID or posaconazole equivalent to 300 mg daily (delayed release oral tab). It is at the discretion of the treating attending physician to change agents as clinically indicated.

Patients post matched related donor transplantation will be maintained on HSV prophylaxis, usually valacyclovir 500 mg BID or its equivalent. 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 encapsulated organism and pneumocystis jiroveci 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.

**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.

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 matched related donor transplantation. G-CSF 5μg/$m^2$ can be substituted for GM-CSF in the event of a GM-CSF shortage or if a patient has a deleterious reaction to GM-CSF as determined by the BMTU attending physician.

Red cell growth factors are permissible after transplantation.

# 7 Evaluation of Safety

## 7.1 Specification of Safety Parameters

### 7.1.1 Unanticipated Problems

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

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

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

### 7.1.2 Adverse Events

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

### 7.1.3 Serious Adverse Events

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

- Results in death

- Is life-threatening (places the participant at immediate risk of death from the event as it occurred)
- Is disabling or incapacitating
- Results in inpatient hospitalization or prolongation of existing hospitalization
- Results in a persistent or significant disability or incapacity
- Results in a congenital anomaly or birth defect
- An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the participant or may require intervention to prevent one of the outcomes listed in this definition.

### 7.1.4 Adverse Event Reporting Specific This Protocol

All patients will be followed for adverse experiences (AEs) (serious and non-serious) by the PI or a Co-I regardless of relationships to study treatment, from the time of HSCT admission until day +100 post-transplant. The following events are expected side effects of high-dose chemotherapy and transplant and will be recorded but will not be reported except as noted:

- Alopecia, dry skin
- Emesis from chemotherapy or other agents unless refractory to standard supportive care, nausea, and anorexia
- Weight loss, cough, dry mouth, headache
- Neutropenia/uncomplicated neutropenic fever, grades 1-3 infectious sequellae
- Thrombocytopenia, petechiae, ecchymosis, 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
- Grade I - III Mucositis
- Grade I - III Diarrhea
- Grades 1-3 allergic or other common reactions to drugs used for supportive care
- Fluid and electrolyte disturbances not associated with instability
- Fluid overload resulting in grade 3 or less hypoxia
- After d + 100, only events that are considered by the investigator to be possibly or probably associated with the treatment regimen will be reported.

- All grade 4 and 5 adverse events will be reported

## 7.2 Safety Assessment and Follow-Up

Events will be followed for outcome information until d+90 or if the event has been resolved (noted on AE log).

## 7.3 Recording Adverse Events

The following subsections detail what information must be documented for each adverse event occurring during the time period specified in Section 7.2 Safety Assessment and Follow-Up.

### 7.3.1 Relationship to Study Intervention

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

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

1. Related (Possible, Probable, Definite)
    a. The event is known to occur with the study intervention.
    b. There is a temporal relationship between the intervention and event onset.
    c. The event abates when the intervention is discontinued.
    d. The event reappears upon a re-challenge with the intervention.
2. Not Related (Unlikely, Not Related)
    a. There is no temporal relationship between the intervention and event onset.
    b. An alternate etiology has been established.

### 7.3.2 Expectedness

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

### 7.3.3 Severity of Event

Adverse events will be graded for severity according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

#### 7.3.4 Intervention

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

## 7.4 Safety Reporting

### 7.4.1 Reporting to IRB Unanticipated Problems

All incidents or events that meet criteria for unanticipated problems (UAPs) as defined in Section Unanticipated Problems require the creation and completion of an unanticipated problem report form (OHR-20).

UAPs that pose risk to participants or others, and that are not AEs, will be submitted to the IRB on an OHR-20 form via the eazUP system within 10 working days of the investigator becoming aware of the event.

UAPs that do not pose risk to participants or others will be submitted to the IRB at the next continuing review.

### 7.4.2 Adverse Event Reporting to the IRB

Grade 1 AEs will be reported to the IRB at continuing review.

Grade 2 AEs will be reported to the IRB at the time of continuing review.

### 7.4.3 Serious Adverse Event Reporting to the IRB

SAEs will be reported to the IRB on OHR-10 forms via the electronic reporting system (eSAEy) according to the required time frames described below, except as noted in Section 7.1.4.

Grade 3-4 AEs that are unexpected and deemed to be at least possibly related to the study will be reported to the IRB within 2 working days of knowledge of the event.

Grade 3-4 AEs that are deemed unrelated to the study will be reported to the IRB within 5 working days.

Grade 5 AEs will be reported to the IRB within one working day of knowledge of the event.

All SAEs will be submitted to the IRB at continuing review, including those that were reported previously.

### 7.4.4 Reporting to SKCC DSMC

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

For expedited reporting requirements, see table below:

DSMC AE/SAE Reporting Requirements

| | Grade 1 | Grade 2 | | Grade 3 | | | | Grades 4 and 5 |
|---|---|---|---|---|---|---|---|---|
| | | | | Unexpected | | Expected | | |
| | Unexpected and Expected | Unexpected | Expected | With Hospitalization | Without Hospitalization | With Hospitalization | Without Hospitalization | Unexpected and Expected |
| Unrelated Unlikely | Reviewed at Quarterly DSMC Meeting and IRB Annual Review | Reviewed at Quarterly DSMC Meeting and IRB Annual Review | Reviewed at Quarterly DSMC Meeting and IRB Annual Review | 5 Working Days | Reviewed at Quarterly DSMC Meeting and IRB Annual Review | 5 Working Days | Reviewed at Quarterly DSMC Meeting and IRB Annual Review | Phase I - 48 Hours<br>(Death: 24 Hours)<br>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<br>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<br>(Death: 24 Hours) |

#### 7.4.5 Reporting to FDA

All grade 3-5 infusion reactions and all unexpected SAEs as defined in 21 CFR 312.32 will be reported to the FDA in an expedited fashion. Specific to the CliniMacs® use at TJUH, the FDA requests expedited reporting of biologic product deviations in addition to grade 3-5 infusion reactions.

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.

#### 7.4.6 Reporting of Pregnancy

Negative pregnancy will be documented prior to admission in women of childbearing potential. Radiation in this protocol will preclude pregnancy.

### 7.5 Halting Rules

Based on prior experience using a 2 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 (grade 3 or 4) should be less than 20%, and the non-relapse mortality should be less than 20% at 100 days. If at any point incidences higher than these thresholds are seen, that would trigger a protocol review to assess whether there are any obvious reasons for the inferior outcomes observed. Depending on the results of the review, enrollment may continue on a limited basis with careful further observation, the protocol may be revised, or the protocol may be terminated. Incidences will be calculated starting after 10 patients are treated on this trial in order to have a sufficient denominator in which to examine outcomes based on percentages.

## 8 Clinical Site Monitoring and Auditing

Clinical site monitoring and auditing is conducted to ensure that the rights of human participants are protected, that the study is implemented in accordance with the protocol and/or other operating procedures, and that the quality and integrity of study data and data collection methods are maintained. Monitoring and auditing for this study will be performed in accordance with the SKCC’s Data and Safety Monitoring Plan (DSMP) developed by the SKCC Data and Safety Monitoring Committee (DSMC). The DSMP specifies the frequency of monitoring, monitoring procedures, the level of clinical site monitoring activities (e.g., the percentage of participant data to be reviewed), and the distribution of monitoring reports. Some monitoring activities may be performed remotely, while others will take place at the study site. Appropriate staff will conduct monitoring activities and provide reports of the findings and associated action items in accordance with the details described in the SKCC DSMP.

## 9 Statistical Considerations

### 9.1 Study Hypotheses

#### 9.1.1 Primary Objective:

The primary objective of this study is to demonstrate that the provision of "5+5" dosing will result in the achievement of 98% donor-derived T cell chimerism at d+28 in a proportion of HSCT recipients that is significantly higher than 50%. In TJU 08D.85, only 50% of patients reached 98% peripheral blood T cell chimerism or higher at d+28.

### 9.1.2 Secondary Objectives

- Evaluate the proportion of donor T cell chimerism at d+90.

- Evaluate the relapse rates at 1-year post HSCT.

- Evaluate the incidence of grades II-IV GVHD occurring within 1 year of HSCT.

- Evaluate the TRM at 1-year post HSCT.

### 9.1.3 Study design

We design this trial with the potential for early termination in case the rate of recipients achieving high donor chimerism (defined as 98% or greater) at d+28 with "5+5" dosing is similar to historic rate of 50%. The design will be a two-stage design using the method of Simon. Choice of design is guided by a desire to stop the trial early if the actual number of patients achieving high donor chimerism at d+28 is 50% or less. If the number of patients achieving a high donor chimerism rate at d+28 is 75% or greater, we would like to have a low probability of failing to conclude effective. Our chosen design is:

| Look after this number of patients | Stop if number of successes is less than |
|---|---|
| 11 | 7 |
| 34 | 22 |

## 9.2 Analysis Plans

All estimates of rates (primary and secondary endpoints and toxicity) will be presented with corresponding 95% confidence intervals. For chimerism rates, the method of Atkinson and Brown[48] will be used to allow for the two-stage design.[49]

## 9.3 Interim Analyses and Stopping Rules

The trial will be stopped after the first 11 patients if the number of patients with < 98% donor T cell chimerism at d+28 is 6 or less.

## 9.4 Sample Size Considerations

With the chosen Simon two-stage design, we have no more than a 0.146 probability of concluding "5+5" dosing ineffective (≤50% patients achieving a high donor T cell chimerism rate of 98% or more) when the rate of recipients achieving a high donor chimerism (98% or more donor T cell chimerism at d+28) is at least 75% (the target for this value was 0.150). Similarly, we have no more than a 0.045 probability of concluding "5+5" dosing effective (≥75% of

recipients achieving a high donor T cell chimerism at d+28 of 98% or more) when it is ineffective (the target for this value was 0.05). If the actual percentage of patients achieving a high donor T cell chimerism rate is 50% or worse, we have at least a 0.726 probability that the trial will stop after the first 11 subjects.

### 9.4.1 Replacement Policy

Patients in whom d+ 28 chimerism studies are not obtained for reasons of death, rejection, or sample failure, may be replaced on study. Patients whose donor cell doses do not meet defined criteria will also be replaced on study.

## 10 Source Documents and Access to Source Data/Documents

Study staff will maintain appropriate medical and research records for this study, in compliance with ICH E6, and regulatory and institutional requirements for the protection of confidentiality of participant information. Study staff will permit authorized representatives of SKCC and regulatory agencies to examine (and when required by applicable law, to copy) research records for the purposes of quality assurance reviews, audits, and evaluation of the study safety, progress and data validity.

# 11 Quality Control and Quality Assurance

This study will be conducted only at the TJUH Center City Campus and as such, adheres to the SKCC quality plan.

# 12 Ethics/Protection of Human Participants

## 12.1 Ethical Standard

The investigator will ensure that this study is conducted in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, as drafted by the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 41979) and codified in 45 CFR Part 46 and/or the ICH E6.

## 12.2 Institutional Review Board

The protocol and informed consent form will be submitted to the TJUH IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented in the study. There are no recruitment materials or participant materials.

## 12.3 Informed Consent Process

Informed consent is a process that is initiated prior to the individual agreeing to participate in the study and continues throughout study participation. Extensive discussion of risks and possible benefits of study participation will be provided to participants and their families, if applicable. A consent form describing in detail the study procedures and risks will be given to the participant. Consent forms will be IRB-approved, and the participant is required to read and review the document or have the document read to him or her. The investigator or designee will explain the research study to the participant and answer any questions that may arise. The participant will sign the informed consent document prior to any study-related assessments or procedures. Participants will be given the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. They may withdraw consent at any time throughout the course of the study. A copy of the signed informed consent document will be given to participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their clinical care will not be adversely affected if they decline to participate in this study. The consent process will be documented in the clinical or research record.

## 12.4 Exclusion of Women, Minorities, and Children (Special Populations)

Patients under the age of 18 are excluded from study. The treating clinicians are knowledgeable in adult care only and as such, patients under the age of 18 will be treated at a pediatric HSCT center. No other special population is excluded.

## 12.5 Participant Confidentiality

Participant confidentiality is strictly held in trust by the investigators, study staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to any study information relating to participants.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The study monitor or other authorized representatives of the sponsor may inspect all study documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) for the study participants. The clinical study site will permit access to such records.

### 12.6 Future Use of Stored Specimens and Other Identifiable Data

Stored specimens are not required by this protocol.

## 13 Data Handling and Record Keeping

The investigators are responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. All source documents must be completed in a neat, legible manner to ensure accurate interpretation of data. The investigators will maintain adequate case histories of study participants, including accurate case report forms (CRFs), and source documentation. CRFs and source documentation may be in electronic form.

### 13.1 Data Management Responsibilities

Data collection and accurate documentation are the responsibility of the study staff under the supervision of the investigators. All source documents and laboratory reports must be reviewed by the study team and data entry staff, who will ensure that they are accurate and complete. Unanticipated problems and adverse events must be reviewed by the investigator or designee.

### 13.2 Data Capture Methods

Study data will be recorded on paper CRFs and electronically on BMT specific spreadsheets and data analysis program. The paper CRFs are kept at TJUH in a designated room that is locked. Electronic data capture will also occur to facilitate statistical analysis. Electronic data will be stored on a TJUH password protected central drive. Handling of electronic data will comply with TJUH HIPPAA compliance policies.

### 13.3 Types of Data

Data regarding patient demographics, risk assessment, performance status, diagnosis, health history, previous treatment, HSCT inpatient stay treatment and events, and post HSCT outcome data such as immune reconstitution, chimerism, disease status, performance status, and other pertinent health history will be collected.

### 13.4 Study Records Retention

Study records will be maintained for at least three years from the date that the grant federal financial report (FFR) is submitted to the NIH.

Study documents must be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing

applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents will be retained for a longer period, however, if required by local regulations. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

### 13.5 Protocol Deviations

A protocol deviation is any noncompliance with the clinical study protocol, Good Clinical Practice, or Manual of Procedures requirements. The noncompliance may be on the part of the participant, the investigator, or study staff. As a result of deviations, corrective actions are to be developed by the study staff and implemented promptly.

All deviations from the protocol must be addressed in study participant source documents and promptly reported to the IRB and other regulatory bodies according to their requirements.

## 14 Study Finances

This study is **unfunded**. The costs of the approach are consistent with what is typically covered by a patient’s insurance company for allogeneic HSCT.

### 14.1 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All Jefferson University Investigators will follow the TJU Conflicts of Interest Policy for Employees (107.03).

### 14.2 Participant Stipends or Payments

There is no payment for participation in this study.

## 15 Publication and Data Sharing Policy

The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a clinical trials registration policy as a condition for publication. The ICMJE defines a clinical trial as any research project that prospectively assigns human participants to intervention or concurrent comparison or control groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Medical interventions include drugs, surgical procedures, devices, behavioral treatments, process-of-care changes, and the like. Health outcomes include any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and adverse events. The ICMJE policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies. The ICMJE does not review specific studies to determine whether registration is

necessary; instead, the committee recommends that researchers who have questions about the need to register err on the side of registration or consult the editorial office of the journal in which they wish to publish.

U.S. Public Law 110-85 (Food and Drug Administration Amendments Act of 2007 or FDAAA), Title VIII, Section 801 mandates that a "responsible party" (i.e., the sponsor or designated principal investigator) register and report results of certain "applicable clinical trials:"

Trials of Drugs and Biologics: Controlled, clinical investigations, other than Phase I investigations, of a product subject to FDA regulation;

Trials of Devices: Controlled trials with health outcomes of a product subject to FDA regulation (other than small feasibility studies) and pediatric postmarket surveillance studies.

NIH grantees must take specific steps to ensure compliance with NIH implementation of FDAAA.

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# 16 Appendices

## Appendix A

## Guidelines for Total Body Irradiation

Modality

Photon irradiation is to be used for the TBI in all patients. Areas beneath lung blocks will be supplemented with electrons to maintain the homogeneity criteria.

Energy

A linear accelerator with energy ≥ 4 MV. 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 the entire 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.

Diagnostic Determination

CT scans through the chest and abdomen will be done prior to initiating irradiation. An average chest wall thickness (both anteriorly and posteriorly) will be calculated and used in determination of electron energy for supplementing the chest wall beneath the lung

blocks. The abdominal scan, renal ultrasound, or intravenous pyelogram will be used to localize the kidneys for proper placement of renal shielding.

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 12.0 Gy. A hyperfractionated regimen over 4 consecutive days, (1.5 Gy BID) shall be used.

Time-Dose Considerations

Hyperfractionation

For patients receiving 2 fractions per day, there is a required minimum time interval of 6 hours between the fractions.

Chest Wall Supplement

Supplementing the chest wall dose with electrons (both anteriorly and posteriorly) shall be done once a day on 2 treatment days, immediately preceding or following treatment to the entire body. The area beneath the lung blocks shall receive an additional 6.0 Gy to $d_{max}$ in a total of 2 fractions.

Total Number of Treatment Days

There shall be a total 4 consecutive treatment days.

Treatment Interruptions

An interruption in the radiotherapy regimen shall not be allowed.

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

Customized blocking to the lungs is required. Customized blocking to the liver and/or kidneys is optional, at the discretion of each participating center with the approval of the coordinating center radiation oncologist.

Patient specific, individually fabricated shielding blocks are required for the lung from both the anterior and posterior directions. A partial transmission block corresponding to a total dose of 8.0 Gy at midplane of the patient under the blocks shall be used. No corrections for inhomogeneity shall be used.

Patient specific, individually fabricated shielding blocks are optional for the liver from both the anterior and posterior directions. A partial transmission block corresponding to a dose reduction to 90% of the central axis dose shall be utilized.

Patient specific, individually fabricated shielding blocks are optional for the kidneys from the posterior direction only. A partial transmission block yielding a total dose of 10.8 Gy +/- 10% to the midplane of the kidney shall be used.

Customized electron cut-outs shall also be constructed corresponding to the size of the lung block plus appropriate margins in all directions.

Superficial Tissue Supplement Technique

The portion of the chest wall shielded by the partial transmission lung blocks will be supplemented with customized (or shaped) low energy electron fields. A total of 6.0 Gy to $d_{max}$ in 2 fractions will be given to the anterior and posterior chest wall. Electron energy will be determined by chest wall thickness as determined by a chest CT scan, with the depth of the 90% dose relative to $d_{max}$ used to determine the electron energy. The dose prescription point will be at $d_{max}$.

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.

Normal Tissue Sparing-Lung Dose

Lung Dose

Each patient must have a calculation performed which shows that with the lung shielding and chest wall supplement, the TBI delivers between 8.0 Gy +/- 10% (defined at midplane at level of carina).

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.
- Simulation films documenting lung, liver and kidney blocks in both the anterior and/or posterior projections shall be taken.
- Portal films (both AP & PA) verifying the position of the lung, liver and kidney blocks shall be taken and must be approved by the supervising radiation oncologist prior to delivery of the first TBI treatment.