Winship Cancer Center
Blood and Marrow Stem Cell Transplantation Protocol

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Current Submission:

Phase II Trial of Low Toxicity GVHD Prevention and Enhanced Immune Recovery with Tacrolimus, Bortezomib and Thymoglobulin®TBT

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Protocol Synopsis

Study Design: The study is designed as a Phase II, single center trial of a low toxicity immune prophylaxis combination of tacrolimus(Tac), bortezomib and thymoglobulin (Thymo)® (TBT) given as GVHD prophylaxis for allogeneic peripheral hematopoietic stem cell transplant (allo-HSCT) recepients.

Primary Objective:
1- To determine a composite end point of alive and severe acute GVHD free at 6 months following HLA matched related or unrelated donor hematopoietic peripheral blood transplantion in patients with hematologic malignancies who receive the immunosuppressive combination TBT as GVHD prophylaxis.
2- To determine the safety of this combination in the first six months post-transplant.

Secondary Objectives: To determine: incidence and severity of aGVHD (grade II-IV), time to platelet and absolute neutrophil recovery (engraftment), the cumulative incidence of grade III-IV aGVHD, days 30, 60 , 90, 180, natural killer cell and gamma delta T-cell recovery and phenotype, Thymo pharmacokinetics and it's correlation with IL-15 levels and natural killer (NK) cell and gamma delta (γδ) T-cell recovery on day 30 post-transplant, donor cell engraftment, incidence of infections-including CMV and EBV reactivation including lymphoproliferative disorders PTLD, incidence of veno-occlusive disease, incidence and severity of chronic GVHD, rates of Grade $\geq$ 3 non-hematologic toxicity according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0, non-relapse mortality (NRM), disease relapse or progression overall and disease free survival at one year, immunosuppression-free survival at one year, and KPS both pre-transplant and at various points post-transplant.

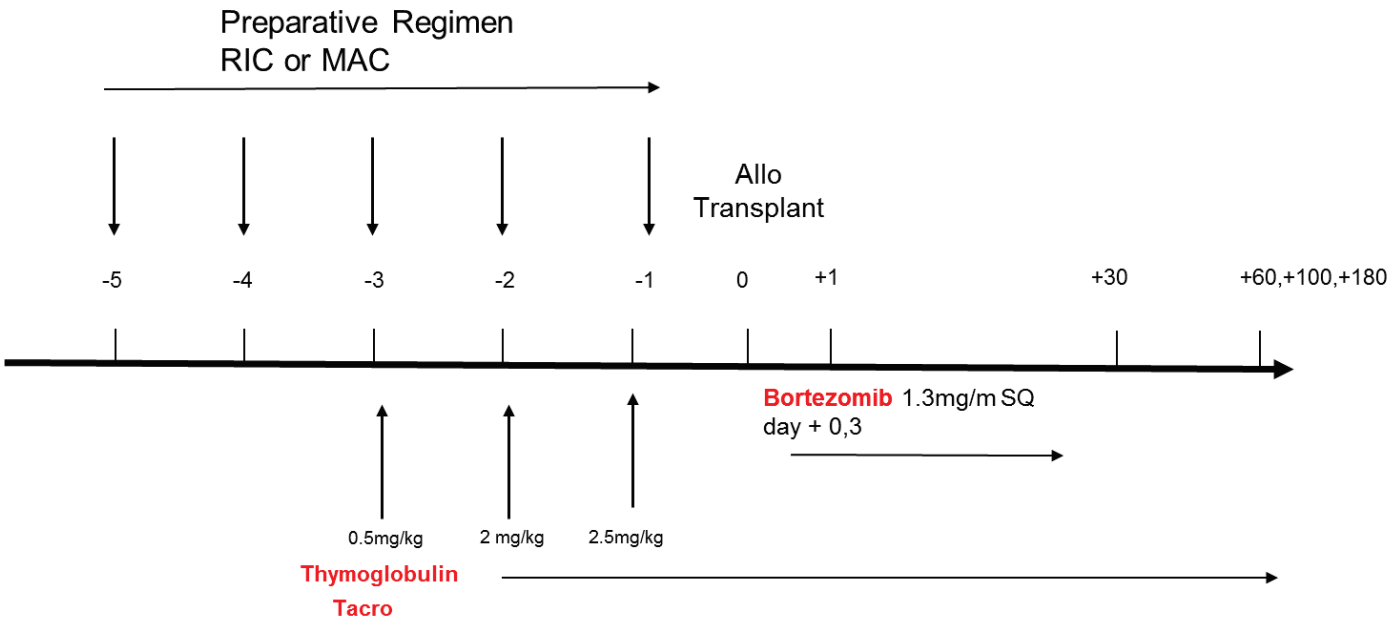
Eligibility Criteria: Eligible patients are between 18 and 75 years undergoing allo-HSCT for treatment of acute leukemia, chronic myeloid leukemia, myelodysplasia, myelofibrosis, chronic lymphocytic leukemia/small lymphocytic lymphoma, chemosensetive lymphoma; and multiple myeloma who are eligible for an allogeneic transplant. Patients must have an HLA matched (8/8) related or unrelated donor, who is willing to donate peripheral blood stem cells and meet institutional criteria for donation. Patients are eligible to receiving a reduced intensity conditioning (RIC) or myeloablative conditioning (MAC) regimens.

Treatment Description: Patients will receive a combination of triple immune suppressive medications TBT as follows: Tacrolimus IV infusion to start on day -3, bortezomib 1.3 mg/m^2 IV on days 0, and +3 post-transplant, and thymoglobulin® IV infusions on days -3 0.5 mg/kg,-2 (2 mg/kg), and -1 (2.5 mg/kg).

Accrual Objective: The clinical trial will enroll 38 patients over 24 months.

Study Duration: Patients will be followed for 6 months and 2 years following allo-HSCT.

Outline of Treatment Plan



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1.0 HYPOTHESES:

1.1 We hypothesize that the combination of TBT will provide effective prevention of aGVHD in patients with hematologic malignancies undergoing allo-HSCT.
1.2 The TBT combination is safe and does not add to the preparative regimen related toxicity.
1.3 The TBT regimen will result in an enhanced mean recovery of natural killer (NK) cells compared to historical controls at day 30 post-transplant.
1.4 Post-transplant Thymo area under the curve (AUC) correlate with higher IL-15 level peak post-transplant and with higher NK/ γδ T-cell recovery on day 30 post-transplant.

2.0 OBJECTIVES:

Primary:

2.1 To determine a composite end point of alive and severe acute GVHD free at 6 months following HLA matched related or unrelated donor hematopoietic peripheral blood transplant in patients with hematologic malignancies who receive the immunosuppressive combination TBT as GVHD prophylaxis.
2.2 To determine the safety of this combination in the first six months post-transplant.

Secondary:
To determine: incidence and severity of aGVHD (grade II-IV), time to platelet and absolute neutrophil recovery (engraftment), the cumulative incidence of grade III-IV aGVHD, days 30, 60 , 90, 180, natural killer cell and gamma delta T-cell recovery and phenotype, Thymo pharmacokinetics and it's correlation with IL-15 levels and natural killer (NK) cell and gamma delta (γδ) T-cell recovery on day 30 post-transplant, donor cell engraftment, incidence of infections-including CMV and EBV reactivation including lymphoproliferative disorders (PTLD), incidence of veno-occlusive disease, incidence and severity of chronic GVHD, rates of Grade $\geq$ 3 non-hematologic toxicity according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0, non-relapse mortality NRM, disease relapse or progression overall and disease free survival at one year, immunosuppression-free survival at one year, and KPS both pre-transplant and at various points post-transplant.

3.0 BACKGROUND AND RATIONALE:

3.1 Graft versus host disease continues to be a major limitation of allo-HSCT outcomes

Graft versus host disease (GVHD) is the major complication of allogeneic hematopoietic stem cell transplantation (allo-HSCT) and a leading cause of morbidity and mortality post-transplant. Factors which influence the incidence and severity of GVHD include the use of: peripheral blood stem cells, unrelated donors, and transplants from human leukocyte antigen (HLA) mismatched donors.[1-4] Methods to decrease the incidence of GVHD have relied primarily on pharmacologic immunosuppression which at most centers consists of a combination of methotrexate (MTX) and a calcineurin inhibitor (CNI) either cyclosporine A (CSA) or tacrolimus (Tac). In spite of the use of these agents, the rate of grade II-IV aGVHD ranges from 35-50% with HLA matched

sibling donors (SD) and up to 70% in unrelated donors (UD).[1-3] Additionally, MTX is associated with significant mucositis, delayed engraftment, pulmonary, renal and hepatic toxicities.[4-7] At Winship cancer center, Langston et al. reported grade II-IV aGVHD 44%, III-IV aGVHD incidence of 23% with an NRM of about 38% at 36 months in patients who underwent allo-HSCT from UD using RIC and Tac/MTX or Tac/mycophenolate mofetel (MMF). Intermediate dose Thymo (6mg/kg) was added for recipients of HLA 7/8 matched donors[8]. Therefore, safer and more efficacious agents for the prevention of GVHD are needed for improved patient outcomes.

3.2 Intermediate dose Thymoglobulin® in GVHD prevention

3.2.1 Intermediate doses of thymoglobulin (Thymo) when combined with CNI have showed encouraging results in improving the rates of both acute and chronic GVHD as well as NRM Devillier et al. reported on 206 patients who were transplanted from an HLA matched donor (SD & UD) with a combination of Tac and Thymo (5mg/kg total dose). Transplant outcomes were as follows: aGVHD II-IV was 23%, III-IV was 9%, NRM 22% at 4 years.[9] The same group showed that ATG dose < 6mg/kg was associated with less risk of relapse in RIC AML patients.[10] Mohty et al. recently reported on a large phase II trial of 80 elderly or less fit patients who underwent transplant from HLA matched (C mismatch was allowed) using an intermediate intensity preparative regimen (3 days of busulfan at 130mg/m2 and fludarabine) with ATG total dose of 5mg/kg(on days -2, -1) and Tac alone or with Tac+ mycophenolate (for C antigen mismatch). He showed a grade III-IV aGVHD incidence of 7.5%, cGVHD of 33% and NRM of 11% at 2 years.[11]

3.2.2 Thymoglobulin: Pharmacology

Thymoglobulin® is a purified, pasteurized, gamma immune globulin, obtained by immunization of rabbits with human thymocytes (Thymoglobulin® Prescribing Information). This immunosuppression product, a polyclonal antibody, contains cytotoxic antibodies of defined specificity against functional molecules on T lymphocytes. To minimize the risk of transmission of infective agents from this biological product, viral testing is performed during production, and a viral inactivation step (pasteurization, i.e., heat treatment of the active ingredient at 60˚C/10 hours) is an integral part of the Thymoglobulin manufacturing process. The potency of the product is determined by lymphocytotoxicity assay. In each lot, antibody activity against human red blood cells and platelets is analyzed and determined to be within acceptable limits before release. Additionally, before release for clinical use, each lot of Thymoglobulin is tested to ensure its ability to inhibit E-rosette formation between human peripheral lymphocytes and sheep red blood cells *in vitro*. Only sterile lots that test negative for anti-human serum protein antibody, anti-glomerular basement membrane antibody, fibroblast toxicity and pyrogens are released.

3.2.3 Thymoglobulin: Mechanism of action

The *in vitro* mechanism of action by which polyclonal anti-lymphocyte preparations suppress immune responses is not fully understood. Thymoglobulin includes antibodies against T-cell markers such as CD2, CD3, CD4, CD8, CD11a, CD18, CD25, CD44, CD45, HLA-DR, HLA Class I heavy chains and ß2 microglobulin [12-16] as well as dendritic cells adhesion molecules[17] and B-cell markers such as CD20.[12,18] *In vitro*, Thymoglobulin (concentrations >0.1 mg/ml) mediates T-cell suppressive effects via inhibition of proliferative responses to several mitogens, with post-transcription blockade of interferon gamma and CD25 synthesis.[19] The *in vivo* mechanism of action of Thymoglobulin is also not fully understood. Possible mechanisms by which Thymoglobulin may induce immunosuppression *in vivo* include T cell clearance from the circulation and modulation of T-cell activation, homing and cytotoxic activities. T-lymphocyte depletion is promptly observed in transplant patients following administration of

Thymoglobulin.[20] This may result from the complement dependent lysis in the intravascular space or the opsonization and subsequent phagocytosis by macrophages of antibody-coated T cells.[19] Monitoring reveals that T lymphocyte depletion in peripheral blood persists for several days to several months following cessation of Thymoglobulin therapy.[21]

Thymoglobulin is a potent immunosuppressive agent that demonstrates a rapid and profound effect resulting in lower white blood cells (WBC), primarily from a decrease in T-cell counts. The magnitude and duration of lymphopenia have been studied. Reductions of circulating lymphocytes by 83% to 92% from pretreatment values were seen after a single dose of Thymoglobulin and were sustained throughout the daily dosing period in 4 clinical pharmacology studies. The recovery from Thymoglobulin-induced lymphocyte depletion was gradual, beginning soon after cessation of therapy, with most recovery by 3 months. Recovery may take longer than 3 months, but persistent lymphocyte depletion that has been seen but has not resulted in any morbidity. T-cell subsets determined by flow cytometry also demonstrate similar dramatic decreases. In a phase III randomized trial, T-cell monitoring was performed in 26 patients. T-cell depletion was greater in the Thymoglobulin patients as compared with the Atgam® (Pharmacia-Upjohn, Kalamazoo, MI) patients. T-cell depletion lasted for at least 90 days after the cessation of therapy. At that time, T-cell counts remained 40% lower in Thymoglobulin-treated patients than in Atgam-treated patients. [21]Thymoglobulin also induces apoptosis in all B-cell lineages, particularly at higher doses.[19]

3.2.4 Thymoglobulin Pharmacokinetics:

General PK: Only few studies evaluated the full pharmacokinetics of rabbit anti-thymocyte globulin R-ATG in stem cell transplant.[22-25] These studies have demonstrated few important observations about R-ATG PK: (1)Total R-ATG (measured by ELISA) have a longer half-life than active R-ATG (capable of binding human lymphocytes and measured by flowcytometry). (2)One pediatric study showed a constant half-life and linear correlation between dose (7.5-20 mg/kg) and maximum concentration (C max), whereas higher doses (30-40mg/kg) accumulated in the body.[24] (3)Clearance of active R-ATG is variable with a median half-life of about 6 days for the active form and about 14 days for total R-ATG. Sub-therapeutic levels of active R-ATG can be detected in a median of about 15 days. (4) R-ATG clearance has a bi-phasic clearance pattern consistent with an initial fast clearance of target bound antibody followed by a slower degradation of unbound targets. (5)ATG-F PK in one study had a slightly shorter half-life of the active component (4 days) compared to R-ATG.[26] (6)Two studies showed that ATG (R-ATG and ATG-F) persist post-transplant with variable reactivity to different lymphoid subsets compared to fresh R-ATG. There was less avidity to NK cells compared to other immune cells.[23,27] This may be explained in part by faster recovery of NK cells which leads to faster clearance of the active component of the antibody.

Factors affecting PK-The ATG PK is variable depending on multiple factors: Host factors including size or body weight, lymphocyte counts at the time of ATG administration, and the presence of anti ATG anti bodies. Transplant factors include: ATG dose, type and Timing of infusion. Admiral et al. proposed a R-ATG PK model in which higher weight and lower lymphocyte counts pre ATG can predict higher ATG exposure.[25] What is not known today is which weight dosing formula would produce a consistent & predictable ATG PK. All therapeutic trials have used total body weight. Since being overweight does not increase the size of the lymphoid tissue in the body, then theoretically this will lead to higher ATG exposure and potentially worse side effects. The other unknown is the effect of graft lymphocyte content on the ATG clearance. The presence of anti-Thymoglobulin anti-bodies will accelerate the clearance and reduce GVHD prevention efficacy.[28] The last factor is timing of ATG infusion in relation to the day of transplant. Early pre transplant ATG infusion (day -10 or -7) can lead to effective host T-cell and APC depletion with little graft T-cell depletion. Which can lead to robust

engraftment and possibly more GVHD.[22] While late ATG infusion (closer to day 0 or after) can lead to more graft T-cell depletion, less GVHD, poor immune reconstitution and possibly more infections and relapses post-transplant.

3.2.5 Thymoglobulin: Safety profile

Thymoglobulin presents a consistent AE profile, with AEs that are generally manageable or reversible. Adverse events with Thymoglobulin occur with similar or less frequency than those reported with other polyclonal anti-T-cell agents. The most frequently reported AEs include fever, chills, leukopenia, thrombocytopenia, headache, diarrhea and abdominal pain. In the US Phase III controlled clinical trial (n=163) comparing the efficacy and safety of Thymoglobulin and Atgam for the treatment of acute renal allograft rejection, AEs (using the COSTART system) were similar in both treatment groups and were generally manageable or reversible.[21] Below is an overview of the safety profile of Thymoglobulin.

3.2.5.1 Overview of Safety

Total patient exposure worldwide since 1984 when Thymoglobulin was first licensed in France, is approximately 2.1 million vials, equivalent to over 54,000 courses. Approximately 60% of patients can be expected to experience adverse reactions with the use of Thymoglobulin. These usually occur after the first infusion. The mechanism of some of those adverse reactions is probably related to cytokine release. Pre-medication with corticosteroids and antihistamines and a decrease in the infusion rate may enable the incidence and severity of certain adverse reactions to be reduced. While anaphylactic hypersensitivity reactions are rare, delayed allergic responses in the form of serum sickness have been reported. Infection and PTLD are associated with use of immunosuppressive agents, including Thymoglobulin. The adverse events seen typically with Thymoglobulin can be grouped.

Adverse events reported during and after Thymoglobulin infusions:

The most common systemic adverse reactions frequently reported in clinical trials which present are fever (63%), chills (57%), pain (46%) headache (40%), abdominal pain (38%), diarrhea (37%), hypertension (37%), nausea (37%), peripheral edema (34%), dyspnea (28%), asthenia (27%), tachycardia (26%). Hypotension, vomiting and malaise have also been commonly reported. The likely mechanism of action for these events is the release of cytokines during the first dose administration, particularly if appropriate pre-medications were not administered. Local adverse reactions such as pain at the infusion site and peripheral thrombophlebitis have also been reported.

Rare delayed allergic reactions such as serum sickness (fever, pruritus, rash associated with joint and muscle pain) may occur 7 to 15 days post-treatment initiation. Immediate serious allergic reactions or anaphylaxis are exceptional.

Adverse events associated with undesirable cross-reacting antibodies:

Adverse reactions associated with the presence of antibodies inducing cross-reactions such as leukopenia (57%) and thrombocytopenia (37%), have been reported during and subsequent to treatment with rabbit anti-human thymocyte globulin. The reactions may emerge during the first 2 days of treatment or after the end of treatment. The mechanism of action likely causing these effects involves the presence of antibodies cross-reacting with shared antigens of lymphocytes, neutrophils and platelets, or additive effects from concomitant medication. Monitoring of the white blood cell and platelet counts enables the severity and frequency of such reactions to be reduced.

Adverse effects associated with over-immunosuppression:

Adverse effects associated with over-immunosuppression including infectious complications (bacterial, fungal, viral and protozoal) and malignancies (particularly lymphoproliferative syndrome) have been reported. It is important to note that concomitant or previous immunosuppressive treatments may contribute to the over-immunosuppression observed. Appropriate anti-infective prophylaxis will significantly reduce the incidence of infections; EBV monitoring and pre-emptive treatment will possibly decrease the incidence of PTLD.

3.2.5.2. Particular comments related to Adverse Events Seen in Hematology

Some transient abnormalities of liver function tests have been described in patients with aplastic anemia treated by Thymoglobulin or other ATG's. It is not clear whether these abnormalities are related to the disease and pre-existing liver dysfunction, or to the treatment.[29] When Thymoglobulin is used during conditioning prior to hematopoietic stem cell transplantation (allo-HSCT) or in the first 3 months after the transplantation to treat acute GVHD, the ability of the patient to form antibodies against rabbit immunoglobulins is abolished. Serum sickness thus, does not appear to occur, however alert observation is recommended. Although an initial study[30] noted an increase in CML relapse with Thymoglobulin compared to other T depletion agents when dosed at 10mg/kg, subsequent studies with lower doses did not have an increase in CML relapse. No increase in relapse was seen in other studies or other disease cohorts.[31] The incidence of PTLD is mainly influenced by the HLA disparity, the T-cell depletion and both the occurrence of and the treatment of acute GVHD.[32] PTLD cases have rarely been reported in patients who were conditioned with Thymoglobulin: In the Karolinska Institute, Sweden, Remberger diagnosed no cases of PTLD in the 61 MUD BMT patients who received Thymoglobulin 2 mg/kg/day from day –5 to –1 prior to transplant.[30] At City of Hope, there were no cases of PTLD in the 20 UD patients who received thymoglobulin 7.5 mg/kg, despite a relatively high rate of EBV reactivation (6/20). Treatment with rituximab was successful in eliminating EBV reactivation in all patients.[33]

3.3 The benefit of bortezomib in GVHD prophylaxis

3.3.1 In the allogeneic allo-HSCT context, a Phase I/II clinical trial of a regimen of bortezomib plus standard tacrolimus/methotrexate for GVHD prophylaxis after fludarabine/busulfan-based RIC allo-HSCT with 1-2 locus HLA-mismatched donors (HLA-A, -B, -C; -DQB1, -DRB1). Bortezomib MTD was 1.3 mg/m^2 administered on Days +1, +4 and +7 after stem cell infusion. Phase I results indicated minimal toxicity (rapid engraftment, zero non-relapse mortality in the first year) and excellent acute GVHD control with the bortezomib-based regimen.[34] Phase I/II results demonstrated that the grade II-IV acute GVHD rate at Day 180 was 22% and no patients developed grade IV acute GVHD. Additionally, 2-year probabilities of non-relapse mortality and relapse were 11% and 38% respectively in a population receiving HLA-mismatched unrelated donor allo-HSCT. Furthermore, a significant improvement in day 30 NK recovery was observed compared with previous Tac/Sirolimus cohort.[35] The same GVHD prevention regimen was tested in the context of full intensity preparative regimen with similar low rates of GVHD and NRM.[36] Al-Homsi et al. showed effective GVHD prevention by combining post-transplant cyclophosphamide with 2 doses of bortezomib 1.3 mg/m^2 on days 0 and +3 post-transplant.[37]

3.3.2 Bortezomib pharmacology

Bortezomib for Injection is a small-molecule proteasome inhibitor developed by Millennium Pharmaceuticals, Inc., (Millennium) as a novel agent to treat human malignancies. Bortezomib is currently approved by the United States Food and Drug Administration (US FDA) for the treatment of patients with multiple myeloma (MM). It is also indicated for the treatment of patients with mantle cell lymphoma (MCL) who have received at least 1 prior therapy.

Bortezomib Mechanism of Action

By inhibiting a single molecular target, the proteasome, bortezomib affects multiple signaling pathways. The antineoplastic effect of bortezomib likely involves several distinct mechanisms, including inhibition of cell growth and survival pathways, induction of apoptosis, and inhibition of expression of genes that control cellular adhesion, migration, and angiogenesis. Thus, the

mechanisms by which bortezomib elicits its antitumor activity may vary among tumor types, and the extent to which each affected pathway is critical to the inhibition of tumor growth could also differ. Bortezomib has a novel pattern of cytotoxicity in National Cancer Institute (NCI) in vitro and in vivo assays. [38] In addition, bortezomib has cytotoxic activity in a variety of xenograft tumor models, both as a single agent and in combination with chemotherapy and radiation.[39-51] Notably, bortezomib induces apoptosis in cells that over express bcl-2, a genetic trait that confers unregulated growth and resistance to conventional chemotherapeutics.[52]

The mechanisms of action leading up to apoptosis have been more clearly defined and include initiation of the unfolded protein response and direct/indirect effects on various molecular targets including cell cycle control proteins p27 and p21, cyclins, signal transduction molecules, transcription factors c-jun and HIF1-α, tumor suppressor protein p53, angiogenesis factors, and many others. Bortezomib is thought to be efficacious in multiple myeloma via its inhibition of nuclear factor κB (NF-κB) activation, its attenuation of interleukin-6 (IL-6)-mediated cell growth, a direct apoptotic effect, and possibly anti-angiogenic and other effects.[53-60]

Bortezomib is thought to be efficacious in multiple myeloma via its inhibition of nuclear factor κB (NF-κB) activation, its attenuation of interleukin-6 (IL-6) mediated cell growth, a direct apoptotic effect, and possibly anti-angiogenic and other effects.[53] Bortezomib also has immunomodulatory effects relevant to allogeneic allo-HSCT. The proteasome, acting via NF-kB, plays an important role in cytokine signaling and the generation of cell mediated immune responses via T cell activation, proliferation and apoptosis. [61-63] Bortezomib also attenuates TLR4 mediated antigen-presenting cell activation, with reduced cytokine production and immunostimulatory activity.[64] In the allogeneic setting, bortezomib preferentially and specifically depletes alloreactive T lymphocytes.[65] It however spares human regulatory T cells (Treg) that act to suppress inappropriate immune responses underlying GVHD.[66] Given the immunomodulatory effects of bortezomib on APCs, alloreactive T lymphocytes and Tregs, as well as its anti-tumor effects and its lack of hematopoietic stem cell toxicity, it is an attractive candidate for control of GVHD after allogeneic transplantation.

Bortezomib Clinical Pharmacokinetics and Pharmacodynamics

The clinical pharmacology characterization of bortezomib has been determined from Phase 1 studies in subjects with solid tumors and hematological malignancies, and confirmed in Phase 2 studies in subjects with multiple myeloma.

Bortezomib demonstrates multi-compartmental pharmacokinetics. Following intravenous administration of 1.0 mg/m^2 and 1.3 mg/m^2 dose, the mean first-dose maximum observed plasma concentrations of bortezomib were 57 and 112 ng/mL, respectively in 11 patients with multiple myeloma and creatinine clearance values >50 mL/min participating in a pharmacokinetics study. In subsequent doses, mean maximum observed plasma concentrations ranged from 67 to 106 ng/mL for the 1.0 mg/m^2 dose and 89 to 120 ng/mL for the 1.3 mg/m^2 dose. The mean elimination half-life of bortezomib upon multiple dosing ranged from 40 to 193 hours. Bortezomib is eliminated more rapidly following the first dose. Mean Total Body Clearances were 102 and 112 L/h following the first dose for doses of 1.0 mg/m^2 and 1.3 mg/m^2, respectively, and ranged from 15 to 32 L/h following subsequent doses for doses of 1.0 and 1.3 mg/m^2, respectively. Clinical experience has shown that the change in clearance does not result in overt toxicity from accumulation in this multi-dose regimen in humans.

In subjects with advanced malignancies, the maximum pharmacodynamic effect (inhibition of 20S activity) occurred within 1-hour post dose. At the therapeutic dose of 1.3 mg/m^2 in subjects

with multiple myeloma, the mean proteasome inhibition at 1-hour post dose was approximately 61%.

The time course of proteasome inhibition in subjects is characterized by maximum inhibition observed within the first hour after administration, followed by partial recovery of proteasome activity over the next 6 to 24 hours to within 50% of the pretreatment activity. On the Day 1, 4, 8, and 11 schedule variable (10%–30%) levels of proteasome inhibition have been observed at next scheduled dosing. In theory, this advantage allows cells to recover proteasome activity for normal cellular housekeeping functions between doses.

The relationship between bortezomib plasma concentrations and proteasome inhibition can be described by a maximum effect (E_{max}) model. The E_{max} curve is initially very steep, with small changes in plasma bortezomib concentration over the range of 0.5 to 2.0 ng/mL relating to large increases in the percent inhibition (0–60%). After that, a plateau occurs where marginal increases of proteasome inhibition are observed in spite of large changes in plasma bortezomib concentrations.[67]

Clinical Experience with Bortezomib: To date, more than 436,000 patients have been treated with bortezomib, including patients treated through Millennium-sponsored clinical trials, Investigator-Initiated Studies, the US NCI Cancer Therapy Evaluation Program (CTEP), and with commercially available drug. Bortezomib has been commercially available since 13 May 2003.

3.3.4 Bortezomib and Graft-versus-Host-Disease: Preclinical Data

In murine models of severe acute GVHD involving fully-HLA mismatched myeloablative allo-HSCT, bortezomib administered early after stem cell infusion protected against GVHD without impairing engraftment. [68,69] In a model intended to induce less severe GVHD, mice receiving bortezomib had 100% survival with no animal developing GVHD, while control animals all succumbed to GVHD prior to Day 50 post-transplantation.[68] Futrthermore administration of bortezomib beyond day 5 post-transplant resulted in acceleration of gut aGVHD in a CD4 dependent mechanism through activation of TLR4 and IL-1β.[70-72].

3.4 Immunologic changes post allogeneic HCT.

3.4.1 Immune reconstitution NK and Gamma Delta T-cells. Following hematopoietic stem cell transplant (allo-HSCT), there is a prolonged period of profound immune deficiency, which includes defects in thymopoiesis. This immune deficiency contributes to the high incidence of opportunistic infection, which continues for years after HCT (40, 41).[73,74] In the early post transplantation period, most peripheral blood lymphocytes are NK cells which can mediate cytotoxicity without prior sensitization and may be responsible for early GVL effects.[75,76] Savani et al. reported that patients who had higher(>150/mm^3) NK cell counts on day 30 after transplantation achieved rapid molecular remission in CML and significantly improved transplant outcomes not only by reduced relapse but also by reduced NRM and GVHD.[77] Ruggeri et al. demonstrated that NK cell allo-reactivity in haplo-identical transplants not only induced a strong GVL effect, but also prevented GVHD reaction through killing of host antigen presenting cells.[78]. Furthermore, haploidentical transplants with α/β T-cell receptor +CD19 B cell depletion, resulted in enrichment of γδ T-cells & NK cell early after transplant with low incidence of GVHD and infections.[79] Godder et al. showed that higher γδ T-cells in the 1st year post haploidentical transplant correlated with less risk of relapse.[80] Both NK and γδ T-cells where shown to acquire

a mature phenotype upon activation with (cytomegalovirus viremia). These activated innate immune cells were capable of killing viral infected targets as well as leukemic blasts, demonstrating their GVL effect.[80-83]. Finally Koreth et al. showed in his Tac/MTX/ bortezomib combination a significant improvement in day 30 NK cell recovery compared with a retrospective Tac/sirolimus cohort.[35]

3.4.2 GVHD prevention agents with favorable innate immune cell reconstitution and function.

Animal models suggest that CNI has a more selective immune suppressor pressure on T-cells than NK & gamma delta T cells.[84-86] Furthermore, Bosch et al. compared two cohorts of patients (pts) with Tac/MTX vs. Tac/MTX/Thymo and showed enhanced day 30 NK recovery in the second group of pts.[87] It is believed that T-cell depletion provides space for faster innate immune cell reconstitution from CD34+ stem cells than adaptive immune cells. Pical-Izard et al. evaluated NK cell reconstitution and phenotype after RIC conditioning regimen (Fludarabine/Busulfan) and Tacro/Thymo GVHD prophylaxis. She reported a mean NK cell of 115/mm^3 (confidence interval 75-145) at day 30 post allo-HSCT. She also showed that earlier development of mature NK cell phenotype markers (CD 56 dim, CD107) correlated with better survival and less relapse.[88] Bortezomib has shown pre-clinical evidence of tumor cell sensitization to NK cell activity through multiple potential mechanisms, including the induction of expression and activity of: DR 5, NKG2D ligands, Caspase 8, DNAM-1, and down regulation of HLA class I expression on target tumor cells.[89-94] Furthermore, Koreth et al. showed in his Tac/MTX/ bortezomib combination a significant improvement in day 30 NK cell recovery compared with retrospective Tac/sirolimus cohort. He showed a median day 30 post-transplant NK cell of 120/mm^3 for RIC and 252/mm^3 for MA preparative regimen.[35,36]

3.4.3 ATG pharmacokinetics and IL-15 levels post-transplant:

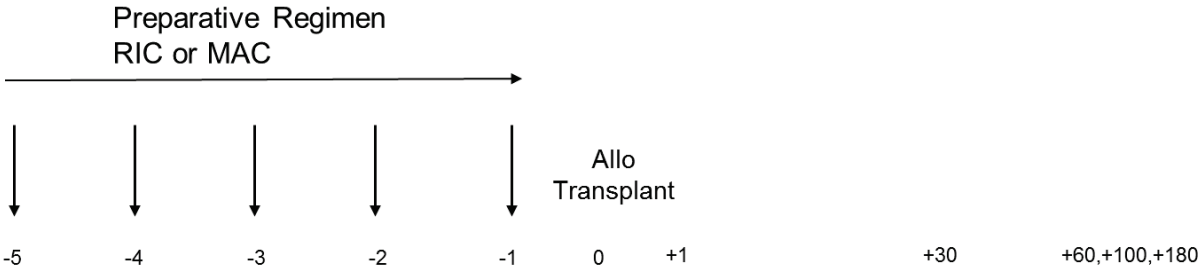
IL-15 is one of the main drivers for NK cell/ γδ T-cells homeostasis and expansion *in vivo*.[95-97] Boyiadzis et al. showed in a group of patients undergoing allo-HSCT with pre-transplant induced lymphopenea and RIC preparative regimen, a significant rise in IL-15 level at the peak of lymphopenia which was on day 0 of transplant. Furthermore, he demonstrated an inverse correlation between the rapid drop of IL-15 level at day 14 with the expansion of NK cells on day 30 post-transplant.[98] Admiraal et al. showed that higher Thymo AUC post-transplant (≥100 AU × day/mL; p=0·0024) Correlated with more graft T-cell depletion and less CD4 reconstitution post-transplant.[99] Therefore, it is likely that the more intense lymphodepletion caused by Thymo will lead to higher IL-15 levels and hence better recovery of NK/ γδ T-cells on day 30 post-transplant.

3.5 Study rationale.

This proposal combines the three least toxic and most effective GVHD prevention agents with favorable impact on NK cell reconstitution. Both Tac and Thymo have been extensively used in GVHD prevention before. Bort has been safely combined with Tac/MTX in >100 patients in multiple phase I-II trials with suggestions of efficacy. Furthermore, Bort is being tested in a large national randomized phase II trial with Tac/MTX, which just finished accrual. There is no perceived overlap in toxicity/side effects between the three agents Bort, Thymo and Tac. The goal is to add no extra toxicity from the GVHD regimen to the preparative regimen toxicity, leading to low NRM. Additionally the enhanced innate immune cell reconstitution will help in preventing infection and relapse, hence improving overall survival. The rationale behind the bortezomib timing is to match the timing schedule by Al-Homsi et al. and to avoid the remote chance of gut GVHD exacerbation when bortezomib given late post-transplant in animal models as described above. [70-72]

4. EXPERIMENTAL PLAN

4.1 Treatment outline



4.2 Study Design

This is a prospective, Phase II study. The study will use preparative regimens based on diagnosis and regimen intensity. These regimens are the standard of care at Winship Cancer Institute:

1- Fludarabine/Busulfan Flu/Bu full intensity (Bu AUC 5000) 4 doses.
2- Busulfan/Cytoxan full intensity (Bu AUC 5000) X 4 doses.
3- Total body radiation (TBI) (radiation dose >9G) combined with Cytoxan or etoposide.
4- Fludarabine/Melphalan (dose ≥140 mg/m^2) as a reduced intensity preparative regimen.

4.2 Study Endpoints

4.2.1 Primary Endpoints

1- To determine a composite end point of alive and severe acute GVHD free at 6 months following HLA matched related or unrelated donor hematopoietic peripheral blood transplant in patients with hematologic malignancies who receive the immunosuppressive combination TBT as GVHD prophylaxis.
2- Safety defined by serious adverse events (SAE) and adverse events (AE) related to this immunosuppressive regimen in the first six months post-transplant

4.2.2 Secondary Endpoints

1- Incidence and severity of aGVHD (grade II-IV)
2- Time to platelet and absolute neutrophil recovery (engraftment)
3- The cumulative incidence of grade III-IV aGVHD
4- Day 30 NK, γδ T-cell recovery and phenotype
5- Thymo pharmacokinetics and it's correlation with IL-15 levels and natural killer (NK) cell and gamma delta (γδ) T-cell recovery on day 30 post-transplant
6- Donor cell chimerism
7- Incidence of infections-including CMV and EBV reactivation including lymphoproliferative disorders PTLD
8- Incidence of veno-occlusive disease
9- Incidence of chronic GVHD
10- Rates of Grade ≥ 3 non-hematologic toxicity according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0
11- Non-relapse mortality NRM
12- Disease relapse or progression
13- Overall and disease free survival at one year
14- Immunosuppression-free survival at one year
15- KPS both pre-transplant and at various points post-transplant.

4.3 Number of Patients
Up to thirty eight patients will be enrolled on this study.

4.4 Study Duration
The duration of active patient participation is 24 months. The primary endpoints will be assessed during the first 6 months post-transplant. We expect 24 months to completion of patient enrollment and 30 months to study completion for the primary endpoints. For secondary endpoint analyses, patients will be followed for up to two years post-transplant.

4.5 Definitions:
4.5.1 Acute and Chronic GVHD:
Acute GVHD usually develops within the first 6 months post-transplantation and appears as a characteristic dermatitis often accompanied by cholestasis and enteritis and based on NIH definition of aGVHD.[100] Initial symptoms of chronic GVHD frequently include nausea, and anorexia with ocular and oral sicca. Rash characteristically appears with changes in skin pigment progressing to sclerosis and contractures. Other organs may be involved. Symptoms may mimic those seen in patients with scleroderma and other autoimmune disorders. Chronic GVHD typically does not occur until three months after transplantation. The modified Keystone Conference 1994 will be used to grade acute GVHD (Appendix A). The Chronic GVHD NIH Consensus Project will be used for grading severity of chronic GVHD (Appendix G).

4.5.3 Clinical Response, Relapse and Residual Disease
Clinical response is a decrease in the amount of disease that was present before the conditioning therapy, by whatever objective measurements were used to determine extent of disease such as physical examination; radiographic/nuclear scans or bone marrow/cytogenetic evaluation.

The term relapse is used to describe the recurrence of malignancy after stem cell transplantation, utilizing the techniques employed pre-transplant to determine extent of disease. The time to relapse is the time to the first observation of radiographic and/or histologic/cytogenetic change which results in characterization as relapse. Molecular relapse (as diagnosed for example by the presence of bcr-abl transcripts by RT-PCR analysis on repeat testing) will be documented but not be included in the overall figures for relapse. For example:

Acute Leukemia: Relapse will be diagnosed when leukemic blasts (>5%) are documented in the blood or bone marrow after transplantation or when there is detection of disease at an extramedullary site. The diagnosis of hematologic relapse will be supported by the reappearance of host cells using short tandem repeats (STR) or other method (e.g., FISH) and confirmed by the reappearance of cytogenetic abnormalities previously documented before transplantation (if applicable).

Lymphoma: The IWG-PET based response criteria will be used for lymphomas (Appendix G)

Response criteria for other diseases will be used when available.

Residual disease is defined as failure to eradicate original disease without prior documentation of remission.

4.5.4 Disease-Free Survival
Disease-free survival is defined as the minimum time interval from the day of transplant to -relapse, -death, or -last contact. For patients with documented residual disease at the start of the study, the time interval will be considered 0 days from transplant.

4.5.5 Cause of death
When defining the cause of death, physicians should classify deaths occurring from infection and from multiple organ failure in the presence of grade ≥ II acute GVHD (Glucksberg), as being caused by acute graft versus host disease. Similarly deaths occurring in the presence of graft failure or secondary rejection, should be classified as being due to graft failure, and deaths in

the presence of relapse of the primary malignancy as being due to relapse (even if other pathology contributing to the death is present).

4.5.6 Non relapse mortality (NRM)

This is defined as death in continuous or complete remission, measured from the date of transplant to the date of death.

5.0 PATIENT ELIGIBILITY

5.1 Study Population

All patients age ≥18 who present with hematological malignancies and are scheduled for peripheral blood HCT from an HLA-matched or -mismatched unrelated donor will be screened for eligibility.

5.2 Inclusion Criteria

1. Age 18-75 years
2. Patients with acute leukemia, chronic myelogenous leukemia, myeloproliferative disorder and myelodysplasia with no circulating blasts and with less than 5% blasts in the bone marrow within 4 weeks of the start of transplant conditioning regimen.
3. Patients with chronic lymphocytic leukemia/small lymphocytic lymphoma; follicular, marginal zone, diffuse large B-cell or mantle cell lymphoma with chemo-sensitive disease at time of transplant.
4. Patients must have a related or unrelated peripheral blood stem cell donor. Sibling donor must be a 6/6 match for HLA-A and -B at intermediate (or higher) resolution, and -DRB1 at high resolution using DNA-based typing, and must be willing to donate peripheral blood stem cells and meet institutional criteria for donation. Unrelated donor must be 8/8 match at HLA-A, -B, -C and –DRB1 at high resolution using DNA-based typing. Unrelated donor must be willing to donate peripheral blood stem cells and be medically eligible to donate stem cells according to NMDP criteria.
5. Cardiac function: Ejection fraction >40%
6. Estimated creatinine clearance greater than 50 mL/minute (using the Cockcroft-Gault formula and actual body weight)
7. Pulmonary function: DLCO ≥40% (adjusted for hemoglobin) and FEV1≥50%
8. Liver function: total bilirubin < 1.5x the upper limit of normal and ALT/AST < 2.5x the upper normal limit. Patients who have been diagnosed with Gilbert's Disease are allowed to exceed the defined bilirubin value of 1.5x the upper limit of normal.
9. Female subjects (unless postmenopausal for at least 1 year before the screening visit, or surgically sterilized), agree to practice two effective methods of contraception or agree to complete abstain from heterosexual intercourse from the time of signing the informed consent through 12 months post-transplant.
10. Male subjects (even if surgically sterilized), of partners of women of childbearing potential must agree to practice effective barrier contraception or abstain from heterosexual intercourse from the time of signing the informed consent through 12 months post-transplant.
11. Signed informed consent.

5.3 Exclusion Criteria

1. Prior allogeneic transplant
2. Karnofsky Performance Score < 70%
3. Active CNS involvement by malignant cells
4. Patients with uncontrolled bacterial, viral or fungal infections (currently taking medication and with progression or no clinical improvement) at time of enrollment.

5. Patients with transformed lymphoma (e.g., Richters transformation arising in follicular lymphoma or chronic lymphocytic leukemia).
6.
7. Patients seropositive for the human immunodeficiency virus (HIV).
8. Patient with active Hepatitis B or C.
9. Patients with hypersensitivity to bortezomib, boron or mannitol.
10. Patients with > grade 2 sensory peripheral neuropathy.
11. Myocardial infarction within 6 months prior to enrollment or New York Heart Association (NYHA) Class III or IV heart failure (see Appendix D), uncontrolled angina, severe uncontrolled ventricular arrhythmias, or electrocardiographic evidence of acute ischemia or active conduction system abnormalities. Prior to study entry, any ECG abnormality at screening must be documented by the investigator as not medically relevant
12. Female patients who are lactating or pregnant
13. Patients with a serious medical or psychiatric illness likely to interfere with participation in this clinical study
14. Patients with prior malignancies, except resected basal cell carcinoma or treated cervical carcinoma in situ. Cancer treated with curative intent > 5 years previously will be allowed. Cancer treated with curative intent < 5 years previously will not be allowed unless approved by the Protocol Officer or one of the Protocol Chairs.

6.0 DRUG INFORMATION

6.8 Bortezomib

6.8.1 Mechanism of action: see section 3.3.2

6.8.2 Toxicity: The most common Bortezomib side effects include:

Hematologic: anemia, neutropenia, thrombocytopenia, leucopenia, lymphopenia
Neurologic: asthenia, dizziness, anxiety, syncope, headache, insomnia, fever, rigors, chills, sensory peripheral neuropathy, and leukoencephalopathy, including reversible posterior leukoencephalopathy syndrome
Pulmonary: cough, dyspnea, pleural effusion, pneumonitis, interstitial pneumonia, edema acute respiratory distress syndrome (ARDS)
Cardiovascular: hypotension, tachycardia, atrial fibrillation, palpitation, congestive heart failure, bradycardia, atrial flutter, atrioventricular block, arrhythmia, cardiac failure, cardiac arrest, pericardial effusion, pericarditis
Infectious: reactivations of herpes zoster, opportunistic infections
Gastrointestinal: weight loss, decreased appetite, anorexia, constipation, dehydration, diarrhea, heartburn, dyspepsia, stomatitis, nausea, vomiting, ileus, GI perforation, acute pancreatitis
Metabolic: hyperglycemia, hypoglycemia, hyponatremia, hypokalemia, hypercalcemia
Renal: renal failure
Neuromuscular and skeletal: arthralgias, back pain, bone pain, muscle cramp and myalgias
Miscellaneous: rash, hemorrhage, blurred vision, deafness, hepatitis, Hyperbilirubinemia
Other medical events of interest that are considered not causally related to bortezomib include hepatic failure and QT prolongation. Fatal outcomes have been reported.

Women of childbearing potential should avoid becoming pregnant while being treated with bortezomib. Genotoxicity testing has shown that bortezomib is

negative in the in vitro Ames assay and in the in vivo micronucleus assay, but it is a clastogen in the in vitro chromosomal aberration assay.

Additional details on the potential risks of bortezomib may be found in the current Investigator's Brochure.

Bortezomib Precautions and Restrictions

It is not known what effects bortezomib has on human pregnancy or development of the embryo or fetus. Therefore, female patients participating in this study should avoid becoming pregnant, and male patients should avoid impregnating a female partner. Non-sterilized female patients of reproductive age and male patients should use effective methods of contraception through defined periods during and after study treatment as specified below.

Female patients must meet 1 of the following:

- Postmenopausal for at least 1 year before the screening visit. *Postmenopausal is defined as the time after which a woman has experienced twelve (12) consecutive months without a menstrual period.*
- Surgically sterile
- If they are of childbearing potential (i.e., not postmenopausal or surgically sterile), agree to practice 2 effective methods of contraception from the time of signing the informed consent form through 12 months post-transplant, or agree to completely abstain from heterosexual intercourse. It is strongly recommended that at least 1 of these 2 methods be 'highly effective' (see Table 2.6).

Table 2.6 Methods of Contraception

Highly Effective Methods	Other Effective Methods (barrier methods)
Intra-uterine devices (IUD)	Latex condom
Hormonal contraceptives (birth control pills/oral contraceptives, injectable contraceptives, contraceptive patches, or contraceptive implants)	Diaphragm with spermicide Cervical cap Sponge
If one of the highly effective methods cannot be used, using 2 effective methods at the same time is recommended.	

6.8.3 Packaging, Formulation, Preparation and Storage

VELCADE (*bortezomib*) for Injection is supplied as individually cartoned 10 mL vials
containing 3.5 mg of *bortezomib* as a white to off-white cake or powder

Unopened vials may be stored at controlled room temperature 25º C (77º F); excursions permitted from 15 to 30º C (59 to 86º F) [see USP Controlled Room Temperature]. Retain in original package to protect from light

Unopened vials of VELCADE are stable until the date indicated on the package when stored in the original package protected from light. VELCADE contains no antimicrobial preservative. When reconstituted as directed, VELCADE may be stored at 25ºC (77ºF); excursions permitted from 15 to 30ºC (59 to 86ºF) [see

USP Controlled Room Temperature]. Reconstituted VELCADE should be administered within eight hours of preparation. The reconstituted material may be stored in the original vial and/or the syringe prior to administration. The product may be stored for up to three hours in a syringe, however total storage time for the reconstituted
material must not exceed eight hours when exposed to normal indoor lighting.

Prior to use, the contents of each vial must be reconstituted with 3.5 mL of normal (0.9%) saline, Sodium Chloride Injection, USP. The reconstituted product should be a clear and colorless solution. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit. If any discoloration or particulate matter is observed, the reconstituted product should not be used.

6.8.4 Supplier: Commercially available

6.9 Tacrolimus (FK-506, Prograf®)

6.9.1 Mechanism of Action: Calcineurin inhibitor. Immunosuppressive agent that interferes with IL-2-mediated T-cell activation.

6.9.2 Toxicity: The most commonly reported toxicities are renal insufficiency, tremors, hypomagnesemia, hypertension, hyperglycemia and seizures.

6.9.3 Packaging and Formulation: Supplied as a sterile solution in 1-mL ampoules containing the equivalent of 5 mg of anhydrous tacrolimus per mL, in boxes of 10 ampoules. Also available as 1 mg and 0.5 mg capsules for oral administration.

6.9.4 Storage and Stability: Store between 5°C and 25°C (41°F and 77°F). Prograf capsules are stored at controlled room temperature, 15°C-30°C (59°F-86°F).

6.9.5 Administration: Tacrolimus injection must be diluted with 0.9% Sodium Chloride Injection or 5% Dextrose Injection to a concentration between 0.004 mg/mL and 0.02 mg/mL prior to use. Diluted infusion solution should be stored in glass or polyethylene containers and should be discarded after 24 hours. The diluted infusion solution should not be stored in a PVC container due to decreased stability and the potential for extraction of phthalates. In situations where more dilute solutions are utilized (e.g., pediatric dosing, etc.), PVC-free tubing should likewise be used to minimize the potential for significant drug adsorption onto the tubing. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Due to the chemical instability of tacrolimus in alkaline media, Prograf injection should not be mixed or co-infused with solutions of pH 9 or greater (e.g., ganciclovir or acyclovir).

6.9.6 Supplies: Commercially available.

6.10 Anti-Thymocyte Globulin, Rabbit (Thymoglobulin®)

6.10.1 Mode of action. See section 2.3.2

6.10.2 Toxicity. See section 2.3.3-2.3.5

6.10.3 Packaging and Formulation. Each vial of Thymoglobulin is supplied as 25mg lyophilized powder, packaged together with 5ml of diluent (sterile water for injection). Allow Thymoglobulin and diluent vials to reach room temperature before constituting the lyophilized powder with 5ml of diluent. Rotate the vial gently until the powder is complete dissolved. When the 25mg vial is reconstituted with 5ml of diluent, each ml contains 5mg of thymoglobulin. Visually inspect the solution for particular matter. If some particulate matter

remains, continue to gently rotate the vial until the Thymoglobulin is completely dissolved. Should particulate matter persist, discard this vial. Thymoglobulin is then further diluted with D5W or NS to a concentration of approximately 0.5mg/ml. Attach a 0.22 micron filter to the infusion bag.

6.10.4 Storage and Stability: Intact vials are stored under refrigeration between 2-8°C (36°F-46°F) and protected from light. Do not freeze. Thymoglobulin should be used within 24 hours once reconstituted with 5 ml of diluent. Thymoglobulin further diluted in 0.9% saline or 5% dextrose must be used immediately. Any unused drug remaining after infusion must be discarded. The following is a summary of in-house stability data for Thymoglobulin at various conditions (data on file with sponsor):

- For vials containing the unreconstituted lyophilized powder, the product is stable for 36 months at 5º ± 3ºC (41º± 4ºF) and 12 months at 37ºC (98.6ºF).
- Reconstituted product is stable for 24 hours at room temperature 20º to 25ºC (68º to 77º F).
- Reconstituted product further diluted in NS or D5W is stable for 24 hours at room temperature 20ºC (68ºF).freezing of Thymoglobulin is not recommended. A frozen product should not be used and should be discarded.

6.10.5 Administration: Infuse the first dose over a minimum of 6 hours, and subsequent doses over a minimum of 4 hours via a 0.22 micron in-line filter.

6.10.6 Supplies: Thymoglobulin® is commercially available.

7.0 DONOR AND GRAFT SELECTION

7.1 In selecting a donor, priority should be given to matched related donors and then matched unrelated donors at the A, B, C and DRB1 loci using high resolution typing. Donors may not have a single mismatch at the allele or antigen level. **The use of mismatched donors in which disparity is only in the host versus graft direction (because of recipient homozygosity) is discouraged because of the potentially heightened risk for graft rejection. Extended typing (e.g. DQB1 and DPB1) can be performed according to institutional practices and use these results in selecting donors; however, it is recommended that this extending typing be used only to select between donors who are equally well matched with the recipient at the A, B, C and DRB1.**

7.2 Donors will undergo G-CSF mobilization according to local institutional and donor center practices. PBSC will be collected by apheresis according to local institutional guidelines. Plasma and red cell depletion are allowed for volume reduction or ABO incompatibility but any other form of graft manipulation (including ex-vivo T cell depletion) is not permitted.

7.4 The target stem cell dose is between 2 x 10^6/kg and 10 x 10^6/kg (actual body weight) $CD34^+$ cells. The maximum CD34+ cell dose is 10 x 10^6/kg.

7.5 Up to two leukapheresis procedures may be performed to obtain the minimum $CD34^+$ cell target. If, after two leukapheresis procedures, fewer than 2 x 10^6/kg $CD34^+$ cells have been collected, transplant centers will have the discretion to continue PBSC cell harvesting or to proceed to bone marrow harvesting to obtain sufficient cells. If bone marrow harvesting is needed in order to meet the desired cell dose, the transplant center needs to notify the Protocol Coordinator, Chairs or Officer.

7.6 If more than 10 x 10^6/kg $CD34^+$ stem cells are collected, the excess will either be discarded or cryopreserved for future use, but will not be administered to the patient.

8.0 PRE-TRANSPLANT CONDITIONING:

Treating physicians may select one of four conditioning approaches (outlined below) for use and should choose the regimen that he or she thinks is best suited for the patient and the patient's disease. Patients who have had prior autologous allo-HSCT, however, must be conditioned with melphalan and fludarabine.

8.1 Total body irradiation (TBI) based conditioning:

Centers may employ the myeloablative TBI-based regimens they ordinarily employ for patients with hematologic malignancies as long as these regimens meet the following criteria:

a) The total dose of TBI is at least 900 cGy and not more than 1400 cGy.
b) It is administered in no less than 6 fractions.
c) It includes one or two chemotherapy agents (e.g. cyclophosphamide, etoposide, thiotepa)

Some degree of lung shielding during TBI administration is encouraged, using an electron boost to the chest wall to preserve full dosing to the ribs.

Cranial and testicular boosts may be administered prior to the start of conditioning at the discretion of the treating physician.

TBI VP16 (Etoposide):	
TBI	days -7 to -4
VP16 60mg/kg IV	day -3
TBI/Cy (cyclophosphamide)	
TBI	days -8 to -5
Cy 60mg/kg	days -3 & -2

8.2 Busulfan and Cyclophosphamide:

In recognition of the fact that varying doses of cyclophosphamide are used in combination with high dose busulfan, centers may use the regimen they ordinarily employ for patients with myeloid malignancies as long as the regimen meets the following criteria:

a) The initial regimen (i.e. before adjustments are made for pharmacokinetic testing results) includes the equivalent* of sixteen, ≥0.8 mg/kg IV doses (q 6 hours) of busulfan, administered q 6 hours.
b) The regimen uses two daily 60 mg/kg doses or four daily 50 mg/kg doses of cyclophosphamide.

Cyclophosphamide Ideal Body Weight Dose Adjustments should follow institutional practices.

Initial busulfan dosing may be based on actual or adjusted-ideal body weight based on the discretion of the treating physician.

Busulfan pharmacokinetic testing: Pharmacokinetic testing is recommended, but should be performed according to institutional standards. Pre-transplant test dosing may be used.

***Modifications in busulfan administration**: The busulfan administration may be modified according to institutional practices as follows:

1) Initial doses as high as 1.2 mg/kg q 6 hr (or 4.8 mg/kg/day see #2) may be used for younger children.
2) Daily doses (e.g. 3.2 mg/kg/day or 130 mg/m^2 rather than 0.8 mg/kg/day) may be used in place of q 6hr dosing.
3) The dosing may be adjusted based on the results of pharmacokinetic testing. This can involve a change in the size of the individual doses or for patients who are getting q 6hr dosing where the pharmacokinetic testing result exceeds the upper limit of the targeted range, the targeted total dose (e.g. 16 * targeted per dose AUC) may be apportioned into 14 or 15 doses rather than 16 as part of the adjustment.

Hydration and MESNA administration: The administration of hydration and cyclophosphamide to prevent hemorrhagic cystitis from cyclophosphamide will follow institutional practices.

Seizure prophylaxis: An anti-convulsant will be administered according to institutional guidelines to prevent busulfan-induced seizures.

8.3 Melphalan and Fludarabine Reduced Intensity Conditioning RIC:

Fludarabine	25 mg/m2/dose IV qd (5 doses)	Days -6 to -2
Melphalan*	140 mg/m2/dose IV	Day -2

Fludarabine and Melphalan should each be infused over 30 minutes.
Alternatively Melphalan can be given as 140mg/m2 as a single dose on day -2.
Alternatively Fludarabine can be given as 30 mg/m2 daily X 4 days from day -5 to -2

Fludarabine and melphalan dosing will be based on actual body weight.

8.4 Busulfan and Fludarabine myeloablative conditioning MAC:

Fludarabine	40 mg/m2/dose IV qd-4 doses	Days -5 to -2
Busulfan	0.8 mg/kg/dose IV q6H-16 doses or the equivalent*	Days --5 to -2

Fludarabine should each be infused over 30 minutes and busulfan over two hours for the q6 hour dosing and over 3 hours for the daily dosing.

Fludarabine dosing will be based on actual body weight. Initial busulfan dosing may be based on actual or adjusted-ideal body weight based on the discretion of the treating physician.

Busulfan pharmacokinetic testing: Pharmacokinetic (PK) testing is recommended, but should be performed according to institutional standards. Pre-transplant test dosing may be used or PK from the first infusion of buslfan can be used to adjust the remaining doses . The recommended final daily AUC range is close to 5000.

***Modifications in busulfan administration**: The busulfan administration may be modified according to institutional practices as follows:

1) Daily doses (e.g. 3.2 mg/kg/day or 130mg/m^2/day rather than 0.8 mg/kg/day) may be used in place of q 6hr dosing.
2) The dosing may be adjusted based on the results of pharmacokinetic testing. This can involve a change in the size of the individual doses or for patients who are getting q 6hr dosing where the pharmacokinetic testing result exceeds the upper limit of the targeted range, the targeted total dose (e.g. 16 * targeted per dose AUC) may be apportioned into 14 or 15 doses rather than 16 as part of the adjustment.

Seizure prophylaxis: An anti-convulsant will be administered according to institutional guidelines to prevent busulfan-induced seizures.

9. Allogeneic Stem Cell Infusion:

PBSC will be administered on Day 0 to all patients according to individual institutional guidelines after appropriate processing and quantification has been performed by the local laboratory. Stem cells are administered through an indwelling central venous catheter. If infusion occurs over two days, Day 0 is the day the last infusion is completed.

10.0 Anti-Infective Prophylaxis

10.1 It is mandatory once weekly CMV PCR screening beginning around day 0 and until day 120 post-transplant be performed and preemptive therapy be given for positive cultures, according to institutional guidelines.

10.2 EBV monitoring by PCR is also mandatory at weekly intervals from day 0 to around day 100; treatment with Rituximab 375 mg/m^2 shall be given for PCR levels of >1000 genome copies/ml. EBV PCR will be repeated once a week and a second dose of Rituximab can be considered if there was no drop in EBV PCR.

10.3 Antibacterial prophylaxis will be administered based on institutional standards of care. The following guidelines are recommended at physician discretion.

10.4 If solumedrol is used for engraftment syndrome or acute skin graft versus host disease in the first 30 days post-transplant, the recommended dose is 1 mg/kg/day and the recommended taper would be 50% down every three days.

11.0 GVHD Prophylaxis

The GVHD prophylactic regimen will consist of the following:

Tacrolimus 0.03 mg/kd/d CIV, beginning day -3 (IBW)
Thymoglobulin® 0.5 mg/kg day-3 (adjusted body weight ABW)
Thymoglobulin® 2 mg/kg day -2
Thymoglobulin® 2.5 mg/kg day -1
Bortezomib 1.3 mg/m2 IV day 0 (after the graft),and day +3. (ABW)

11.1 Tacrolimus

11.1.1 Tacrolimus Dosing

Tacrolimus will be administered intravenously at a dose of 0.03 mg/kg (ideal body weight) q 24h by continuous infusion starting on Day –3. Intravenous tacrolimus will be discontinued once the patient starts eating and the drug will then be given orally at a dose of approximately 4 times the intravenous dose.

11.1.2 Dose Modifications of Tacrolimus.

If vomiting occurs within twenty minutes after an oral dose of tacrolimus, the dose should be repeated. Anti-nausea medications may be given as needed. Intractable nausea and vomiting may require intravenous administration of tacrolimus at 1/4 of the oral dose. Tacrolimus may cause impaired renal function, hyperbilirubinemia, increased serum transaminase levels, hypertension, tremor, and seizures. Impaired renal function may require tacrolimus dose reductions. Severe toxicities may require temporary suspension or discontinuation of treatment.

Tacrolimus dose adjustments should not be based exclusively on serum levels. Tacrolimus levels should be used as a guide in conjunction with clinical observations of the biologic effects of the drug, i.e., toxicity and immunosuppression.
Suggested Tacrolimus Dose Adjustment Guidelines

Plasma Levels	Toxicity Grade	Tacrolimus Dose
<5ng/ml	0	Increase 50%
5-12ng/ml	0,1	No change
“ “	II	Decrease 25%
“ “	III	Decrease 50%
“ “	IV	Stop 100%
>15ng/ml	0	Decrease 25% every 3-4 days
>20ng/ml	0	Stop 100%

11.1.4 Tacrolimus Blood Levels

Tacrolimus levels should be measured at least weekly during the first 50 days post-transplant. **Target levels of 5-12 ng/ml** are acceptable in patients who manifest no evidence of toxicity or GVHD. While the patients are getting Tacrolimus continuous I.V infusion the levels are checked on Monday, Wednesday, Friday and PRN with am labs.

When the patients switch to oral Tacrolimus trough levels will be checked. Trough plasma levels should be drawn 10-12 hours after the last dose if possible.

11.1.5 Drug Interactions

Drugs that may increase tacrolimus levels: Fluconazole, Voriconazole, posaconazole Itraconazole, Ketoconazole, erythromycin, H2 blockers, Verapamil, Diltiazem, nicardipine, danazol, bromocriptine, metoclopramide, methylprednisolone, somatostatin (octreotide).

Drug that may decrease tacrolimus levels: Rifampin, Phenobarbital, phenytoin, carbamazepne, Octreotide (may lower serum levels by decreasing intestinal absorption of the oral drug).

Drugs that may result in additive nephrotoxicity: Aminoglycosides, Amphotericin B, Acyclovir, Furosemide, TMP-SMX, and grape fruit juice. These drugs are to be used with caution and close attention in anticipation of tacro level change.

11.2 Bortizomib

11.2.1 Bortezomib will be administered at the dose of 1.3 mg/m^2 (based upon actual body weight) as an approximately 3-5 second IV push on Days +0 (after graft infusion),and + 1post transplant. There must be at least 72 hours between each dose of bortezomib. Subcutaneous administration of bortezomib is not allowed on this protocol.

11.2.2 Bortezomib dose modifications:

Before each drug dose, the patient will be evaluated for possible toxicities that may have occurred after the previous dose(s). Toxicities are to be assessed according to the NCI Common Toxicity Criteria for Adverse Events (CTCAE), Version 4.0

Neuropathic pain and peripheral sensory neuropathy are to be managed as described in Table 2.4b.

Table: Management of Patients With VELCADE-Related Neuropathic Pain and/or Peripheral Sensory or Motor Neuropathy

Severity of Peripheral Neuropathy Signs and Symptoms[a]	Modification of Dose and Regimen
Grade 1 (asymptomatic; loss of deep tendon reflexes or parasthesias) without pain or loss of function	No action
Grade 1 with pain or Grade 2 (moderate symptoms; limiting instrumental Activities or Daily Living [ADL][b])	Reduce VELCADE to 1.0 mg/m^2
Grade 2 with pain or Grade 3 (severe symptoms; limiting self care ADL[c])	Withhold VELCADE therapy until toxicity resolves. When toxicity resolves reinitiate with a reduced dose of VELCADE at 0.7 mg/m^2 (*missed dose days will not be made up*).
Grade 4 (life-threatening consequence; urgent intervention indicated)	Discontinue VELCADE

Source: VELCADE USPI issued January 2012.
Abbreviations: ADL = activities of daily living
a Grading based on NCI Common Terminology Criteria for Adverse Events (CTCAE) v4.0.
b Instrumental ADL: refers to preparing meals, shopping for groceries or clothes, using telephone, managing money, etc
c Self care ADL: refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

11.2.4 Patients with mild hepatic impairment (bilirubin ≤ 1.5 × ULN) do not require dose adjustment. If a patient develops moderate or severe hepatic impairment with bilirubin ≥ Grade 2 (> 1.5 -3.0 X ULN) after exposure to Bortezomib, the dose should be held until the toxicity returns to < Grade 2. Restarting Bortezomib must be done at the next lower dose level as below.

Once bortezomib is reduced for any toxicity, the dose may not be re-escalated.

If after bortezomib has been held, the toxicity does not resolve, then bortezomib must be discontinued.

If the toxicity resolves, as described above, bortezomib may be restarted at the same schedule the patient was on prior to holding therapy, and the dose must be reduced by approximately 25% as follows:

- If the patient was receiving 1.3 mg/m^2, reduce the dose to 1 mg/m^2.
- If the patient was receiving 1 mg/**m^2, reduce the dose to 0.7 mg/m^2.**

11.3 Thymoglobulin

11.3.1 Thymoglobulin Pre-medication

One hour prior to the first Thymoglobulin dose, solumedrol 0.5 mg/kg will be given intravenously; 3 hours past the start of Thymoglobulin, solumedrol 0.5 mg/kg will be repeated intravenously. Acetaminophen 650mg PO, and diphenhydramine 50mg IV should be given within 30 minutes prior to thymoglobulin infusion.

It is recommended to consider B1 blockers (metoprolol or atenolol) to start one day before the Thymo infusion and continue until after day 0 of transplant to protect patients at risk for CAD from Thymo induced tachycardia.

11.3.2 Thymoglobulin Dosing

The total dose chosen for this protocol is 5 mg/kg because of a high rate of EBV reactivation with a recent experience (personal Communication from City of Hope) using 7.5 mg/kg. Thymoglobulin will be dosed according to an escalated regimen to minimize infusional toxicities, as follows: 0.5 mg/kg on day –3, 2 mg/kg on day –2, and 2.5 mg/kg day –1.

11.3.3 Thymoglobulin Administration

After the patient is pre-medicated, Thymoglobulin will be infused using a 0.22-micron in-line filter. Thymoglobulin should not be co-administered with blood products or other biologic agents. All Thymoglobulin doses will be infused over 6 hours. Avoid simultaneous infusion of Thymoglobulin with any other solution, particularly lipids, in the same infusion line. Patients will be observed for AEs during all Thymoglobulin infusions. All patients will receive the second Thymoglobulin dose approximately 24 hours (no sooner than 12 hours and no greater than 36 hours) after the start of the first Thymoglobulin dose. Subsequent doses should be administered at similar time intervals unless dose interruption is required (see below).

11.3.4 Thymoglobulin Discontinuation or Interruption Guidelines

Thymoglobulin treatment may be ***discontinued*** **or** ***interrupted*** at any time for the following reasons:

a). Patient refuses to continue treatment

b). Infusion of study drug related SAE

Thymoglobulin may be re-instituted if, in the judgment of the investigator, the primary clinical cause(s) for dose adjustment have been resolved.

The initial dose of Thymoglobulin may be associated with a reaction typically associated with fever and chills commonly referred to as a cytokine release syndrome. Often such symptoms are mild and can be reduced by decreasing the rate of infusion so that the drug is administered over 12 hours. Therapy such as additional Benadryl, corticosteroids, or adrenergic agents may be required. For severe cytokine release symptoms such as dyspnea or symptoms of capillary leak syndrome, infusion should be interrupted while appropriate clinical therapy is administered. Clinical therapy for cytokine release symptoms includes additional corticosteroids and antihistamines. Additional resuscitative measures for severe symptoms or anaphylaxis (e.g. 0.3 ml aqueous epinephrine (1:1000 dilution), pressor agents, oxygen, intravenous fluids and airway management) should be provided as clinically indicated.

Hypersensitivity type III, such as arthralgia, myalgia, skin rash or other symptoms of immune complex disease may occur, and usually responds within 24-48 hours to 100mg of IV hydrocortisone or oral prednisone equivalent (20mg). If, in the opinion of the physician, additional therapy is required for treatment of immune complex symptoms, additional corticosteroid therapy (e.g. a prednisone taper over the next 7-10 days) may be given as necessary. Ranitidine 150mg BID or other H-2 blockade or antacid prophylaxis should be given while patients are on corticosteroid therapy and for 2 weeks thereafter.

11.3.5 Guidelines for Tapering of Immunosuppression

In general, no evidence of GVHD should be present and the following guidelines should be observed. Tacrolimus taper commenced after 12 weeks, with the goal of having the patient no longer receiving immune suppression by 6 months in the absence of GVHD

12.0 Immuno-correlative and pharmacokinetic studies:

This section is for immunologic correlative studies in patients who participate in the protocol.

12.1 Correlative immunologic study objectives:

In this study, we plan to evaluate the following immunologic assays (biomarkers) to better understand mechanisms involved in graft-versus-host disease and immune reconstitution in allogeneic donor transplants performed in this protocol with the introduction of a new GVHD prophylaxis combination of tacrolimus, bortezomib, and thymo. These markers are:

(1) NK cell recovery and phenotype post-transplant. CD3, CD56, KIR (four antibodies one color), NKG2A, NKG2C, & functional phenotype (TNFα, CD107, IFNγ after exposure to K562).

(2) (2) γδ T-cells recovery and phenotype post-transplant. CD3, Gamma delta Pan TCR

T cell immunophenotyping and absolute subset number quantification will be performed in bulk for each patient using PBMC samples isolated and frozen at each time point. Absolute leukocyte counts will be determined on each sample and will be used to convert the percentage of each subset in samples determined below into absolute cell numbers of each subset. Percentage of each subset in samples will be determined using multi-parameter flow cytometry. The mean numbers and phenotype of NK cell and γδ T-cells at days 30, 60,100,180 post-transplant will be compared with historical published French NK cell reconstitution data with tacrolimus/Thymo,[88] in addition to our local NK/ γδ T-cells reconstitution numbers in the first 3 months after transplant.

12.3 Pharmacokinetic studies

Plasma samples were (10 ml in red top tube) obtained daily from the day preceding Thymo administration (day-4) to day +4 post-transplantation, then on days+7,+14,and 30+/-2 post-

transplant. Plasma will be frozen at -80°for future active R-ATG and IL-15 level measurements. Measurement of active R-ATG (the fraction which retains the capacity to bind to human lymphocytes) will be done according to the methods described by Regan et al.[101]. The IL-15 QuantiGlo ELISA (R&D Systems) will be used to measure IL-15 level.

12.4 Immunocorrelative Studies

Patients will have blood draws at 30 mls into CPT tubes Becton Dickinson Vacutainer Systems Franklin Lakes, NJ) on days +14,+30 (+/-2),+60(+/-4),+100 (+/-7),+180(+/-7) post-transplant. PBMCs were isolated from CPT tubes after centrifugation. PBMCs will be used for immunophenotyping studies.

Immunologic correlative and pharmacokinetic study calendar

Test	Time point	Pre Thymo	Post Thymo Days-4, -3,-2,-1, 0,+1,+2,+3,+4	Day+7	Day + 1 4	Day + 30 (+/-2)	Day + 60 (+/-4)	Day + 100 (+/- 7)	Day + 180 (+/- 7)
Active Thymo		X	X	X	X	X	X		
IL-15		X	X	X	X	X	X		
NK cell					X	X	X	X	X
γδ T-cells					X	X	X	X	X

All samples will be sent to the Dr Waller's lab for processing, then, saved for immunologic assays as above.

12.5 Maintenance of Patient related biological material and records.

Dr. Al-Kadhimi (pager number 71101) will be contacted to pick up and start processing all patients' blood samples. Each sample will have a unique identifying number that will link it to the patient's name and medical record number as well as the date of collection. The samples will be stored for a total of 5 years to give ample time after finishing patient accrual to carry out the experiments outlined in the methods and procedures section. Beyond 5 years from the date of starting patient accrual the samples will be destroyed by thawing and bleaching. The cost of storing and maintaining the samples will be covered by Dr. Al-Kadhimi's startup funds. If for any unforeseeable reason the PI runs out of funds to maintain and store these patients' samples or the PI leaves the institution or the study, Dr. Al-Kadhimi will identify another investigator to assume responsibility of the protocol, the maintenance and storage of patient's samples, otherwise the samples will be destroyed.

13.0 Study Procedures: SCREENING AND BASELINE EVALUATION

A flow chart of study procedures and assessments is provided below.

The transplant patients that meet the study eligibility criteria and are consented for the study will undergo baseline evaluations. Much of the information listed below will be obtained at the time of determining eligibility/screening prior to enrollment on the protocol.

The following baseline evaluations should represent those assessments/labs performed within 4 weeks **prior to the start** of the conditioning regimen.

- History, physical examination, height and weight.
- KPS (See Appendix F).
- Donor and recipient serological status for HIV, CMV, HSV, HCV Ab, and HBV, including Hepatitis B core antibody (HbcAb), Hep B surface antibody (HepBsAb) and Hepatitis B surface antigen (HbsAg).

- Serum or urine pregnancy test (females only).
- CBC with differential, platelet count, basic metabolic panel (creatinine, glucose, Na, K, CI, HCO_3, calcium, blood urea nitrogen), liver function tests (Bilirubin, AST, ALT, alkaline phosphatase, LDH), cholesterol and triglycerides
- EKG
- Pulmonary function tests, including DLCO, FEV1 and FVC
- Bone marrow aspirates for pathology and cytogenetics
- Chest-X-Ray
- MUGA or Echocardiogram

14.0 STUDY PROCEDURES: MAINTENANCE PHASE/Post-Transplant PERIOD

The following procedures will be performed post-transplantation, according to Appendix E:

- History and physical examination to assess GVHD and other morbidity weekly through first discharge after transplant, then around day 30, 90, 180 and 360 post-transplant
- Record specific adverse events and opportunistic infections
- KPS Performance Status (see Appendix F)
- Disease specific re-staging based on institutional standards
- Perform laboratory evaluations weekly while in the hospital, then at day 30, 90, 60, 100, and 180 post transplant:

o CBC (hemoglobin, hematocrit, WBC with differential and platelet count)
o Basic metabolic panel: creatinine, glucose, Na, K, CI, HCO_3, calcium, blood urea nitrogen (BUN)
o Liver function tests (bilirubin, AST, ALT, alkaline phosphatase, LDH)
o Tacrolimus level once or twice/week

- Note any serologic change (CMV and EBV)

Study Calendar	Baseline					
Study Day	Prior to start	Post-Transplant Hospitalization	30	60	100	180
Window Days for Study Visit	-30 days	(varies)	+/- 4	+/- 7	+/- 10	+/- 20
Study Month	-	-	1	2	3	6
Informed Consent	X					
Inclusion/Exclusion Criteria, including organ function/staging	X					
Medical History	X					
Concomitant Medications		X	X	X	X	X
Clinical Assessment & KPS Adverse Events Opportunistic Infections Assessment of Engraftment/GVHD Assessment for Patient/Graft Survival	X[1]	X	X	X	X	X
Hematology (complete blood count (CBC) with differential platelets)	X	X	X	X	X	X
BMP[2]	X	X[4]	X	X	X	X
Liver function tests[3]	X	X[5]	X	X	X	X
Pregnancy Test	X					
Tacrolimus Trough Level	NA	X[6]	X	X	X	X
Disease restaging[7]	X	X				
CMV and EBV screening[8]		X	X	X	X	X

[1]Clinical Assessment at screening.
[2] Serum chemistries to include creatinine, glucose, sodium (Na), potassium (K), chloride (Cl), bicarbonate (HCO_3), calcium, blood urea nitrogen (BUN)/Urea.
[3] Liver function tests: bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, LDH
[4] At least three times a week during hospitalization
[5] Liver function tests will be done as clinically indicated based on KCI BMT standards
[6]Tacrolimus levels to be checked 3 times per week and sirolimus levels two times a week and at every clinic visit as per standard operating procedure for the Blood and Marrow Transplant Program.
[7] Disease restaging to be done at discretion of physician in keeping with standard of care.
[8] It is mandatory once or twice weekly CMV shell vial culture or PCR screening beginning around day 21 and until day 100 post-transplant be performed. EBV monitoring by PCR is also mandatory at weekly intervals from day 21 to around day 100.

15.0 GVHD

15.1 Diagnosis

No skin biopsy is required in any patient, but it is recommended in patients with only skin manifestations. A gastro-intestinal tract biopsy is also recommended in patients diagnosed with upper gastro-intestinal acute GvHD alone. Appropriate biopsies are also recommended in patients diagnosed with aGvHD, but without skin signs.

15.2 Staging and Grading

For acute GVHD, the clinical stage of the three organs involved will be assessed, so that an overall grade according to modified Keystone criteria cab be obtained. See Appendix A. For chronic GVHD, the NIH consensus Project (Appendix G) will be used for grading and severity.

15.3 Outcome of acute GVHD

This will be defined as follows:
Complete response – clinical stages in all organ systems are zero.
Partial response – at least one organ system has improved by at least one stage, and none of the other organ systems have worsened.
Mixed response – at least one organ system has improved by at least one stage and at least one organ system has worsened by at least one stage.
Stable disease – no change in the clinical stage of any organ system from baseline.
Progressive disease – at least one organ system has worsened by at least one stage without improvement of any other organ systems from baseline.
Recurrence – complete or partial response followed by recurrence of aGvHD requiring an increase of systemic methylprednisolone or equivalent by at least 2 mg/kg/day, or the introduction of another immunosuppressant agent.

15.4 Treatment of acute GVHD

If acute GVHD grade II or greater develops during therapy, it will be treated with methylprednisolone 1-2 mg/kg/d, or per active protocols. Treatment with tacrolimus should continue. For steroid refractory GVHD, treatment will be at the discretion of the investigator.

16.0 NCI Toxicities

Toxicities will be recorded using the NCI CTCAE 4.0 Scale. The NCI CTCAE 4.0 can be found at http://ctep.cancer.gov/reporting/ctc.html

17.0 Statistical Considerations

17.1 Study Design: Single Arm phase II trial

17.2 Endpoint:

17.2.1 Primary endpoints

1- To determine a composite end point of alive and severe acute GVHD free at 6 months following HLA matched related or unrelated donor hematopoietic peripheral blood transplant in patients with hematologic malignancies who receive the immunosuppressive combination TBT as GVHD prophylaxis.

2- Safety defined by serious adverse events (SAE) and adverse events (AE) related to this immunosuppressive regimen in the first six months post-transplant

17.2.2 Secondary endpoints

The secondary endpoints for this protocol are:

1- Incidence and severity of aGVHD grade II-IV.
2- Time to platelet and absolute neutrophil recovery (engraftment)
3- The cumulative incidence of grade III-IV aGVHD
4- Day 30 NK, γδ T-cell recovery and phenotype
5- Thymo pharmacokinetics and it's correlation with IL-15 levels and natural killer (NK) cell and gamma delta (γδ) T-cell recovery on day 30 post-transplant
6- Donor cell engraftment
7- Incidence of infections-including CMV and EBV reactivation including lymphoproliferative disorders PTLD
8- Incidence of veno-occlusive disease

9- Incidence of chronic GVHD
10- Rates of Grade ≥ 3 non-hematologic toxicity according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0
11- Non-relapse mortality NRM
12- Disease relapse or progression
13- Overall and disease free survival at one year
14- Immunosuppression-free survival at one year
15- KPS both pre-transplant and at various points post-transplant.

17.3 Trial Design and Sample Size: We will use Simon's two stage minimax design for this phase 2 trial using a composite primary end point of unfavorable transplant outcomes defined as death from any cause or alive with a prior history of grade 3-4 aGVHD by 6 months post-transplant. Our current institutional rate of this composite outcome at 6 months is 35% (10% non-fatal grade 3/4 aGvHD+ 25% 6 month mortality). We hypothesize to reduce the incidence of this composite endpoint to ≤17%. We aim to accrue 17 patients for the first stage and 21 patients for the 2nd stage with a total of 38 patients. After the first 17 patients if we observe 6 or more cases of non-lethal grade 3/4 GVHD or 6 month-mortality as defined above, the trial will not advance to stage 2 for futility. After accruing 38 pts, if we observe 9 or more bad outcomes (as defined above), the trial will be deemed not promising for further clinical development. A sample size of 38 will achieve a power of 80% and type one error of 0.045.

To test the hypothesis that this GVHD prevention regimen leads to activation of donor NK cells that may have potent anti-leukemic activity we will measure the number of NK cells at day 30, hypothesizing that the use of the TBT regimen will lead to double the number of peripheral blood NK cells at day 30 to 250/mm^3 compared to a historical mean of 120/mm^3 using Tacrolimus ATG.[88] A sample size of 35 achieves 100% power to detect a difference of -165.0 between the null hypothesis mean of 120.0 and the alternative hypothesis mean of 285.0 with an estimated standard deviation of 100.0 and with a significance level (alpha) of 0.05000 using a two-sided Wilcoxon test assuming that the actual distribution is normal.

17.4 Statistical Analysis: Descriptive statistics will be mainly employed to address primary and secondary endpoints. The incidence of aGVHD incidence will be summarized as percentage and 95% confidence level will be also constructed. SAE and AE will be tabulated with frequency and percentage. Time to platelet and absolute neutrophil recovery will be presented with median and range. Other secondary endpoints, including the cumulative incidence of grade III-IV aGVHD, Day 30 NK, γδ T-cell recovery and phenotype, Thymo pharmacokinetics and it's correlation with IL-15 levels and natural killer (NK) cell and gamma delta (γδ) T-cell recovery on day 30 post-transplant, donor cell engraftment, incidence of infections-including CMV and EBV reactivation, incidence of veno-occlusive disease, incidence of chronic GVHD, rates of Grade ≥ 3 non-hematologic toxicity, non-relapse mortality NRM, will summarized with frequency and percentage. The disease relapse or progression, overall and disease free survival, immunosuppression-free survival will be analyzed with Kaplan Meier method and Logrank test. KPS both pre-transplant and at various points post-transplant will be presented with frequency and percentage.

17.5 Stopping Rules: The stopping rule for safety monitoring is based on non-relapse mortality at 6 months as a grade 5 SAE. Our current 1 year NRM is about 20%. For the first stage of the trial and considering the upper limit of the 90% confidence interval of what our

current experience at year, we would stop the trial if by 6 months post-transplant we observe 5 non relapse deaths in the first 17 patients.

17.6 Accrual and Study Duration: The anticipated patient's accrual for this trial is 2 years. This is based on an expected transplant rate of 50 HLA matched related and unrelated transplant operations per year at the Winship Cancer Institute. Six months observation is needed for primary endpoints. Therefore, the study duration to observe the primary end point is 18 months.

18.0 DATA AND Safety MONITORING

18.1 Monitoring and Personnel Responsible for Monitoring

The Data and Safety Monitoring Committee (DSMC) of the Winship Cancer Institute will provide oversight for the conduct of this study. The DSMC functions independently within Winship Cancer Institute to conduct internal monitoring functions to ensure that research being conducted by Winship Cancer Institute Investigators produces high-quality scientific data in a manner consistent with good clinical practice (GCP) and appropriate regulations that govern clinical research. Depending on the risk level of the protocol, the DSMC review may occur every 6 months or annually. For studies deemed High Risk, initial study monitoring will occur within 6 months from the date of the first subject accrued, with 2 of the first 5 subjects being reviewed. For studies deemed Moderate Risk, initial study monitoring will occur within 1 year from the date of the first subject accrued, with 2 of the first 5 subjects being reviewed. Subsequent monitoring will occur in routine intervals per the Winship Data and Safety Monitoring Plan (DSMP).

The DSMC will review pertinent aspects of the study to assess subject safety, compliance with the protocol, data collection, and risk-benefit ratio. Specifically, the Winship Cancer Institute Internal Monitors assigned to the DSMC may verify informed consent, eligibility, data entry, accuracy and availability of source documents, AEs/SAEs, and essential regulatory documents. Following the monitoring review, monitors will provide a preliminary report of monitoring findings to the PI and other pertinent individuals involved in the conduct of the study. The PI is required to address and respond to all the deficiencies noted in the preliminary report. Prior to the completion of the final summary report, monitors will discuss the preliminary report responses with the PI and other team members (when appropriate). A final monitoring summary report will then be prepared by the monitor. Final DSMC review will include the final monitoring summary report with corresponding PI response, submitted CAPA (when applicable), PI Summary statement, and available aggregate toxicity and safety data.

The DSMC will render a recommendation and rating based on the overall trial conduct. The PI is responsible for ensuring that instances of egregious data insufficiencies are reported to the IRB. Continuing Review submissions will include the DSMC recommendation letter. Should any revisions be made to the protocol-specific monitoring plan after initial DSMC approval, the PI will be responsible for notifying the DSMC of such changes. The Committee reserves the right to conduct additional audits if necessary.

Patient safety, study efficacy and compliance will be reviewed at the BMT working group meeting. The BMT working group will also oversee the conduct of this study. In addition interim analysis will be conducted once after 17 patients have accrued and had minimum of 6 months of follow up for futility assessment. The bone marrow research Team consisting of the PI,

Collaborating Investigators, CRA, protocol nurse, and statistician is responsible for monitoring the data and safety of this study, including implementation of any stopping rules for safety and efficacy.

Scheduled meetings will occur depending upon the activity of the protocol. These meetings will include the Principal Investigator, data managers, and anybody deemed important to attend by the Principal Investigator.

During these meetings the following points will be reviewed and discussed:

1. Safety of protocol participants (AE reporting).
2. Validity and integrity of the data.
3. Enrollment rate relative to expectations and the characteristics of participants.
4. Retention of participants and adherence to the protocol (potential or real protocol violations).
5. Completeness of collected data.

18.2 Adverse Events

18.2.1 Reporting: In adherence to good clinical practice where the rights, safety, and well-being of research protocol participants are protected, the timely reporting of adverse reactions by the sponsor-investigator in accordance to regulation of the Food & Drug Administration (Ref. Title 21, Code of Federal Regulations, Part 312). Adverse reactions shall be reported to Institutional Review Board according to their Policies and Procedures. Completion of adverse reaction reporting forms provides adequate documentation of adverse events.

In concert with the Blood & Marrow Stem Cell Transplant Program clinical personnel, BMT Data Management Research personnel and investigator or subinvestigator shall identify and grade adverse actions using NCI Common Terminology Criteria for Adverse Events v 4.0 (CTAE) that is available at (http://ctep.cancer.gov/forms/CTCAEv4.pdf). Data Management Research staff shall refer to Emory IRB for guidance in IRB reporting requirements for adverse and unexpected events. All unexpected grade 3-5 AEs will be reported to Emory IRB whether it is related or unrelated to study intervention. Non-relapse related deaths, occurring within 30 days, as a grade five AE will be reported to Emory IRB as required.

Study Intervention Relationship:
The investigator will determine which events are associated with the use of the study intervention. For reporting purposes, an AE should be regarded as possibly related to the use of the investigational product if the investigator believes:

- There is a clinically plausible time sequence between onset of the AE and graft manipulation; and/or
- There is a biologically plausible mechanism for graft manipulation to cause or contributing to the AE; and
- The AE cannot be attributed solely to concurrent/underlying illness, other drugs, or procedures.

18.2.2 Procedures:

Blood & Marrow Stem Cell Transplant Program Data Management Office personnel shall:

1. Determine the following:
   a. Did the Adverse Event occur within 100 days from the start of transplant conditioning?

b. Institutional location of the Adverse Event.
c. Did death or immediate life-threatening event occur?
d. Did a serious event occur – if so, was it expected?
e. Can the relationship to the study intervention be ruled out?

Based on information obtained, relevant arms of the flow chart may be employed to complete Emory University Adverse Reaction and Unexpected Event Form.
Principal Investigator shall verify adverse reaction/unexpected event by signing Adverse Reaction and Unexpected Event Form.
Completed Adverse Reaction and Unexpected Event Form shall be submitted to the Emory IRB within appropriate time frame as required.

18.2.3 Adverse Event - An adverse event (AE) is any untoward medical experience or change of an existing condition that occurs during or after treatment, whether or not it is considered to be related to the protocol intervention. All AEs occurring during this study, whether observed by the physician, nurse, or reported by the patient, will be graded per NCI CTCAE version 4.0 and recorded on protocol-specific case report forms.

18.2.4 Serious Adverse Event- A serious adverse event (SAE) is defined as *any expected or unexpected adverse event* (AE, generally equivalent to CTCAE grades 3, 4 or 5) that is *related or unrelated* to the intervention that results in any of the following outcomes:

- Death
- A life-threatening event
- In-patient hospitalization (not required as part of the treatment) or
- prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly/birth defect
- Causes cancer
- Is an overdose

Certain medical events that may not result in death, be life-threatening, or require hospitalization, may also be considered a serious adverse event when appropriate medical or surgical intervention is necessary to prevent one of the outcomes listed above.

18.2.5 Unexpected Adverse Event – Any event in which the severity or specificity is not consistent with the risk information described in the protocol, and the event is not anticipated from the subject's disease history or status.

18.2.6 Expected Adverse Event - Any event in which the severity or specificity is consistent with the risk information described in the protocol, or is anticipated based on the subject's medical history.

18.2.7 Attribution - For reporting purposes, attribution is the assessment of the likelihood that an AE is caused by the research agent or protocol intervention. The attribution is assigned by the Principal Investigator after considering the clinical information, the medical history of the subject, and past experience with the research agent/intervention. This is recorded per institutional guidelines. Adverse events in one of 5 categories will be scored as the following: 5=related, 4=probably related, 3=possibly related, 2=unlikely related and 1=unrelated. The attribution is subject to change as follow-up information becomes available, and it can be changed by the DSMB in the process of review.

18.2.8 Outcome of Serious Adverse Events - The outcome of all SAEs will be graded as resolved; recovering; ongoing; resolved with sequela; fatal.

18.3 Clinical Laboratory Tests and Normal Laboratory Values - Throughout the study, clinical laboratory tests will be performed in the clinical laboratory.

18.4 Case Report Forms - Case report forms are designed to contain information necessary for the evaluation of the patient and investigational agent.

18.5 Reporting and Recording of Data - All information required by the protocol is to be recorded on CRFs based on source data. Source data is defined as all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluations of the trial. If source data are unavailable, an explanation should be given. Concomitant Medications will not be collected for this protocol. Grade 1 and 2 toxicities will only be collected if they are related to the study drug.

18.6 Records Retention - The Principal Investigator will maintain all records related to the study as required by regulations and institutional policy.

18.7 Changes to the Protocol - All protocol amendments will be in writing and signed by the Principal Investigator to document his/her agreement. The Principal Investigator will have all protocol amendments will be approved by the Institutional Review Board prior to their implementation

19.0 Removal of patient from study

The investigator should withdraw a patient from the study treatment whenever:

1-Continued participation is no longer in the patient's best interests. Reasons for discontinuing treatment may include the occurrence of a SAE or an inter-current illness. These patients will be taken off treatment and followed according to the study calendar.
2-The patient requests to end treatment.
3-Patients who could not receive all planed Thymo/Bortezomib doses for GVHD prevention will be removed from the study and replaced by another patient.

20.0 Ethics

20.1 Responsibilities of the Principal Investigator (21 CFR Part 312.60)

The Principal Investigator and his/her staff must be available to fulfill the needs of this trial. The Principal Investigator ensures that the proposed trial will not be disturbed by other possible intervening trials. The Principal Investigator must organize the available facilities and technical structures according to the nature of the trial to ensure filing of documents for the duration of the trial as well as after its completion.

In accordance with Title 21 CFR (Part 50), the Principal Investigator commits to obtaining the informed consent of the patient or guardian by signature and clearly indicates the content of the information given to the patient and the means by which consent is obtained.

The Principal Investigator commits him/herself to conform to GCP, 21 CFR Parts 50, 54, 56 and 312 regulations, concerning his/her duties.

For the entire group of persons involved in carrying out the trial (day, night and emergency personnel), the Principal Investigator is responsible for the following:

1. Ensuring that the personnel are informed of the protocol used and that they understand the part they are responsible for implementing.
2. Training personnel if necessary.
3. Designating individual(s) specifically responsible for the administrative management of the trial.
4. Ensuring that other departments or services involved in this trial are informed of the trial and determining with them the specific operating procedures necessary to conduct the trial.

The Principal Investigator is responsible for ensuring that the protocol and its appendices are scrupulously followed, particularly when other departments are involved in the trial. The Principal Investigator oversees the quality of the data collected in the case reports. The data obtained during the trial are recorded directly, with all modifications of data signed, dated, and justified as stated above. Modifications must conform to the procedures defined for paper and electronic records.

20.2 Institutional Review Board/Ethics Committee Approval

Prior to implementation of this study, the research protocol and the proposed patient consent form must be reviewed and approved by the Winship Cancer Institute Protocol Review and Monitoring Committee and the Emory University Institutional Review Board (Emory IRB). All amendments to the protocol must be approved by the Emory IRB.

16.0 Minorities and Women

All eligible patients from both genders and from all racial/ethnic groups will be recruited equally into this trial, with the only exclusionary criteria being those stated in section 6.0.

References:

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2. Ratanatharathorn V, Nash RA, Przepiorka D, et al. Phase III study comparing methotrexate and tacrolimus (prograf, FK506) with methotrexate and cyclosporine for graft-versus-host disease prophylaxis after HLA-identical sibling bone marrow transplantation. *Blood.* Oct 1 1998;92(7):2303-2314.

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APPENDIX A: GVHD GRADING SCALE

Grading of acute GVHD will be based on the following 3 tables and recorded as I,II,III, or IV as defined in table 2. (Modified Glucksberg Index).

Table 1 Extent of organ involvement

Stage	*Skin*	*Liver (bilirubin)*	*Gut (stool output per day)*
0	No GVHD rash	<2 mg/dl	<500 ml/day or persistent nausea (child: <10 ml/kg/day)
1	Maculopapular rash <25% BSA	2–3 mg/dl	500–999 ml/day (child: 10–19.9 ml/kg/day) or persistent nausea, vomiting or anorexia, with a positive upper GI biopsy
2	Maculopapular rash 25–50% BSA	3.1–6 mg/dl	1000–1500 ml/day (child: 20–30 ml/kg/day)
3	Maculopapular rash >50% BSA	6.1–15 mg/dl	Adult: >1500 ml/day (child: >30 ml/kg/day)
4	Generalized erythroderma plus bullous formation	>15 mg/dl	Severe abdominal pain with or without ileus
Grade			
I	Stages 1–2	None	None
II	Stage 3 or	Stage 1 or	Stage 1
III	—	Stages 2–3 or	Stages 2–4
IV	Stage 4 or	Stage 4	—

Abbreviations: BSA = body surface area; GI = gastrointestinal; GVHD = graft-versus-host disease.

Appendix B: Karnofsky Performance Status

DESCRIP	**PERCENT (%)**
Normal; no complaints; no evidence of disease	100
Able to carry on normal activity; minor signs and symptoms of disease	90

Normal activity with effort; some signs and symptoms of disease	80
Cares for self; unable to carry on normal activity or do work	70
Requires occasional assistance, can care for most personal needs	60
Requires considerable assistance and frequent medical care	50
Disabled; requires special care and assistance	40
Severely disabled; hospitalization indicated although death not imminent	30
Very sick; hospitalization necessary; requires active support treatment	20
Moribund; fatal processes progressing rapidly	10
Dead	0

APPENDIX C: SUGGESTED CRITERIA FOR DETERMINING THE RELATIONSHIP OF ADVERSE EVENTS TO STUDY DRUG

All AEs will be recorded on CRFs with the date of occurrence and disappearance. Their relationship to the study drug and their intensity can be rated according to the following guidelines*:

Unrelated

This category applies to those adverse experiences that, after careful consideration are clearly and incontrovertibly due to extraneous causes (disease, environment, etc.)

Unlikely (two or more)

An AE may be considered “unlikely” if or when two of the following apply:

1. It does not follow a reasonable temporal sequence from administration of the drug.
2. It could readily have been produced by the patient’s clinical state, environmental or toxic factors, or other modes of therapy administered to the patient.
3. It does not follow a known or expected response pattern to the test drug.
4. It does not reappear or worsen when the drug is re-administered.

Possibly (two or more)

An AE may be considered “possible” if or when two of the following apply:

1. It follows a reasonable temporal sequence from administration of the drug.
2. It could readily have been produced by the patient’s clinical state, environmental or toxic factors, or other modes of therapy administered to the patient.
3. It follows a known response pattern to the suspected drug.

Probably (three or more)

An AE may be considered “probable” if or when three of the following apply:

1. It follows a reasonable temporal sequence from administration of the drug.
2. It could not be reasonably explained by the known characteristics of the patient’s clinical state, environmental or toxic factors, or other modes of therapy administered to the patient.
3. It disappears or decreases on cessation or reduction in dose. There are important exceptions when the AE does not disappear upon discontinuation of the drug, yet drug-relatedness clearly exists.
4. It follows a known response pattern to the suspected drug.

Definitely (all four)
An AE may be assigned an attribution of “definite” if or when all four of the following apply:

1. It follows a reasonable temporal sequence from administration of the drug.
2. It could not be reasonably explained by the known characteristics of the patient’s clinical state, environmental or toxic factors, or other modes of therapy administered to the patient.
3. It disappears on cessation or reduction in dose and recurs with reintroduction or increase in dose of the test drug. This is not to be construed as requiring reintroduction of the drug, however, “definite” attribution cannot be made unless the drug has been reintroduced and the adverse reaction has recurred.
4. It follows a known response pattern to the suspected drug.

*These suggested criteria are intended to aid the clinician in the casuality assessment process. The physician should exercise his/her clinical judgment in assessing final causality to a suspected agent.

APPENDIX D: DECLARATION OF HELSINKI

II. WORLD MEDICAL ASSOCIATION

DECLARATION OF HELSINKI

Recommendation guiding physicians
in biomedical research involving human subjects

Adopted by the 18th World Medical Assembly
Helsinki, Finland, June 1964

and amended by the
29th World Medical Assembly, Tokyo, Japan, October 1975
35th World Medical Assembly, Venice, Italy, October 1983
and the 41st World Medical Assembly, Hong Kong, September 1989

Introduction

It is the mission of the physician to safeguard the health of the people. His or her knowledge and conscience are dedicated to the fulfillment of this mission.

The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."

The purpose of biomedical research involving human subjects must be to improve diagnostic, therapeutic and prophylactic procedures and the understanding of the aetiology and pathogenesis of disease.

In current medical practice most diagnostic, therapeutic or prophylactic procedures involve hazards. This applies especially to biomedical research.

Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

In the field of biomedical research a fundamental distinction must be recognized between medical research in which the aim is essentially diagnostic or therapeutic for a patient, and medical research, the essential object of which is purely scientific and without implying direct diagnostic or therapeutic value to the person subjected to the research.

Special caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

Because it is essential that the results of laboratory experiments be applied to human beings to further scientific knowledge and to help suffering humanity, the World Medical Association has prepared the following recommendations as a guide to every physician in biomedical research

involving human subjects. They should be kept under review in the future. It must be stressed that the standards as drafted are only a guide to physicians all over the world. Physicians are not relieved from criminal, civil and ethical responsibilities under the laws of their own countries.

1. Basic principles

Biomedical research involving human subjects must conform to generally accepted scientific principles and should be based on adequately performed laboratory and animal experimentation and on a thorough knowledge of the scientific literature.

The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol which should be transmitted for consideration, comment and guidance to a specially appointed committee independent of the investigator and the sponsor provided that this independent committee is in conformity with the laws and regulations of the country in which the research experiment is performed.

Biomedical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given his or her consent.

Biomedical research involving human subjects cannot legitimately be carried out unless the importance of the objective is in proportion to the inherent risk to the subject.

Every biomedical research project involving human subjects should be preceded by careful assessment of predictable risks in comparison with foreseeable benefits to the subject or to others. Concern for the interests of the subject must always prevail over the interests of science and society.

The right of the research subject to safeguard his or her integrity must always be respected. Every precaution should be taken to respect the privacy of the subject and to minimize the impact of the trial on the subject's physical and mental integrity and on the personality of the subject.

Physicians should abstain from engaging in research projects involving human subjects unless they are satisfied that the hazards involved are believed to be predictable. Physicians should cease any investigation if the hazards are found to outweigh the potential benefits.

In publication of the results of his or her research, the physician is obliged to preserve the accuracy of the results. Reports of experimentation not in accordance with the principles laid down in this Declaration should be not accepted for publication.

In any research on human beings, each potential subject must be adequately informed of the aims, methods, anticipated benefits and potential hazards of the trial and the discomfort it

may entail. He or she should be informed that he or she is at liberty to abstain from participation in the trial and that he or she is free to withdraw his or her consent to participation at any time. The physician should then obtain the subject's freely-given informed consent, preferably in writing.

When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship to him or her or may consent under duress. In that case the informed consent should be obtained by a physician who is not engaged in the investigation and who is completely independent of this official relationship.

In case of legal incompetence, informed consent should be obtained from the legal guardian in accordance with national legislation. Where physical or mental incapacity makes it impossible to obtain informed consent, or when the subject is a minor, permission from the responsible relative replaces that of the subject in accordance with national legislation.

> Whenever the minor child is in fact able to give a consent, the minor's consent must be obtained in addition to the consent of the minor's legal guardian.

The research protocol should always contain a statement of the ethical considerations involved and should indicate that the principles enunciated in the present Declaration are complied with.

2. Medical research combined with professional care (clinical research)

In the treatment of the sick person, the physician must be free to use a new diagnostic and therapeutic measure, if in his or her judgement it offers hope of saving life, re-establishing health or alleviating suffering.

The potential benefits, hazards and discomfort of a new method should be weighed against the advantages of the best current diagnostic and therapeutic methods.

In any medical trial, every patient – including those of a control group, if any – should be assured of the best proven diagnostic and therapeutic method.

The refusal of the patient to participate in a trial must never interfere with the physician-patient relationship.

If the physician considers it essential not to obtain informed consent, the specific reasons for this proposal should be stated in the experimental protocol for transmission to the independent committee (1.2)

The physician can combine medical research with professional care, the objective being the acquisition of new medical knowledge, only to the extent that medical research is justified by its potential diagnostic or therapeutic value for the patient.

3. Non-therapeutic biomedical research involving human subjects (non-biomedical research)

In the purely scientific application of medical research carried out on a human being, it is the duty of the physician to remain the protector of the life and health of that person on whom biomedical research is being carried out.

The subjects should be volunteers – either healthy persons or patients for whom the experimental design is not related to the patient's illness.

The investigator or the investigating team should discontinue the research if in his/her or their judgment it may, if continued, be harmful to the individual.

In research on man, the interest of science and society should never take precedence over considerations related to the well-being of the subject

APPENDIX E: KARNOFSKY / ECOG PERFORMANCE STATUS SCALE

Performance Status Scale

Karnofsky	Definition	ECOG
100	Asymptomatic	0
90	Able to carry on normal activity; minor signs or symptoms of disease	
80	Normal activity with effort; some signs or symptoms of disease	
70	Cares for self; unable to carry on normal activity or to do active work	1
60	Requires occasional care for most needs	
50	Requires considerable assistance and frequent medical care	2
40	Disabled; requires special care and assistance	
30	Severely disabled; hospitalization is indicated though death in not imminent	3
20	Very sick; hospitalization necessary; active supportive treatment is necessary	4
10	Moribund, fatal processes progressing rapidly	
0	Dead	

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APPENDIX F: ASSESSMENT OF LYMPHOMA BY INTEGRATED IWC+PET

Assessment of Lymphoma by Integrated IWC+PET

Table 1. IWC+PET-Based Response Designations Based on the IWC Designations and PET Findings

IWC+PET-Based Response Designations	Description
CR	CR by IWC with a completely negative PET
	CRu, PR, or SD by IWC with a completely negative PET and a negative BMB if positive prior to therapy
	PD by IWC with a completely negative PET and CT abnormalities (new lesion, increasing size of previous lesion) ≥ 1.5 cm (≥ 1.0 cm in the lungs) and negative BMB if positive prior to therapy
CRu	CRu by IWC with a completely negative PET but with an indeterminate BMB
PR	CR, CRu, or PR by IWC with a positive PET at the site of a previously involved node/nodal mass
	CR, CRu, PR, or SD by IWC with a positive PET outside the site of a previously involved node/nodal mass
	SD by IWC with a positive PET at the site of a previously involved node/nodal mass that regressed to < 1.5 cm if previously > 1.5 cm, or < 1 cm if previously 1.1-1.5 cm
SD	SD by IWC with a positive PET at the site of a previously involved node/nodal mass (ie, residual mass)
PD	PD by IWC with a positive PET finding corresponding to the CT abnormality (new lesion, increasing size of previous lesion)
	PD by IWC with a negative PET and a CT abnormality (new lesion, increasing size of previous lesion) of < 1.5 cm (< 1.0 cm in the lungs)

Abbreviations: IWC+PET, International Workshop Criteria plus positron emission tomography; CR, complete response; BMB, bone marrow biopsy; CT, computed tomography; CRu, unconfirmed complete response; PR, partial response; SD, stable disease; PD, progressive disease.

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APPENDIX G: Chronic GVHD NIH Consensus Project

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
PERFORMANCE SCORE: ☐ **KPS ECOG LPS**	☐ Asymptomatic and fully active (ECOG 0; KPS or LPS 100%)	☐ Symptomatic, fully ambulatory, restricted only in physically strenuous activity (ECOG 1, KPS or LPS 80-90%)	☐ Symptomatic, ambulatory, capable of self-care, >50% of waking hours out of bed (ECOG 2, KPS or LPS 60-70%)	☐ Symptomatic, limited self-care, >50% of waking hours in bed (ECOG 3-4, KPS or LPS <60%)
SKIN *Clinical features:* ☐ Maculopapular rash ☐ Lichen planus-like features ☐ Papulosquamous lesions or ichthyosis ☐ Hyperpigmentation ☐ Hypopigmentation ☐ Keratosis pilaris ☐ Erythema ☐ Erythroderma ☐ Poikiloderma ☐ Sclerotic features ☐ Pruritus ☐ Hair involvement ☐ Nail involvement **% BSA involved** ☐	☐ No Symptoms	☐ <18% BSA with disease signs but **NO** sclerotic features	☐ 19-50% BSA **OR** involvement with superficial sclerotic features "not hidebound" (able to pinch)	☐ >50% BSA **OR** deep sclerotic features "hidebound" (unable to pinch) **OR** impaired mobility, ulceration or severe pruritus
MOUTH	☐ No symptoms	☐ Mild symptoms with disease signs but not limiting oral intake significantly	☐ Moderate symptoms with disease signs with partial limitation of oral intake	☐ Severe symptoms with disease signs on examination with major limitation of oral intake
EYES Mean tear test (mm): ☐ >10 ☐ 6-10 ☐ ≤5 ☐ Not done	☐ No symptoms	☐ Mild dry eye symptoms not affecting ADL (requiring eyedrops ≤ 3 x per day) **OR** asymptomatic signs of keratoconjunctivitis sicca	☐ Moderate dry eye symptoms partially affecting ADL (requiring drops > 3 x per day or punctal plugs), **WITHOUT** vision impairment	☐ Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision caused by keratoconjunctivitis sicca
GI TRACT	☐ No symptoms	☐ Symptoms such as dysphagia, anorexia, nausea, vomiting, abdominal pain or diarrhea without significant weight loss (<5%)	☐ Symptoms associated with mild to moderate weight loss (5-15%)	☐ Symptoms associated with significant weight loss >15%, requires nutritional supplement for most calorie needs **OR** esophageal dilation
LIVER	☐ Normal LFT	☐ Elevated Bilirubin, AP*, AST or ALT <2 x ULN	☐ Bilirubin >3 mg/dl or Bilirubin, enzymes 2-5 x ULN	☐ Bilirubin or enzymes > 5 x ULN

Figure 1. Organ scoring of chronic GVHD. *AP may be elevated in growing children, and not reflective of liver dysfunction. †Pulmonary scoring should be performed using both the symptom and pulmonary function testing (PFT) scale whenever possible. When discrepancy exists between pulmonary symptom or PFT scores the higher value should be used for final scoring. Scoring using the Lung Function Score (LFS)