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A PHASE II STUDY OF HIGH-DOSE POST-TRANSPLANT CYCLOPHOSPHAMIDE AND BORTEZOMIB (PTCYBOR) FOR THE PREVENTION OF GRAFT-VERSUS-HOST DISEASE (GVHD) FOLLOWING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)

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Study Product:	cyclophosphamide and bortezomib
Study Product Provider:	; cyclophosphamide and bortezomib covered as standard of care.
ClinicalTrials.gov Number	Pending

Initial version: [July 2018]

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Amended (1.0): April 26, 2019

Amended (1.1): August 28, 2019

Statement of Compliance

This study will be conducted in accordance with the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46), 21 CFR Parts 50, 56, 312, and 812 as applicable, any other applicable US government research regulations, and institutional research policies and procedures. The International Conference on Harmonisation ("ICH") Guideline for Good Clinical Practice ("GCP") (sometimes referred to as "ICH-GCP" or "E6") will be applied only to the extent that it is compatible with FDA and DHHS regulations. The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection Training.

PROTOCOL APPROVAL SIGNATURES

Protocol Title: **A PHASE II STUDY OF HIGH-DOSE POST-TRANSPLANT CYCLOPHOSPHAMIDE AND BORTEZOMIB (PTCYBOR) FOR THE PREVENTION OF GRAFT-VERSUS-HOST DISEASE (GVHD) FOLLOWING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)**

Protocol Number: s18-01185

This study will be conducted in compliance with the clinical study protocol (and amendments), International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines for current Good Clinical Practice, and applicable regulatory requirements.

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Signature

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Date

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List of Abbreviations

ADR	Adverse drug reaction
AE	Adverse Event or Adverse Experience
ALP	Alkaline phosphatase
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
AST	Aspartate Aminotransferase
ATG	Anti-thymocyte globulin
BP	Blood Pressure
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CI	Confidence Interval
CNI	Calcineurin inhibitors
CrCl	Creatinine Clearance
CFR	Code of Federal Regulations
CRF	Case Report Form
CSOC	Clinical Study Oversight Committee
CTO	Clinical Trials Office
DC	Dendritic cell
DSMC	Data Safety Monitoring Committee
ECI	Event of Clinical Interest
GRFS	GvHD and relapse-free survival
GvHD	Graft-versus-Host Disease
GvT	Graft-versus-tumor
HSCT	Hematopoietic stem cell transplantation

LFTs	Liver function tests
LPS	Lipopolysaccharide
MMF	Mycophenolate mofetil
MTX	Methotrexate
OS	Overall survival
PBMC	Peripheral blood mononuclear cells
PI	Proteasome inhibitors
PTCy	Post-Transplant cyclophosphamide
rATG	Rabbit anti-thymocyte globulin
RR	Relapse rate
TRM	Treatment-related mortality

Protocol Summary

Title	A Phase II Study of High-Dose Post-Transplant Cyclophosphamide and Bortezomib (PTCyBor) for the Prevention of Graft-versus-Host Disease (GvHD) Following Allogeneic Hematopoietic Stem Cell Transplantation (HSCT)
Short Title	Cyclophosphamide and bortezomib for GvHD Prevention
Brief Summary	This is a single arm open label phase II clinical trial. Adult patients with hematological malignancies undergoing allogeneic HSCT from matched-related or unrelated donor are eligible for the study if they meet the standard criteria defined in our institutional standard operation procedures (SOPs), meet all inclusion criteria, and do not satisfy any exclusion criteria. Patients will receive reduced-intensity or myeloablative conditioning regimen of fludarabine, busulfan, and rabbit anti-thymocyte globulin (rATG). Patients will receive PTCyBor as GvHD prophylaxis.
Phase	Phase II
Objectives	Primary Objective: • Estimate the incidence of acute GvHD in patients receiving cyclophosphamide and bortezomib as GvHD prophylaxis. • Estimate the incidence of chronic GvHD in patients receiving cyclophosphamide and bortezomib as GvHD prophylaxis Secondary Objectives: • Estimate the incidence of the following: primary graft failure, poor graft function, and secondary graft failure, treatment-related mortality (TRM), relapse rate (RR), GvHD and relapse-free survival (GRFS), and overall survival (OS). • Assess immune reconstitution by quantifying CD3+, CD4+, CD8+, and CD19+ in comparison to established reference data
Methodology	Interventional, non-randomized open label.
Endpoint	Primary: Acute GvHD by day +120 and chronic GvHD by day +365. Secondary: TRM, RR, GRFS, and OS.
Study Duration	Study will complete 2 years after the final participant's date of transplant. Anticipate 3 year to complete enrollment and thus a 5-year total duration.
Participant Duration	Up to 2 years

Enrollment Period	Up to 3 years
Duration of IP administration	Up to 1 week
Study Center and Site	NYU Langone Health
Number of participants	47 participants enrolled
Description of Study Agent/Procedure	Bortezomib 1.3 mg/m^2 IV 6 hours after graft infusion and 72 hours thereafter. Cyclophosphamide 50 mg/kg IV over 2 hours on Day +3 and +4
Key Procedures	Examinations to include toxicity and GvHD assessments and blood draws to assess immune reconstitution.
Statistical Analysis	Sample size considerations: The primary outcome variables for this study include the (1) incidence of acute GvHD by day +120 post-transplant and (2) incidence of chronic GvHD by day +365. If we assume that the rate of acute GvHD grades II-IV with standard of care is 50% as reported in the literature, we can detect a reduction in acute GvHD incidence to 30% for PTCyBor, with alpha = 0.05 (2 sided) and power of 80% with 47 patients using a z test for the test of the observed percentage compared to an expected percentage of 50%. With 47 patients, the observed 95% confidence interval will 35.1% to 64.9% if the observed rate is 50%; if the observed rate is 30%, the corresponding confidence interval will be 17.5% to 45.1%. The primary analyses will include all patients who are transplanted and receive treatment. All primary endpoints will be evaluated using cumulative incidence functions; exact 95% confidence intervals will be provided for the day +120 incidence for acute GvHD and the day +365 incidence for chronic GvHD. Secondary analyses will estimate the occurrence of primary graft failure, poor graft function, secondary graft failure, TRM, RR, GRFS, and OS.

Schematic of Study Design

1. KEY ROLES

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2. BACKGROUND AND RATIONALE

Allogeneic hematopoietic stem cell transplantation (HSCT) remains the only curative therapy for a variety of benign and malignant hematological conditions.[1] According to the Center for International Bone Marrow Transplant Research (CIBMTR), more than 13,000 allogeneic HSCTs are performed annually in the US alone (http://www.cibmtr.org). However, despite the routine use of prophylactic immunosuppression, graft-versus-host disease (GvHD) continues to represent a frequent cause of morbidity and mortality of allogeneic HSCT.[2-4] Furthermore, GvHD impairs the quality of life of patients undergoing allogeneic HSCT and hampers the utility and wide applicability of this treatment strategy. Therefore, innovative approaches to the prevention of GvHD are warranted.

Current GvHD prophylactic regimens are based on different combinations of methotrexate (MTX), mycophenolate mofetil (MMF), calcineurin inhibitors (CNI), mTOR inhibitors, and anti-thymocyte globulin (ATG). In addition to their partial efficacy, the indiscriminate activity of these drugs against T cells results in delayed immune reconstitution and impaired graft-versus-tumor (GvT) effect, thus increasing the incidence of disease relapse.[5] Furthermore, these agents are toxic and burdensome due to their common pharmacological interactions and the need for blood level monitoring and strict patient adherence.

Post-Transplant Cyclophosphamide (PTCy) for GvHD Prevention:

PTCy has recently been introduced for the prevention of GvHD. Contrary to the previously listed agents, PTCy selectively destroys rapidly proliferating host-reactive T cells, fosters regulatory T cells (T_{reg}) expansion, and induces long-lasting tolerance by deleting intra-thymic alloreactive T cells.[6,7] Because of its selective activity, targeting proliferating rather than resting T cells, PTCy has been claimed to nurture rapid immune reconstitution and preserve GvT effect.[8] Originally introduced to circumvent *ex vivo* T cell depletion following haploidentical HSCT, PTCy has now been studied alone in the setting of matched-related and unrelated donor transplants.[9] A recent multi-institutional trial of myeloablative and bone marrow allogeneic HSCT reported an incidence of grades II-IV and grades III-IV acute GvHD of 50% and 15%, respectively. These rates were similar to the rates achieved with CNI-based regimens. On the other hand the incidence of chronic GvHD was more favorable at 14%. Importantly, the prophylactic treatment was complete by day +4 and was well tolerated.[9] These findings were corroborated by several similar studies (reviewed in 10). However, when Alousi et al. tested PTCy alone as GvHD prophylaxis following reduced-intensity peripheral blood (as opposed to bone marrow) allogeneic HSCT, the authors reported excessive incidence of acute GvHD and decreased overall survival (OS) in comparison to a contemporaneous institutional control group.[11] This has led a number of investigators to examine the combination of PTCy with conventional agents.[12-13] The Blood and Marrow Transplant Clinical Trial Network (BMT CTN) recently completed a phase II multicenter trial (PROGRESS I) that randomly assigned patients to 1 of 3 GvHD prophylactic regimens: MTX, tacrolimus, and maraviroc, MTX, tacrolimus, and bortezomib, or MMF, tacrolimus, and PTCy. All patients received reduced-intensity peripheral blood allogeneic HSCT. Each regimen was compared to a nonrandomized prospective contemporaneous control cohort receiving MTX and tacrolimus as GvHD prophylaxis. The cumulative incidence of grade II-IV acute GvHD was not different for each arm versus the control cohort. However, grade III-IV acute GvHD and chronic GHD requiring systemic immunosuppressive therapy were lower in the MMF, tacrolimus, and PTCy arm in comparison to the control cohort. GvHD-free survival was also superior.[14]

Proteasome Inhibitors for GvHD Prevention:

Dendritic cells (DC) play a pivotal role in the pathophysiology of GvHD.[15] While recipient DC are necessary and sufficient for the development of GvHD, donor DC are only important for maximal GvHD.[16] Boeck et al. studied the kinetics of DC engraftment after allogeneic HSCT and demonstrated that the majority of patients lose recipient DC and achieve full donor DC chimerism at engraftment.[17] Consequently, targeting DCs early after allogeneic HSCT seems to represent a sound strategy to prevent GvHD.

Bortezomib, the first-in-class proteasome inhibitors (PI), suppresses DC maturation and function and possesses a variety of immune-modulatory effects.[18-20] Several investigators have shown that bortezomib decreases lipopolysaccharide (LPS)-induced expression of maturation and co-stimulatory markers on the surface of DC generated from the peripheral blood mononuclear cells (PBMNC) of volunteer donors in presence of GM-CSF and IL-4.[18-20] These surface molecules include CD40 ligand, chemokine receptor CCR7, CD54, CD80, CD83, CD86, and DC-SIGN. We have shown similar effect on naïve DCs isolated from the peripheral blood of volunteer donors using a magnetic beads-based selection procedure.[21] Bortezomib also reduces the production of IL-1β, IL-6, IL-23, Il-12p70, and TNF-α of unstimulated DCs.[18-20] *In vivo*, we demonstrated that DCs isolated from the peripheral blood of patients receiving a combination of PTC and bortezomib exhibit impaired LPS-mediated maturation and that T cell proliferation in response to the recipient's irradiated DCs is suppressed. Although the treatment was completed by day +4, the drug effects remained notable on day +21.[22]

In animal studies, bortezomib has been shown to decrease the incidence and severity of GvHD in a major histocompatibility complex (MHC)-mismatched transplantation murine model. The effect was due, at least in part, to DC inhibition.[18-20] Notably, despite this beneficial effect of bortezomib administered early post-transplant, delayed administration of bortezomib was associated with paradoxical worsening of GvHD-associated intestinal injury and sudden death of animals.[23] This phenomenon was attributed to increased serum levels of IL-1β and downstream, TNF-α and INF-γ.[23] Using bioluminescence imaging, we demonstrated that the sudden deterioration in mice condition after delayed proteasome inhibition was associated with rapid accumulation of donor T cells in target organs and suggested that the phenomenon can be prevented by the addition of a lymphocyte depleting agent such as cyclophosphamide (see below).[24-26]

In humans, Koreth el al. conducted a phase I-II study in mismatched-unrelated donor allogenic HSCT. GvHD prophylaxis consisted of a short course of bortezomib (days +1, +4, +7) in addition to MTX and tacrolimus. The incidence of grade II-IV and III-IV acute GvHD in this high-risk group was 22% and 7%, respectively. The incidence of chronic GvHD was 29%. Immune reconstitution was prompt.[27] The same group recently reported the results of a randomized phase II study comparing 3 GvHD prevention regimens (MTX and tacrolimus, MTX, tacrolimus, and bortezomib, and MTX, tacrolimus, and sirolimus) in matched and mismatched-unrelated donor allogeneic HSCT. The incidence of acute and chronic GvHD was similar in the 3 groups. However, a potential benefit of bortezomib was noted in patients receiving matched grafts.[28]

PTCy and proteasome inhibitors in GvHD prevention:

As stated above, calcineurin and mTOR inhibitors have an unfavorable toxicity profile and are burdensome. They are typically administered for 6-9 months following allogeneic HSCT. We adopted a different approach to prevent GvHD using a short course of PTCy and bortezomib. We hypothesized that such combination is safe and effective and omits the need for calcineurin and mTOR inhibitors including in peripheral blood allogeneic HSCT.

Preclinical data. To examine the *in vivo* effect on the combination of PTCy and proteasome inhibitors we performed a series of experiments in a GvHD mouse model. First, we established that ixazomib, a proteasome inhibitor,[29] has similar *in vitro* effects to bortezomib in term of effects on DC maturation and cytokine production.[26] Additionally, in a MHC-mismatch mouse model, ixazomib ameliorated GvHD in a schedule dependent fashion mirroring the effects of bortezomib. Second, we aimed to determine the effectiveness of PTC and ixazomib in mice. Ten-week old BALB/c mice were irradiated (10 grays in two fractions) on day -1 and transplanted with $5x10^6$ BM cells from C57BL6 mice. GvH inocula consisted of $5x10^6$ splenocytes from C57BL6 (some post-CFSE labeling) or B6;FVB-Ptprca Tg(CAG-Luc,-GFP)L2G85Chco Thy1a/J (ubiquitously transgenic for firefly luciferase for bioluminescence imaging (BLI). All mice were kept in a pathogen-free environment. Mice were untreated or received cyclophosphamide (1 mg intraperitoneally) on day +2, ixazomib (30µg, subcutaneously) on days -1, +2, and +5, or both according to the same schedule. Mice were monitored for weight, GvHD score, and survival or were sacrificed on day +6 for cytokine measurement and splenic cell counts. For BLI, mice receiving transgenic splenocytes were injected with 150mg/kg of D-Luciferin intraperitoneally and were imaged 25 minutes later. GvHD worsening and sudden death occurred in a fraction of mice receiving ixazomib following a day +5 injection. The phenomenon was completely prevented when cyclophosphamide was administered. Importantly, the group receiving both cyclophosphamide and ixazomib had significantly improved weight, GvHD score, and survival when compared to the mice left untreated or receiving either drug alone (figure 1). The addition of cyclophosphamide to ixazomib prevented IL-1β increase, was associated with suppression of tumor necrosis factor α (TNFα), and resulted in a profound depletion of splenic total and donor CD4+ cells. Furthermore, proliferating cells (CFSE low) were preferentially depleted as opposed to quiescent cells (CFSE high). BLI imaging showed an increase in signal intensity shortly after the administration of ixazomib on day +5. On the other hand, the mice receiving cyclophosphamide in addition to ixazomib exhibited significantly lower donor T cell proliferation when compared to the other groups (figure 2).[25]

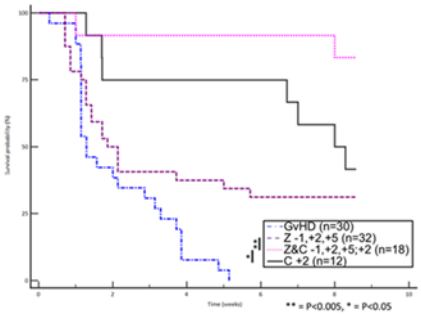


Figure 1

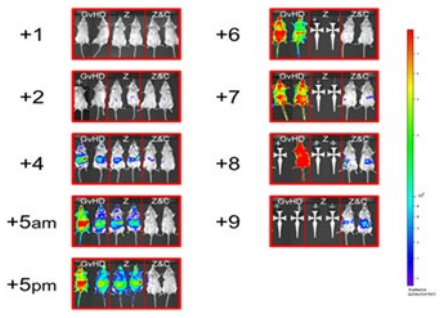


Figure 2

In summary, our preclinical data suggests that the combination of cyclophosphamide and proteasome inhibition suppresses cytokine production *in vivo* and reduces splenic total and donor CD4+ cell expansion with preferential depletion of proliferating cells, as opposed to quiescent cells and prevents the phenomenon of GvHD acceleration associated with delayed and prolonged proteasome inhibition. We also demonstrated that the combination results in improved survival of mice subjected to GvHD when compared to the control group and the groups receiving either drug alone.

Clinical data. We performed a phase I dose escalation study with an expansion cohort in patients undergoing matched-related or unrelated donor allogeneic HSCT with reduced-intensity conditioning and peripheral blood allogeneic HSCT. Patients received a fixed dose of PTCy (days +3 and +4) and an increasing dose of bortezomib in 3 cohorts, from 0.7 to 1 and to 1.3 mg/m2 administered 6 hours after graft infusion and 72 hours thereafter during the phase I. The study was then extended at the higher dose. Overall, 28 patients were enrolled. Patients receiving grafts from unrelated donors also received rabbit ATG (rATG). No graft failure and no unexpected grade ≥3 non-hematological toxicities were encountered. The median times to neutrophil and platelet engraftment were 16 and 27 days, respectively. Day +100 treatment-related mortality (TRM) was 3.6% (95% CI 0.2%-15.7%). The cumulative incidences of grade II-IV and grade III-IV acute GvHD were 35.9% (95% CI 18.6%-53.6 %) and 11.7% (95% CI 2.8%-27.5%), respectively. The incidence of chronic GvHD was 27% (95% CI 11.4%-45.3%). The progression-free survival (PFS), OS, and GvHD & relapse-free survival (GRFS) rates were 50% (95% CI 30.6%-66.6%), 50.8% (95% CI 30.1%-68.2%), and 37.7% (95% CI 20.1%-55.3%), respectively. Immune reconstitution, measured by CD3, CD4, and CD8 recovery was prompt. Notably, there was no negative impact on renal function. Although three patients had decreased glomerular filtration rates (GFR) at enrollment, all patients had normal GFR at last follow-up. The study suggested that the combination of PTCy and bortezomib (PTCyBor) for the prevention of GvHD is feasible and safe and yields promising results warranting further examination.[30,31]

rATG for GvHD Prevention:
The use of rATG following allogeneic HSCT has been extensively studied in systematic reviews and in randomized clinical trials.[30-33] The incidence of acute and chronic GvHD was reduced without a concomitant in the relapse rate, in all studies except for one retrospective study by the CIBMTR. In the latter study, rATG had a negative impact on both disease-free and OS in patients receiving reduced-intensity conditioning from matched siblings or mismatched unrelated.[34] However, the median dose of rATG was 7 mg/kg and the impact of timing of administration was not considered. More recently, a study by Devellier at al. analyzed patients receiving a reduced-intensity conditioning regimen with 2.5 or 5 mg/kg rATG prior to matched sibling donor allogeneic HSCT. The study showed lower rates of acute and chronic GvHD in the group receiving 5 mg/kg, without a negative impact on relapse; thus suggesting that there is a dose-dependent balance between acute and chronic GvHD prevention and long-term anti-tumor effects.[35]

Based on the above, we propose a phase II study using PTC and bortezomib for the prevention of GvHD following allogeneic HSCT in patient with hematological malignancies. Patients will also receive rATG.

2.1. Potential Risks & Benefits

2.1.1. Known Potential Risks

2.1.1.1. Cyclophosphamide

The most common side effects of cyclophosphamide (Cytoxan®):
Hematopoietic system: Neutropenia occurs in patients treated with cyclophosphamide. The degree of neutropenia is particularly important because it correlates with a reduction in resistance to infections. Fever without documented infection has been reported in neutropenic patients.
Gastrointestinal system: Nausea and vomiting occur with cyclophosphamide therapy. Anorexia and, less frequently, abdominal discomfort or pain and diarrhea may occur. There are isolated reports of hemorrhagic colitis, oral mucosal ulceration and jaundice occurring during therapy.
Skin and its structures: Alopecia occurs in patients treated with cyclophosphamide. Skin rash occurs occasionally in patients receiving the drug. Pigmentation of the skin and changes in nails can occur.

2.1.1.2. Bortezomib

The most common side effects of bortezomib (Velcade®):
Most commonly reported (incidence ≥ 20%) in clinical studies include nausea, diarrhea, thrombocytopenia, neutropenia, peripheral neuropathy, fatigue, neuralgia, anemia, leukopenia, constipation, vomiting, lymphopenia, rash, pyrexia, and anorexia.

2.1.2. Other risks of study participation

Additional risks associated with participation in this study, include risks associated with phlebotomy and breach of confidentiality. Risks associated with phlebotomy include weaknesses, redness, pain, bruising, bleeding, or infection at the needle site. Privacy protection procedures are in place and good clinical practice guidelines are followed to minimize the risks associated with research procedures and participation.

2.1.3. Known Potential Benefits

It is possible that some study subjects receiving therapies may experience an improvement during the study. Also, some subjects may not get any benefit from being in this research study. The information learned in this study may benefit future individuals with acute and chronic GvHD.

3. OBJECTIVES AND PURPOSE

STUDY DESIGN:

3.1. Study hypothesis: We hypothesize that the administration of cyclophosphamide and a short-course of bortezomib in the setting of matched-related or unrelated donor allogeneic HSCT peripheral blood transplantation is effective in the prevention of GvHD and allows prompt immune reconstitution.

3.2. Study overview: This is a phase II clinical trial. Adult patients with hematological malignancies undergoing allogeneic HSCT from matched-related or unrelated donor are eligible for the study if they meet the standard criteria defined in our standard operation procedures (SOPs), meet all inclusion criteria, and do not satisfy any exclusion criteria. Patients will receive a reduced-intensity or myeloablative conditioning regimen with fludarabine, busulfan, and rATG. Patients will receive PTCyBor as GvHD prophylaxis (Appendix B).

3.3. Objectives

3.3.1. Primary objectives:

- Estimate the incidence of acute GvHD in patients receiving cyclophosphamide and bortezomib as GvHD prophylaxis.
- Estimate the incidence of chronic GvHD in patients receiving cyclophosphamide and bortezomib as GvHD prophylaxis

3.3.2. Secondary objectives:

- Estimate the incidence of the following: primary graft failure, poor graft function, and secondary graft failure, TRM, RR, GRFS, OS.
- Assess immune reconstitution by quantifying CD3+, CD4+, CD8+, and CD19+ in comparison to established reference data.

3.4. Study Endpoints

3.4.1. Primary study endpoints

Acute GvHD: The first day of acute GvHD of any grade will be used to calculate the cumulative incidence for that grade. This end point will be evaluated through day +120 post-transplant.

Chronic GvHD: The first day of chronic GvHD will be used to calculate the cumulative incidence of chronic GvHD. This end point will be evaluated through day +365 post-transplant.

3.4.2. Secondary study endpoints

Primary graft failure is evaluated to day +45 and as defined below.

Poor graft function is evaluated to day +30 and as defined below.

Secondary graft failure is evaluated following engraftment through day +730, as defined below.

TRM are participant deaths not attributable to disease relapse or progression and will be evaluated to day +730.

RR is evaluated to day +730 and is considered the date in which the disease for which transplant is performed is evident by methods of disease detection.

GRFS is evaluated to day +730 and considers the number of participants that are without reported grade III-IV acute GvHD, chronic GvHD requiring systemic therapy and have not experienced relapse or death.

OS is evaluated to day +730 and considers all participants alive at the end of the study's evaluation period.

3.5. Definitions

3.5.1. **Engraftment of Neutrophils:** Absolute neutrophil count (ANC) recovery is defined as an ANC of ≥ 0.5 x 10^9/L for three consecutive laboratory values obtained on different days. The day used as neutrophil engraftment is the date of the first of three laboratory values.

3.5.2. **Engraftment of Platelets:** Platelet engraftment is defined as a platelet count ≥ 20 x 10^9/L for 3 consecutive measurements obtained on different days. The patient must not have received a platelet infusion for seven consecutive days prior to the first day being considered. The day used as platelet engraftment is the date of the first of three laboratory values.

3.5.3. **Primary graft Failure:** Graft failure is defined as failure to achieve neutrophil engraftment by day +28 or lack of donor chimerism > 50% by day 45 not due to the underlying malignancy.

3.5.4. **Poor graft function** is defined by at least 2 of the following 3 criteria: Hemoglobin < 8 g/dL, ANC < 0.5 10^9/L, and platelets < 20 10^9/L. The cytopenia must be unexplained (such as by disease relapse) and unresponsive to cytokines and must last at least 4 weeks.

3.5.5. **Secondary graft failure** is defined as poor graft function associated with donor chimerism < 5%.

3.5.6. **Acute GvHD**: The first day of acute GvHD of any grade will be used to calculate the cumulative incidence for that grade (e.g., onset of grade III 70 days post-transplant - time to grade III is 70 days). This end point will be evaluated through day +120 post-transplant. The diagnosis of acute GvHD is based on clinical and pathological evaluation by the principal investigator in collaboration with the treating physician. The acute GvHD grading system is described in appendix B.

3.5.7. **Chronic GvHD:** The first day of chronic GvHD will be used to calculate the cumulative incidence of chronic GvHD. The diagnosis of chronic GvHD is based on clinical and pathological evaluation by the principal investigator in collaboration with the treating physician. The chronic GvHD grading system is described in appendix C.

4. STUDY ENROLLMENT AND WITHDRAWAL

ELIGIBILITY: Patients with hematological malignancies undergoing an allogeneic transplant with a fludarabine, busulfan, and rATG conditioning regimen, using an eligible or suitable ≥ 8 out of 8 match (high resolution match at loci A, B, C, and DRB1) related or unrelated donor are eligible to participate. Donors are excluded in case of donor-specific HLA antibodies or positive cross-matching. Patients must meet all of the following inclusion criteria and none of the exclusion criteria.

4.1. Inclusion Criteria

4.1.1. Age ≥ 18 years

4.1.2. Karnofsky score ≥ 70%

4.1.3. No evidence of progressive bacterial, viral, or fungal infection

4.1.4. Creatinine clearance > 50 mL/min/1.72m^2

4.1.5. Total bilirubin, ALT and AST < 2 x the upper limit of normal (except for Gilbert's syndrome)

4.1.6. Alkaline phosphatase ≤ 250 IU/L

4.1.7. Left Ventricular Ejection Fraction (LVEF) > 45%

4.1.8. Adjusted Carbon Monoxide Diffusing Capacity (DLCO) > 60%

4.1.9. Negative HIV serology

4.1.10. Negative pregnancy test: confirmation per negative serum β-human chorionic gonadotropin (β-hCG)

4.2. Exclusion Criteria:

4.2.1. Pregnant or nursing females or women of reproductive capability who are unwilling to completely abstain from heterosexual sex or practice 2 effective methods of contraception from the first dose of bortezomib through 90 days after the last dose. A woman of

reproductive capability is one who has not undergone a hysterectomy (removal of the womb), has not had both ovaries removed, or has not been post-menopausal (stopped menstrual periods) for more than 24 months in a row.

4.2.2. Male subjects who refuse to practice effective barrier contraception during the entire study treatment period and through a minimum of 90 days after the last dose of study drug, or completely abstain from heterosexual intercourse. This must be done even if they are surgically sterilized (i.e., post-vasectomy).

4.2.3. Inability to provide informed consent.

4.2.4. Patient had myocardial infarction within 6 months prior to enrollment or has New York Heart Association (NYHA) Class III or IV heart failure (see Appendix E), 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.

4.2.5. Known allergies to any of the components of the investigational treatment regimen.

4.2.6. Serious medical or psychiatric illness likely to interfere with participation in this clinical study.

4.2.7. Diagnosed or treated for another malignancy within 3 years of enrollment, with the exception of complete resection of basal cell carcinoma or squamous cell carcinoma, an in-situ malignancy, or low-risk prostate cancer after curative therapy.

4.2.8. Participation in clinical trials with other investigational agents not included in this trial, within 14 days of the start of this trial and throughout the duration of this trial.

4.2.9. Prisoners

4.3. Inclusion of Women, Minorities and Vulnerable Subjects

Both men and women of all races and ethnic groups are eligible for this trial.

Vulnerable subjects will not be eligible for enrollment into this study. Pregnant women are not included in this study, due to the unknown safety effects to the developing fetus. Prisoners are not eligible.

4.4. Strategies for Recruitment and Retention

4.4.1. The inclusion and exclusion criteria in this study should not have a negative effect on the enrollment of the desired populations.

4.4.2. Patients will be recruited from physicians participating in this study. Consenting, screening, and treatment will take place at the NYU Langone Health PCC under the supervision of the principal investigator, Dr. Al Homsi. Prospective subjects will receive detailed information regarding this study; its investigational nature, required study procedures, alternative treatments, risks and potential benefits of the study. They will also receive the informed consent document to read. All questions will be answered by the PI or qualified research personnel.

4.4.3. The Principal Investigator will:

1. Obtain signed and dated informed consent from the potential subject before any study specific procedures are performed.
2. Determine patient eligibility; see Section 4.1 and 4.2

3. Submit registration to NYU Langone Health PCC CTO.
4. Receive registration confirmation from the NYU Langone Health PCC CTO, including a unique study identification number assigned to the patient that will be distributed to the study team upon registration of the patient.

4.4.4. Recruitment and consenting will take place in a private area such as an exam room to protect the patient's privacy. The informed consent process and documentation follows that established procedures of the NYU Langone Health PCC CTO.

4.5. Registration Procedures

4.5.1. General Guidelines

Each patient must sign and date an informed consent form before undergoing any study specific procedure unless a procedure is being performed as part of the patient's standard of care.

Enrollment in the study requires that all inclusion and exclusion criteria have been met. Enrollment occurs upon confirmation of registration from the NYULH PCC CTO. The following materials must be submitted to the CTO for subject registration:

1. Complete signed and dated informed consent form
2. Complete signed and dated eligibility checklist
3. All supporting documentation verifying each eligibility criterion has been met

Registration will occur within 48 hours of research coordinator receipt of all of the above documents. A written confirmation of enrollment including a unique study identification number assigned by the research coordinator will be disbursed to the study team upon registration.

Once eligibility is verified, a unique patient study number will be issued within 24 hours of receiving all required registration material. The patient will not be identified by name. This is the point, at which, the patient is considered accrued on study.

4.5.2. Data Reporting to the Center for International Blood and Transplant Research (CIBMTR)

All transplant centers are required to register patients with the Center for International Blood and Marrow Transplant Research (CIBMTR) and complete pre-transplant essential data forms, day 100 report forms and follow-up forms. CIBMTR data is covered under NYULH IRB: S11-00861.

4.6 Duration of Study Participation

Subjects may remain on study for as long as 2 years, with treatment as detailed in the protocol. Withdrawal due to toxicity, withdrawal of consent, or at the discretion of the investigator may occur.

4.7 Total Number of Participants and Sites

There will be one site participating in the study, with a total of 47 patients enrolled, accrued over 3 years.

4.8 Participants Withdrawal or Termination

4.8.1. Reasons for Withdrawal or Termination

A subject has the right to voluntarily discontinue study treatment or withdraw from the study at any time, for any reason, and without repercussion. The investigator has the right to discontinue a patient from study treatment or withdraw a patient from the study at any time.

Reasons for subject withdrawal from the study may include, but are not limited to:

- Subject withdrawal of consent at any time.
- Disease progression (if applicable)
- Intolerable toxicity (If applicable)
- Any medical condition that the investigator determines may jeopardize the patient's safety if s/he continues in the study or continues treatment with study drug.
- The investigator determines it is in the best interest of the patient.
- Failure of the subject to adhere to protocol procedure requirements
- Pregnancy (if applicable)

4.8.2. Handling of Participant Withdrawals or Termination
Withdrawal from the study can be made in writing to the Principal Investigator, Dr. A Samer Al-Homsi.

4.9 Premature Termination or Suspension of Study

This study may be temporarily suspended or prematurely terminated if there is sufficient, reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to Dr. A Samer Al-Homsi and any regulatory authorities. If the study is prematurely terminated or suspended, the PI will promptly inform the IRB and will provide the reason(s) for the termination or suspension.

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

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete or evaluable
- Determination of futility

Study may resume once concerns about safety, protocol compliance, data quality are addressed and satisfy the IRB.

4.10. Stopping for excess TRM

With 47 patients planned, we will test the hypothesis that the TRM rate is ≤ 20% versus the alternative hypothesis that the TRM rate is ≥ 40% with one sided α=0.05 and power of 90%, using an O'Brien Fleming group sequential alpha stopping boundary with three (3) interim and one (1) final look as summarized in Table 1 below.

Table 1: Exact Stopping Boundaries for Excess Transplant Related Mortality (TRM) (1 sided α=0.05, power=80%, Ho: TRM≤0.20, Ha: TRM≥0.40) [Calculations from EAST 6.4 Cytel, Inc.]

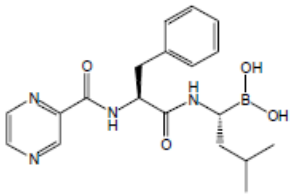
Interim Look	Number of Patients	Cumulative Alpha Spent	Stopping Boundaries: Reject TRM≤0.20	
			≥Number Events	≥Proportion Observed
1	12	0	9	0.75
2	24	0.006	11	0.46
3	35	0.023	13	0.37
Final	47	0.05	15	0.32

5. STUDY AGENTS

5.1. Bortezomib (Velcade®) – Millennium Pharmaceuticals

5.1.1. Therapeutic Class: Proteasome Inhibitor

5.1.2. Description: An antineoplastic agent available for intravenous 6 injection (IV) use only. Each single dose vial contains 3.5 mg of bortezomib as a sterile lyophilized powder. Inactive ingredient: 35 mg mannitol, USP. Bortezomib is a modified dipeptidyl boronic acid. The product is provided as a mannitol boronic ester which, in reconstituted form, consists of the mannitol ester in equilibrium with its hydrolysis product, the monomeric boronic acid. The drug substance exists in its cyclic anhydride form as a trimeric boroxine. The chemical name for bortezomib, the monomeric boronic acid, is [(1R)-3-methyl-1-[[(2S)-1-15 oxo-3-phenyl-2-[(pyrazinylcarbonyl) amino]propyl]amino]butyl] boronic acid. Bortezomib has the following chemical structure:



The molecular weight is 384.24. The molecular formula is C19H25BN4O4. The solubility of bortezomib, as the monomeric boronic acid, in water is 3.3 to 3.8 mg/mL in a pH range of 2 to 6.5.

5.1.3. Indications: Treatment of multiple myeloma patients who have received at least 1 prior therapy.

5.1.4. Mechanism of Action: Bortezomib is a reversible inhibitor of the chymotrypsin-like activity of the 26S proteasome in mammalian cells. The 26S proteasome is a large protein complex that degrades ubiquitinated proteins. The ubiquitin-proteasome pathway plays an essential role in regulating the intracellular concentration of specific proteins, thereby maintaining homeostasis within cells. Inhibition of the 26S proteasome prevents this targeted proteolysis, which can affect multiple signaling cascades within the cell. This disruption of normal homeostatic mechanisms can lead to cell death. Experiments have demonstrated that bortezomib is cytotoxic to a variety of cancer cell types in vitro. Bortezomib causes a delay in tumor growth in vivo in nonclinical tumor models, including multiple myeloma.

5.1.5. Pharmacokinetics: Following intravenous administration of a 1.3 mg/m2 dose, the median estimated maximum plasma concentration of bortezomib was 509 ng/mL (range=109 to 1300 ng/mL) in 8 patients with multiple myeloma and creatinine clearance values ranging from 31 to 169 mL/min. The mean elimination half-life of bortezomib after first dose ranged from 9 to 15 hours at doses ranging from 1.45 to 2.00 mg/m2 in patients with advanced malignancies. The pharmacokinetics of bortezomib as a single agent have not been fully characterized at the recommended dose in multiple myeloma patients.

5.1.5.1. Absorption and Distribution: The distribution volume of bortezomib as a single agent was not assessed at the recommended dose in patients with multiple myeloma. The binding of bortezomib to human plasma proteins averaged 83% over the concentration range of 100 to 1000 ng/mL.

5.1.5.2. Metabolism and excretion: In vitro studies with human liver microsomes and human cDNA-expressed cytochrome P450 isozymes indicate that bortezomib is primarily oxidatively metabolized via cytochrome P450 enzymes 3A4, 2C19, and 1A2. Bortezomib metabolism by CYP 2D6 and 2C9 enzymes is minor. The major metabolic pathway is deboronation to form 2 deboronated metabolites that subsequently undergo hydroxylation to several metabolites. Deboronated bortezomib metabolites are inactive as 26S proteasome inhibitors. Pooled plasma data from 8 patients at 10 min and 30 min after dosing indicate that the plasma levels of metabolites are low compared to the parent drug. The pathways of elimination of bortezomib have not been characterized in humans.

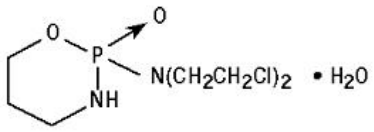
5.1.6. Administration and Storage: Local skin irritation was reported in 5% of patients, but extravasation of was not associated with tissue damage. Prior to use, the contents of each vial must be reconstituted with 3.5 mL of normal (0.9%) saline, Sodium Chloride Injection, USP. The reconstituted product 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. Unopened vials are stable until the date indicated on the package when stored in the original package protected from light. Bortezomib contains no antimicrobial preservative. When reconstituted as directed, it may be stored at 25ºC (77ºF). After reconstitution bortezomib should be administered within 8 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 8 hours in a syringe; however total storage time for the reconstituted material must not exceed 8 hours when exposed to normal indoor lighting. Unopened vials may be stored at controlled room temperature 25ºC (77ºF); excursions permitted from 15 to 30ºC (59 to 86ºF). Retain in original package to protect from light.

5.2. Cytoxan® (cyclophosphamide) – Bristol-Myers Squibb

5.2.1. Therapeutic Class: nitrogen mustard alkylating agent

5.2.2. Description: CYTOXAN® (cyclophosphamide for injection, USP) is a sterile white powder containing cyclophosphamide monohydrate. CYTOXAN Tablets (cyclophosphamide tablets, USP) are for oral use and contain 25 mg or 50 mg cyclophosphamide (anhydrous). Inactive ingredients in CYTOXAN Tablets are: acacia, FD&C Blue No. 1, D&C Yellow No. 10 Aluminum Lake, lactose, magnesium stearate, starch, stearic acid and talc.

Cyclophosphamide is a synthetic antineoplastic drug chemically related to the nitrogen mustards. Cyclophosphamide is a white crystalline powder with the molecular formula $C_7H_{15}Cl_2N_2O_2P•H_2O$ and a molecular weight of 279.1. The chemical name for cyclophosphamide is 2-[bis (2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide monohydrate. Cyclophosphamide is soluble in water, saline, or ethanol and has the following structural formula:



5.2.3. Indications: Treatment of malignant lymphomas, Hodgkin's disease, lymphocytic lymphoma, mixed-cell type or histiocytic lymphoma, Burkitt's lymphoma, multiple myeloma, chronic lymphocytic leukemia, chronic granulocytic leukemia, acute myelogenous and monocytic leukemia, acute lymphoblastic leukemia in children, mycosis fungoides, neuroblastoma, ovary adenocarcinoma, retinoblastoma, breast carcinoma. Treatment of biopsy proven "minimal change" nephrotic syndrome in children, but not as primary therapy.

5.2.4. Mechanism of Action: Nitrogen mustard alkylating agent; exerts action by cross linking of tumor cell DNA.

5.2.5. Pharmacokinetics:

5.2.5.1. Absorption: Well absorbed; bioavailability (≥75%).

5.2.5.2. Distribution: Plasma protein binding (≥60% as metabolites).

5.2.5.3. Metabolism: Liver; active metabolites.

5.2.5.4. Elimination: Urine (5-25% unchanged); $T_{1/2}$=3-12 hrs.

5.2.6. How Supplied: Inj (Lyophilized): 500mg, 1g, 2g; Tab: 25mg, 50mg

5.2.7. Administration and Storage:

5.2.7.1. Administration: Oral, IV, IM, intraperitoneal, intrapleural route. Inspect drug product visually for particulate matter and discoloration prior to parenteral administration.

5.2.7.2. Storage: Vial: Below 25°C (77°F). Tab: Below 25°C (77°F); excursions permitted to 30°C (86°F); protect from temperatures above 30°C (86°F).

5.3. Study Agent Accountability Procedures

Cyclophosphamide and bortezomib will both be purchased per standard of practice from a commercial source and compounded according to the package insert by the main hospital pharmacy.

5.4. Product Complaints: A product complaint is a verbal, written, or electronic expression which implies dissatisfaction regarding the identity, strength, purity, quality, or stability of a drug product. Individuals who identify a potential product complaint situation should immediately contact the PI and report the event. Whenever possible, the associated product should be maintained in accordance with the label instructions.

6. REQUIRED STUDY ACTIVITIES (APPENDIX A)

6.1. Recipient Pre-Transplant Work-Up and Eligibility for Transplant: The patient will undergo pre-transplant work-up per standard of care and in timeframes defined by standard operating procedures (SOPs). This will include but is not limited to: history and physical examination, ABO and Rh type, CBC, CMP, PT-INR, PTT, creatinine clearance, high resolution HLA typing (including KIR for patients with myeloid malignancies), infectious disease markers (IDMs), β-HCG serum pregnancy test (females of reproductive capability only – refer to exclusion criteria), chest x-ray (CXR), ECG, pulmonary function tests (PFT) with DLCO, ECHO to measure left ventricular ejection fraction (LVEF), informed consent for transplant, and final medical clearance by their treating physician.

6.2. Donor Work-Up: The donor will also undergo a pre-transplant donor evaluation and eligibility per institutional SOPs or by donor center. Study procedures will not impact the donor. The attending physician will review all donor eligibility prior to the initiation of the conditioning chemotherapy.

6.3. Pre-Transplant

6.3.1. Work up as outlined in 6.1.

6.3.2. Daily, from Day -7 through day 0: CBC, CMP, liver function tests (LFTs), and a physical exam, including toxicity assessment.

6.4. Post-Transplant

6.4.1. CBC and CMP daily until neutrophil engraftment, then at least weekly until day +100 (+/- 3 days), and at least monthly until day +180 (+/- 7 days).

6.4.2. LFT twice weekly until engraftment, then at least weekly until day +100 (+/- 3 days), and at least monthly until day +180 (+/- 7 days).

6.4.3. Physical examination including toxicity and GvHD assessments daily until discharge, then weekly un

6.4.4. til day +100 (+/- 3 days), and at least monthly until day +180 (+/- 7 days).

6.4.5. From day +180 until day +730: CBC, CMP, LFTs, and a physical exam including toxicity and GvHD assessments will be done at least every three months (+/- 7 days).

6.4.6. Post-chimerism: at engraftment, then at least monthly until day +180, and every 3 months until day +730 (+/- 7 days).

6.4.7. Immune reconstitution studies will be drawn on days +30, +100, +180, and +365 (+/- 7 days), 10-20mL of blood for CD3, CD4, CD8 and CD19.

7. TREATMENT PLAN

7.1. Conditioning Regimen (Study Schema): Eligible patients will receive one of the following conditioning regimens, both of which are standard of care, on an inpatient basis. The choice of the conditioning regimen is determined by the treating physician based on host factors and

disease nature and status. As described by BMT standard operating policy (procedure) (SOP) CLNTX007: Selection of Conditioning Regimens for Blood and Marrow Transplantation – Adult.

7.1.1. Myeloablative:

7.1.1.1. Fludarabine 40 mg/m^2 IV once daily on days -5, -4, -3, and -2.

7.1.1.2. Busulfan IV, q6 hours, on days -5, -4, -3, and -2. The busulfan dose will be determined based on pharmacokinetic (PK) studies performed on a one time test dose administered 1-3 weeks before the beginning of conditioning regimen. The target systemic exposure will be 900-1500 micoMol/min. PK will be sent to (Pharmacokinetics Laboratory, Mayo Medical Laboratories, 3050 Superior Drive NW, Rochester, MN 55901). See appendix F: Busulfan Information: Mail-In Specimen Instructions. Once the PK samples are received by Mayo Medical Laboratory, a report is generated and returned to the institution detailing the recommended dose. This recommended dose will be the dose used for conditioning.

7.1.1.3. rATG (Thymoglobulin®) 1 mg/kg, 1.5 mg/kg, and 2.5 mg/kg IV, daily, on days -4, -3 and -2, respectively.

7.1.1.4. Filgrastim (Neupogen®) will begin on day +5. Filgrastim will be stopped when patients achieve an ANC > 1.5 x 10^9/L on two consecutive days.

7.1.2. Reduced intensity

7.1.2.1. Fludarabine 30 mg/m^2 IV once daily on days -7, -6, -5, -4, -3 and -2.

7.1.2.2. Busulfan 130 mg/m^2 IV, once daily, on days -3, -2.

7.1.2.3. rATG (Thymoglobulin®) 1 mg/kg, 1.5 mg/kg, and 2.5 mg/kg IV, daily, on days -4, -3 and -2, respectively.

7.1.2.4. Filgrastim (Neupogen®) will begin on day +7. Filgrastim will be stopped when patients achieve an ANC > 1.5 x 10^9/L on two consecutive days.

7.2. **Graft Infusion:** At least 2 x 10^6/kg peripheral blood CD34$^+$ cells (HPC-A) will be infused on day 0 per institutional protocol. The goal is to infuse 4.9 - 9 x 10^6/kg CD34$^+$ cells. Stem cells are collected by apheresis after mobilization with 10 µg/kg of filgrastim (G-CSF or Neupogen®) per the National Marrow Donor Program and institutional program practice.

7.3. **GvHD prophylaxis:** All patients will investigational GvHD prophylaxis based on a combination of PTCy and bortezomib. No other prophylactic treatment will be given.

7.3.1. **Cyclophosphamide** 50 mg/kg IV over 1 hour on days +3 and +4 with concomitant hydration. The hydration will be NS with or without 20 mEq/L KCl at 250 mL/hr starting 4 hours before and continuing until 24 hours after the second dose is complete. Furosemide is also given on an as needed basis to maintain fluid balance.

7.3.2. **Bortezomib** 1.3 mg/m^2 IV 6 hours after graft infusion and 72 hours thereafter.

7.4. **Drug Preparation:** The drugs will be prepared under the supervision of a pharmacist, or appropriately qualified and trained personnel. Fludarabine, busulfan, rATG, and cyclophosphamide doses will be based on adjusted body surface area if actual body weight is > 125% the ideal body weight.

7.5. **Supportive care*:*** Steroids will **not** be allowed after graft infusion. All supportive care including anti-emetics, anti-seizure, sinusoidal obstruction syndrome (SOS) prophylaxis, transfusion support, and infection prophylaxis will be administered per established routine institutional SOPs.

7.6. **Management of acute and chronic GvHD:** Patients who develop acute or chronic GvHD will be managed by the treating physician according to his or her standard practice and as defined by BMT SOP CLNAL005: Definition, Prevention, Diagnosis, Grading, and Management of Graft-versus-Host-Disease (GvHD) – Adults.

7.7. . Patients will remain on study for follow-up and data collection.

7.8. **Ancillary Therapy:** Patients will continue to receive any other therapy that the treating physician feels necessary according to the routine program practices. Management of shared toxicities and adverse events associated with cyclophosphamide and bortezomib such as nausea, vomiting, diarrhea, etc. will be routinely managed according to the routine practices.

8. ASSESSMENT OF SAFETY

8.1. Specification of Safety Parameters

8.2. Adverse Events (AE) and Serious Adverse Events (SAE) are unexpected toxicities that occur in the transplant setting (i.e. hematologic, gastrointestinal toxicities are expected). The Common Terminology Criteria for Adverse Events, Version 5.0, will be used for assessing the severity of adverse events.

8.2.1. The following will be reported and monitored:

- Grade 3 non-hematologic toxicity directly related to study drugs (such as peripheral neuropathy).
- Grade 2 or higher hepatic bilirubin.

8.2.2. Time Period and Frequency for Event Assessment and Follow-up

The investigator or site staff will be responsible for detecting, documenting and reporting events that meet the definition of an adverse event (AE) or serious adverse event (SAE). AEs and SAEs will be followed from the start of conditioning regimen to 30 days after the last study drug administration and/or until treatment-related adverse events resolve or stabilize, whichever occurs first.

8.3. Adverse Events (AE)

8.3.1. Definitions of Adverse Events (AE)

An adverse event (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

8.3.2. Reporting an Adverse Event: All adverse events, whether observed by the investigator or reported by the patient, must be recorded with details. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), duration of episode, intensity of episode, action taken with respect to the test drug, time of resolution/stabilization of the event and patient outcome. The investigator must evaluate each adverse event for its relationship to the test drug and for its seriousness. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate report forms. All unexpected AEs occurring while on study must be documented appropriately regardless of relationship.

8.4. Serious Adverse Events (SAE)

8.4.1. Definition of SAE: events are classified as serious or non-serious. A *serious adverse event* is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as *non-serious adverse events*.

8.4.2. Reporting a Serious Adverse Event: The Principal Investigator (PI), Dr. A. Samer Al-Homsi, (who may also sometimes be referred to as the sponsor-investigator), is conducting the study and acting as the sponsor. Therefore, the ethical and legal obligations of the principal investigator include both those of a sponsor and those of an investigator.

All SAEs, regardless of expectedness or relationship with any study drug, must be reported to the sponsor-investigator, as soon as possible, but no later than 24 hours of the investigator's observation or awareness of the event.

The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

SAEs that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE. UPs will be recorded in the data collection system throughout the study.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

Events should be reported using the NYU CTO Medical Events Form.

Adverse events that do not fit the above immediately reportable criteria must still be reported to the IRB at each annual review, either in a summary or tabular format.

Incidents or events that meet the OHRP criteria for UPs require the creation and completion of an UP report form. It is the site investigator's responsibility to report UPs to their IRB and to the overall principal investigator. The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are SAEs will be reported to the IRB and to the DCC within 24 hours of the investigator becoming aware of the event.
- Any other UP will be reported to the IRB and to the DCC within 24 hours of the investigator becoming aware of the problem.

- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and OHRP within 5 days of the IR's receipt of the report of the problem from the investigator.

SAE reporting will begin in conjunction with the date of treatment administration. Any SAEs that the investigator believes may have been caused by a protocol procedure must be reported immediately to the principal investigator, with a notification email sent to NYUPCCsafetyreports@nyulangone.org and recorded on the case report form.

All fatal or life-threatening adverse events must be immediately reported to the Principal Investigator, via appropriate reporting mechanism and the NYU Langone Health IRB by telephone or e-mail. Within 24 hours of the event, the Serious Adverse Event Form must be emailed to NYUPCCsafetyreports@nyulangone.org whether full information regarding the event is known or not.

Additional follow-up by the investigator will be required if complete information is not known. De-identified source documentation of all examinations, diagnostic procedures, etc. which were completed with respect to the event should be included with the SAE form. For laboratory results, include the laboratory normal ranges.

The Serious Adverse Event Form must also be emailed to the principal investigator and Clinical Trials Office within 24-hours (NYUPCCsafetyreports@nyulangone.org), this documentation will be forwarded to the DSMC's appointed medical monitor within 24 hours of the event whether full information regarding the event is known or not. Additional follow-up by the investigator will be required if complete information is not known.

Current contact information shall be maintained at the site within the regulatory binder.

All SAEs will be evaluated by the DSMC. The investigator is responsible for reporting all SAEs to the appropriate IRB and DSMC.

8.5. Overdose

An overdose is defined as the accidental or intentional administration of any dose of a product that is considered both excessive and medically important. All occurrences of overdose must be reported as an SAE.

8.6. Other Safety Considerations

Any significant worsening noted during interim or final physical examinations, electrocardiograms, X-rays, and any other potential safety assessments, whether or not these procedures are required by the protocol, should also be recorded as a non-serious or serious AE, as appropriate, and reported accordingly.

8.7. Definition of Unanticipated Problems (UP)

Unanticipated Problems Involving Risk to Subjects or Others

Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc)

- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

8.8. Classification of an Adverse Event

8.8.1. Severity of Event

As described, AEs will be graded by criteria established by CTCAE, version 5.0. For AEs not able to be defined by this grading system, the following guidelines will be used to describe severity.

- **Mild:** Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate:** Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe:** Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating.

8.8.2. Relationship to Study Agent

For all collected AEs, the clinician who examines and evaluates the participant will determine the AE's causality based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below.

- **Definitely Related:** There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to drug administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related:** There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the drug, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- **Possibly Related:** There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related," as appropriate.
- **Unlikely to be related:** A clinical event, including an abnormal laboratory test result, whose temporal relationship to drug administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the trial medication) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).

- **Not Related:** The AE is completely independent of study drug administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

8.9. Non-serious Adverse Event Collection Reporting

The collection of non-serious AE information should begin from the start of conditioning regimen. All non-serious adverse events (not only those deemed to be treatment-related) should be collected continuously during the treatment period and for a minimum of 30 days following the last dose of study drug.

Non-serious AEs should be followed to resolution or stabilization, or reported as SAEs if they become serious. Follow-up is also required for non-serious AEs that cause interruption or discontinuation of study drug and for those present at the end of study treatment as appropriate.

8.10. Expectedness

The Principal investigator and/or sub-investigator(s) will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study agent.

8.11. Reporting of Pregnancy

Procedures for Reporting Drug Exposure during Pregnancy and Birth Events: If a woman becomes pregnant or suspects that she is pregnant while participating in this study, she must inform the investigator immediately and permanently discontinue study drug(s). The pregnancy must be followed for the final pregnancy outcome (i.e., delivery, still birth, miscarriage) and PI will request this information from the investigator. If a female partner of a male patient becomes pregnant during the male patient's participation in this study, this must be reported to PI immediately. Every effort should be made to follow the pregnancy for the final pregnancy outcome.

The pregnancy must be reported immediately to the principal investigator, and the Clinical Trials Office, by emailing: NYUPCCsafetyreports@nyulangone.org. Pregnancy in itself is not regarded as an adverse event unless there is a suspicion that the investigational product may have interfered with the effectiveness of a contraceptive medication.

8.12. Reporting Procedures - Notifying the IRB

Federal regulations require timely reporting by investigators to their local IRB of unanticipated problems posing risks to subjects or others. The following describes the NYULH IRB reporting requirements, though Investigators at participating sites are responsible for meeting the specific requirements of their IRB of record.

Report promptly, but no later than 5 working days:

Researchers are required to submit reports of the following problems promptly but no later than 5 working days from the time the investigator becomes aware of the event:

- Unanticipated problems including adverse events that are unexpected and related
  - **Unexpected**: An event is "unexpected" when its specificity and

severity are not accurately reflected in the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document and other relevant sources of information, such as product labeling and package inserts.

- **Related to the research procedures**: An event is related to the research procedures if in the opinion of the principal investigator, the event was more likely than not to be caused by the research procedures.
- **Harmful**: either caused harm to subjects or others, or placed them at increased risk

Other Reportable events:

The following events also require prompt reporting to the IRB, though no later than 5 working days:

- Complaint of a research subject when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- Protocol deviations or violations (includes intentional and accidental/unintentional deviations from the IRB approved protocol) for any of the following situations:
  - one or more participants were placed at increased risk of harm
  - the event has the potential to occur again
  - the deviation was necessary to protect a subject from immediate harm
- Breach of confidentiality

- Incarceration of a participant when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
  New Information indicating a change to the risks or potential benefits of the research, in terms of severity or frequency. (e.g. analysis indicates lower-than-expected response rate or a more severe or frequent side effect; Other research finds arm of study has no therapeutic value; FDA labeling change or withdrawal from market)

8.13. Reporting Process

The reportable events noted above will be reported to the IRB using the form: “Reportable Event Form” or as a written report of the event (including a description of the event with information regarding its fulfillment of the above criteria, follow-up/resolution and need for revision to consent form and/or other study documentation).

Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator’s study file.

8.14. Other Reportable events:

- **Exceptions to the study protocol**
  Exceptions to the protocol must investigator’s IRB approval before they are initiated. Any protocol deviations initiated without the investigator’s IRB approval that may affect the scientific soundness of the study, or affect the

rights, safety, or welfare of study subjects, must be reported to the investigator's IRB as soon as a possible, but ***no later than 5 working days*** of the protocol deviation.

8.15. Study Halting Rules

8.15.1. Removal of Subjects from Study

8.15.1.1. The investigator will make every reasonable effort to keep each patient in the study until all planned treatments and assessments have been performed. Unplanned discontinuation may occur for any of the following reasons:

- Patient develops allergy to either study drug.
- Treating physician feels that continuation in study is not appropriate. Such a determination may be made if the patient experiences adverse events related to the study protocol of ≥ grade 3 other than hematologic or infection-related toxicity that the physician or PI determines the study is no longer in the subject's best interest.
- Patient withdraws informed consent.
- Patient is not meeting the follow up requirements.

8.15.1.2. If patients fail to complete the study doses of cyclophosphamide and bortezomib, alternative GVHD prophylaxis will be instituted immediately at the discretion of the treating physician.

8.15.1.3. The PI can decide to replace patients withdrawn from the study at his discretion. All patients who receive the study drugs will be evaluated. The study will enroll patients for a total of 47 evaluable patients, expected to be accrued over three years.

8.15.1.4. **Premature Closure of Study:** This study may be prematurely terminated, if in the opinion of the sponsor-investigator, there is sufficient reasonable cause. Written notification documenting the reason for study termination will be provided by the terminating party. Circumstances that may warrant termination include but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to patients,
- Failure to enter patients at an acceptable rate,
- Failure Insufficient adherence to protocol requirements,
- Insufficient complete or evaluable data,
- Plans to modify, suspend, or discontinue the development of the drug.

8.16. Safety Oversight

All Internal SAEs reported by the CTO, occurring to patients on clinical trials that are not monitored by any other institution or agency, are reported via email: NYUPCCsafetyreports@nyulangone.org and reviewed within 48 hours by the medical monitor. Based on the review, one of three determinations will be made:

SAE report is considered to be adequate
Queries for clarification to PI regarding treatment attribution and/or resolution of SAE or completeness of other information. The committee may request a cumulative review of all SAEs on the study to date.

Request for full DSMC committee review of protocol at the next scheduled meeting.

The DSMC Coordinator will record the committee's decision and incorporate it into the study summary for the next scheduled study review.

9. CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of human subjects are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with GCP, and with applicable regulatory requirement(s).

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan detailed below. Adverse events are evaluated regularly by the principal investigator in conjugation with the research team, the DSMC is notified of adverse events via email initially, and then reviewed at the next DSMC monthly meeting. The Data Safety and Monitoring Committee (DSMC) will review the study at least *quarterly*. Medical monitoring will include a regular assessment of the number and type of serious adverse events.

9.1. Data Monitoring Committee

This investigator-initiated study will be monitored by the Data Safety Monitoring Committee (DSMC) of the New York University (NYU) Perlmutter Cancer Center (PCC). The DSMC operates based on the 2017 National Cancer Institute approved Charter. It is an existing and multidisciplinary committee (consisting of clinical investigators/oncologists, biostatisticians, nurses, and research administration staff knowledgeable of research methodology and design and in proper conduct of clinical trials) that is responsible for monitoring safety, conduct and compliance in accordance with protocol data monitoring plans for interventional clinical trials conducted in the NYULH Perlmutter Cancer Center that are not monitored by another institution or agency. The DSMC reports to the Director of the NYULH PCC.

Per the NYU PCC Institutional Data Safety and Monitoring Plan, this Phase II trial will be monitored by DSMC *quarterly* (from the date the first patient is enrolled), at protocol-specified interim time points, and at the completion of the study prior to study closure. This review includes accrual data, subject demographics and adverse events. Principal Investigators are required to attend the review of their studies. Additional reviews can be scheduled based on SAE reports, investigator identified issues, external information, etc. DSMC summary reports are available to facilitate the review and monitoring of this study. These reports include the following: patient listings and summary reports that describe study enrollment and accrual, eligibility, demographic characteristics, dose modifications, adverse experiences, subject's death and additional external published data if applicable to the study. Cumulative toxicities, SAEs, and AEs are reviewed, to identify possible adverse events with elevated frequency that is unexpected. Once a recommendation is made if further action is required, the Investigator's must respond within the timeframe specified by the DSMC.

10 STATISTICAL CONSIDERATIONS

10.1 Sample size: The primary outcome variables for this study include the incidence of acute and chronic GvHD. For sample size considerations, we focus on the rate of acute GvHD. If we assume that the rate of acute GvHD grades II-IV with standard of care is 50% as reported in the literature and 30% in the experimental group, we can detect a reduction in acute GvHD incidence to 30% for high-dose post-transplant cyclophosphamide and bortezomib (CyBor) , with alpha = 0.05 (2 sided) and power of 80% with 47 patients using a z test for the test of the observed percentage compared to an expected percentage of 50%. With 47 patients, the observed 95% confidence interval will 35.1% to 64.9% if the observed rate is 50%; if the observed rate is 30%, the corresponding confidence interval will be 17.5% to 45.1%.

The corresponding 95% confidence interval for the rate of chronic GvHD, expected to be 20% is 9.8% to 34.2%.

Calculations from PASS 2014, NCSS, J. Hintze,Kaysville, Ut.

10.2 Statistical Analysis:

Baseline Descriptive Statistics: Patient characteristics will be summarized using descriptive statistics. For qualitative characteristics (such as gender, co-morbid conditions, donor source, CMV status amongst others) proportions and frequency distributions will be provided. For quantitative characteristics including hematologic parameters, summary and graphical displays will be provided.

10.3 Analysis of the Primary Efficacy Endpoint(s):

10.3.1 Acute GvHD: The first day of acute GvHD of any grade will be recorded for that grade. This end point will be evaluated through day +120 post-transplant. The diagnosis of acute GvHD is based on clinical and pathological evaluation by the principal investigator in collaboration with the treating physician. The acute GvHD grading system is described in appendix B. All participants that received a transplant and received any prophylactic treatment will be included in the analysis. An additional analysis will be carried out for all patients that received a transplant and completed prophylactic treatment with evaluation from date of completion of prophylactic treatment. Cumulative incidence curves will be provided along with 95% confidence intervals for the 120 day cumulative incidence.

10.3.2 Chronic GvHD: The first day of chronic GvHD will be recorded. The diagnosis of chronic GvHD is based on clinical and pathological evaluation by the principal investigator in collaboration with the treating physician. The chronic GvHD system is described in appendix C. The analysis will be based on the maximum grade of chronic GvHD. All participants that completed transplant and any prophylactic treatment will be included in the analysis. An additional analysis will be carried out for all patients that received a transplant and completed prophylactic treatment with evalution from date of completion of prophylactic treatment. Cumulative incidence curves will be provided along with 95% confidence intervals for the day 365 cumulative incidence.

Additional descriptive analysis will be provided for any GvHD.

10.4 Analysis of the Secondary Endpoint(s):

All analyses of secondary endpoints will be based on cumulative incidence curves of Kaplan Meier curves to estimate the rates of occurrence as defined for each endpoint.

10.4.1 Primary graft failure is evaluated to day +45 and incidence of graft failure will be calculated from date of transplant to failure for all patients who receive a transplant and any prophylactic treatment and from date of completion of prophylactic treatment for all participants that completed treatment.

10.4.2 Poor graft function is evaluated to day +30 incidence of poor graft function will be calculated, from date of transplant to failure for all patients who receive a transplant and any prophylactic treatment and from date of completion of prophylactic treatment for all participants that completed treatment.

10.4.3 Secondary graft failure is evaluated after engraftment is achieved will be calculated from date of engraftment for all patients with engraftment.

10.4.4 TRM will be analyzed based on participants that who received a transplant with any prophylactic treatment and for all patients who received a transplant and completed prophylactic treatment.

10.4.5 RR is evaluated to day +730 and will be analyzed for all patients who received a transplant and for all transplanted patients that completed treatment.

10.4.6 GRFS is evaluated to day +730 and considers as successes participants that are without reported GvHD III-IV acute GvHD, chronic GvHD requiring systemic therapy and have not experienced relapse or death after transplant.

10.4.7 OS is evaluated to day +730 and considers all participants who received a transplant and for all transplanted patients who completed prophylactic treatment as described above. .

10.5 Missing data: Distribution of patients and disease characteristics will be summarized for patients with missing data regarding key endpoints or who are non-compliant. Comparisons will be made to patients who complete the study to evaluate bias.

10.6 Safety Analyses: All adverse events (expected and unexpected) will be tracked and graded per CTCAE, version 5.0 over time. In addition, the attributed relationship to study drug will be summarized. All adverse events will be reviewed by the primary investigator, in conjunction with the sub-investigators to determine safety.

11 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA AND DOCUMENTS

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial. It is acceptable to use CRFs as source documents. If this is the case, it should be stated in this section what data will be collected on CRFs and what data will be collected from other sources.

TrialMaster, an electronic database capture system will be created to record the data for this trial. Research coordinators will input clinical trial data into the database. This database is password protected and only the PI, assigned study team members, and CTO staff will have access to the database. DataCore, a core resource of the institution, will provide the primary data collection instrument for the study. All data requested in the system must be reported. All missing data must be explained. The quality assurance (QA) specialists will monitor this trial every 4-6 weeks for data entry accuracy.

Source documentation should be consistent with data entered into any electronic medical record or TrialMaster. Relevant source documentation to be reviewed by the QA specialists throughout the study includes but are not limited to:

1. Baseline measures to assess pre-transplant status
2. Treatment records
3. Adverse events

12 ADMINISTRATIVE REQUIREMENTS

12.1 Good Clinical Practice: The study will be conducted in accordance with the International Conference on Harmonization (ICH) for Good Clinical Practice (GCP) and the appropriate regulatory requirement(s).[85] The investigator will be thoroughly familiar with the appropriate use of the study drugs as described in the protocol and Investigator's Brochures. Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study, and retained according to the appropriate regulations.

12.1.1 Ethical Considerations: The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki. The IRB will review all appropriate study documentation in order to safeguard the rights, safety and well-being of the patients. The study will only be conducted at sites where IRB approval has been obtained. The protocol, Investigator's Brochure, informed consent, advertisements (if applicable), written information given to the patients, safety updates, annual progress reports, and any revisions to these documents will be provided to the IRB by the investigator.

Protocol Compliance: The investigator will conduct the study in compliance with the protocol, and given approval by the IRB, and the appropriate regulatory authority(ies). Any departures from the protocol must be fully documented in the source documents

12.2 Patient Information and Informed Consent: After the study has been fully explained, written informed consent will be obtained from the patient. The method of obtaining and documenting the informed consent and the contents of the consent will comply with ICH-GCP and all applicable regulatory requirement(s).

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation will be provided to the participants

and their families. Consent forms will be IRB-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. The participants may withdraw consent at any time throughout the course of the trial. A copy of the signed informed consent document will be given to the participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

A copy of the signed informed consent document will be stored in the subject's research record. The consent process, including the name of the individual obtaining consent, will be thoroughly documented in the subject's research record. Any alteration to the standard consent process (e.g. use of a translator, consent from a legally authorized representative, consent document presented orally, etc.) and the justification for such alteration will likewise be documented.

The consenting process and documentation will follow Standard Operating Procedures (Obtaining Informed Consent for Clinical Trials) of the NYULH PCC CTO.

12.2.1. Informed Consent

Consent will be obtained only by a participating investigator who has completed requisite training for human subject research and has been instructed by the Principal Investigator about the research study and consent process. Investigators will review the informed consent form with patients and address any questions or concerns prior to obtaining written informed consent for participation and HIPAA authorization.

For non-English speaking patients, institutional translation services will be utilized. All procedures for consenting non-English speaking patients will be in accordance with NYU Langone Health PCC CTO guidelines and policies.

For patients who cannot read; a witness, not related to the research study will be present. The consent will be read to the patient. The patient will also be allowed to ask any questions s/he may have. The investigator will ask the patient questions to ensure s/he understands the study. If the investigator determines the subject understands the study, the patient will mark an X where his/her name would go and the witness will sign the consent form.

12.2.2. Documentation of Consent

The Principal Investigator or IRB approved sub-investigator will be responsible for documentation in the medical record that consent has been obtained from all participants. A signed copy of the consent form will be given to each participant. Original consent forms will be stored in the subject's medical chart.

12.2.3. Patient and Data Confidentiality: In order to maintain patient privacy, all information obtained in connection with this study which identifies the patient will remain confidential in accordance with state and federal law.

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

Participant confidentiality is strictly held in trust by the participating investigators and their staff. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence.

Representatives of the IRB may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by local IRB and Institutional regulations.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at NYU Langone Health. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by NYU Langone Health research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the NYU Langone Health.

13 DATA HANDLING AND RECORD KEEPING

13.1. Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site PI. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL.

Data reported in the eCRF derived from source documents should be consistent with the source documents or the discrepancies should be explained and captured in a progress note and maintained in the participant's official electronic study record.

Clinical data (including AEs, concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into Trialmaster, a 21 CFR Part 11-compliant data capture system provided by DataCore. The data system includes password protection and internal quality checks to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

13.2. Study Records Retention

The sponsor-investigator will maintain all study records according to ICH-GCP and applicable regulatory requirement(s).

13.3. Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

Protocol deviations must be reported to the local IRB per their guidelines. The site PI and study staff is responsible for knowing and adhering to their IRB requirements. All protocol deviations must be addressed in study source documents and reported to IRB Program Official at the time of annual continuing review. If a protocol deviation is determined to be reportable new information, the IRB will be notified immediately.

13.4. Publication and Data Sharing Policy

This study will comply with the NIH Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a clinical trials registration policy as a condition for publication. The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or concurrent comparison or control groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Medical interventions include drugs, surgical procedures, devices, behavioral treatments, process-of-care changes, and the like. Health outcomes include any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and adverse events. The ICMJE policy, and the Section 801 of the Food and Drug Administration Amendments Act of 2007, requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies. For interventional clinical trials performed under NIH IC grants and cooperative agreements, it is the grantee's responsibility to register the trial in an acceptable registry, so the research results may be considered for publication in ICMJE member journals. The ICMJE does not review specific studies to determine whether registration is necessary; instead, the committee recommends that researchers who have questions about the need to register err on the side of registration or consult the editorial office of the journal in which they wish to publish.

Study results may be published, and patients may access the results of this study via clinicaltrails.gov or any published media. However, no results will be sent directly to subjects. The study will be available on clinicaltrials.gov.

14 STUDY FINANCES

14.1 Funding Source

All study elements are standard of care and will be billed to third parties. The data management cost will be covered by the Disease Management Group (DMG).

14.2. Costs to the Participant

All aspects of care, including conditioning regimen and GvHD prevention is considered standard of care and will be billed to the insurance company or patient.

14.3 Participant Reimbursements or Payments

No subject will receive payments or stipends for participation in this research study.

15 CONFLICT OF INTEREST POLICY

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by

a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan prior to participation in this study. All NYULH investigators will follow the applicable University conflict of interest policies.

16 REFERENCES

1. Appelbaum FR. Hematopoietic cell transplantation at 50. *N Engl J Med* 2007;357:1472-1475.
2. Welniak LA, Blazar BR, Murphy WJ. Immunobiology of allogeneic hematopoietic stem cell transplantation. *Annu Rev Immunol* 2007;25:139–170.
3. Remberger M, Ackefors M, Berglund S, Blennow O, Dahlöf G, Dlugosz A, at al. Improved survival after allogeneic hematopoietic stem cell transplantation in recent years. A single-center study. *BBMT* 2011; 17:1688-1697.
4. Arai S, Arora M, Wang T, Spellman S, He W, Couriel D, et al. Increasing incidence of chronic graft-versus-host disease in allogeneic transplantation- A report from CIBMTR. *BBMT* 2014; 21:266-274.
5. Cutler C, Li S, Ho VT, Koreth J, Alyea E, Soiffer RJ, et al. Extended follow-up of methotrexate-free immunosuppression using sirolimus and tacrolimus in related and unrelated donor peripheral blood stem cell transplantation. *Blood* 2007; 109:3108–3114.
6. Luznik L, O'Donnell PV, Fuchs EJ. Post-transplantation cyclophosphamide for tolerance induction in HLA-haploidentical BMT. *Semin Oncol* 2012; 39:683:693.
7. Ross D, Jones M, Komanduri K, Levy RB. Antigen and lymphopenia-driven donor T cells are differentially diminished by post-transplantation administration of cyclophosphamide after hematopoietic cell transplantation. *BBMT* 2013; 19:1430-1438.
8. Luznik L, Bolaños-Meade J, Zahurak M, et al. High-dose cyclophosphamide as single-agent, short-course prophylaxis of graft-versus-host disease. *Blood* 2010; 115: 3224-3230.
9. Kankary GK, O'Donnell PV, Furlong T, de Lima MJ, Wei W, Medeot M, et al. Multi-institutional study of post-transplant cyclophosphamide as single-agent graft-versus-host disease prophylaxis after allogeneic bone marrow transplantation using myeloablative busulfan and fludarabine conditioning. *Journal Clin Oncol* 2014; 32:3497-3505.
10. Al-Homsi AS, Roy TS, Cole K, Feng Y, Duffner U. Post-transplant high-dose cyclophosphamide for the prevention of graft-versus-host disease. *BBMT* 2015; 21:604-611.
11. Alousi AM, Brammer JE, Saliba RM, Andersson B, Popat Y, Hosing C, et al. Phase II trial of graft-versus-host disease prophylaxis with post-transplant cyclophosphamide after reduced-intensity busulfan/fludarabine conditioning for hematological malignancies. *BBMT*; 2015: 21:906-912.
12. Solomon SR, Sanacore M, Zhang X, Brown S, Holland K, Morris LE, Bashey A. Calcineurin inhibitor-free graft –versus-host disease with post-transplantation cyclophosphamide and brief course sirolimus following reduced-intensity peripheral blood stem cell transplantation. *BBMT* 2014; 20:1828-1834.
13. Carnevale-Schiannca F, Caravelli D, Gallo S, Coha v, D'Ambrosio L, Vassallo E, Fizzotti M, Nesi F, Gioeni L, Berger M, Polo A, Gammaitoni L, Becco P, Giraudo L, Mangioni M, Sangiolo D, Grignani G, Rota-Scalabrini D, Aglietta M. Post-transplant cyclophosphamide and tacrolimus-mycophenolate mofetil combination prevents graft-versus-host disease in allogeneic peripheral blood hematopoietic cell transplantation from HLA-matched donors. *BBMT* 2017; 3:459-466.
14. Bolaños-Meade J, Reshef R, Fraser R, Fei M, Abhyankar S, Al-Khadimi Z, Alousi AM, Antin JH, Bickett K, Damon LE, Efebera YA, Geller N, Giralt SA, hari P, Holtan SG, Horowitz MM, Jacobson

D, Jones RJ, Liesveld J, Logan BR, MacMilan ML, Mielcarek MB, Noel P, Pidala JA, Porter DL, Weisdorf DJ, Wu J, Pasquini MC, Koreth J. Novel approaches for graft-versus-host disease (GvHD) prophylaxis: primary results of Progress I multicenter trial of matched allogeneic hematopoietic cell transplantation (alloHCT) using reduced intensity conditioning (RIC) BMT CTN 1203. BBMT 2018 Epub ahead of print.

15. Markey KA, MacDonald KPA, Hill GR. The biology of graft-versus-host disease: experimental systems instructing clinical practice. *Blood* 2014; 124:345-362.
16. Matte CC, Liu J, Cormier J, Anderson BE, Athannasiadis I, et al. Donor APCs are required and are sufficient for the development of GvHD but not GvL. *Nature Medicine* 2004; 10:978-992.
17. Boeck S, Hamann M, Pihusch V, Heller T, Diem H, Rolf B, et al. Kinetics of dendritic cee chimerism and T cell chimerism in allogeneic hematopoietic stem cell recipients. *Bone Marrow Transplantation* 2006; 37:57-64.
18. Tao Y, Zhang W, Fang Y, Yang D, Wang L, Zhou H, Wang J. Bortezomib attenuates acute graft-vs.-host disease through interfering with host immature dendritic cells. *Exp Hematol* 2011; 39:710-720.
19. Sun K, Welniak LA, Panoskaltsis-Mortari A, O'Shaughnessy MJ, Liu H, Barao I, Riordan W, Sitcheran R, Wysocki C, Serody JS, Blazar BR, Sayers TJ, Murphy WJ. Inhibition of acute graft-versus-host disease with retention of graft-versus-tumor effects by the proteasome inhibitor bortezomib. *Proc Natl Acad, Sci U S A* 2004; 101:8120-8125.
20. Nencioni A, Grünebach F, Patrone F, Ballestretro A, Brossart R. The proteasome and its inhibitors in immune regulation and immune disorders. *Critical Reviews in Immunology* 2006; 26:487-497.
21. Al-Homsi AS, Lai Z, Roy T, Kouttab N. Effect of novel proteasome inhibitors on dendritic cell maturation, function, and expression of IκB and NF-κB. *Transpl Immunol* 2013, 29:1-6.
22. Al-Homsi As, Lai Z, Roy TS, Al-Malki MM, Koutab N, Junghans RP. Post-transplant cyclophosphamide and bortezomib inhibit dendritic cell maturation and function and alter their IκB and NF-κB expression. *Transpl Immunol* 2014; 30:40-45.
23. Sun K, Wilkins DEC, Anver MR, Sayers TJ, Panoskaltsis-Mortaari A, Blazar BR, Welniak LA, Murphy WJ. Differential effects of proteasome inhibition by bortezomib on murine acute graft-versus-host disease (GVHD): delayed administration of bortezomib results in increased GVHD-dependent gastrointestinal toxicity. *Blood* 2005, 106:3293-3299.
24. Al-Homsi AS, Feng Y, Duffner U, Al-Malki MM, Goodyke A, Cole K, Muilenburg M, Mageed AA. Bortezomib for the prevention and treatment of graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. *Exp Hem* 2016; 44: 771-777.
25. Al-Homsi As, Goodyke A, McLane M, Abdel-Mageed S, Cole K, Muilenburg M, Feng Y. Post-transplant cyclophosphamide and ixazomib combination rescues mice subjected to experimental graft-versus-host disease and is superior to either agent alone. *BBMT* 2016;23:255-261.
26. Al-Homsi As, Goodyke A, Cole K, Muilenburg M, McLane M, Abdel-Mageed S, Feng Y. Ixazomib Suppresses human dendritic cell and modulates murine graft-versus-host disease in a schedule-dependent fashion. *Exp Hem* 2106; 48:50-57.
27. Koreth J, Stevenson KE, Kim HT, McDonough SM, Bindra B, Armand P, et al. Bortezomib-based graft-versus-host disease prophylaxis in HLA-mismatched unrelated donor transplantation. *J Clin Oncol* 2012; 30:3202-3208.
28. Koreth J, Kim HT, Lange PB, Poryanda SJ, Reynolds CG, Rai SC, Armand P, Cutler CS, Ho VT, Glotzbecker B, Yusuf R, Nikiforow S, Chen YB, Dey B, McMasters M, Ritz J, Blazar BR, Soiffer RJ, Antin JH, Alyea EP. Bortezomib-based immunosuppression after reduced-intensity conditioning

hematopoietic stem cell transplantation: randomized phase II results. *Haematologica* 2018; 103:522-530.

29. Kupperman E, Lee EC, Cao Y, Bannerman B, Fitzgerald M, Berger A, et al. Evaluation of the Proteasome Inhibitor MLN9708 in Preclinical Models of Human Cancer. Cancer Research. 2010; 70:1970-1980.
30. Al-Homsi AS, Cole K, Bogema M, Duffner U, Williams S, and Mageed A. Short course of post-transplant cyclophosphamide and bortezomib for graft-versus-host disease prevention after allogeneic peripheral blood stem cell transplantation is feasible and yields favorable results: a phase I study. *BBMT* 2015; 21:1315-1320.
31. Al-Homsi AS, Cole K, Muilenberg M, Duffner U, Abidi M, Willaims S, Mageed A. Phase I-II study of calcineurin inhibitor- and mTOR inhibitor-free post-transplant cyclophosphamide and bortezomib (CyBor) for the prevention of graft-versus-host disease. *BBMT* 2017; 23: 1651-1657.
32. Mohty M. A bit of antithymocyte globulins can take you a long way! *BBMT 2012; 47:617-618.*
33. Finke J, Bethge WA, Schomoor C, Ottinger HD, Steljes M, Zander AR, Volin L, Ruutu T, Heim DA, Schwerdfeger R, Kolbe K, Mayer J, Maertens J, Linkesch W, Holler E, Koza V, Bornhäusser M, Einsele H, kolb HJ, Bertz H, Egger M, Socié G, . Standard graft-versus-host disease prophylaxis with or without anti-T cell globulin in haematopoietic cell transplantation from HLA-matched unrelated donors: a randomized, open-label, multicenter phase 3 trail. *Lancet Oncol* 2009; 10:855-864.
34. Mohty M, Labopin M, Balere ML, Scoié G, Milpied N, Tabrizi R, Ifrah N, Hicheri Y, Dhedin N, Michallet M, Buzyn A, Cahn JY, Bourhis JH, Blaise D, Raffoux C, Espérou H, Yakoub-Agha I. Antithymocyte globulins and chronic graft-vs-host disease after myeloablative allogeneic stem cell transplantation from HLA-matched unrelated donors: a report from the Societe Francaise de Greffe de Moelle et de Therapie Cellulaire. *Leukemia* 2010; 24: 1867-1874.
35. Kumar A, Mhaskar AR, Reljic, Mhaskar RS, Hharfan-Dabaja MA, Anasetti C, Mohty M, Djulbegovic B. Antithymocyte globulin for acute graft-versus-host disease prophylaxis in patients undergoing allogeneic hematopoietic cell transplantation: a systematic review. *Leukemia* 2012; 26:582-588
36. Soiffer RJ, Lerademacher J, Ho V, Kan F, Artz A, Champlin RE, Devine S, Isola L, Lazarus HM, Marks DI, Porter DL, Waller EK, Horowitz MM, Eapen M. Impact of immune modulation with anti-T cell antibodies on the outcome of reduced-intensity allogeneic hematopoietic stem cell transplantation for hematologic malignancies. *Blood* 2011; 117: 6963-6970.
37. Devellier R, Crocchiolo R, Castagna, Fürst S, El Chiekh J, Faucher C, Prebet T, Etienne A, Chabannon C, Vey N, Estemi B, Blaise D. The increase from 2.5 to 5 mg/kg of rabbit anti-thymocyte globulin dose in reduced-intensity conditioning reduces acute and chronic GvHD for patients with myeloid malignancies undergoing all-SCT. *BMT* 2012; 47: 639-645.

17 APPENDICES

17.1 Appendix A: Required Study Activities
17.2 Appendix B: Acute Graft-Versus-Host Disease Assessment (aGVHD)
17.3 Appendix C: Chronic Graft-Versus-Host Disease Assessment (cGVHD)
17.4 Appendix D: New York Heart Association (NYHA) Classification of Cardiac Disease
17.5 Appendix E: Busulfan Pharmacokinetics Requisition

17.1 APPENDIX A: REQUIRED STUDY ACTIVITIES

Activity	Pre-Study[1]	Day -7 to Day -1[2]	Day 0	Day +3	Day +4	Daily until Neutrophil Engraftment	Daily until discharge	Twice a week until Engraftment	Weekly until Day +100 (+/- 3 days)	Monthly until Day +180 (+/- 7 days)	Every 3 months until Day+730 (2 years) (+/- 7days)
Study team procedures											
Consent	X										
Medical History	X										
Physical Exam	X	X	X			X	X		X	X	X
Height	X										
Weight	X	X									
Vital Signs	X	X				X	X		X	X	X
Cychlophophamide[3]				X	X						
Bortezomib[4]			X	X							
rATG[5]		X									
Toxicity Assessment		X	X			X	X		X	X	X
GVHD Assessment						X	X		X	X	X
Chimerism[6]						X				X	X
Immune Reconstitution Samples[7]											
Laboratory Assessments											
CBC,CMP	X	X	X			X			X	X	X
LFTs		X	X					X	X	X	X

[1]Pre-transplant: per standard of care
[2]Pre-tranplant: collect CBC, CMP, liver function tests, and physical exam, including toxicity assessments
[3] Cyclophosphamide administered Day +3 and Day +4
[4] Bortezomib administered on Day 0 (6 hours after graft infusion) and 72 hours thereafter
[5]rATG administered Days -4, -3, and -2
[6]Post-chimerism: at neutrophil engraftment, then at least monthly (+/- 7 days) until day +180, and every 3 months (+/- 7 days) until day +730.
[7]Blood samples for immune reconstitution will be collected on days +30, +100, +180, and +365 (+/- 7 days) after transplant.

17.2 APPENDIX B: ACUTE GRAFT VERSUS HOST DISEASE ASSESSMENT (aGvHD)

Clinical staging of aGVHD			
Stage	**Skin**	**Liver (bilirubin)**	**Gastro-intestinal tract**
0	No rash	< 2.0 mg/dL	Diarrhea ≤ 500 ml/day
1	Maculopapular rash < 25% of body surface	2.0-3.0 mg/dL	Diarrhea 501-1000 ml/day or nausea with biopsy proof
2	Maculopapular rash 25-50% body surface	3.1 – 6.0 mg/dL	Diarrhea 1001-1500 ml/day
3	Generalized erythroderma	6.1 – 15 mg/d	Diarrhea > 1500 ml/day
4	Generalized erythro-derma with blister or bullous formation and desquamation	>15 mg/dL	Severe abdominal pain without or with

Overall Grade aGvHD			
	Skin	**Liver**	**Gut**
0 none	0	0	0
1 mild	1-2	0	0
2 moderate	1-3	1	1
3 severe	2-3	2-3	2-3
4 life threatening	2-4	2-4	2-4

17.3 APPENDIX C: CHRONIC GRAFT VERSUS HOST DISEASE ASSESSMENT (cGVHD)

The involved organs are scored as follows:

KPS: Karnofsky's performance status. BSA: body surface area. GI: gastrointestinal. LFT: liver function tests. AP: alkaline phosphatase. SOB: shortness of breath. LFS: lung function score (FEV1 and DLCO are converted into numeric scores and added to each other to get the LFS: > 80% = 1, 70-79% = 2, 60-69% = 3, 50-59% = 4, 40-49% 5, < 40% 6). ADL: activities of daily life.

	Score			
Organ	**0**	**1**	**2**	**3**
KPS	100%	80-90%	60-70%	< 60%
Skin	No symptoms	< 18% BSA	19-50% BSA or sclerotic features but able to pinch	> 50% BSA or sclerotic changes and unable to pinch or with impaired mobility
Mouth	No symptoms	Not limiting oral intake significantly	Limiting oral intake partially	Limiting oral intake severely
Eyes	No symptoms	Dryness, requiring eye-drops ≤ 3/day or asymptomatic keratoconjunctivitis sicca (KCS)	Dryness, requiring eye-drops > 3/day without visual impairment	Unable to work because of ocular symptoms or vision loss
GI tract	No symptoms	< 5% weight loss	5-15% weight loss	> 15% weight loss requiring calorie supplements or esophageal dilation
Liver	Normal LFT	Elevated bilirubin, AP, AST, or ALT < 2 X normal	Elevated bilirubin, AP, AST, or ALT 2-5 X normal	Elevated bilirubin, AP, AST, or ALT . 5 X normal
Lungs	No symptoms, FEV1 > 80% or LFS 2	SOB after 1 flight of steps FEV1 60-70% or LFS 3-6	SOB walking flat, FEV1 40-59% or LFS 6-9	SOB at rest or requiring O_2, FEV1 ≤ 39% or LFS 10-12
Joints and fascia	No symptoms	Mild decrease in range of motion not affection ADL	Moderate decrease in range of motion with moderate impairment of ADL	Significant de-crease in range of motion with significant impairment of ADL
Genital tract	No symptoms	Symptoms and with mild signs on exam and no effect on coitus	Symptoms and with moderate signs on exam with discomfort on coitus	Symptoms and with advanced signs on exam such as strictures or ulcerations with severe pain on coitus

The following is checked and scored (0-3) as applicable:

Esophageal stricture or web
Pleural effusions
Pericardial effusion
Ascites
Platelets < 100 109/L
Eosinophils > 0.5 109/L
Peripheral neuropathy
Polymyositis
Myasthenia gravis
Coronary artery disease
Cardiac conduction defects
Cardiomyopathy

Based on the organ scoring the severity of cGVHD is defined as follows:
Mild cGVHD: involvement of 1-2 organs with maximum scores of 1 in all affected organs or sites.
Moderate cGVHD: 1. involvement of 1-2 organ involvement with a maximum score of 2 in any affected organ or site (except for lungs). 2. involvement of 3 or more organs with maximum score of 1 in all affected organs or sites.
Severe cGVHD: a lung score of 2 or a score 3 in any organ or site.

17.4 APPENDIX D: NEW YORK HEART ASSOCIATION CLASSIFICATION OF CARDIAC DISEASE

The following table presents the NYHA classification of cardiac disease.

Class	Functional Capacity	Objective Assessment
I	Patients with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.	No objective evidence of cardiovascular disease.
II	Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of minimal cardiovascular disease.
III	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of moderately severe cardiovascular disease.
IV	Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.	Objective evidence of severe cardiovascular disease.

Source: The Criteria Committee of New York Heart Association. Nomenclature and Criteria for Diagnosis of Diseases of the Heart and Great Vessels. 9th Ed. Boston, MA: Little, Brown & Co; 1994:253-256.

17.5 APPENDIX E: BUSULFAN PHARMACOKINETICS REQUISITION

Please complete, print and submit. Reset Form

MAYO CLINIC
Mayo Medical Laboratories

Busulfan Information: Mail-In Specimen Instructions

Questions: Phone 800-533-1710.
International clients: + 1 507 266 5700 or email mmlglobal@mayo.edu

Patient Information

Patient Name	Birth Date (Month dd, yyyy)	Body Weight (kg)

Dosage Information

Dosage administration date (Month dd, yyyy)
Dosage (mg q 6 hr)

Specimen Collection Times

Infusion start time	:	☐ a.m. ☐ p.m.
Infusion stop time/Collect immediate post infusion specimen - BU2H, DOSE (typically 2 hours after infusion start)	:	☐ a.m. ☐ p.m.
Second specimen draw time	:	☐ a.m. ☐ p.m.
Third specimen draw time	:	☐ a.m. ☐ p.m.
Fourth specimen draw time	:	☐ a.m. ☐ p.m.

BUAUC / Busulfan, Intravenous Dose, Area Under the Curve (AUC), Plasma

Four plasma specimens are required for this test

1. The first specimen (BU2H, DOSE) should be drawn immediately after termination of an intravenous infusion of 0.8 mh/kg busulfan. Additional specimens should also be drawn at 1 hour (BU3H), 2 hours (BU4H), and 4 hours (BU6H) after termination of infusion.
2. Draw blood in a green top (sodium heparin) tube. Spin down and send 1 mL of sodium heparin plasma frozen in plastic vial.
3. Label each specimen appropriately (exact time of draw).
4. Complete all sections of form.
5. Send all 4 specimens and form together under 1 order.

Print

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Summary of Changes:

Summary of Changes from Version 1.0 dtd 2019 Apr 26 to Version 1.1 dtd 2019 Aug 27

Section 7.1.1.2	**Busulfan can be given q6 added.**