A Two Step Approach To Matched-Sibling Allogeneic Hematopoietic Stem Cell Transplantation for High-Risk Hematological Malignancies

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1.0 Objectives

This research protocol has been developed for patients undergoing matched-sibling hematopoietic stem cell transplant (HSCT). The patients who are treated according to this 2 step allogeneic HSCT protocol will receive cyclophosphamide to induce in-vivo tolerization of both autologous and allogeneic lymphocytes, followed by an allogeneic CD34-selected HSCT. The primary research questions relate to immune reconstitution, incidence of GVHD, and relapse in patients who receive lymphocyte treatment of this type in allogeneic HSCT and how it impacts overall survival. Our objectives are:

Primary Objective

1. To determine the overall survival in patients undergoing allogeneic HSCT on this protocol at one year post HSCT.

Secondary Objectives

2. To determine the incidence and severity of GVHD in patients undergoing allogeneic HSCT on this protocol.

3. To assess the pace of lymphoid recovery in patients undergoing allogeneic HSCT on this protocol.

2.0 Introduction and Rationale

High-dose chemoradiotherapy followed by hematopoietic allogeneic stem cell transplantation (HSCT) is a potentially curative modality for a variety of hematologic disorders, including acute and chronic leukemia, myelodysplastic syndrome (MDS), multiple myeloma (MM), and lymphoma that are incurable with conventional dose chemotherapy[1]. There are three major therapeutic components of a conventional allogeneic HSCT. The first component is the use of a high-dose myeloablative-conditioning regimen to eradicate the underlying malignancy and to suppress the host immune system in preparation to receive the donor stem cell graft. This is followed by the infusion of donor stem cells to both rescue the host from the lethality of the conditioning regimen as well as to eliminate residual tumor cells and host resistance to donor stem cells by graft-versus-tumor reactions (GVT). The third component is pre-grafting T-cell depletion of donor stem cells or post-grafting immunosuppression to regulate the development of graft-versus-host disease (GVHD).[2,3] The consequence to one degree or another, of all types of transplants is a period of immune system vulnerability either from post-grafting immunosuppressant medication or the removal of T-cells from the donor inoculum. During this period, patients are at risk for infection and relapse. Patients undergoing matched sibling allogeneic HSCT have the lowest rates of GVHD, and consequentially can be removed from immunosuppressive medications sooner. Decreased incidence of GVHD and more rapid immune reconstitution contribute

to greater overall survival rates after matched sibling HSCT as compared to transplants utilizing alternate donor sources.

Overall Survival in Matched-Sibling Transplantation

Although overall survival rates after matched-sibling allogeneic HSCT are superior to matched unrelated donor (MUD) HSCT, and partially-matched HSCT, there continues to be obstacles to long-term survival in this patient group. Age > 40 – 50 years at the time of HSCT[4,5] and consequently older sibling donor age,[6] disease type,[7] advanced disease,[4,5] patient comorbidities,[6,8,9,10] and an ECOG performance score of > 1 at the time of transplant[6,9] can have adverse effects on survival after HSCT regardless of donor source.

Marks et al[11] analyzed 298 adult patients with acute lymphoblastic leukemia (ALL) in first or second remission (CR1 or CR2). The patients were conditioned with cyclophophamide and total body irradiation (Cy/TBI) or etoposide and TBI. At one year, the best overall survival rate was 80% in patients transplanted in CR1 with TBI/etoposide. For patients transplanted in CR2, the best one year overall survival was 77% in the Cy/TBI group. For patients transplanted in CR1, the best leukemia free survival (LFS) rate for one year was in patients conditioned with TBI/etoposide, and was 79%. For patients transplanted in CR2, the best LFS rate one year was 68%, and was achieved in the group of patients receiving TBI/etoposide. The major single cause of death in this study was disease recurrence. GVHD, infection, interstitial pneumonia, and organ failure were other major causes of treatment failure. In addition, the presence of cytogenetic abnormalities, including t (9:22), was associated with a greater risk of relapse.

Lee et al[12] examined survival rates in patients with acute myelogenous leukemia (AML) in first remission after matched-sibling HSCT. The patients were stratified by the dose of $CD34^+$ cells they received at transplant ($<2.5 \times 10^6$/kg versus $>2.5 \times 10^6$/kg). At one year, patients receiving the higher cell dose had an overall survival rate of over 80% and a LFS rate of 80%. Patients transplanted with the lower $CD34^+$ cell dose had an overall survival rate of over 75% at one year, and a LFS rate of over 65% at one year. In contrast to this study, Cook et al[13] examined the survival of 44 patients post matched-sibling HSCT who had a history of primary induction failure AML. The study patients obtained first remission only after 2 to 5 courses of chemotherapy. In this higher-risk group of patients with AML, the one year overall survival rate was 67%, with adverse cytogenetics being significantly associated with poorer survival. GVHD, infection and pulmonary toxicity were the major sources of treatment-related death.

Runde et al[14] analyzed the outcomes of 131 MDS patients reported to the Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation (EBMT). All of the patients were treated with bone marrow from matched-sibling donors and the majority (70%) received a TBI based conditioning regimen. T-cell depletion was

the GVHD prophylaxis in 12%. The majority of patients had low or high-risk MDS. Low risk MDS was defined as refractory anemia or refractory anemia with ringed sideroblasts. Higher-risk MDS was defined as refractory anemia with excessive blasts, blasts in transformation, or secondary AML. Four patients had chronic myelomonocytic leukemia (CMML). Median patient age was 33 years. At one year, the best disease-free survival DFS was about 60% in the low-risk MDS patients. Overall survival at one year was about 60% for patients transplanted within 4 months of diagnosis, but only about 30% for patients transplanted between 4 and 12 months after diagnosis. In the other disease stages, DFS at one year was around 50% or lower. Two of the 4 patients with CMML died within 2 months of HSCT. The third patient with CMML died at 24 months, and the fourth patient was alive and disease-free at 62 months. Pretransplant diagnosis was significantly associated with outcome, and acute GVHD and infection were the major causes of treatment related mortality. Two of the four CMML patients died of relapsed disease. In a large multicenter retrospective study of patients with MDS, Guardiola et al[15] compared the outcomes of patients undergoing matched-sibling HSCT who received either peripheral blood progenitor cells (PBPC) versus bone marrow from their donors. The International Prognostic Score (IPS) for the majority of patients in this study was intermediate-2 or higher, and less than half the patients received pretransplant chemotherapy. Most patients were older than 35 years. Conditioning regimens were variable with slightly less than half the patients receiving a TBI-based regimen. The overall survival at one year for patients receiving PBPC versus bone marrow grafts was 60% or greater for both groups, and the event free survival at one year was 60% and 45% respectively. Although the use of PBPCs was associated with a 10% increased early treatment mortality due to acute GVHD, the 2-year outcome was significantly better with the use of a PBPC graft.

Several studies have examined the outcome of patients with myeloproliferative disorders, excluding CML, after HSCT. In a retrospective study from the EBMT and Fred Hutchinson Cancer Center, Guardiola et al[16] examined the outcomes of 61 patients, the majority of whom underwent a matched-sibling HSCT (6 patients received matched unrelated donor grafts), with agnogenic myeloid metaplasia (AMM). Patients without extensive marrow fibrosis had a one year overall survival of 85%, in contrast to 55% in patients with more significant marrow fibrosis. GVHD was a significant contributor to transplant-related mortality. Daly et al[17] examined the outcome of 25 patients with AMM and essential thrombocytosis (ET) who underwent HSCT mostly with Cy/TBI conditioning. The stem cell source was mostly bone marrow; with half of the patients receiving cells from a matched sibling donor (most other patients received cells from a matched unrelated donor). The one year non-relapse mortality rate was 48.3% for this group. Although source of stem cells was not found to have a significant effect on outcome, it is conceivable that the overall survival in this study was poorer than the one in the Guardiola study because the use of unrelated donors was associated with a higher incidence of GVHD. Like the Guardiola study, GVHD was a contributor to toxicity, and was significantly associated with prior splenectomy. Finally, Kerbauy et al[18] reported on

the outcomes of 43 patients with CMML undergoing HSCT at the Fred Hutchison Cancer Center. About half of the patients received a TBI-based conditioning regimen, and about half the patients received progenitor cells from a matched-sibling donor. Older age and poorer performance status were associated with increased mortality. Overall survival at one year was close to 60% for those with a better performance status and only about 25% for the rest. Patients who received unrelated donor grafts were the largest group alive at the time of publication, although stem cell source was not reported to have an impact on overall survival. GVHD, multi-organ failure and pulmonary toxicity were the major causes of death.

In a report from the International Bone Marrow Transplant Registry (IBMTR), Freytes et al[19] examined the outcomes of 114 patients with low, intermediate, and high-grade Non-Hodgkin Lymphoma (NHL) or Hodgkin Lymphoma (HL) who underwent allogeneic HSCT for relapse disease after autologous transplant. Sixty-one percent of the patients received stem cells from a matched-related donor. The one year overall survival for this group of patients was close to 43% and was significantly better than patients who received grafts from other sources. TBI-based conditioning regimens and complete remission at the time of HSCT were also significantly associated with a better outcome. Peniket et al[20] reported on an EBMT registry matched study that compared outcomes of 1185 allogeneic stem cell transplants to 14,687 autologous transplants from 1982 to 1998 in patients with low grade NHL, intermediate grade NHL, high-grade NHL, Burkitt's lymphoma, and Hodgkin disease. In each disease subcategory, more than 81% of the donors were matched-siblings. The majority of patients in the allogeneic arm had advanced disease. A matched analysis showed that overall survival was superior in the autologous arm for every disease category despite better relapse rates in many categories of lymphoma. Poorer outcomes in the allogeneic arms were attributed to advanced disease stage and chemo-resistance at the time of HSCT, grades III and IV GVHD, and treatment related mortality. Overall survival at 1 year was about 60% for low grade lymphoma, about 50% for the other lymphomas, and about 40% for Hodgkin disease.

Akpek et al[21] examined the long-term results of 157 patients with relapsed Hodgkin disease who underwent autologous or matched-sibling allogeneic transplant using various conditioning regimens. Fifty-three patients received matched-sibling allografts and had a 42% probability of survival at one year and a 36% probability of event-free survival at one year. GVHD was the most common cause of treatment-related mortality, and having disease that was still sensitive to chemotherapy was associated with a better event free survival.

Several large studies have examined outcomes of patients after matched-sibling HSCT with heterogeneous diagnoses. Retrospective data reported to the IBMTR and EBMTR was analyzed by Champlin et al[22] for patients undergoing matched sibling HSCT for ALL, AML, and CML. The purpose of the study was to compare outcomes for 536 patients receiving bone marrow versus 288 patients receiving blood stem cells from their

donors. All patients with acute leukemia were in first or second CR, and patients with CML were in chronic or accelerated phase. The patients received a variety of conditioning regimens, with 40% of the patients receiving TBI. The probability of LFS for patients with acute leukemia in first CR at one year was 70% with blood stem cells and 61% with bone marrow. Probability of LFS among patients with acute leukemia in 2nd CR was 77% with blood stem cells and 57% with bone marrow. The probability of LFS among patients with CML transplanted in chronic phase was 63% with blood stem cells and 74% with bone marrow. Patients with CML transplanted in accelerated phase or second chronic phase had a probability of LFS at one year of 68% with blood stem cells and 23% with bone marrow. For patients with advanced disease, blood stem cell products were associated with a better outcome.

The Stem Cell Trialists' Collaborative Group[23] performed an individual patient data meta-analysis of nine randomized trials involving 1,111 adult patients undergoing HSCT who received peripheral blood stem cells versus bone marrow from their donors. All of the studies required an HLA-matched sibling donor except for one of the smaller studies (N=60) which allowed a one mismatched family donor. Diagnoses included both early and late stage AML, ALL, CML, myeloma, NHL, Hodgkin disease, MDS, and myelofibrosis. Disease-free survival (DFS) at one year was between 60 and 70% for both stem cell and bone marrow recipients. In early stage disease, one year overall survival for both groups was between 75 and 80%. Patients who had later stage disease had a one year overall survival of about 58% for blood stem cell recipients versus 48% for bone marrow recipients. This was related to a higher incidence of relapse among patients receiving stem cells from bone marrow. At 3 years, relapse rates, DFS, and OS were improved for patients with late stage disease receiving blood stem cell transplants. The use of blood stem cells was associated with a significantly increased risk of acute and extensive chronic GVHD.

Finally, Alyea et al[24] examined the outcomes of 152 patients over the age of 50 undergoing HLA-matched HSCT who received a myeloablative versus a non-myeloablative transplant. There were 81 patients in the myeloablative arm, 64% of whom had matched-sibling donors. Diagnoses included AML, CML, ALL, MDS, NHL, and CMML. Patients had early and late stage disease, and almost all of the patients received CY/TBI for conditioning. Overall survival at one year was 38%, and progression-free survival at one year was 36%. GVHD, pulmonary complications, and infection were major contributors to transplant-related mortality.

Based upon the results discussed above, younger patients with early stage leukemia can expect a one year DFS of 70% to 80%. Older patients, patients in later stage remission or relapse, patients with lymphoma, later stage MDS or myelofibrosis have much lower survival rates and should be considered a high-risk group. With the rare exception of disease status, none of these adverse risk characteristics can be changed. As reflected in the above literature review, one year DFS percentages for these higher-risk patients are

between 35-60%. As opposed to pre-transplant risk factors, post-transplant complications have the potential to be avoided or abrogated with improved conditioning regimens. Three major sources of post-HSCT mortality are infection, relapse and GVHD. All three of these complications are to some extent related to T-cell function post-transplant. New methods of transplantation that lead to earlier lymphoid recovery without significant GVHD post HSCT may attenuate these complications and result in better outcomes. At one year post transplant, a regimen that could improve DFS percentages in high-risk patients to at least 60% would demonstrate efficacy in terms of decreasing treatment-related mortality, early infectious death, early relapse, and acute GVHD.

Strategies to Improve HLA-Matched Related HSCT

If there was a mechanism to manipulate the donor lymphocyte content of the graft in such a way that the donor lymphocytes were made tolerable to the host, morbidity from GVHD would likely decrease without affecting the benefits of lymphoid reconstitution relating to infection and relapse. There is data regarding the immune modulating effects of many drugs, such as cyclophosphamide (CTX) and GM-CSF that may be exploited to create immune tolerance of donor lymphocytes in the transplant inoculum. CTX has been a backbone of allogeneic conditioning for years, however most of its use has been prior to the infusion of donor cells, and thus tolerizing effects on donor T lymphocytes have not been realized. An HSCT regimen that allows donor cells to be given in two steps, the lymphocyte infusion and the stem cell infusion, would provide an opportunity to tolerized donor lymphocytes without affecting donor stem cells.

Cyclophosphamide

CTX is an alkylating agent used extensively in the treatment of malignancies in both transplant and non-transplant settings. Athough CTX has direct effects on tumor cells, its modulation of T-cell responses to tumor have also documented. CTX was shown to cause immunologically mediated regression of CTX-resistant L5178Y lymphoma in mice by eliminating tumor-induced suppressor T-cells which resulted in an increased number of Lyt-2^+ T-cells capable of augmenting immunity and causing tumor regression.[25,26] The direct cytotoxic effect of CTX on malignant cells, as well as the adoptive immunotherapeutic properties of CTX against tumor cells, make it an effective anti-cancer therapy.

CTX is also known to be preferentially cytotoxic to activated T-cells and to induce clonal deletion of these cells in the periphery and thymus.[27] It is this aspect of CTX's immunomodulatory effects that may prove beneficial in HSCT.

Much of what is known about the immunomodulatory effects of CTX come from murine systems of CTX-induced tolerance. In one such model, called a "cells-followed-by-CTX system,"[28-30] CTX is given 1 to 3 days after antigenic stimulation with allogeneic cells (usually spleen cells). In this type of system, tolerance to tumor, skin, or other solid

organs can be induced.[31] The infusion of allogeneic cells results in a proliferative response in mature T and B cells after exposure to alloantigen. The DNA in the proliferating cells is especially sensitive to CTX, therefore alloreactive clones are preferentially destroyed, including donor cells which might mediate GVHD and host cells which might mediate rejection.[32] In the cells-followed-by CTX murine system, intrathymic chimerism also occurs when donor type cells arising from stem cells contained in the infused spleen or marrow cells [33] are regenerated. As a result, subsequent intrathymic clonal deletion of donor-antigen reactive T-cells occurs. Thus, alloantigenicity and hematopoietic regenerative capability are necessary for long-lasting tolerance.[33,34] One possible difficulty in applying this type of immunological approach to human matched-sibling HSCT is that in the murine haploidentical models tolerance induction relies on a disparity between donor and host lymphocytes.[34-38] In the matched sibling setting, this disparity is not as great and therefore the activation and subsequent deletion of reactive lymphocytes may not be as profound. The effectiveness of CTX given after lymphocytes to induce tolerance in a human matched-sibling setting is unknown.

The establishment of tolerance to allografts with the use of CTX has been demonstrated by many other investigators. Allogeneic tolerance was established in diabetic mice when simultaneous transplantation of islet cell and bone marrow was performed just after treatment with fludarabine and cyclophophamide.[39] Zhang et al, [40] demonstrated CTX induced tolerance in C3H mice to skin and heart allografts from H-2 matched AKR mice after priming the recipient mice with donor spleen cells followed by intraperotineal CTX. In this study, the clonal destruction of MIs-1a-reactive CD4+ Vbeta 6^+ T-cells in the periphery, and the clonal deletion of Vbeta 6^+ thymocytes were observed. Luznik et al, [41] demonstrated that the dose of TBI in allogeneic marrow transplantation in mice could be reduced from 500 cGy to $\leq$ 200 cGy with the addition of post-transplant CTX. Animals that were conditioned with TBI alone or given post-transplant CTX alone failed to achieve engraftment and contained host anti-donor cytotoxic T-cells.

CTX-induced tolerance in rat transplantation has also been demonstrated when CTX is given after allogeneic stimulation. Okano, S et al, [42] gave Lewis recipients spleen and bone marrow cells from Dark Agouti (DA) donors followed by CTX 2 days later. DA livers were then grafted on d+25. Rats survived for more than 165 days with donor-specific tolerance confirmed by second skin grafts. In other studies with rats, fetal small bowel, heart, and kidney allografts were successfully placed with the establishment of CTX-induced donor tolerance. [43-45]

In human T-cells responding to LCL cell lines, CTX induced apoptosis and modulation of activation and effector functions. Strauss et al, [27] examined the mechanisms of action of various immunosuppressive agents on LCL line 721.221, CIR, and the mouse mastocytoma cell line P1.HTR. In these studies, CTX was found to induce massive apoptosis in activated T-cells. In the same study, CTX was found to accelerate CD95-

mediated apoptosis in human T-cells and suppress activation of naïve T-cells at the initiation step of an allo-response without affecting cytokine production. This finding suggests that CTX may induce deletion of antigen-specific T-cells.

In human clinical treatment, CTX has been used successfully with and without other immunosuppressive agents to treat a variety of autoimmune disorders. CTX has selective immune effects in multiple sclerosis by suppressing IL-12 and Th1-type responses and is effective in worsening MS that has an inflammatory component due to its ability to ablate autoaggressive lymphocytes.[46] Traynor et al used CTX in the setting of autologous HSCT to obtain remission in patients with end-stage lupus.[47] In this study, patients had spontaneous decline or normalization of the T-cell activation marker, CD 69^{+} after the therapy without an excessive rise of this marker upon stimulation. CTX has also been used successfully to treat acquired hemophilia,[48] rheumatoid arthritis, [49] and autoimmune hepatitis.[50] High-dose CTX has also been used in the treatment of aplastic anemia because of its ablative effect on activated lymphocytes. In this setting hematopoietic stem cells are spared because they contain aldehyde dehydrogenase, an enzyme which degrades cyclophosphamide thus providing partial protection to the stem cells from the cytotoxic effects of CTX.[51]

Data and experience with CTX-induced tolerance in human allogeneic transplantation is limited. Luznik et al, [52] reported on 46 patients who received high dose CTX after matched related and unrelated HSCT as the sole agent for GVHD prophylaxis. The cumulative incidence of grades III-IV GVHD in this group was only 9%. In partially-matched related HSCT, O'Donnell et al, [53] reported on 10 patients receiving CTX after the infusion of donor cells. Although follow up was short, four patients were alive without significant GVHD.

These human studies provide proof of principal of the tolerizing effects of CTX. In both studies, CTX was given **after** the infusion of the donor product. One potential deleterious effect of this method is the effect that CTX may have on the progenitor cells contained in the product. Aldehyde dehydrogenase contained in progenitor cells is thought to be protective against the effects of CTX, and unquestionably, count recovery is possible after high doses of CTX. However there is data that demonstrates both negative effects of CTX on progenitor cells and variable patient response to the effects of CTX. Studies examining the effects of 4-hydroperoxycyclophosphamide (4HC) on human progenitor cells in marrow purging demonstrate dose-related inhibition of hematopoietic colony formation, [54] significantly increased times to leukocyte recovery after transplant, [55] and interpatient variability to the effects of 4HC on CFU-GM recovery. [56] In addition to the short term effects of CTX on progenitor cells, this cytotoxic agent has also been implicated in the development of serious late effects on hematopoietic progenitor cells, including acute leukemia [57] and MDS. Le Beau et al [58] reviewed the clinical course of 63 patients with therapy-related MDS or AML. Twenty-one of these patients developed their secondary malignancy after chemotherapy alone.

Seven of the 21 patients had received therapy with CTX, including one patient who had received CTX alone. In contrast, secondary lymphomas have been far less common.

Based on these studies, an optimal method to establish donor tolerance without exposing donor progenitor cells to CTX in allogeneic HSCT may be to establish a 2-step transplant process. In this scenario, donor lymphocytes would be given first, followed three days later (after activation) by CTX similar to the "cells-followed-by-CTX" model discussed above. As a second step, CD34+ selected progenitor cells would be infused after CTX has been metabolized. Thus the benefit of CTX elimination of alloreactive lymphocytes could be realized, while the hematopoietic progenitors would be spared CTX exposure. Here at Jefferson, this 2 step model of HSCT has been introduced for patients undergoing partially-matched related HSCT. Of the 7 patients treated so far, there has only been one case of serious (grade III) GVHD, which as promptly responded to steroids.

Growth Factors

Another potential strategy to manipulate donor lymphocytes is through the use of growth factors which are known to affect their cytokine secretion profile as they mature after recognizing antigen. This has been demonstrated in $CD4^+$ and $CD8^+$ subsets of T-lymphocytes as well as in NK-cells in humans and murine models.[59-62] The differentiation takes place either towards a type-1 or a type-2 cytokine secretion profile. G-CSF (granulocyte-colony stimulating factor) has been shown to skew lymphocytes toward a type 2 secretion profile whereas GM-CSF (granulocyte-macrophage-colony stimulating factor) skews them toward a type 1 profile.

It has been shown for $CD4^+$ and $CD8^+$ subsets of T-cells that type-1 cytokine-secreting cells (termed Th1 & Tc1 cells) predominantly secrete γ-interferon and mediate and escalate GVHD by recruiting secondary effector cells that include cytotoxic T lymphocytes (CTLs), NK-cells and macrophages that kill host cells. In addition, these type-1 cytokine-secreting T-cells directly mediate host cell apoptosis by utilizing the perforin-granzyme pathway as well as by engaging the Fas (CD95) receptor on the host target cell membrane. On the other hand, type-2 cytokine secreting cells (Th-2 and Tc-2) that predominantly secrete IL-4, IL-5 and IL-10 tend to inhibit GVHD.[63]

G-CSF

Peripheral blood stem cell products from granulocyte-colony stimulating factor-mobilized donors contain a ten-fold higher dose of T-cells than products obtained from bone marrow harvest. Despite this difference in T-cell content, the rates of acute GHVD are the same for recipients of PBSC products as they are for those who receive marrow products. Arpinati et al [64] enumerated dendritic cell subsets in peripheral blood products from normal donors treated with G-CSF versus bone marrow products from unstimulated donors. They demonstrated that DC-2 dendritic cells were found in much higher numbers in the G-CSF –mobilized product, and that this resulted in polarization of T-cells toward a Th2 response. DC1 content was the same for both products. Franzke and

colleagues [65] showed that a G-CSF receptor can be induced on T-cells, and have functional activity, when T-cells are stimulated with G-CSF. Thus G-CSF's effects on polarization of T-cells towards a Th-2 subtype is both direct and indirect. Th-2 cells are associated with less cytotoxic activity and proliferative response than Th-1 cells. It is this imbalance in Th-1 and Th-2 subsets that is thought to be responsible for the mitigation of GVHD despite the use of these T-cell rich grafts.

In the setting of allogeneic HSCT, one could argue that the use of a G-CSF-primed donor product may be optimal to decrease the incidence of GVHD. The use of this product is a double-edged sword however. Volpi and colleagues, [66] compared the lymphocyte profiles of 2 groups of patients post T –cell depleted partially-matched related donor HSCT. The first group contained 36 patients who received post-transplant G-CSF. The second group (43 patients), were not given G-CSF at any time. The investigators found that the patients receiving post-transplant G-CSF had impaired production of interleukin 12 by dendritic cells and delayed recovery of T-cells with functional Th1 reactivity. This impairment of functional immune recovery was long-lasting and was expected due to the use of G-CSF. Although not reaching statistical significance, the group receiving post-transplant G-CSF had an infection-related mortality rate of 35% as compared to the 25% infection-related mortality rate of the group that did not receive G-CSF. GVHD was not significantly affected in either group. In HSCT, early immune function is dependent upon the expansion of a donor lymphocyte population infused with the graft until de novo production of naïve T-cells occurs. Skewing the available T-cell population towards a Th2 subtype may magnify defects in reconstitution Therefore, it may be reasonable to assume that the benefits of using G-CSF to decrease GVHD in this setting may be outweighed by the risks of infection related to the lack of T-cell cytotoxic activity.

GM-CSF

GM-CSF induces the growth and differentiation of several different cell lines including those of neutrophils, eosinophils, and monocytic colonies. [67] The use of GM-CSF has been shown to decrease the duration of severe neutropenia, and consequently decrease fatal infections after AML induction therapy. [68] In allogeneic transplantation, GM-CSF therapy significantly shortened the duration of neutropenia, decreased length of stay, and reduced the use of antimicrobials. [69]

Whereas G-CSF does not have a significant effect on monocytes or macrophages, GM-CSF has a stimulatory effect on Th1 lymphocyte-inducing dendritic cells which facilitates cell-mediated immune responses. It is this property of GM-CSF that seems to convey superior infectious protection versus G-CSF, beyond that of simply decreasing the period of neutropenia. [70] For example, Peters et al [71] found that patients undergoing high-dose chemotherapy with or without auto PBSC who received filgrastim or no growth factor had a 4.2 times greater risk of developing a fungal infection than those patients who received GM-CSF. Because GM-CSF is known to enhance the functional

effects of monocytes, macrophages and dendritic cells, [72] its use has also been associated with anti-tumor activity both alone [73] and in combination with monoclonal antibodies[74,75] and vaccine therapy.[76]

In the setting of allogeneic HSCT, the use of GM-CSF in donor PBSC mobilization would be expected to produce a skewing of TH1/Th2 balance of the donor lymphocytes contained therein toward a TH1 pattern, reciprocal to G-CSF's effects. Presumably, this would result in a greater ability of donor lymphocytes to mount an inflammatory response which may help fight infection. However, a GM-CSF induced imbalance may favor the development of GVHD. Therefore, the avoidance of any growth factor effect during collection of the lymphocytic portion of the transplant product may be the most beneficial option. Post-transplant, the use of GM-CSF appears to convey a degree of infectious protection and anti-tumor activity that is not afforded by G-CSF by virtue of its stimulation and enhancement of cell lines other than neutrophils. Therefore, in patients receiving allogeneic HSCT, post-transplant GM-CSF may help reduce the morbidity associated with this type of procedure.

Strategy For the Current Protocol - A Two-Step Approach to Partially-Matched Related Donor HSCT

In the following protocol, we seek to decrease complications of matched sibling HSCT in high-risk patient groups and increase disease-free and overall survival. In order to accomplish this, patients on this protocol will receive their transplant in 2 steps. Many of these patients have been heavily pretreated and may be chemotherapy-resistant. Therefore, a TBI-based regimen will be used in this protocol. The combination of TBI and cyclophosphamide is one of the most durable and successful transplant conditioning regimens and will be used as the basis of this therapy. TBI will be administered first in this protocol. Next, a fixed dose of donor lymphocytes, collected prior to any donor growth factor exposure, will be infused just after TBI in order to avoid graft rejection and post-transplant infection. The starting dose of lymphocytes will be 2 X 10e8. This dose is based upon our already established experience with this type of transplant in partially-matched related HSCT and as of this writing, has successfully obtained engraftment in 7 patients. Because the donor will have lymphocyte collection prior to G-CSF administration, the infused lymphocytes will express a balanced cytokine profile which will hopefully allow a better immune response to infection. Three days after the donor's lymphocytes are infused we will attempt to establish donor-host tolerance, minimizing the risks of both graft rejection and severe GVHD by administering two doses of cyclophosphamide to the patient. After collection of the lymphocytes, donors will then begin priming with G-CSF, in preparation for a PBSC collection which will allow a higher $CD34^+$ content than that obtainable through marrow harvest. The last step of the transplant process, the infusion of donor progenitor cells, will occur two days after cyclophosphamide. In this way, donor progenitor cells will not be exposed to the toxic effects of CTX. These PBSC will be $CD34^+$ selected to remove contamination by any

additional T cells which would not be in vivo treated with CTX by virtue of their infusion with the CD34+ cells after CTX therapy.

An important aspect of this two step approach is that it separates the dosing of T cells and hematopoietic stem cells in ways not possible with other approaches. Normally these two cellular populations are contained in the same transplant product and the doses of these cells cannot be independently controlled. This two step approach allows us to give the entire innoculum of hematopoietic stem cells, but to vary the T cell dose using the method of continuous reassessment to define the optimal T cell dose which will maximize engraftment while minimizing GVHD. This will hopefully allow the outcome of therapy to become more reproducible one patient to the next.

In summary, this two-step transplant process will:

Allow us to define and then consistently utilize a fixed, optimized dose of lymphocytes
Avoid the effects of G-CSF on donor lymphocytes
Promote the establishment of donor-recipient tolerance by in vivo CTX treatment
Increase the amount of donor progenitor cells collected by using PBSC mobilization as opposed to OR harvest
Prevent the exposure of donor progenitor cells to cyclophosphamide

This is a phase II protocol with the primary objective being an increase in disease-free survival at on year in a high-risk patient population. Any patient who has failed standard treatment for their disease may be treated on this protocol. However, only patients designated as high-risk will be assessed in terms of efficacy of this treatment.

3.0 Patient Selection

Inclusion Criteria

1) Any patient with a hematologic or oncologic diagnosis in which allogeneic HSCT is thought to be beneficial, and in whom front-line therapy has already been applied. Patients will be considered high-risk if they have any of the following:
    a. Age > 50 years
    b. ECOG Performance status of $\leq$2
    c. Acute leukemia: requiring more than one chemotherapy regimen to obtain 1st CR; second or greater CR, 1st relapse; any ph+ ALL
    d. CML 2nd chronic phase, accelerated phase, or blastic phase
    e. MDS with IPS of Intermediate 2 or greater
    f. Any myeloproliferative disorder
    g. Hodgkin lymphoma: relapsed, refractory, or primary induction failure

h. Non-Hodgkin lymphoma: relapsed, refractory, primary treatment failure, or not eligible for an autologous HSCT
i. Other conditions not listed will be assessed as high-risk by the PI

2) Patients must have a related donor who is either HLA-identical or a one antigen mismatch at the HLA- A; B; C; and DR loci.

3) Patients must adequate organ function:

a. LVEF of $\geq$45%

b. DLCO (adjusted for hemoglobin) $\geq$45% of predicted

c. Adequate liver function as defined by a serum bilirubin $\leq$1.8, AST or ALT $\leq$ 2.5X upper limit of normal

d. Creatinine clearance of $\geq$ 60 ml/min

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

5) Able to give informed consent

Patient Exclusion Criteria

1) ECOG performance status of 3 or 4.

2) HIV positive

3) Active involvement of the central nervous system with malignancy

4) Psychiatric disorder that would preclude patients from signing an informed consent

5) Pregnancy

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

Donor Selection

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

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

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

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

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

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

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

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

4.0 Informed Consent

Patients referred for the trial will have their eligibility criteria verified. On meeting the eligibility for the trial as outlined, informed consent will be obtained

using forms approved by the Thomas Jefferson University Hospital Institutional Review Board and following guidelines related to the use of human subjects in research. The risks and hazards of the procedure, as well as alternative forms of therapy will be presented to the patient in detail. Patients will receive a signed copy of the consent form after the consent interview.

5.0 Treatment Plan

Proposed Schema **- Patient**

	-9 Tues	-8 Wed	-7 Thu	-6 Fri	-5 Sat	-4 Sun	-3 Mon	-2 Tues	-1 Wed	0 Thu
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 Thu	-6 Fri	-5 Sat	-4 Sun	-3 Mon	-2 Tue	-1 Wed
AM	Lymphocyte Collection	Lymphocyte Collection *(If Needed)*	Neup**	Neup	Neup	Neup PBSC Collection	Neup PBSC Collection
PM			Neup	Neup	Neup	Neup	

*Mycophenolate Mofetil (MMF)
**Neupogen (Neup)

There should be no administration of agents that suppress lymphocyte reactivity from admission until day -1 in this protocol. This includes steroids, calcineurin inhibitors, MMF, or monoclonal antibodies that affect lymphocyte number or function. If patients have previously required steroids as a premedication for transfusion, they may receive a dose of steroid equivalent to 5 mg of prednisone through day -7. After day -7, the majority of TBI is complete. At this time, the immune system response to alloantigens should be somewhat attenuated. Diphenhydramine and meperidine may be used if necessary. Any use of steroids from day -6 through day 0 should not be administered without approval from the PI.

5.1 TBI

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 unless they have received prior irradiation. Prior irradiation will be evaluated by the radiation

oncologist to define eligibility for this TBI schedule. In addition there may be technical or patient related factors which will require some minor modification in the TBI technique utilized. Selected patients may require local boosting of certain organ sites prior to conditioning therapy. Deviations from the guidelines described here may only be performed with the approval of the radiation oncologist and the investigators. See Appendix A for radiation guidelines.

5.2 Donor Lymphocyte Infusion

The dose of the donor lymphocyte infusion (DLI) will be 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 (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. An exact dose of 2 X 10e8/kg lymphocytes will be infused. Donors will be apheresed for lymphocytes on days -7 and -6.

For the donor lymphocyte apheresis, total blood volumes to be processed will be determined using the following calculation:

Recipient weight in kg: _____kg
Multiply by desired CD3+ cells/kg: x _____x10(7)/kg
Total CD3+ cells requested: = _____x10(7)
Multiply by 2 x 2
TOTAL mononuclear cells (TMC) = _____x10(7)
Divided by 100 x 10(&) TMC/L = _____ Liters processed

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

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

An aliquot of the apheresis product will be assessed for CD3 content by flow cytometry. The following cell panel will be used:

ITC	PE
gG1	IgG1
gG1	IgG2a
D45	CD14 + CD13
D3	CD4
D3	CD8
D3	CD16 + CD56
D3	CD19
D4	CD8

A gate is drawn around the entire CD45+ population. %WBC/total events = the percentage of CD45+ cells within this gate corrected for the isotype control. CD3 percentages are calculated, corrected for the isotype control, based on the total white cell (CD45+) gate, not based on a "lymphocyte gate". There are 4 CD3 counts performed in the panel. The two median values are averaged to determine the final raw CD3 count. The raw CD3 count is then corrected for any counted events which are not WBC (i.e. CD45-), as follows:

Corrected %CD3 = (raw CD3 count)/ (%WBC/total events).

Total T-cells required for the initial infusion = ($2x10^8$ T-cells/kg) * (Weight in kg)
T-cells/ml of product
T-cells/ml of product = (WBC) * (Corrected %CD3)
Volume to be infused = (Total T-cells required for the initial infusion)/(T-cells/ml of product)

Lymphocyte apheresis will be performed at Thomas Jefferson University Hospital or the American Red Cross, by trained apheresis personnel using standard techniques. No hematopoietic growth factors will be administered to apheresis donors prior to lymphocyte collection. The donor will have venous catheters placed in each arm for the purposes of undergoing leukopheresis. Leukocyte collections will be performed using a standard apheresis machine such as the

Cobe Spectra apheresis instrument (Cobe Laboratories Inc., Lakewood, CO).

During the infusion, the patient will be monitored for any untoward reactions. Each infusion will take place in the Bone Marrow Transplant Unit. Donor lymphocyte infusions will be administered by nursing staff experienced in the administration of blood products.

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

5.3 Cyclophosphamide

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

Voriconazole can block the conversion of CTX to its active metabolite, 4-hydroxycyclophosphamide. For this reason, no voriconazole will be administered to any patient from admission (or the beginning of conditioning) until day -1. Voriconazole may be started on day -1. There are no restrictions on the use of ambisome.

5.4 Collection and Infusion of Progenitor Cells

The dose of progenitor cells will be based on CD 34^+ cells per kilogram of recipient body weight. Donors will begin G-CSF, 5µg/kg bid, on day -5. The donor will return for neupogen-primed progenitor cell collection on days -2 and -1. Each day, 18-27 liters will be processed. CD34+ cell enrichment will be performed via the closed system method using the CliniMACS® CD34 Reagent System (Miltenyi Biotec Inc., Auburn, CA). The CliniMACS system utilizes super-paramagnetic particles composed of iron oxide and dextran conjugated to monoclonal antibodies. These antibodies bind to target cells with the corresponding cell surface antigen (in this case, CD34). After magnetic labeling, the cells are separated using a high-gradient magnetic separation column. The magnetically labeled cells are retained in the column and separated from the unlabeled cells. Removing the magnetic field from the separation column elutes the retained cells. Eluted cells will be characterized using fluorescent-activated cell sorting (FACS) analysis. All procedures will be performed in a sterile environment with strict adherence to all applicable regulations regarding the processing and use of human stem cells. The use of this device will conform to TJU BMT Laboratory standard operating procedures.The dose of $CD34^+$ cells/kg

is not to exceed 10 X10e6/kg. A minimum dose of 2.0 X 10e6/kg should be collected. Failure to meet this target may result in a third day of collection.

In our experience, the ideal amount of T-cells left in the $CD34^+$ product is no greater than $5x10^4$/kg, so that every effort will be made to keep T-cell amounts to below this threshold. It is recognized that because of donor heterogenicity, every product will have varying percentages of cells. Thus, patients will be advised during the informed consent process that an excess amount of residual T-lymphocytes in the $CD\ 34^+$ product may increase the risk of GVHD.

Progenitor cell apheresis will be performed at Thomas Jefferson University Hospital or the American Red Cross, by trained apheresis personnel using standard techniques. The donor will have venous catheters placed in each arm for the purposes of undergoing leukopheresis. 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.

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

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

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

Benadryl, epinephrine, and hydrocortisone should be available for emergency use if necessary. Oxygen with nasal cannula should be set-up and available in the patient room.

5.5 GVHD Prophylaxis

The day -1 tacrolimus dose is a loading dose and will be 0.03 mg/kg IV in a divided dose whether the patient is on voriconazole or posaconazole. Starting on day 0, tacrolimus will be maintained at a dose of 0.015 mg/kg in divided doses IV if given simultaneously with voriconazole or posaconazole. If the patient is not receiving voriconazole or posaconazole, the dose of tacrolimus will remain at 0.03 mg/kg in divided doses IV. Tacrolimus levels will be checked daily starting on day 0. Tacrolimus dosing should be titrated to maintain a target level of 8ng/ml +/- 2.

MMF will be dosed at 1 gram IV BID beginning on day -1. MMF oral dosing will be initiated at least 2 to 3 days prior to discharge to assure that patients tolerate the oral drug prior to going home.

Tacrolimus oral dosing will be initiated at least 2 to 3 days prior to discharge. This is to assure that stable, therapeutic levels are maintained after hospitalization.

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

Patients without active GVHD will be maintained on tacrolimus until day +42. Tacrolimus will be weaned starting at day +42 in the absence of GVHD (mild, localized skin GVHD treated with topical steroids is excluded), and completed by d +56 if tolerated. Once tacrolimus levels are less that 5 ng/ml levels, they no longer have to be checked unless there is a clinical concern to do so.

MMF will be discontinued beginning at day +28 +/- 3 days in the absence of GVHD. MMF may be discontinued earlier if there is count suppression thought to be due to the drug.

6.0 Study Measurements**

All post-allogeneic transplant patients have physical assessments, laboratory studies and pathology studies performed as per the TJUH BMT Guidelines for Post-Transplant Allogeneic Assessments found on the BMT link on the TJUH Intranet.

The table below outlines the mandatory measurements/time points for this study.

	Day + 28	Day +90	Day +180	Day + 270	1 Year	
GVHD Assessment Presence and degree of skin rash, presence and amount of diarrhea, LFT's	X	X	X	X	X	
Chimerism/ Disease Assessment						
Peripheral blood for CD3+ chimerism & Buffy coat chimerism (and/or FISH for xx/xy if opposite-sex donor)	X	X	X	X	X	
Bone marrow exam (morphology, flow cytometry, cytogenetics, BC chimerism)	X	X	X	X	X	
Immune Reconstitution Studies						
Flow cytometry for lymphocyte subsets	X	X	X	X	X	

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 day +90, +180, +270, and 1 year marrows can be obtained within the time period of 1 month before or 1 month after the targeted time to account for patient scheduling factors. This table represents a <u>minimum</u> recommended sampling and visit strategy.

6.1 Hematopoietic engraftment. Will be defined as

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

6.2 Toxicity Criteria. Regimen-related toxicity will be graded according to the NCI Common Toxicity Criteria, version 3.0. The NCI Common Toxicity Criteria can also be found at the following WEB address:

http://ctep.cancer.gov/reporting/ctcnew.html

6.3 Disease Response:

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

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

6.4 GVHD Scoring

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

6.5 Adverse event reporting. All patients will be followed for serious adverse experiences (SAEs), regardless of relationships to study treatment, from the time of enrollment. The following events are expected side effects of high-dose chemotherapy and transplant and will not be reported except as noted:

- Alopecia, dry skin, headache
- Emesis from chemotherapy or other agents unless refractory to standard supportive care, nausea, anorexia, weight loss, dry mouth
- Neutropenia/uncomplicated neutropenic fever –Only infection associated with shock will be reported. Other infections will be recorded.
- Neutropenia/uncomplicated neutropenic fever, grades 1-3 infectious sequellae
- Thrombocytopenia, Petechiae, ecchymoses, minor vaginal bleeding, epistaxis, hemorrhoidal bleeding, or other similar bleeding events will not be reported. (Bleeding events requiring transfusion and/or intervention such as endoscopy or radiologic evaluation will be reported.)
- Anemia

- Grade I - III Mucositis
- Grades I-III Diarrhea Allergic or other common reactions to drugs used for supportive care unless grade 3 or higher

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

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

6.6 Reports to the Federal Drug Administration (FDA)

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

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

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

6.7 Study Endpoint

The endpoint of this study is OS at 1 year.

7.0 Supportive Care

7.1 Avoidance of Infection

Patients who are post allogeneic HSCT will follow the same guidelines as patients who are neutropenic until advised differently by their attending physician. Infectious prophylaxis and treatment of infection will be as per the "TJUH Guidelines for Infectious Prophylaxis and Management of Febrile Neutropenia". These guidelines can be found on the BMT link of the TJUH Intranet.

The TJUH document "**Preparing To Go Home After Bone Marrow Transplant**" will be reviewed with each patient and family ideally prior to admission, but before discharge.
Central venous catheters will be removed as soon as clinically manageable.

Patients who are post allogeneic HSCT may be given documentation which identifies them at high risk for infection and GVHD if they desire. This documentation will be signed by their attending physician and may be used for ED visits or documentation of transplant. An example of this type documentation is contained in Appendix C.

7.2 Infectious Prophylaxis-General Guidelines

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

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

Patients post allogeneic HSCT will be maintained on PCP prophylaxis, usually Bactrim DS 1 BID, 3x weekly. It is at the discretion of the treating attending physician to change agents based on culture results, drug intolerance.

Prophylactic medications **may** be discontinued when the patient is no longer on immunosuppressive medications, and the CD4 count is $\geq$ 100/µl.

7.3 Growth Factor and Transfusion Support

To prevent inadvertent lymphoid engraftment, all mature blood cell products must be irradiated to >/=2500cGy.

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

White cell growth factor will be administered beginning on day +1. After white count recovery, discontinuation of the white cell growth factor is at the discretion of the attending physician; however, every effort should be made to keep the ANC $\geq$ 1000 for all patients post HSCT.
Red cell growth factors are permissible after transplantation.

8.0 Drug Information and Administration

8.1 Cyclophosphamide

Mechanism: A multistep process activates it by conversion to 4-hydroxycyclophosphamide by the liver microsomal oxidase system and to aldophosohamide by tautomerization in the peripheral tissues. Aldophosphamide spontaneously degrades into acrolein and phosporamide mustard, which cause cellular glutathione depletion and DNA alkylation. This results in inhibition of DNA replication and transcription. Cells expressing high levels of aldehyde dehydrogenase (e.g. stem cells, L1210 leukemia cells) resist cyclophosphamide-mediated cytotoxicity as aldophosphamide is inactivated by this enzyme. The drug also does not affect quiescent cells and therefore stem cells are generally protected, an important factor if autologous hematopoietic recovery is relied on in the event of graft failure.

Metabolism: Cyclophosphamide is broken down as described above and the break down products are excreted by the kidneys.

Incompatibilties: Phenobarbital or rifampin may increase the toxicity of cyclophosphamide. Concurrent allopurinol or thiazide diuretics may exaggerate bone marrow depression May prolong neuromuscular blockade from succinylcholine Cardiotoxicity may be additive with other cardiotoxic agents (cytarabine, daunorubicin, doxorubicin). May decrease serum digoxin levels. Additive bone marrow depression with other antineoplastics or radiation therapy. May potentiate the effects of warfarin. May decrease antibody response to live-virus vaccines and increase the risk of adverse reactions. Prolongs the effects of cocaine.

Toxicity: Nausea, vomiting, water retention due to inappropriate secretion of anti-diuretic hormone (SIADH), cardiomyopathy with myocardial necrosis and congestive heart failure, hemorrhagic cystitis, alopecia, skin rash, pulmonary fibrosis, sterility and secondary malignancies.

Administration: Patients will receive a dose of cyclophosphamide 60 mg/kg IV, on days –3 and -2. The dose of cyclophosphamide will be calculated according to the dosing body weight. The cyclophosphamide dose is dissolved in saline and administered as a 2 hour IV infusion. Patients shall receive hydration consisting of normal saline solution at 3 ml/kg/hour (actual weight) for 2 hours before and 8 hours after the cyclophosphamide infusion. MESNA (sodium-2-mercaptoethane sulfonate) will be administered as a 60 mg/kg/continuous IV infusion over 24 hours starting 30 minutes prior to cyclophosphamide infusion and ending 24 hours after the last dose of cyclophosphamide. The dose of MESNA will also be calculated based on dosing body weight.

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

8.2 Donor Leukocyte Infusion (DLI)

Administration: All patients will receive a dose of $CD3^+$ T cells per kilogram of dosing body weight as outlined in the treatment design. Details of the apheresis procedure to obtain white blood cells, quantification of T cells by flow cytometry, and administration of the white cell product to the recipient are provided in the treatment section. All drugs that may cause lymphocyte suppression are held prior to lymphocyte infusion (day -6), through day 0 as detailed in the treatment section. Every effort will be made to administer the donor lymphocytes around or as close to the designated day of lymphocyte infusion. Moreover the viability of the lymphocytes will be tested by flow cytometry and the number of viable $CD3^+$ T cells will be used to dose the DLI.
Toxicity: GVHD, delayed myelosuppression, infusion reactions.

8.3 G-CSF

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

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

8.4 GM-CSF

Mechanism: GM-CSF is a recombinant human granulocyte-colony stimulating factor produced by recombinant DNA technology in a yeast expression system. It supports survival, clonal expansion, and differentiation of hematopoietic cells. GM-CSF is also capable of activating mature granulocytes and macrophages, and

is a multilineage factor with effects on the myelomonocytic, erythroid, and megarkaryocytic lines.
Metabolism: GM-CSF is detected in the serum at 15 minutes after injection. Peak levels occur about 1 to 3 hours after injection, and it is detectable in the serum for up to 6 hours after injection.
Incompatibilities: Interactions between GM-CSF and other drugs have not been fully evaluated. Drugs which may potentiate the myeloproliferative effects of GM-CSF, such as lithium and corticosteroids, should be used with caution.
Toxicities: Allergic and anaphylactic reactions have been reported. A syndrome characterized by respiratory distress, hypoxia, flushing, hypotension, syncope and or tachycardia has been associated with the first administration of GM-CSF in a cycle. These signs have resolved with treatment.
Administration: In this protocol, GM-CSF will be given to the patients beginning on Day +1 in the PM. The drug should continue until the patient has a self-sustaining ANC of 1500.

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

8.5 Mycophenolate Mofetil (MMF)
Mechanism: Inhibits the enzyme inosine monophosphate dehydrogenase, which is involved in purine synthesis. This inhibition results in suppression of T- and B-lymphocyte proliferation.
Metabolism: Following oral and IV administration, mycophenolate is rapidly hydrolyzed to mycophenolic acid (MPA), its active metabolite. Distribution is unknown. MPA is extensively metabolized; <1% excreted unchanged in urine. Some enterohepatic recirculation of MPA occurs. Half Life: *MPA*¾17.9 hr.
Incompatibilities: Combined use with azathioprine is not recommended (effects unknown) · Acyclovir and ganciclovir compete with MPA for renal excretion and, in patients with renal failure, may increase each other's toxicity. · Magnesium and aluminum hydroxide antacids decrease the absorption of MPA (avoid simultaneous administration). Cholestyramine and colestipol decrease the absorption of MPA (avoid concurrent use). Toxicity may be increased by salicylates. · May interfere with the action of oral contraceptives (additional contraceptive method should be used). · May decrease the antibody response to and increase risk of adverse reactions from live-virus vaccines, although influenza vaccine may be useful. ·When administered with food, peak blood levels of MPA are significantly decreased.
Toxicities: GI: Bleeding, diarrhea, vomiting. ·
Hematopoietic: leukopenia ·
Miscellaneous: sepsis, increased risk of malignancy
Administration: In this protocol, MMF will be administered at a dose of 1 gram IV BID beginning on day -1. MMF will be discontinued on day +28 +/- 3 days,

in the absence of GVHD. MMF may be discontinued earlier if there is count suppression from the drug.

8.6 Tacrolimus

Mechanism: Tacrolimus, it is a macrolide immunosuppressant. It inhibits lymphocytes by forming a complex with FKBP-12, calcium, calmodulin leading to the decrease in the phosphatase activity of calcineurin. This in turn prevents generation of NF-AT, a nuclear factor for initiating gene transcription for lymphokines like interleukin-2 and interferon-γ^{99}. This drug is used with corticosteroids for prophylaxis of organ rejection in patients receiving allogeneic liver transplants. Its use is also currently being investigated in kidney, bone marrow, cardiac, pancreas, pancreatic island cell and small bowel transplantation.
Metabolism: This drug is well absorbed orally. It is metabolized in the liver by unknown mechanisms and demethylation and hydroxylation has been proposed based on *in vitro* studies. The metabolized products are excreted in the urine.
Incompatibilities: Nephrotoxic drugs, antifungals (azoles), calcium-channel blockers, cimetidine, danazol, erythromycin, methylprednisone and metoclopramide increase the bioavailabilty of tacrolimus. On the other hand phenobarbital, phenytoin, rifamycins and carbamazepine decrease tacrolimus levels.
Toxicities: Adverse reactions include:

1. tremor, headache, neurotoxicity
2. diarrhea, nausea,
3. hypertension
4. TTP and renal dysfunction.

Administration: The day -1 tacrolimus dose is a loading dose and will be 0.03 mg/kg IV in a divided dose whether the patient is on voriconazole or posaconazole. Starting on day 0, tacrolimus will be maintained at a dose of 0.015 mg/kg in divided doses IV if given simultaneously with voriconazole or posaconazole. If the patient is not receiving voriconazole or posaconazole, the dose of tacrolimus will remain at 0.03 mg/kg in divided doses IV. Tacrolimus levels will be checked daily starting on day 0. Tacrolimus dosing should be titrated to maintain a target level of 8ng/ml +/- 2.

9.0 Patient Safety

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

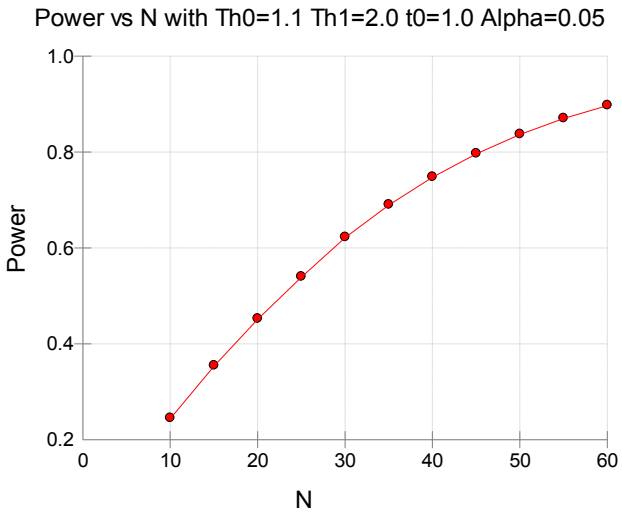
The study will be monitored monthly by the Principal Investigator (PI) and the study medical monitor. Monitoring reports will be submitted to the Clinical Research Organization (CRO), Protocol Review Committee (PRC), and the Data Safety and Monitoring Committee (DSMC). The PI will submit all unexpected on-site adverse events (AE) and serious adverse events (SAE) to the TJU IRB utilizing the electronic Kimmel Cancer Center Clinical Trials Adverse Event Reporting system, with hard copies also submitted to the Office of Scientific Affairs within 48 hours of occurrence. Unexpected deaths due related to this protocol will be reported within 24 hours.

In addition to the Cancer Center's DSMC, the TJU BMT program has instituted an internal DSMC which additionally reviews safety data. Recommendations from the internal DSMC will be submitted to the Cancer Center DMSB and IRB with study reports.

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

10.0 Statistical Considerations

The primary outcome of interest is one year survival in the patient population defined as high-risk in section 3.0. We use an exponential survival model for one arm, where we assume the rate of survival at one year is 60%. Using the relationship $\theta = -t_0 / \ln\left(1 - P\left(Failure\right)\right)$, where t_0 is one year, and $P\left(Failure\right) = 40\%$ at one year. The resulting θ is 1.96 (exponential parameter 0.510). Using a similar approach, and assuming that under the null, the probability of failure at 1 year is 60%, yields a θ 1.09. Assuming a 2-sided test and alpha=0.05, yields approximately 80% power for n=45 patients and 84% power for n=50 patients. The figure below illustrates the power under other sample sizes. Power computations were completed using PASS2002.



The trial will enroll up to 50 participants onto this trial, and participants enrolled will be included in the analysis. The minimum number of participants needing to power the trial is 45.

Analysis Plan

Formal sequential analysis with survival analysis is complicated. Due to the relatively small sample size and anticipated number of events, we propose interim monitoring following approximately every 10 events using the B value approach (Lan and Wittes). This will allow for monitoring of the trial without a formal determination of the number of analyses and timing of analyses given that accrual rates, event rates, and other information can only be estimated at this time. While not a formal sequential procedure, it could provide evidence (either pro or con) that the results of the study at interim monitoring times are not likely to change even if the study is carried to completion.

Analysis will be completed using a Kaplan-Meier plot with 95% point-wise confidence intervals. One-year event rates will be estimated from this plot (the survival distribution function), and point-wise confidence interval at one year used to test the hypothesis of one-year survival exceeding 60% with this treatment. The point-wise confidence interval will be computed using an arcsine-square root transformation, as this was shown (Borgan and Liestol) to have better properties in small sample sizes. This will also allow for a Z-test which can then be appropriately transformed to a B score for interim monitoring of the study.

Secondary Objectives

A descriptional analysis will be performed to address the secondary objectives:

- To determine the incidence and severity of GVHD in patients undergoing allogeneic HSCT on this protocol and
- To assess the pace of lymphoid recovery in patients undergoing allogeneic HSCT on this protocol

Assessment for GVHD and immune reconstitution analysis will be performed according the table in Section 6.0.

Analysis for Safety

Patient outcomes are routinely monitored in an ongoing fashion for all patients on investigational trials, beyond their formal endpoints. Based on prior experience using a two step approach similar to that described in this trial, we anticipate that the incidence of graft failure should be less than 10%, the incidence of severe GVHD should be less than 10%, and the non-relapse mortality should be less than 20%. 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.

References

Lan KKG, Wittes J. The B Value: a tool for monitoring data, Biometrics 44, 579-585, 1988.

Borgan O, Liestol K. A note on confidence intervals and confidence bands for the survival curve based on transformations. Scandinavian Journal of Statistics, 17:35-41, 1990.

11.0 References

1. Thomas ED: Karnofsky Memorial Lecture. Marrow transplantation for malignant diseases. Journal of Clinical Oncology 1:517, 1983.

2. Weiden PL, Flournoy N, Thomas ED, Prentice R, Fefer A, Buckner CD, Storb R: Antileukemic effect of graft-versus-host disease in human recipients of allogeneic-marrow grafts. New England Journal of Medicine 300:1068, 1979.

3. Weiden PL, Sullivan KM, Fournoy M, Storb R, Thomas ED: Antileukemic effect of chronic graft-versus-host disease: contribution to improved survival after allogeneic marrow transplantation. New England Journal of Medicine 304:1529, 1981.

4. van Besien K, Deeg HJ. Hematopoietic stem cell transplantation for myelofibrosis. Seminars in Oncology 32: 414-421, 2005.

5. Gratwohl A, Brand R, Apperley J, Crawley C, Ruutu T, Corradini P, Carreras E, Devergie A, Guglielmi C, Kolb HJ, Niederwieser D. Allogeneic hematopoietic stem cell transplantation for chronic myeloid leukemia in Europe 2006: transplant activity, long term data and current results. An analysis by the chronic leukemia working party of the European group for blood and marrow transplantation. Haematologica 91: 513-521, 2006.

6. Mehta L, Gordon L, Tallman M, Winter J, Evens A, Frankfurt O, Williams S, Grinblatt D, Kaminer L, Meagher R, Ginghal S. Does younger donor age affect the outcome of reduced-intensity allogeneic hematopoietic stem cell transplantation for hematologic malignancies beneficially? Bone Marrow Transplantation 38: 95-100, 2006.

7. Imamura M, Asano S, Harada M, Ikedo Y, Kato K, Kato S, Kawa K, Kojima S, Morishima Y, Morishita M, Nakahata T, Okamura J, Okoamoto S, Shiobara S, Tanimoto M, Tsuchida M, Atsuta Y, Yamamoto K, Tanaka J, Hamajima N, Kodera Y. Current status of hematopoietic cell transplantation for adult patients with hematologic diseases and solid tumors in japan. International Journal of Hematology 83: 164-178, 2006.

8. Alamo J, Shahjahan M, Lazarus H, de Lima M, Giralt S. Comorbidity indices in hematopoietic stem cell transplantation: a new report card. Bone Marrow Transplantation 3: 475-479, 2005.

9. Artz A, Pollyea D, Koherginsky, Stock W, Rich E, Odenike O, Zimmerman T, Smith S, Godley L, Thirman M, Daugherty C, Extermann M, Larson R, van

Besien K. Performance status and comorbidity predict transplant-related mortality after allogeneic hematopoietic cell transplantation. Biology of Blood and Marrow Transplantation 12: 954-964, 2006.

10. Hows J, Passweg J, Tichelli A, Locasciulli A, Szydlo R, Bacigalupo A, Jacobson N, Ljungman P, Cornish J, Nunn A, Bradley B, Socié G. Comparison of long-term outcomes after allogeneic hematopoietic stem cell transplantation from matched sibling and unrelated donors. Bone Marrow Transplantation 38: 799-805, 2006.

11. Marks D, Forman S, Blume K, Pérez W, Weisdorf D, Keating A, Gale R, Cairo M, Copelan E, Horan J, Lazarus H, Litzow M, McCarthy P, Schultz K, Smith D, Trigg M, Zhang M, Horowitz M. A comparison of cyclophosphamide and total body irradiation with etoposide and total body irradiation as conditioning regimens for patients undergoing sibling allografting for acute lymphoblastic leukemia in first or second complete remission. Biology of Blood and Marrow Transplantation. 12: 438-453, 2006.

12. Lee S-H, Lee M, Lee J-H, Min Y, Lee K-H, Cheong J-W, Lee J, Park K, Kang J, Kim K, Kim W, Jung C, Choi S-J, Lee J-H, Park K. Infused CD34+ cell dose predicts long-term survival in acute myelogenous leukemia patients who received allogeneic bone marrow transplantation from matched sibling donors in first complete remission. Biology of Blood and Marrow Transplantation. 11: 122-128, 2005.

13. Cook G, Clark R, Crawley C, Mackinnon S, Russell N, Thomson K, Pearce R, Towlson K, Marks D. The outcome of sibling and unrelated donor allogeneic stem cell transplantation in adult patients with acute myeloid leukemia in first remission who were initially refractory to first induction chemotherapy. Biology of Blood and Marrow Transplantation 12: 293-300, 2006.

14. Runde V, de Witte T, Arnold R, Gratwohl A, Hermans J, van Biezen A, Niederwieser D, Labopin M, Walter-Noel M, Bacigalupo A, Jacobsen N, Ljungman P, Carreras E, Kolb H, Aul C, Apperley J. Bone marrow transplantation from HLA-identical siblings as first-line treatment in patients with myelodysplastic syndromes: early transplantation is associated with improved outcome. Bone Marrow Transplantation 21, 255-261, 1998.

15. Guardiola P, Runde V, Bacigalupo A, Ruutu T, Locatelli F, Boogaerts M, Pagliuca A, Cornelissen J, Schouten H, Carrera E, Finke J, Biezen A, Brand R, Niederwiesser D, Gluckman E, deWitte T. Retrospective comparison of bone marrow and granulocyte-stimulating factor-mobilized peripheral blood

progenitor cells for allogeneic stem cell transplantation using HLA identical sibling donors in myelodysplastic syndromes. Blood 99: 4370-4378, 2002.

16. Guardiola P, Anderson J, Bandini G, Cervantes F, Runde V, Arcese W, Bacigalupo A, Przepiorka D, O'Donnell M, Polchi P, Buzyn A, Sutton L, Cazals-Hatem D, Sale G, de Witte T, Deeg HJ, Gluckman E. Allogeneic stem cell transplantation for agnogenic myeloid metaplasia: a European Group for Blood and Marrow Transplantation, Société Française de Greffe de Moelle, Gruppo Italiano per il Trapianto del Midollo Osseo, and Fred Hutchinson Cancer Research Center collaborative study. Blood 93: 2831-2838, 1999.

17. Daly A, Song K, Nevill T, Nantel S, Toze C, Hogge D, Forrest D, Lavoie J, Sutherland H, Sheperd J, Hasegawa W, Lipton J, Messner H, Kiss T. Stem cell transplantation for myelofibrosis: a report from two Canadian centers. Bone Marrow Transplantation 32: 35-40, 2003.

18. Kerbauy D, Chyou F, Gooley T, Sorror M, Scott B, Pagel J, Myerson D, Appelbaum F, Storb R, Deeg HJ. Allogeneic hematopoietic cell transplantation for chronic myelomonocytic leukemia. Biology of Blood and Marrow Transplantation 11: 713-720, 2005.

19. Freytes C, Loberiza F, Rizzo D, Bashey A, Bredeson C, Cairo M, Gale R, Horowitz M, Klumpp T, Martino R, McCarthy P, Molina A, Pavlovsky S, Pecora A, Serna D, Tsai T, Zhang M, Vose J, Lazarus H, van Besien K. Myeloablative allogeneic hematopoietic stem cell transplantation in patients who experience relapse after autologous stem cell transplantation for lymphoma: a report of the International Bone Marrow Transplant Registry. Blood 104: 3797-3803, 2004.

20. Peniket A, Ruiz de Elvira M, Taghipour G, Cordonnier C, Gluckman E, de Witte T, Santini G, Blaise D, Greinix H, Ferrant A, Cornelissen J, Schmitz N, Goldstone A. An EBMTR registry matched study of allogeneic stem cell transplants for lymphoma: allogeneic transplantation is associated with a lower relapse rate but a higher procedure-related mortality rate than autologous transplantation. Bone Marrow Transplantation 31: 667-678, 2003.

21. Alpek G, Ambinder R, Piantadosi S, Abrams R, Brodsky R, Vogelsang G, Zahurak M, Fuller D, Miller C, Noga S, Fuchs E, Flinn I, O'Donnell P, Seifter E, Mann R, Jones R. Long-term results of blood and marrow transplantation for hodgin's lymphoma. Journal of Clinical Oncology 19: 4314-4321, 2001.

22. Champlin R, Schmitz N, Horowitz M, Chapuis B, Chopra R, Cornelissen J, Gale R, Goldman J, Loberiza F, Hertenstein B, Klein J, Montserrat E, Zhang

M, Ringdén O, Tomany S, Rowlings P, Van Hoef M, Gratwohl A. Blood stem cells compared with bone marrow as a source of hematopoietic cells for allogeneic transplantation. Blood 95: 3702-3709, 2000.

23. Stem Cells Trialists' Collaborative Group. Allogeneic peripheral blood stem-cell compared with bone marrow transplantation in the management of hematologic malignancies: an individual patient data meta-analysis of nine randomized trials. Journal of Clinical Oncology 23: 5074-5087, 2005.

24. Alyea E, Kim H, Ho V, Cutler C, Gribben J, DeAngelo D, Lee S, Windawi S, Ritz J, Stone J, Antin J, Soiffer R. Comparative outcome of nonmyeloablative and myeloablative allogeneic hematopoietic cell transplantation for patients older than 50 years of age. Blood 105: 1810-1814, 2005.

25. Awwad M., North RJ. Cyclophosphamide (Cy)-facilitated adoptive immunotherapy of a Cy-resistant tumour. Evidence that Cy permits the expression of adoptive T-cell mediated immunity by removing suppressor T cells rather than by reducing tumour burden. Immunology. 65:87-92, 1988.

26. Awwad M., North RJ. Cyclophosphamide-induced immunologically mediated regression of a cyclophosphamide-resistant murine tumor: a consequence of eliminating precursor L3T4+ suppressor T-cells. Cancer Research. 49:1649-1654, 1989.

27. Strauss G, Osen W, Debatin K-M. Induction of apoptosis and modulation of activation and effector function in T cells by immunosuppressive drugs. Clinical & Experimental Immunology, 128: 255-266, 2002.

28. Mayumi H, Umesue M, Nomoto K. Cyclophosphamide-induced immunological tolerance: an overview. Immunobiology, 195: 129-139, 1996.

29. Fontalin LN, Chernyakhovskaya I, Mayumi H, Good RA. Recent state of cyclophosphamide-induced skin allograft tolerance in mice. Immunobiology 182: 323-333, 1991.

30. Mayumi H, Good RA: Long-lasting skin allograft tolerance in adult mice induced across fully allogeneic (multimajor H-2 plus multiminor histocompatibility) antigen barriers by a tolerance-inducing method using cyclophosphamide. Journal of Experimental Medicine. 169: 213, 1989.

31. Prigozhina TB, Gurevitch O, Zhu J, Slavin S. Permanent and specific transplantation tolerance induced by a nonmyeloablative treatment to a wide variety of allogeneic tissues. Transplantation 10: 1394-1399, 1997.

32. Maeda T, Eto M, Nishimura Y, Nomoto KE, Kong YY, Nomoto KI. Direct evidence for clonal destructive of allo-reactive T cells in mice treated with cyclophosphamide after allo-priming. Immunology 78: 113-121, 1993.

33. Mayumi H, Good RA. The necessity of both allogeneic antigens and stem cells for cyclophosphamide-induced skin allograft tolerance in mice. Immunobiology 178: 287-304, 1989.

34. Mayumi H, Himeno K, Tanaka K, Tokuda J, Fan L, Nomoto K. Drug-induced tolerance to allografts in mice. IX. Establishment of complete chimerism by allogeneic spleen cell transplantation from donors made tolerant to H-2-identical recipients. Transplantation 42: 417-422, 1986.

35. Mayumi H, Himeno K, Shin T, Nomoto K. Drug-induced tolerance to allografts in mice. IV. Mechanisms and kinetics of cyclophosphamide-induced tolerance. Transplantation 39: 209-215, 1985.

36. Mayumi H, Himeno K, Tokuda J, Fan L, Nomoto K. Drug-induced tolerance to allografts in mice. X. Augmentation of split tolerance in murine combinations disparate at both H-2 and non H-2 antigens by the use of spleen cells from donors preimmunized with recipient antigens. Immunobiology 174: 274-291, 1987

37. Mayumi H, Himeno K, Shin T, Nomoto K. Drug-induced tolerance to allografts in mice. VI. Tolerance induction in H-2 haplotype-identical strain combinations in mice. Transplantation 40: 188-194, 1985.

38. Mayumi H, Good RA. Induction of tolerance across major barriers using a two-step method with genetic analysis of tolerance induction. Immunobiology 179: 86-108, 1989.

39. Wu T, Levay-Young B, Heuss N, Sozen H, Kirchhof N, Sutherland DER, Hering B, Guo Z. Inducing tolerance to MHC-matched allogeneic islet grafts in Diabetic NOD mice by simultaneous islet and bone marrow transplantation under nonirradiative and nonmyeloablative conditioning therapy. Transplantation 74: 22-27, 2002.

40. Zhang QW, Tomita Y, Matsuzaki G, Yoshikawa M, Shimizu I, Nakashima Y, Sueishi K, Nomoto K, Yasui H. Mixed chimerism, heart, and skin allograft tolerance in cyclophosphamide-induced tolerance. Transplantation 70: 906-916, 2000.

41. Luznik L, Engstrom LW, Iannone R, Fuchs EJ. Posttransplantation cyclophosphamide facilitates engraftment of major histocompatibility complex-identical allogeneic marrow in mice conditioned with low-dose total body irradiation. Biology of Blood & Marrow Transplantation 8: 131-138, 2002.

42. Okano S, Eto M, Tomita Y, Yoshizumi T, Yamada H, Minagawa R, Nomoto K, Sugimachi K, Nomoto K. Cyclophosphamide-induced tolerance in rat orthotopic liver transplantation. Transplantation 71: 44-456, 2001.

43. Nakao M, Taguchi T, Ogita K, Nishimoto Y, Suita S. Immune tolerance induced by donor antigen and cyclophophosphamide in rat fetal small bowel transplantation. Fukuoka Igaku Zasshi-Fukuaka Acta Medica 96: 49-57, 2005.

44. Okayama J, Ko S, Kanehiro H, Kanokogi H, Hisanaga M, Ohashi K, Sho M, Nagao M, Ikeda N, Kanamura T, Akashi S, Nakajima Y. Bone marrow chimerism and tolerance induced by single-dose cyclophosphamide. Journal of Surgical Research 120: 102-110, 2004.

45. Eto M, Nishmura Y, Matsuo K, Omoto K, Goto K, Kumuzawa J, Nomoto K. Prolongation of kidney graft survival by cyclophosphamide-induced tolerance in rats. Journal of Urology 153: 1693-1696, 1995.

46. Weiner HL, Cohen JA. Treatment of multiple sclerosis with cyclophosphamide: a critical review of clinical and immunological effects. Multiple Sclerosis 8: 142-154, 2002.

47. Traynor AE, Schroeder J. Rosa RM. Cheng D. Stefka J. Mujais S. Baker S. Burt RK. Treatment of severe systemic lupus erythematosus with high-dose chemotherapy and haemopoietic stem-cell transplantation: a phase I study. Lancet. 356: 701-7, 2000.

48. Nemes L, Pitlik E. New protocol for immune tolerance induction in acquired hemophilia. Haematologica 85: 64-68, 2000.

49. Suarez-Almazor ME, Belseck E, Shea B, Wells G, Tugwell P. Cyclophosphamide for treating rheumatoid arthritis. Cochrane Database of Systematic Reviews 4: CD001157, 2000.

50. Kanzler S, Gerken G, Dienes HP, Meyer zum Buschenfelde KH, Lohse AW. Cyclophosphamide as alternative immunosuppressive therapy for autoimmune

hepatitis-report of three cases. Zeitschrift fur Gastroenterologie 35: 571-578, 1997.

51. Brodsky RA, Sensenbrenner LL, Jones RJ. Complete remission in severe aplastic anemia after high-dose cyclophosphamide without bone marrow transplantation. Blood 87: 491-4, 1996.

52. Luznik L, Chen A, Kaup M, Bright E, Bolanos-Meade J, Thorburn C, Kos F, Hess A, Jones R, and Fuchs E. Post-transplantation high-dose cyclophosphamide (Cy) is effective single agent GVHD prophylaxis that permits prompt immune reconstitution after myeloablative HLA matched related and unrelated bone marrow transplantation (BMT). Blood (ASH Annual Meeting Abstracts) 108: 2891, 2006.

53. O'Donnell PV, Luznik L, Jones RJ, Vogelsang GB, Leffell MS, Phelps M, Rhubart P, Cowan K, Piantadosi S, Fuchs EJ. Nonmyeloablative bone marrow transplantation from partially HLA-mismatched related donors using posttransplantation cyclophosphamide. Biology of Blood & Marrow Transplantation 8: 377-386, 2002.

54. Ratajczak MZ, Ratajczak J, Kuczynski W, Light B, Lusk EJ, Gewirtz AM. In vitro sensitivity of human hematopoietic progenitor cells to 4-hydroperoxycyclophosphamide. Experimental Hematology 21: 1663-1667, 1993.

55. Gorin NC, Aegerter P, Auvert B, Meloni G, Goldstone AH, Burnett A, Carella A, Korbling M, Herve P, Marininchi D, Löwenberg R, Verdonck LF, dePlanque M, Hermans J, Helbig W, Porcellini A, Rizzoli V, Alesandro EP, Franklin IM, Reiffers J, Colleselli P, Goldman JM. Autologous bone marrow transplantation for acute myelocytic leukemia in first remission: a European survey of the role of marrow purging. Blood 75: 1606-1614, 1990.

56. Jones RJ, Zuehlsdorf M, Rowley SD, Hilton J, Santos GW, Sensenbrenner LL, Colvin OM. Variability in 4-hydroxyperoxycyclophosphamide activity during clinical purging for autologous bone marrow transplantation. Blood 70: 1490-1494, 1987.

57. Karp J, Sarkodee-Adoo CB. Therapy-related acute leukemia. Clinics in Laboratory Medicine 20: 71-81, 2000.

58. Le Beau MM, Albain KS, Larson RA, Vardiman JW, Davis EM, Blough RR, Golomb HM, Rowley JD. Clinical and cytogenetic correlations in 63 patients with therapy-related myelodysplastic syndromes and acute nonlymphocytic

leukemia: further evidence for characteristic abnormalities in chromosomes no. 5 and 7. Journal of Clinical Oncology 4: 325-245, 1986.

59. Mosmann TR, Cherwinski HM, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T-cell clones. Definition according to the profiles of lymphokine activities and secreted proteins. Journal of Immunology 136: 2348-2357, 1986

60. Romangnani S. T cell subsets (Th1 versus Th2). Annals of Allergy Asthma Immunology 85; 9-18, 2000.

61. Mosmann TR, Li L, Sad S, Functions of CD8 T-cell subsets secreting different cytokine patterns. Seminars in Immunology 9: 87-92, 1997.

62. Godfrey DI, Hammond KJ, Poulton LD, Smyth MJ, Baxter AG. NK & T cell facts, functions and fallacies. Immunology Today 21: 573-583, 2000.

63. Fowler DH, Breglio J, Nagel G, Eckhaus MA, Gress RE. Allospecific $CD8^+$ Tc1 and Tc2 populations in graft-versus-leukemia effect and graft-versus-host disease. Journal of Immunology 157: 4811-4821, 1996.

64. Arpinati M, Green CL, Heimfeld S, Heuser JE, Anasetti C. Granulocyte-colony stimulating factor mobilizes T helper 2-inducing dendritic cells. Blood 95: 2484-2490, 2000.

65. Franzke A, Piao W, Lauber J, Gatzlaff P, Könecke C, Hansen W, Schmitt-Thomsen A, Hertenstein B, Buer J, Ganser A. G-CSF as immune regulator in T cells expressing the G-CSF receptor: implications for transplantation and autoimmune diseases. Blood 102: 734-739, 2003.

66. Volpi I, Perruccio K, Tosti A, Capanni M, Ruggeri L, Posati S, Aversa F, Tabilio A, Romani L, Martelli MF, Velardi A. Postgrafting administration of granulocyte-colony stimulating factor impairs functional immune recovery in recipients of human leukocyte antigen haplotype-mismatched hematopoietic transplants. Blood 97: 2514-2521, 2001.

67. Metcalf D. The molecular biology and functions of the granulocyte-macrophage colony-stimulating factors. Blood 67: 257-267, 1986.

68. Rowe JM, Andersen JW, Mazza JJ, Bennett JM, Paietta E, Hayes FA, Oette D, Cassileth PA, Stadtmauer EA, Wiernik PH. A randomized placebo-controlled phase III study of granulocyte-macrophage colony-stimulating factor in adult patients (> 55 to 70 years of age) with acute myelogenous

leukemia: a study of the Eastern Cooperative Oncology Group (E1490). Blood. 86: 457-62, 1995.

69. Klumpp TR. Goldberg SL. Mangan KF. Effect of granulocyte colony-stimulating factor on the rate of neutrophil engraftment following peripheral-blood stem-cell transplantation. Journal of Clinical Oncology. 13: 2144, 1995.

70. Stull DM. Colony-stimulating factors: beyond the effects on hematopoiesis. American Journal of Health-System Pharmacy 59: S12-S20, 2002.

71. Peters BG. Adkins DR. Harrison BR. Velasquez WS. Dunphy FR. Petruska PJ. Bowers CE. Niemeyer R. McIntyre W. Vrahnos D. Auberry SE. Spitzer G. Antifungal effects of yeast-derived rhu-GM-CSF in patients receiving high-dose chemotherapy given with or without autologous stem cell transplantation: a retrospective analysis. Bone Marrow Transplantation 18: 93-102, 1996.

72. Schiller JH, Hank JA, Khorsand M, Storer B, Borchert A, Huseby-Moore K, Burns D, Wesly O, Albertini MR, Wilding G, Sondel PM. Clinical and immunological effects of granulocyte-macrophage colony-stimulating factor coadministered with interleukin 2: a phase IB study. Clinical Cancer Research. 2: 319-30, 1996.

73. Chachoua A, Oratz R, Hoogmoed R, Caron D, Peace D, Liebes L, Blum RH, Vilcek J. Monocyte activation following systemic administration of granulocyte-macrophage colony-stimulating factor. Journal of Immunotherapy with Emphasis on Tumor Immunology. 15: 217-24, 1994.

74. Chachoua A, Oratz R, Liebes L, Alter RS, Felice A, Peace D, Vilcek J, Blum RH. Phase Ib trial of granulocyte-macrophage colony-stimulating factor combined with murine monoclonal antibody R24 in patients with metastatic melanoma. Journal of Immunotherapy with Emphasis on Tumor Immunology. 16:132-41, 1994.

75. Yu AL, Batova A, Alvarado C, Rao VJ, Castleberry RP. Usefulness of a chimeric anti-GD2 and GM-CSF for refractory neuroblastoma: a POG phase II study. Proceedings of the American Society of Clinical Oncology 16: 513a (abs), 1997.

76. Leong SPL, Enders-Zohr P, Zhou YM, Allen RE, Sagebiel RW, Glassberg AB, Hayes FA. Active specific immunotherapy with GM-CSF as an adjuvant to autologous melanoma (AM) vaccine in metastatic melanoma. Proceedings of the American Society of Clinical Oncology 15: 1360 (abs), 1996.

12.0 Appendices

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

Modality

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

Energy

Either a linear accelerator or Cobalt source may be used. Dose to superficial tissues near skin surface will be increased by using a beam "spoiler" lucite plate close to the patient.

Since neoplastic infiltrates may be found in the skin, it is necessary for the superficial dose to satisfy the same total dose requirements as other locations.

Geometry

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

Dose Rate

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

Calibration & Beam Data Verification

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

Treatment Volume

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

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 – 13.5 Gy.
A hyperfractionated regimen over 4 consecutive days 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 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 9.0 Gy and 10.0 Gy to the mid-lung region without inhomogeneity corrections. The calculation will be repeated using inhomogeneity corrections approved by the physicist at the coordinating center.

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

12.2 **Appendix B** GVHD Grading System Grade

Clinical Staging of Acute Graft-Versus-Host Disease

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

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

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

12.3 **Appendix C** Patient Identification Card

Patient Name _____ Attending Physician _____

Allergies: _____

I have undergone a related-donor bone marrow transplant on _____. My treating physician has advised me that development of an infection is life-threatening to me. If I have a suspected infection (fever > 100.4F, rigors or chills), I am to receive the following as soon as possible:

_____ ceftazidime 2 grams IV

_____ ciprofloxicin 400 mg IV

_____ vancomycin I gram IV.

Please call 215-955-8874 for the hematology fellow on call for further instructions. If there is difficulty contacting the fellow on call, please do not hesitate to call the page operator and have the BMTU attending physician on-call paged on my behalf for further instructions.

I am also at high-risk for a condition called graft-versus-host disease (GVHD). This condition is also life-threatening and is manifested by a skin rash, liver inflammation, nausea, vomiting or severe diarrhea. If I report to the emergency room with these symptoms please call the Hematology fellow on-call for further instructions as soon as possible.

Attending Signature _____

Attending Name (printed) _____