A Study of Granix to Disrupt the Bone Marrow Microenvironment in Patients with Multiple Myeloma Undergoing Autologous Stem Cell Transplantation

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Protocol#: 201405057
Teva Protocol#: TV44688-ONC-60114
Version Date: 06/19/2017

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Study Drug(s): tbo-filgrastim (Granix)
Melphalan (Alkeran)

IND#: 122470
clinicaltrials.gov #: NCT02112045

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Protocol Revision History

Initial Approval Version	**05/14/2014**
Amendment 1	**08/12/2014**
Amendment 2 (PRMC approved only)	**01/07/2015**
Amendment 2	**01/08/2015**
Amendment 3	**02/06/2015**
Amendment 4	**04/28/2015**
Amendment 5	**05/27/2015**
Amendment 6	**01/12/2016**
Amendment 7	**06/19/2017**

STUDY SCHEMA

Overview

Patients will be randomized (1:1) to receive either Granix & HDM or HDM alone for ASCT conditioning. Granix will be administered Day -7 through Day -2 at a dose of 480 or 960 mcg/day (based on actual body weight). Melphalan will be administered on Day -2 at a dose of 140 or 200 mg/m^2 .

Experimental Arm

Day -7 Granix (480 or 960 mcg)
Day -6 Granix (480 or 960 mcg)
Day -5 Granix (480 or 960 mcg)
Day -4 Granix (480 or 960 mcg)
Day -3 Granix (480 or 960 mcg)
Day -2 Granix (480 or 960 mcg) Melphalan (140 or 200 mg/m^2)
Day -1 Rest Day
Day 0 Stem Cell Infusion

Control Arm

Day -2 Melphalan (140 or 200 mg/m^2)
Day -1 Rest Day
Day 0 Stem Cell Infusion

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1.0 BACKGROUND AND RATIONALE

1.1 High Dose Melphalan Followed by Autologous Stem Cell Rescue in Multiple Myeloma

Multiple myeloma (MM) is a plasma cell neoplasm arising from clonal expansion of plasma cells in the bone marrow. The disease is characterized by the presence of a serum or urine monoclonal protein which can be used to follow disease progression, and patients with MM are at risk of complication from lytic skeletal lesions, renal failure, hypercalcemia, anemia, and recurrent infection due to aberrant immune function. MM is the second most common hematologic malignancy in the United States with over 19,000 new cases in 2010 and nearly 10,000 deaths.[1] In recent years, several novel agents have been approved to treat MM, such as proteosome inhibitors and immunomodulatory agents, with dramatic improvements in response and overall survival. Nevertheless, outside of allogeneic stem cell transplantation, the disease is still considered incurable in most cases and new treatment strategies are urgently needed.

High dose melphalan (HDM) followed by autologous hematopoietic stem cell transplant (ASCT) has been a mainstay of MM treatment for many years and currently is considered standard of care for patients who are transplant candidates. Transplantation may be performed either as salvage after relapse or as consolidation therapy after initial treatment. In the latter setting, the initial therapy to reduce disease burden is chosen to minimize and effects on the ability to harvest stem cells. Subsequently, stem cells are mobilized using G-CSF alone or in combination with other mobilizing agents, stored, and reinfused following treatment with HDM (200mg/m^2).

The efficacy of HDM has been demonstrated in a number of prospective clinical trials. The Myeloma VII trial randomized 401 patients to HDM plus ASCT versus standard dose chemotherapy and found improved complete response (CR) (44% vs 8%), with improvements in both progression-free survival (PFS) and overall survival (OS).[2] Similarly, the Intergroupe Francais du Myelome randomized 200 patients with intermediate to high stage myeloma to HDM plus ASCT versus standard chemotherapy and found improved CR (22% versus 5%) as well as improved OS.[3] Treatment-related mortality was similar in each group. While no prospective trials have compared HDM plus ASCT to standard therapy alone in the era of novel agents, HDM plus ASCT remains the standard of care with more than 5,300 transplants performed in 2012.[4]

Given the effectiveness of HDM in this setting, several studies have examined whether adding other active drugs to the conditioning regimen would result in better outcomes. Unfortunately, to date these attempts have either not demonstrated improved efficacy or have added unacceptable toxicity. Allegre et al reported a single-arm phase II study of busulfan plus melphalan, showing an improved CR rate compared to historical controls

but at the cost of increased mucositis and hematologic toxicity.[5] Similarly, adding total body irradiation to melphalan does not improve CR rate but significantly increases mucositis.[6] Finally, several retrospective studies have failed to show an advantage to adding agents such as cyclophosphamide, thiotepa, or idarubicin to HDM in the setting of ASCT.[7-9] Of note, these trials primarily enrolled younger, fitter patients, while "real world" transplant centers face increasing numbers of patients aged 65 or older with significant co-morbidities.[10] There is considerable interest, therefore, in improving transplant protocols to improve efficacy without increasing toxicity.

1.2 The Multiple Myeloma Microenvironment

Besides hematopoietic cells, the bone marrow microenvironment—collectively termed "stroma"—is composed of non-hematopoietic elements such as osteoblasts and osteoblast lineage cells, osteoclasts, endothelial cells, CXCL12-abundant reticular cells (CAR cells), and the extracellular matrix (ECM). A growing number of pre-clinical studies suggest that this microenvironment plays a key role in supporting the proliferation and survival of MM cells (reviewed in[11]). For example, MM cells adhere to ECM and stromal cells via integrins such as VLA-4 and these interactions promote cell cycling and antiapoptotic pathways in MM cells.[12] Similarly, interactions between Notch expressed on MM cells and Notch ligand presented by stromal cells leads to activation of pathways promoting MM cell survival and proliferation.[13] Other pathways implicated in MM cell/stromal cell signaling include P-selectin/PSGL-1 interactions, the Hedgehog pathway, and the WNT pathway. [14-16] These findings suggest that physical proximity of MM cells to the bone marrow stromal elements provides cues for the growth and survival of MM cells.

Besides these cell-cell and cell-ECM interactions, the bone marrow microenvironment provides a rich supply of cytokines that support MM cell proliferation and survival. IL-6 is a pro-inflammatory cytokine that acts as a potent mitogen for myeloma cells in vitro and in vivo.[17] Elevated serum IL-6 levels are associated with poor prognosis in patients with MM, and treatment with anti-IL-6 neutralizing antibody leads to decreased tumor burden in animal models of MM.[18] Besides IL-6, numerous other cytokines and growth factors have been implicated in MM survival and progression, including IGF-1 and VEGF.[19, 20]

Of note, several factors that support normal B lymphopoiesis also appear to play a role in the growth of malignant MM cells in the bone marrow. B-cell activating factor (BAFF) is a cytokine necessary for normal B cell development that is upregulated in patients with MM. BAFF signaling induces NF-κB, PI-3/AKT and MAPK pathways in MM cells, promoting proliferation and survival.[21, 22] Similarly, another key regulator of B lymphocyte development, IL-7, activates the oncogene MUC1 in MM cells, which in turn leads to activation of growth signals through activation of the beta catenin pathway. [23] Finally, the chemokine stromal derived factor 1 (SDF-1, CXCL12) is a chemokine highly expressed

by CAR cells and osteoblasts in the bone marrow that plays a key role in B-cell survival primarily by promoting retention of B lineage precursors in the bone marrow.[24, 25] Blocking SDF-1 signaling leads to MM cell release from the bone marrow as well as disruption of MM cell homing to the marrow.[26]

Taken together, these findings suggest a model wherein MM cells co-opt normal pro-survival signaling mechanisms present in the bone marrow microenvironment for their own survival and growth. This raises the possibility that pharmacologic targeting of MM cell-stromal interactions may deprive MM cells of pro-survival signals and sensitize MM cells to cytotoxic agents.

1.3 G-CSF Treatment Leads to Remodeling of the Bone Marrow Microenvironment

G-CSF is a glycoprotein member of the hematopoietic growth factor family that is the principal regulator of granulopoiesis. Clinically, G-CSF is widely used to shorten the duration of neutropenia following chemotherapy and to mobilize hematopoietic stem cells prior to transplant. The mechanisms of G-CSF-induced stem cell mobilization have not been fully elucidated, but it appears to function at least in part by modifying the bone marrow microenvironment, disrupting key physical and signaling interactions between bone marrow stromal cells and both normal hematopoietic progenitors as well as malignant cells, promoting their egress to the peripheral blood (reviewed in[27]). For instance, G-CSF treatment leads to downregulation of SDF-1 in the bone marrow, which results in loss of a key retention signal for hematopoietic cells and their subsequent egress.[28, 29]

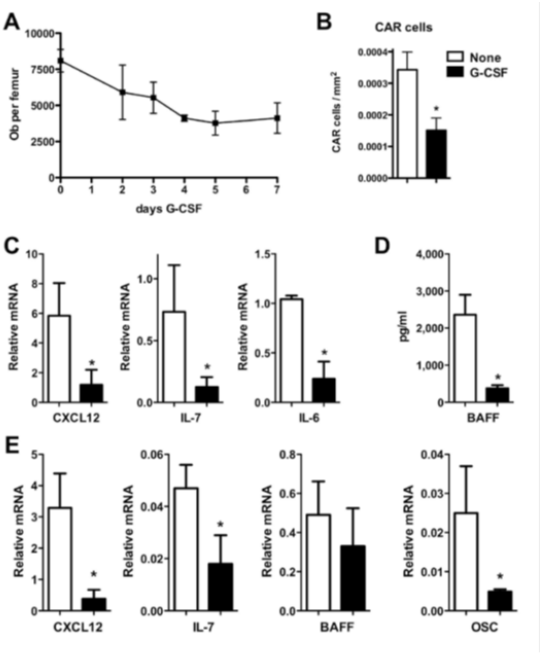


Figure 1. Alteration in the bone marrow microenvironment by G-CSF. **A). C57Bl/6 mice were treated with G-CSF for the indicated period of time and the number of mature osteoblasts (Ob) determined by histomorphometry. B) Mice were treated with G-CSF for 5 days and the number of CAR cells (per mm of trabecular bone surface) measured by histomorphometry. C). Bone marrow RNA was isolated from the bone marrow and real time RT-PCR for the indicated gene was performed. Expression relative to β-actin mRNA is shown. D) Bone marrow was harvested, cells pelleted, and ELISAs for BAFF protein performed on the extracellular fluid. E) Bone marrow core biopsies were obtained from 3 healthy volunteers and from 3 healthy donor for allogeneic stem cell transplantation treated with G-CSF (5 μg/kg) for 4 days. Real time RT-PCR for the indicated gene was performed. RNA expression relative to β-actin mRNA is shown. Data represent the mean ± SEM. *P < 0.05.**

Preclinical studies have shown that mobilization of malignant cells from the bone marrow sensitizes them to chemotherapy, presumably through the loss of pro-survival signals provided by the bone marrow microenvironment. For instance, Nervi et al. used AMD3100, a small molecule inhibitor of SDF-1 signaling, to mobilize leukemic cells in a mouse model of APL and showed enhanced killing with cytotoxic chemotherapy in mobilized cells.[30] Zeng et al. treated leukemic mice with G-CSF to mobilize blasts and similarly found increased sensitivity to chemotherapy.[31] Currently, several clinical trials are underway to test this approach in MDS and acute leukemias.[30-32] Of note, Azab et al. showed that disruption of MM cell-stromal cell interaction by treatment with AMD3100 led to increased sensitivity to chemotherapy in a small animal xenograft model of MM.[33] Together, these findings raise the possibility that G-CSF-induced mobilization of myeloma cells could sensitize them to chemotherapy by physically disrupting pro-survival signals from stromal cells in the bone marrow microenvironment.

In addition to mobilizing myeloma cells from a protective bone marrow niche, preclinical data from our group suggests that G-CSF treatment could lead to overall reduction of pro-survival signals for myeloma cells in the bone marrow. G-CSF treatment causes a transient remodeling of the bone marrow microenvironment to suppress the lymphoid compartment, including plasma cells. G-CSF treatment targets certain bone marrow stromal cells to suppress their production of factors that support lymphopoiesis and plasmablast maintenance. Specifically, G-CSF targets CXCL12-abundant reticular (CAR) cells and osteoblasts (Figure 1A-B), both of which have been implicated in B lymphopoiesis [25, 34]. Expression of CXCL12, c-kit, IL-6, IL-7, and insulin-like growth factor-1 is markedly suppressed by G-CSF treatment in CAR cells and osteoblasts (Figure 1C-E). The loss of these supportive signals may lead to decreased myeloma cell survival and increased sensitivity to chemotherapy. Indeed, in mice, G-CSF treatment results in an 8-fold reduction of plasmablasts in the bone marrow (Figure 2).

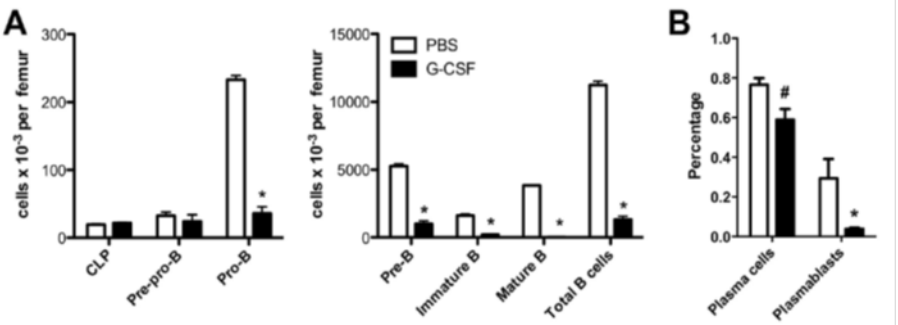


Figure 2. B cell suppression by G-CSF in mice. **C57Bl/6 mice were treated with G-CSF (125 µg/kg twice daily) for five days, bone marrow cell harvested and analyzed by flow cytometry for the indicated B cell population. The absolute number (A) or percentage (B) of the indicated cell population in the femur is shown. Data represent the mean ± SEM. *P < 0.001; #P < 0.05. CLP: common lymphoid progenitor.**

1.4 Granix

The development of recombinant G-CSF has allowed for its widespread clinical use in the settings described above. Until recently, the most commonly used recombinant G-CSF have been Filgrastim, manufactured using an Escherichia Coli expression system, and lenograstim, which is derived from Chinese hamster ovary cells. Granix (also known as as Biograstim, Filgrastim ratiopharm, and Ratiograstim) has been FDA approved for use in febrile neutropenia prophylaxis after chemotherapy. It has been shown to have equivalent efficacy and safety to the most commonly used recombinant G-CSF, filgrastim, in numerous *in vitro*, *in vivo*, and pharmacokinetic studies.[35-39]

1.5 Rationale for Study

In the era of novel agents such as proteasome inhibitors and immunomodulatory agents, overall survival in MM has improved dramatically. Nevertheless, cures are rare and

patients are typically managed with a succession of treatments designed to decrease disease burden and improve survival. HDM followed by ASCT remains a potent tool in this regard; however, attempts to improve upon HDM by adding other active agents to transplant protocols have largely resulted in unacceptable increases in toxicity. Current evidence suggests a major role for interactions between MM cells and the local bone marrow microenvironment in promoting expansion and survival of MM cells in the bone marrow. G-CSF treatment causes the profound, transient changes in the bone marrow microenvironment that result in loss of many of the local signals that are postulated to support MM cell survival. We hypothesize, therefore, that G-CSF will sensitize MM cells to HDM in the setting of ASCT, resulting in improved response rates while not increasing transplant-related toxicity. We propose to test this hypothesis with a single center, phase II randomized study to test the safety and efficacy of the recombinant G-CSF Granix plus HDM versus HDM alone prior to ASCT.

2.0 OBJECTIVES

2.1 Primary Objective

To compare the complete response rate (CR+sCR) at day 100 of patients treated with Granix + HDM followed by ASCT to patients treated with HDM alone followed by ASCT.

2.2 Secondary Objectives

1. To compare the toxicity of Granix + HDM to HDM alone.

2. To compare the overall response rate (CR+sCR+VGPR+PR) at day 100 of patients treated with Granix + HDM followed by ASCT to patients treated with HDM alone followed by ASCT.

3. To compare the overall survival of patients treated with Granix + HDM followed by ASCT to patients treated with HDM alone followed by ASCT.

4. To compare the progression-free survival of patients treated with Granix + HDM followed by ASCT to patients treated with HDM alone followed by ASCT.

5. To compare the rate of neutrophil engraftment of patients treated with Granix + HDM followed by ASCT to patients treated with HDM alone followed by ASCT.

6. To compare the rate of platelet engraftment of patients treated with Granix + HDM followed by ASCT to patients treated with HDM alone followed by ASCT.

2.3 Exploratory Objective

To describe the biological effects of Granix on the bone marrow and changes in the bone marrow cytokine and chemokine levels. This will include:

a. Quantification of marrow osteoblasts and CAR cells
b. Measurement of SDF-1 (CXCL12), IL-6 and BAFF
c. Assessment of myeloma cell proliferation and survival in the bone marrow.
d. Assessment of myeloma cell mobilization into the blood by G-CSF

3.0 PATIENT SELECTION

3.1 Inclusion Criteria

1. Symptomatic multiple myeloma requiring treatment

2. Received at least two cycles of any regimen as initial systemic therapy for multiple myeloma and are within 2-12 months of the first dose of initial therapy

3. At least 18 years of age

4. Adequate autologous stem cell collection, defined as an unmanipulated, cryopreserved, peripheral blood stem cell collection containing at least 2×10^6 CD34+ cells/kg based on patient body weight.

5. Adequate organ function as measured by:
   a. Cardiac function: Left ventricular ejection fraction at rest $\geq$40%
   b. Hepatic function: Bilirubin $\leq 2 \times$ ULN and aspartate amino transferase/alanine amino transferase (AST/ALT) $\leq 3 \times$ ULN
   c. Renal function: Creatinine clearance $\geq$40 mL/minute (measured or calculated/estimated)
   d. Pulmonary function: Carbon monoxide diffusing capacity (DLCO; corrected for hemoglobin [Hgb]), forced expiratory volume in 1 second (FEV1), forced expiratory vital capacity (FVC) $\geq$50% of predicted value
   e. Oxygen saturation $\geq$92% on room air

6. Eastern Cooperative Oncology Group (ECOG) performance status 0, 1, or 2 (see Appendix A)

7. Able to understand and willing to sign an IRB-approved written informed consent document

3.2 Exclusion Criteria

1. Evidence of multiple myeloma disease progression (as defined by IMWG) any time prior to ASCT
2. Prior stem cell transplant (autologous or allogeneic)
3. Smoldering MM not requiring therapy
4. Plasma cell leukemia
5. Systemic amyloid light chain amyloidosis
6. Active bacterial, viral, or fungal infection
7. Seropositive for human immunodeficiency virus (HIV)
8. Known, active hepatitis A, B, or C Infection
9. Pregnant or breastfeeding.
10. Receiving other concurrent anticancer therapy (including chemotherapy, radiation, hormonal treatment, or immunotherapy, but excluding corticosteroids) within 7 days prior to the ASCT or planning to receive any of these treatments prior to the last study visit on Day +100.
11. Hypersensitive or intolerant to any component of the study drug(s) formulation
12. Receiving growth factors (filgrastim, XM02-filgrastim, peg-filgrastim, plerixafor, etc) or undergoing apheresis < 7 days prior to the start of treatment on protocol (Day -7).

3.3 Inclusion of Women and Minorities

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

4.0 REGISTRATION PROCEDURES

Patients must not start any protocol intervention prior to registration through the Siteman Cancer Center.

The following steps must be taken before registering patients to this study:

1. Confirmation of patient eligibility
2. Registration of patient in the Siteman Cancer Center OnCore database
3. Assignment of unique patient number (UPN)

4.1 Confirmation of Patient Eligibility

Confirm patient eligibility collecting the information listed below:

1. The registering MD's name
2. Patient's race, sex, and DOB
3. Three letters (or two letters and a dash) for the patient's initials
4. Copy of signed consent form
5. Completed eligibility checklist, signed and dated by a member of the study team
6. Copy of appropriate source documentation confirming patient eligibility

4.2 Patient Registration in the Siteman Cancer Center OnCore Database

All patients must be registered through the Siteman Cancer Center OnCore database.

4.3 Assignment of UPN

Each patient will be identified with a unique patient number (UPN) for this study. All data will be recorded with this identification number on the appropriate CRFs.

4.4 Randomization

Eligible and consenting patients will be randomized on a 1:1 basis to receive either HDM + Granix followed by ASCT or HDM alone followed by ASCT. Patients will be stratified based on response status at randomization (CR/VGPR v. PR/SD).

Randomization will be in blocks of random size. The randomization table will be uploaded in our REDCap system. Randomization will occur via an online form with entry of the patient ID number and stratum information. Once all information is entered, randomization is carried out via a submit button through REDCap. The randomization scheme will be created using a formal probability model implemented in SAS (version 9.3 or higher).

5.0 TREATMENT PLAN

5.1 Overview

Patients will be randomized (1:1) to receive either Granix plus HDM or HDM alone for ASCT conditioning. Patients randomized to receive Granix will be dosed daily on Days -7 through -2 at a dose of 480 or 960mcg/day (dependent on weight). Melphalan will be given to all patients on Day -2 at a dose of 140 or 200mg/m^2.

5.2 Autologous Stem Cell Mobilization and Collection

Patients will undergo autologous stem cell mobilization following institutional guidelines prior to study entry. To prevent the potentially confounding effect of growth factors, stem cell mobilization and apheresis must be performed > 7 days prior to the start of treatment on protocol (Day -7).

5.3 Agent Administration

5.3.1 Granix

Granix will be administered subcutaneously (SQ) starting on Day -7 and continuing through Day -2 (6 total doses). Granix is supplied in single-use syringes with 480 mcg of drug. Patients who weigh less than or equal to 100 kg will receive a dose of 480 mcg per day; patients who weigh more than 100 kg will receive a dose of 960 mcg per day. Granix dose should be calculated on using actual weight from the screening visit; however, the dose should be recalculated if the patient's weight changes by more than 10% at the Day -7 visit. On Day -2 Granix administration should occur at least approximately 1 hour prior to melphalan administration.

5.3.2 Melphalan

Melphalan will be administered intravenously (IV) via a central venous catheter over approximately 30 minutes on Day -2 at a dose of 140 or 200 mg/m^2. Full dose melphalan (200mg/m^2) should be administered to all patients unless contradicted due to advanced age, renal disease, etc.

For the calculation of BSA, actual body weight should be used for patients who weigh less than 100% of their ideal body weight (IBW). Patients who weigh 100-120% of their IBW, BSA should be calculated based on IBW. For patients who weigh more than 120% of their IBW, BSA should be calculated based on corrected

body weight (CBW). Formulas for calculating IBW and CBW are provided in Appendix B.

5.3.3 Dose Modifications and Delays

5.3.3.1 Granix

Blood counts will be monitored on Day -7 and Day -2 while on study. Granix should be held if WBC ≥ 100,000 /µl. Once WBC improves to < 100,000 /µl, Granix dosing may be resumed.

For grade 3 or 4 non-hematologic toxicity related to Granix, hold Granix and monitor daily. Once toxicity resolves to ≤ grade 2, Granix dosing may be resumed.

Granix dose schedule should not be altered for reasons other than toxicity. Missed doses for any reason, including toxicity, will not be made up.

5.3.3.2 Melphalan

Melphalan doses may be delayed for adverse events at the discretion of the treating physician with consultation of the Principal Investigator. Delayed doses can be made up.

5.3.4 Pharmaceutical Information

5.3.4.1 Granix

Granix is a recombinant methionyl human granulocyte colony stimulating factor. It will be provided for study use by Teva Pharmaceuticals.

5.3.4.2 Melphalan

Melphalan, an alkylating agent, is a phenylalanine derivative of nitrogen mustard. It will be sourced from commercial supply. Prior to administration, melphalan will be diluted with 250mL of normal saline, to a final concentration of less than or equal to 2 mg/mL.

5.4 Stem Cell Transplantation

Autologous peripheral blood stem cells (≥ $2x10^6$ CD34+ cells/kg) will be given per institutional guidelines on Day 0.

5.5 General Concomitant Medication and Supportive Care Guidelines

Starting on Day +7, filgrastim (Neupogen) will be administered daily SC at a dose of 5mcg/kg/day until neutrophil engraftment, per institutional guidelines.

Supportive treatment, including anti-emetics, hydration, transfusions, and antibiotics will be per discretion of the treating physician.

5.6 Women of Childbearing Potential

Women of childbearing potential (defined as women with regular menses, women with amenorrhea, women with irregular cycles, women using a contraceptive method that precludes withdrawal bleeding, and women who have had a tubal ligation) are required to have a negative serum pregnancy test within 14 days prior to Day -7.

Female and male patients (along with their female partners) are required to use two forms of acceptable contraception, including one barrier method, during participation in the study and for 1 month following the administration of study treatment.

5.7 Consolidation/Maintenance Therapy

To prevent confounding effects, consolidation or maintenance therapy after transplant is not permitted for the duration of the study, through the Day +100 visit. Any patient who begins consolidation or maintenance therapy prior to the Day +100 visit will be removed from the study.

6.0 SCHEDULE OF ASSESSMENTS

Table 1. Overview of Study Activities	Screening[1]	Treatment								D+30 (+/-7)	D+100 (+/-21)	F/U[2]
		D-7	D-6	D-5	D-4	D-3	D-2	D-1	D 0			
Informed Consent	X											
Medical History	X											
Physical Exam including ECOG PS	X									X	X	X[7]
CBC w/ diff	X	X[9]					X	X	X[3]			
Chemistry[4]	X									X	X	
SPEP and immunofixation	X									X	X	
Serum free light chains	X									X	X	
24 Hour Urine (Total Protein, UPEP and immunofixation)	X									X[8]	X[8]	
Serum β-hCG[5]	X											
ECHO or MUGA	X											
PFT	X											
BMBx morphology		X[10]					X[10]				X	
Correlative Studies[6]		X[10]					X[10]					
Adverse event assessment[11]		X ---------- X										

[1] Within 42 days prior to first study drug dose, with exception of ECHO/MUGA and PFT which are required within 180 days prior to first study drug dose.
[2] Every 6 months for 2 years after Day 0, for relapse and survival
[3] CBC to be performed daily until engraftment then at least weekly until platelet engraftment or Day +100 (whichever occurs first)
[4] Chemistry includes Na, K, CO_2, glucose, BUN, creatinine, Ca, total protein, albumin, bilirubin, alkaline phosphatase, AST, ALT, LDH. Phosphorus to be included only on day 30.
[5] Required only in women of childbearing potential.
[6] See Section 8.0 for required samples
[7] A telephone visit to assess survival and disease status may be done in lieu of a physical exam.
[8] 24 hour urine not required for patients without detectable urine protein on screening exam.
[9] Granix arm only
[10] Required only for the first 10 patients randomized to the Granix arm with measureable monoclonal paraprotein in the blood (by electrophoresis or serum free light chain)
[11] Adverse events will be followed until clinical resolution

6.1 Duration of Study and Follow Up

If the constraints of this protocol are considered to be detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, the protocol therapy must be discontinued and the reason(s) for discontinuation documented in the case report forms.

Patients will be removed from the study any of the following reasons:

- Death
- Adverse event(s) that, in the judgment of the investigator, may cause severe or permanent harm or which rule out continuation of study drug
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Suspected pregnancy
- Serious noncompliance with the study protocol
- Lost to follow-up
- Patient withdraws consent

- Investigator removes the patient from study
- The Siteman Cancer Center decides to close the study

Patients who prematurely discontinue treatment for any reason other than withdrawal of consent or loss to follow-up will be followed as indicated in the schedule of assessments (Section 6.0).

In addition to follow-up visits on Days +30 and +100, patients will be followed every 6 months for 2 years or until death, whichever occurs first.

7.0 DATA SUBMISSION SCHEDULE

Case report forms with appropriate source documentation will be completed according to the schedule listed in this section.

Case Report Form	Submission Schedule
Original Consent Form	Prior to registration
Registration Form Eligibility Form Myeloma Staging Form Treatment History Form Medical History Form	Prior to starting treatment
Study Drug Dosing Form	Day 0
Count Recovery Form	Engraftment
Follow Up Form	Day 30 Day 100 Every 6 months for 2 years after Day 0
Disease Assessment Form	Baseline Day 100
MedWatch Form	See Section 11.0 for reporting requirements

8.0 CORRELATIVE STUDIES

The specific objectives of the correlative studies are to measure the effect of G-CSF on: 1) mobilization of myeloma cells into the blood 2) osteoblast and CAR cell number in the bone marrow; 3) expression of CXCL12, IL-6, and BAFF in the bone marrow; and 4) myeloma cell apoptosis and cell cycle status in the bone marrow.

To accomplish these objectives, up to ten patients randomized to the Granix arm with measureable monoclonal paraprotein in the blood (by electrophoresis or serum free light chain)

will have the following specimens collected prior to beginning treatment on Day -7 and subsequently before the first dose of melphalan on Day -2:

- bone marrow aspirate: ~5mL in EDTA tube(s)
- bone marrow core section: ~5-10mm in sterile PBS
- peripheral blood: 20cc collected in EDTA tube(s)

These samples are optional and only patients who indicate they wish to participate in the optional testing in the informed consent form will be included.

8.1 Sample Processing

Samples should be maintained at room temperature and delivered to the laboratory of Daniel C. Link located at Room 613 Southwest Tower of the Washington University Medical Center.

a) Myeloma cell mobilization. Mobilization of myeloma cells into the peripheral circulation following treatment with G-CSF is a surrogate for disruption of myeloma-stromal cell interactions. The magnitude of mobilization will be determined by comparing Day -7 and Day -2 samples by flow cytometry. Cells will be stained with CD38 and CD138 using protocols established in the clinical flow cytometry lab. Plasma cells will be identified as CD38high CD138+ cells.

b) Osteoblast and CAR cell enumeration. Bone marrow core biopsies will be divided and the portion targeted for immunostaining will be fixed in zinc formalin overnight, decalcified, paraffin embedded and sectioned using the Anatomic and Molecular Pathology (AMP) Core lab at Washington University. Immunostaining protocols for human CXCL1231 (R&D systems, #MAB350) and osteocalcin32 (Biotrend Chemikalien GmBH) have been published. We have successfully used the CXCL12 antibody to stain human bone marrow sections (data not shown). Consistent with a previous report, we see CXCL12 expression in bone-lining osteoblasts and CAR cells. The sections will be analyzed independently in a blinded fashion by two individuals using standard histomorphometry tools to quantify the number of osteoblasts.

c) Bone marrow cytokine / chemokine levels. To measure the effect of G-CSF on marrow cytokine/chemokine levels, we will analyze a portion of the core biopsy at baseline (Day -7) and on Day -2. The core biopsy will be mechanically disrupted and divided into cellular and cell-free fractions by centrifugation. Total RNA will be extracted from the cellular fraction and real time RT-PCR assays for CXCL12, IL-6, and BAFF will be performed in triplicate. The cell-free supernatant will be used to quantify CXCL12, IL-6, and BAFF protein expression (in triplicate) using commercially available ELISAs. Chemokine and cytokine protein levels from ELISAs will be normalized to total protein content.

d) Cell cycle analysis and apoptosis. The cell cycle status of plasma cells will be assessed based on Ki67 expression. Ki67 staining have been used assess cell cycle status in B and T cell lymphoproliferative disorders, and it has been shown to correlate well with cycle status in human tumors. Expression of activated caspase 3 is widely used to assess apoptosis. Blood mononuclear cells and bone marrow aspirate cells will be stained with CD38, CD138, and either Ki67 or activated caspase 3. Cycling plasma cells will be identified as CD38high CD138+ Ki67+ cells. Apoptotic cells will be identified as CD38high CD138+ activated-caspase 3+ cells.

9.0 STATISTICAL CONSIDERATIONS

9.1 Study Design

This is an open label, single institution, two-arm, unstratified, randomized phase II study to test the safety and efficacy of Granix in combination with HDM compared with HDM alone in patients with MM undergoing ASCT for first-line treatment. The primary endpoint of the study is CR at Day +100 after ASCT.

9.2 Sample Size

The sample size was determined assuming a two-sided binomial test for difference of proportions with power=.80 and significance level=.05. The null hypothesis is H0: CR in Granix + HDM arm = CR in HDM-alone arm, against the alternative hypothesis H1: CR in Granix + HDM arm $\neq$ CR in HDM-alone arm. Historical data suggests an expected CR in the HDM-alone group of around 55%. Eighty-eight patients in each arm will provide 80% power at a 5% significance level to detect a 20% difference in the proportion of patients with CR.

9.3 Accrual Rate

The target enrollment is 176 patients. Expected accrual is approximately two years, given our institutional HDM+ASCT rate of 150-160 per year.

9.4 Data Analysis

Demographic and clinical characteristics will be summarized using descriptive statistics (frequency or percentage with 95% confidence interval, mean, standard deviation, quartiles and interquartile range). Responses will be evaluated at Day +100 after transplant. The CR at Day +100 after ACST will be calculated, accompanied with 95% confidence interval (CI), and the CR in the Granix plus HDM arm will be compared with the CR in the HDM-alone arm using Fisher's Exact test to evaluate the efficacy of Granix

plus HDM. Comparisons will be 2-sided, using P value less than 0.05 as significance level. PFS and OS will be analyzed by Kaplan-Meier (KM) method, illustrated by KM curves with 95% CI.

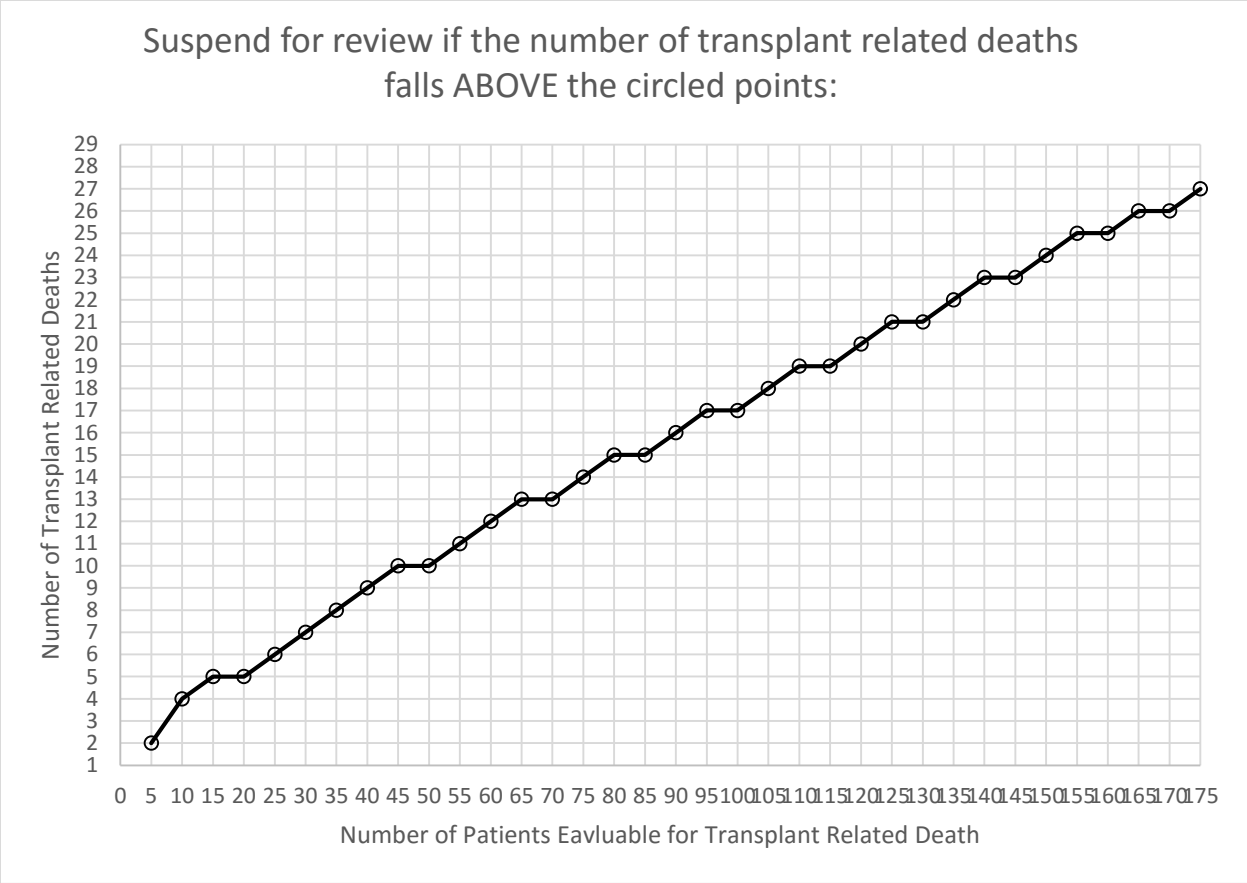
9.4.1 Interim Analysis

One interim analysis to test for efficacy and futility will occur after 88 treated patients, 44 in each study arm, have completed the day +100 visit. Two one-sided tests for difference of proportions will be used. O'Brien-Fleming sequential stopping boundaries indicate that the study will be stopped for futility if the standardized Z value < .668 and for benefit if the value > 2.68.

9.5 Toxicity and Plans for Data and Safety Monitoring

Toxicity will be reviewed on a continuous basis. Early stopping of this trial will be based on unacceptable toxicity, defined as excess mortality at Day +30, by assuming that Day +30 mortality rate of 10% or less is acceptable and that a toxicity rate of 20% or more would be unacceptable. The plot and table below describe a continuous toxicity monitoring rule with 80% power and 0.05 significance level. If the TRM rate is as high as 20% this rule has a high probability of correctly stopping the study early (probability = .95). If the TRM rate is a low as 10% it has a low probability of incorrect early stopping (probability = .051).

Adverse events will be assessed using NCI CTCAE v4.0 and summarized by patient, type, grade and frequency.



Suspend for review if the number of transplant related deaths EXCEEDS:	In the following number of patients:
2	5
4	10
5	15
5	20
6	25
7	30
8	35
9	40
10	45
10	50
11	55
12	60

13	65
13	70
14	75
15	80
15	85
16	90
17	95
17	100
18	105
19	110
19	115
20	120
21	125
21	130
22	135
23	140
23	145
24	150
25	155
25	160
26	165
26	170
27	176

9.6 Correlative Studies

For correlative studies, measurements collected on the patients enrolled in the Granix plus HDM arm will be compared pre-Granix and post-Granix to evaluate Granix effect. All measurements will be summarized using descriptive statistics. Paired t-test or Wilcoxon signed rank test (when normality assumption is violated) will be used to test for pre- to post- treatment change. Nominal or binary categorical variables will be tabulated at baseline and a later time point while the association will be assessed by Fisher's exact test. Ordinal and short scales with repeated observations of the same values will be compared using an ordinal test for trend such as Jonckheere's test.

In addition, since osteoblast and CAR cell enumeration are not established assays, we will assess intra-rater variability by computing intra class correlations (ICC), if reasonably

Gaussian, and utilize Bland-Altman plots to describe any observable disagreement between different raters.

10.0 RESPONSE EVALUATION

Response will be assessed per the International Myeloma Working Group (IMWG) Response Criteria[41] as defined below.

10.1 Complete Response

Complete response (CR) requires all of the following:

- Disappearance of monoclonal protein by both protein electrophoresis and immunofixation studies from the blood and urine
- <5% plasma cells in the bone marrow
- Disappearance of soft tissue plasmacytomas

10.2 Stringent Complete Response

Stringent complete response (sCR) requires all of the following:

- CR as defined above
- Normal free light chain ratio
- Absence of clonal cells in the bone marrow by immunohistochemistry or immunofluorescence

10.3 Very Good Partial Response

Very good partial response (VGPR) requires all of the following:

- Serum and urine monoclonal protein detectable by immunofixation but not on electrophoresis
  OR
  > 90% reduction in serum monoclonal protein with urine monoclonal protein < 100 mg per 24 hours
- If present, > 50% reduction in the size of soft tissue plasmacytomas (by clinical or radiographic examinations)

10.4 Partial Response

Partial response (PR) requires all of the following:

- > 50% reduction in the level of the serum monoclonal protein.
- Reduction in urine monoclonal protein by either > 90% or to < 200 mg

- If present, > 50% reduction in the size of soft tissue plasmacytomas (by clinical or radiographic examinations)
- If serum and urine monoclonal protein are unmeasurable, a >50% decrease in difference between the involved and uninvolved free light chain levels is required in place of monoclonal protein criteria (The absolute decrease must be > 10 mg/dl)
- If serum and urine monoclonal protein are unmeasurable and serum free light chain is unmeasurable, a > 50% reduction in plasma cells is required in place of monoclonal protein provided that baseline bone marrow plasma cell percentage was > 30%

10.5 Stable Disease

Stable disease (SD) is defined as not meeting criteria for any other response as defined in this section.

10.6 Progressive Disease

Progressive disease (PD) requires one or more of the following:

- > 25% increase in the level of serum monoclonal protein, which must also be an absolute increase of at least 0.5 g/dL, and confirmed on a repeat investigation.
- > 25% increase in 24-hour urine monoclonal protein, which must also be an absolute increase of at least 200 mg/24hr and confirmed on a repeat investigation.
- > 25% increase in plasma cells in a bone marrow aspirate or on trephine biopsy, which must also be an absolute increase of at least 10%.
- > 25% increase in the difference between involved and uninvolved free light chain levels (The absolute increase must be >10 mg/dl) (only in patients without measurable serum and urine monoclonal proteins)
- Definite increase in the size of existing lytic bone lesions or soft tissue plasmacytomas. A definite increase is defined as at least 50% (and at least 1 cm) increase as measured serially as the sum of the products of the cross-diameters of the lesions.
- Development of new bone lesions or soft tissue plasmacytomas (not including compression fracture).
- Development of hypercalcemia (corrected serum calcium > 11.5 mg/dL or 2.8 mmol/L not attributable to any cause other than progressive multiple myeloma).

Note: A response of progressive disease nullifies any other concurrent response. For example, at a given time point a participant meets criteria for VGPR but has development of new bone lesions the response is PD not VGPR.

10.7 Clinical Relapse

Clinical relapse (i.e. progressive disease requiring alternate myeloma treatment) requires one or more of the following:

- Decrease in hemoglobin > 2 g/dl not attributable to any cause other than progressive multiple myeloma
- Increase in creatinine by > 2 mg/dl not attributable to any cause other than progressive multiple myeloma
- Other worsening laboratory result, or clinical condition that the treating physician determines is not attributable to any cause other than progressive multiple myeloma

10.8 Progression-Free Survival

Progression-free survival (PFS) is defined as the duration from time of transplant Day 0 to time of first progression/clinical relapse, death, or the date the patient was last known to be in remission.

10.9 Overall Survival

Overall survival (OS) is defined as the duration from the time of transplant Day 0 to death or last follow-up.

10.10 Treatment-Related Mortality

Treatment-related mortality (TRM), which is defined as death not due to disease progression before Day +100, will be calculated. This outcome measure will be summarized by the cumulative incidence estimated with 95% confidence intervals.

10.11 Engraftment

The rates of engraftment (neutrophil and platelets) and non-engraftment in the study population will be determined using the following definitions:

Neutrophil engraftment is defined as ANC $\geq 0.5 \times 10^9$/L × 3 consecutive daily assessments. The first of 3 consecutive days for which ANC $\geq 0.5 \times 10^9$/L will be recorded as the date of neutrophil engraftment. Time to neutrophil engraftment will be calculated as the time from the date of the ASCT to the date of neutrophil engraftment.

Non-engraftment is defined as failure to reach an ANC > 0.5×10^9/L × 3 consecutive daily assessments by Day +30.

Platelet engraftment is defined as an untransfused platelet measurement >20,000/mm^3 × 2 consecutive assessments separated by at least 3 days. The first day for which the untransfused platelet measurement is >20,000/mm^3 will be recorded as the date of platelet engraftment. Time to platelet engraftment will be calculated as the time from receiving the date of ASCT to the date of platelet engraftment. Untransfused is defined as no transfusions within 7 days.

Non-engraftment is defined as failure to reach platelets > 20,000 × 10^9/L × 3 consecutive assessments by Day +100.

Time to neutrophil and platelet engraftments will be calculated for each patient and summarized using Kaplan-Meier estimates.

11.0 REGULATORY AND REPORTING REQUIREMENTS

The entities providing oversight of safety and compliance with the protocol require reporting as outline below.

The Washington University Human Research Protection Office (HRPO) requires that all events meeting the definition of unanticipated problem or serious noncompliance be reported as outlined in Section 11.2.

The FDA requires that all serious and unexpected adverse events be reported as outlined in Section 11.4. In addition, any fatal or life-threatening adverse experiences where there is a reasonable possibility of relationship to study intervention must be reported.

11.1 Definitions

11.1.1 Adverse Events (AES)

Definition: any unfavorable medical occurrence in a human subject including any abnormal sign, symptom, or disease.

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website.

Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health

and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website:
http://www.hhs.gov/ohrp/policy/advevntguid.html

11.1.2 Serious Adverse Event (SAE)

Definition: any adverse drug experience occurring at any dose that results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity (i.e., a substantial disruption of a person's ability to conduct normal life functions)
- A congenital anomaly/birth defect
- Any other experience which, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

All unexpected SAEs must be reported to the FDA.

11.1.3 Unexpected Adverse Experience

Definition: any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure (or risk information, if an IB is not required or available).

Events that are both serious AND unexpected must be reported to the FDA.

11.1.4 Life-Threatening Adverse Experience

Definition: any adverse drug experience that places the subject (in the view of the investigator) at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.

Life-threatening adverse experiences must be reported to the FDA.

11.1.5 Unanticipated Problems

Definition:

- unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- related or possibly related to participation in the research (in this guidance document, 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); and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

11.1.6 Noncompliance

Definition: failure to follow any applicable regulation or institutional policies that govern human subjects research or failure to follow the determinations of the IRB. Noncompliance may occur due to lack of knowledge or due to deliberate choice to ignore regulations, institutional policies, or determinations of the IRB.

11.1.7 Serious Noncompliance

Definition: noncompliance that materially increases risks, that results in substantial harm to subjects or others, or that materially compromises the rights or welfare of participants.

11.1.8 Protocol Exceptions

Definition: A planned deviation from the approved protocol that are under the research team's control. Exceptions apply only to a single participant or a singular situation.

Local IRB Pre-approval of all protocol exceptions must be obtained prior to the event.

11.2 Reporting to the Human Research Protection Office (HRPO) at Washington University

The PI is required to promptly notify the IRB of the following events:

- Any unanticipated problems involving risks to participants or others which occur at WU, any BJH or SLCH institution, or that impacts participants or the conduct of the study.

- Noncompliance with federal regulations or the requirements or determinations of the IRB.
- Receipt of new information that may impact the willingness of participants to participate or continue participation in the research study.

These events must be reported to the IRB within 10 working days of the occurrence of the event or notification to the PI of the event. The death of a research participant that qualifies as a reportable event should be reported within 1 working day of the occurrence of the event or notification to the PI of the event.

11.3 Reporting to the Quality Assurance and Safety Monitoring Committee (QASMC) at Washington University

The PI is required to notify the QASMC of any unanticipated problem occurring at WU or any BJH or SLCH institution that has been reported to and acknowledged by HRPO as reportable. (Unanticipated problems reported to HRPO and withdrawn during the review process need not be reported to QASMC.)

QASMC must be notified within 10 days of receipt of IRB acknowledgment via email to a QASMC auditor.

11.4 Reporting to the FDA

The conduct of the study will comply with all FDA safety reporting requirements. **PLEASE NOTE THAT REPORTING REQUIREMENTS FOR THE FDA DIFFER FROM REPORTING REQUIREMENTS FOR HRPO/QASMC.** It is the responsibility of the investigator to report any unanticipated problem to the FDA as follows:

- Report any unexpected fatal or life-threatening adverse experiences (Section 11.1.4) associated with use of the drug by telephone or fax no later than **7 calendar days** after initial receipt of the information.
- Report any serious, unexpected adverse experiences (Section 11.1.2), as well as results from animal studies that suggest significant clinical risk within **15 calendar days** after initial receipt of this information. All MedWatch forms will be sent by the investigator or investigator's team to the FDA at the following address or by fax:

Center for Drug Evaluation and Research
Division of Hematology Products
5901-B Ammendale Rd.
Beltsville, MD 20705-1266
FAX: 301-796-9845

11.5 Timeframe for Reporting Required Events

Reportable adverse events will be collected from first dose of study treatment (Day -7) through the Day +30 visit. For the purposes of this protocol, reportable adverse events are events grade 2 or greater events. Reportable adverse events will be followed until clinical resolution. Numerous grade 1 events are expected following high dose chemotherapy and stem cell transplantation and therefore will not be reported. Hematologic toxicities are expected and therefore the following adverse events will not be reported regardless of grade: anemia, white blood cell decreased, neutrophil count decreased, lymphocyte count decreased, and platelet count decreased. In addition, the following grade 2 non-hematologic adverse events are expected and will not to be reported as adverse events: nausea, vomiting, diarrhea, anorexia, fatigue or alopecia.

12.0 DATA AND SAFETY MONITORING

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, the Principal Investigator will provide a Data and Safety Monitoring (DSM) report to the Washington University Quality Assurance and Safety Monitoring Committee (QASMC) semi-annually beginning six months after accrual has opened (if at least five patients have been enrolled) or one year after accrual has opened (if fewer than five patients have been enrolled at the six-month mark).

The Principal Investigator will review all patient data at least every six months, and provide a semi-annual report to the QASMC. This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual
- Protocol activation date
- Average rate of accrual observed in year 1, year 2, and subsequent years

- Expected accrual end date
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Measures of efficacy
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Summary of toxicities
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

The study principal investigator and Research Patient Coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or Research Patient Coordinator becomes aware of an adverse event, the AE will be reported to the HRPO and QASMC according to institutional guidelines.

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APPENDIX A: ECOG Performance Status Scale

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

APPENDIX B: Ideal Body Weight and Corrected Body Weight Formulas

Ideal Body Weight Formula for Patients Over 5 Feet in Height
Male IBW (kg) = 50 kg + 2.3 kg for each inch over 5 feet
Female IBW (kg) = 45.5 kg + 2.3 kg for each inch over 5 feet

Ideal Body Weight Formula for Patients Under 5 Feet in Height
Male IBW (kg) = 50 kg – 2.3 kg for each inch under 5 feet
Female IBW (kg) = 45.5 kg – 2.3 kg for each inch under 5 feet

Corrected Body Weight
CBW (kg) = IBW in kg + [0.2 x (actual body weight in kg – IBW in kg)]