Local Protocol #: 11-12-434

TITLE: "A Phase II study Assessing the Efficacy and Toxicity of PK-directed Intravenous Busulfan in Combination with High-Dose Melphalan and Bortezomib as Conditioning Regimen for First-line Autologous Hematopoietic Stem Cell Transplantation in Patients with Multiple Myeloma"

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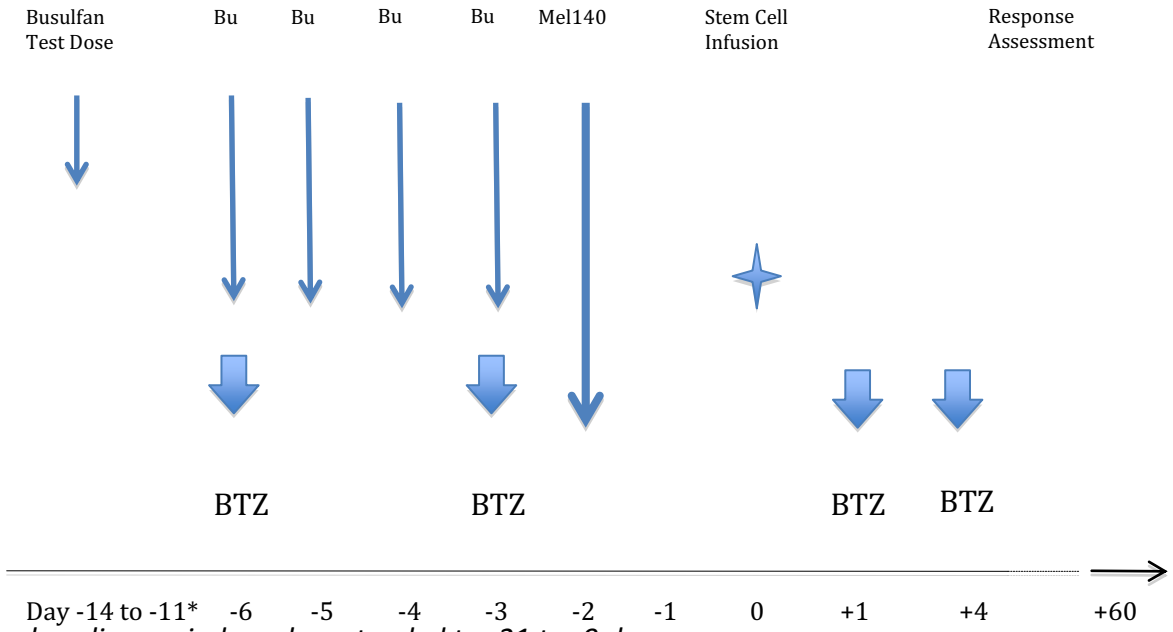
Protocol Type / Version # / Version Date: Version 4.0/ 25 February 2014

SYNOPSIS

BACKGROUND: High dose therapy followed by autologous hematopoietic stem cell transplantation (ASCT) has an established role in the treatment of patients with multiple myeloma. The most commonly used conditioning (or preparative) regimen in this setting is high-dose melphalan (200mg/$m^{2;}$ Mel200), which has been shown to result in improved progression free and overall survival. Achievement of a complete response following ASCT is a powerful indicator for freedom from relapse, as well as progression free and overall survival. The complete response (CR) rate observed after Mel200 followed by ASCT is between 10-35% depending on the induction regimen used. There is evidence that the combination of busulfan (Bu) and melphalan (Mel) results in longer progression free and overall survival compared to melphalan alone. Additionally, the use of bortezomib (Btz) during conditioning with either high dose melphalan alone or the combination of melphalan and IV busulfan has shown to be both safe and to have very promising efficacy. We hypothesize that the combination of Bu/Btz/Mel140 will lead to a 20% improved complete response rate compared with historical controls.

The primary objective is to determine the complete response rate of PK directed iv Busulfan, Bortezomib and Melphalan (Bu/BTZ/Mel140) conditioning followed by autologous hematopoietic stem cell transplantation (ASCT)
Secondary Objectives are to determine the overall response rate of the regimen Bu/BTZ/Mel140, to determine the treatment related toxicity and mortality of the regimen, including 100-day mortality rates, to determine the duration of response, time to progression, event-free and overall survival, and to determine whether there is a gender or race difference in the pharmacokinetic profile of IV busulfan.

TREATMENT SCHEMA



** The baseline period can be extended to -21 to -9 days*

Abbreviations: Bu, busulfan – PK directed; Mel140; melphalan at 140mg/m^2; BTZ, bortezomib at 1mg/m2

Eligibility Criteria: Patients must have active multiple myeloma (MM) requiring treatment, measurable disease, be age 18-72, have adequate heart, lung, and renal function, ECOG performance status $\leq$ 2, and a life expectancy of > 12 months. Patients will be excluded if they have received more than 12 months of prior chemotherapy for this disease, >Grade 2 peripheral neuropathy, hypersensitivity to bortezomib, boron, or mannitol, unresolved grade >2 non-hematologic toxicity from previous therapy, previous autologous or allogeneic stem cell transplant, uncontrolled infection, significant co-morbid medical conditions or uncontrolled life threatening infection, prior malignancy other than adequately treated non-melanoma skin cancer.

Statistical Considerations: The primary endpoint of the trial will be the complete response rate 100 days after the ASCT as defined by the International Myeloma Working Group uniform response criteria. We aim to demonstrate that the complete response rate (CRR) 100 Days after the ASCT is at least 35%. This would demonstrate at least the same complete response rate as the reported complete response rates of the Mel200 conditioning regimen. If the true response rate for the new regimen (Bu/Mel/Btz) is 55%, with 28 patients in the study, we will be able to construct a 95% confidence interval for the true response rate with the lower bound no less than 35%.
The secondary endpoints to be explored will be the overall response (very good partial & partial response, stable disease and progression), toxicity as assessed by the Common Terminology Criteria for Adverse Events (CTCAE) v4.0,[2] mortality at days 30, 100 and 360 post transplant, time to progression, median progression, event free and overall survival, and possibly associations of the pharmacokinetic profile of iv busulfan as determined by the test dose and the variables gender and race. Methylation and gene expression signatures of bone marrow pre-treatment plasma cells will be correlated with outcome.

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1. OBJECTIVES

1.1. Primary Objectives

To determine the complete response rate as defined by the International Myeloma Working Group (IMWG) criteria[3] for patients with Multiple Myeloma treated with high dose chemotherapy with PK directed iv Busulfan, Bortezomib and Melphalan (Bu/BTZ/Mel140) followed by autologous hematopoietic stem cell transplantation (ASCT) as first line therapy

1.2. Secondary Objectives

1.2.1 To determine the overall response rate of the regimen Bu/BTZ/Mel140

1.2.2 To determine the treatment related toxicity and mortality of the regimen, including 100-day mortality rates

1.2.3 To determine the duration of response, time to progression, progression-free survival, event-free survival and overall survival for this conditioning regimen

1.2.4. To determine whether there is a gender or race difference in the pharmacokinetic profile of IV busulfan

1.2.5 To determine methylation and gene expression signatures of pre-treatment bone marrow plasma cells and explore associations of these signatures with outcome

2. BACKGROUND

2.1 High Dose Therapy in Multiple Myeloma

High dose therapy followed by autologous hematopoietic stem cell transplantation (ASCT) has an established role in the treatment of patients with multiple myeloma. Since the early 1990s, several studies have compared first-line treatment including ASCT versus standard combination chemotherapy. Most trials have comprised patients of a younger age category, essentially up to the age of 65 years.[4] The first randomized study to demonstrate the superiority of ASCT was the French IFM90 study on 200 patients, published in 1996: median OS and event-free survival (EFS) were 57 and 44 months, respectively, for patients who underwent ASCT, compared with 44 and 18 months in the chemotherapy group,[5] and the difference was most strongly pronounced in patients under the age of 60 years. However, other controlled randomized studies only showed improvement in EFS but not in OS, or no benefit in outcomes was observed. Still, high-dose treatment with ASCT is considered as the gold standard for myeloma patients at least up to 60 to 65 years of age, based on its demonstrated efficacy, and the benefits in quality of life, with long treatment-free periods of good disease control.[4]

The most commonly used conditioning (or preparative) regimen in this setting is high-dose

melphalan (200mg/m$^{2;}$ Mel200). This intensive therapy has been shown to result in improved progression free and overall survival compared to other treatment strategies not involving high-dose chemotherapy.[6-8] However, multiple myeloma remains an incurable disease and even after achieving a complete response patients will eventually relapse unless other competing events occur first. Better therapies, that prevent relapse for a longer time, are needed. There is convincing evidence that achievement of a complete response following autologous hematopoietic stem cell transplantation (ASCT) is a powerful indicator for freedom from relapse, as well as progression free and overall survival.[9] Improved conditioning regimens associated with better therapeutic indexes that result in higher response rates, less morbidity and mortality are needed.

2.2 IV Busulfan (Busulfex™)

Introduction

Busulfan, a potent, cytotoxic, bifunctional alkylating agent that causes myeloablation, has been used clinically since the 1950s to treat hematological malignancies and myeloproliferative syndromes. Busulfan produces deoxyribonucleic acid (DNA) cross-linking and chromosomal damage that can be lethal to rapidly dividing cells. At the low end of the active dose range, busulfan causes a selective depression of granulocytopoiesis. Increasing doses lead to progressive general myelotoxicity culminating in marrow ablation due to cell death. High doses cause significant DNA damage and are myeloablative.[10] The selectivity of busulfan for marrow cells has several explanations. One is based on the susceptibility of relatively undifferentiated stem cells during the G-phase of the cell mitotic cycle to alkylation by busulfan. Busulfan treatment during the G-phase halts further differentiation and progression of the cell through the cell cycle. Microscopic examination of stem cell populations indicates arrested cell division with polyploidy and cell death.[10]

Busulfan was used initially at low oral doses for palliative care, then at high oral doses in combination with other alkylating agents having immunosuppressive properties to promote the engraftment of bone marrow following allogeneic or autologous transplantation. In more recent years, busulfan has been used as one component of a chemo- plus radiotherapy or chemotherapeutic-based conditioning regimen prior to transplant. Busulfan's narrow therapeutic window as well as unpredictable intestinal absorption and erratic bioavailability led to the development of a parenteral formulation.[11] Subsequently, intravenous (IV) busulfan (under the name of Busulfex™) was approved in 1999 in the US and Canada as a pretransplant conditioning agent.[12]

Pharmacokinetics and Clinical Data

Following doses as 3-hour infusions, daily for 4 days, drug clearance and systemic exposure (area under the concentration-time curve, AUC) were reported to be linear and dose proportional over a dose rate range of 0.4 to 1.2 mg/kg/h. The maximum concentration (C_{max}) of busulfan increased in proportion with dose. Dose proportional kinetics were also observed for 3-hour infusions compared to 2-hour infusions. A dose of 130 mg/m$_2$ infused over 3 hours produced a mean C_{max} of 3.6 μg/ml. The mean C_{max} observed for a 0.8-mg/kg/dose (~32 mg/m$_2$) infused over 2 hours was 1.2 μg/ml. Similarly, the median AUC for the 130 mg/m$_2$ dose, 3-hour infusion was 4873 μM**x**min (CV 21.8%) and the median AUC for the ~32 mg/m$_2$ dose, 2-hour

infusion was 1292 **µ**M**.**min (CV 25%). Intrapatient variability in day-to-day estimated clearance was <20%. No drug accumulation was observed over the 4-day course of administration. Clearance values were similar for both dose regimens.[13]
Busulfan exposure (AUC) has been shown to be correlated with clinical outcome and manifestation of adverse events. Andersson et al[14] compared outcomes in subjects with busulfan exposure in the optimal range (AUC between 950 and 1520 µM**x**min) to those with lower (AUC < 950 µM**x**min) or higher (AUC >1520 µM**x**min) exposures in allogeneic HSCT for chronic myelogenous leukemia. The optimally-dosed group had a significantly better survival rate than those with lower or higher exposures. A retrospective study comparing the fixed-dose oral busulfan-based regimen and PK-directed IV busulfan-based regimen showed the improvement in clinical outcomes of NHL: non-relapsed mortality, IV 3% vs. oral 28%; progression free survival, IV 50% vs. oral 17%.[15]

Therefore, PK-directed dose optimization of busulfan in autologous HSCT is relevant in order to achieve the optimal therapeutic AUC window, to eliminate unnecessary underexposure to the drug caused by variability in busulfan metabolism, and to potentially improve the survival in the patient population who undergo autologous HSCT in lymphoma. Instead of adjusting later busulfan doses based on the exposure (AUC) observed on the first day of dosing in a conditioning regimen, it was proposed to administer a test dose of busulfan to obtain busulfan PK parameters prior to implementation of the conditioning regimen. Kletzel et al[16] implemented a test dose method in 30 pediatric patients with malignant and nonmalignant conditions. A 0.8 mg/kg test dose of IV busulfan (target AUC range of 800 to 1200 µM**x**min) was followed one to two weeks later by two daily doses of a PK-directed dose to target an AUC range of 3200 to 4800 µM**x**min. Twenty-six of the patients had evaluable results; two of the sample sets showed sampling errors and two were corrupted by shipping problems. The conditioning regimen dose was adjusted in 20 patients based on the test dose PK; eight patients received lower doses (2.5 to 3.1 mg/kg) and twelve patients received higher doses (3.3 to 7.2 kg/mg). The median dose administered was 3.2 mg/kg (2.5 to 7.2 mg/kg) and the target AUC was achieved in 23 of 30 patients, six of the seven patients failing to meet the target were less than two years old. Test dose and first PK-directed dose clearance were the same with the exception of two patients; one with higher clearance and one with lower clearance relative to the first dose. The reason for the discrepancy is not known. The authors concluded that the test dose provided information for subsequent dosing and the method was feasible, safe, and convenient for administration of IV busulfan to children.

The pharmacodynamics of non-cell-cycle specific agents, including alkylating agents such as busulfan, tend to be associated with total exposure rather than dosing schedule. As a consequence, a number of investigators have evaluated the pharmacokinetics, safety, and efficacy of a dose of 3.2 mg/kg (or the equivalent of roughly130 mg/m2) QD administered as a 3-hour infusion as an alternative to the labeled 0.8 mg/kg 2-hour infusions four times per day. Clinical outcomes were similar for a higher dose given QD and a lower dose given four-times a day.[17-19]

Busulfan, as an alkylating agent, has been shown to be an effective reagent for lymphoma in the autograft setting as well as in allogeneic HSCT. The clinical data using busulfan and

cyclophosphamide with or without etoposide have yielded clinical results that are comparable to the other preparative regimens for autologous HSCT both in NHL and HL.[15,20,21]

In patients with multiple myeloma, the use of intravenous busulfan in combination with melphalan as high dose chemotherapy has also demonstrated promising efficacy. The studies showed safety and indicated improved outcomes compared to historical controls that were treated with melphalan alone both in the upfront and relapsed setting.[22,23] In a retrospective case-control study the combination of busulfan and melphalan resulted in longer progression free and overall survival compared to melphalan alone.[24] This has as yet not been studied prospectively.

2.3. Melphalan

Melphalan, also known as L-phenylalanine mustard, phenylalanine mustard, L-PAM, or L-sarcolysin, is a phenylalanine derivative of nitrogen mustard. Melphalan is a bifunctional alkylating agent that is active against selected human neoplastic diseases. It is known chemically as 4-[bis(2-chloroethyl)amino]-L-phenylalanine. As a result, its cytotoxicity appears to be related to the extent of its interstrand cross-linking with DNA, probably by binding at the N7 position of guanine. Like other bifunctional alkylating agents, it is active against both resting and rapidly dividing tumor cells.

While in its first trials high-dose melphalan was administered orally, the current use of melphalan is largely currently restricted to the intravenous (i.v.) route, which avoids the problem of its inconsistent oral absorption. Melphalan does not require metabolic activation. It undergoes spontaneous hydrolysis in the plasma, with inactive monohydroxy and dihydroxy metabolites appearing within minutes of the drug administration. About 15% of the drug is excreted intact in the urine. It is highly bound to proteins, mainly albumin. Melphalan is actively transported into cells, mostly by the high-affinity L-amino-acid transport system, which also carries glutamine and leucine.
Melphalan PKs remain linear when delivered at high doses, in a range from 140 to 220 mg/m2. Its distribution fits a two-compartment model, with an elimination half-life ($t_{1/2\beta}$) of 45–60 min, which allows the infusion of stem cells within 8–24 h of melphalan administration. After high-dose therapy, mean peak cerebrospinal fluid concentrations may reach 10% of the corresponding plasma concentrations. The dose-limiting toxicity (DLT) of high-dose melphalan is gastrointestinal mucositis and myelosuppression.[25]

Melphalan at a dose of 200mg/m2 intravenously (Mel200) is the most commonly used conditioning for multiple myeloma. Traditionally, the complete response (CR) rate observed after Mel200 followed by ASCT is between 10-35% depending on the induction regimen used.[6-8]

2.4. Bortezomib

Bortezomib (Velcade, PS-341), the prototypical clinically relevant proteasome inhibitor, is a

dipeptidyl boronic acid derivative that has recently been approved for use in myeloma and mantle cell lymphoma and has also shown activity in other hematologic malignancies.[26-28] Bortezomib inhibits the chymotryptic activity of the 20S proteasome, and in so doing, disrupts the proteasome mediated degradation of diverse cellular proteins.[29] Bortezomib activity has been postulated to reflect interference with NF-**κ**B signaling secondary to inhibition of degradation of the NF-**κ**B inhibitory protein I**κ**B**α**..[30] However, multiple other mechanisms of lethality have been proposed, including increased generation of reactive oxygen species (ROS), which has been demonstrated in lung cancer and leukemia cells.[31] Moreover, proteasome inhibitors have been shown to kill cells in association with induction of ER stress, presumably by interfering with the disposition of unfolded proteins. For reasons that are incompletely understood, proteasome inhibitors selectively induce apoptosis in tumor as compared with normal cells. [32,33]
For patients, who are not transplant candidates, bortezomib is considered at present one of the most active agents, either by itself or when combined with other agents.[27,34,35]

Additionally, the combination of bortezomib with either high dose melphalan alone or high dose melphalan and IV busulfan as part of a preparative regimen for autologous stem cell transplantation in multiple myeloma has been shown to be both safe and to have very promising efficacy in early trials.[8,36]

2.5 Rationale

High dose therapy followed by autologous hematopoietic stem cell transplantation (ASCT) has an established role in the treatment of patients with multiple myeloma. The most commonly used conditioning (or preparative) regimen in this setting is high-dose melphalan (200mg/m$^{2;}$ Mel200). This intensive therapy has been shown to result in improved progression free and overall survival compared to other treatment strategies not involving high-dose chemotherapy. Traditionally, the complete response (CR) rate observed after Mel200 followed by ASCT is between 10-35% depending on the induction regimen used.[6-8]

However, multiple myeloma remains an incurable disease and even after achieving a complete response patients will eventually relapse unless other competing events occur first. Better therapies, that prevent relapse for a longer time, are needed. There is convincing evidence that achievement of a complete response following autologous hematopoietic stem cell transplantation (ASCT) is a powerful indicator for freedom from relapse, as well as progression free and overall survival.[9] Better conditioning regimens associated with improved therapeutic indexes that result in higher response rates, less morbidity and mortality are needed.

Oral preparations of busulfan have traditionally been used as preparation for hematopoietic stem cell transplantation. Its use has been limited by unreliable and erratic bioavailability that resulted in severe toxicity. The intravenous formulation of busulfan (Busulfex™) has a much more favorable safety profile secondary to its more reliable pharmacokinetic properties and bioavailability. Dosing based on a pharmacokinetic (PK) profile following a test dose results in very reliable and reproducible serum levels.[13] This has not only led to less toxicity, but also resulted in improved outcomes.[14,37]

The use of intravenous busulfan in combination with melphalan as high dose chemotherapy has been explored in patients with multiple myeloma. The studies demonstrated safety and indicated improved outcomes compared to historical controls that were treated with Melphalan alone both in the upfront and relapsed setting.[22,23] In a retrospective case-control study the combination of busulfan and melphalan resulted in longer progression free and overall survival compared to melphalan alone.[24]
The addition of bortezomib to a preparative regimen with either high dose melphalan alone or the combination of melphalan and IV busulfan has shown to be both safe and to have very promising efficacy in early trials.[8,36]

While high-dose chemotherapy followed by autologous stem cell transplantation (ASCT) is an established treatment modality for multiple myeloma, the superiority of one regimen over another has not been established. Based on these observations we hypothesize that a conditioning regimen consisting of intravenous PK-directed busulfan in addition to melphalan and bortezomib will be safe and result in a high response rate. The proposed regimen differs from a similar study by Rodriguez et al[36] by using a) PK-directed dosing of iv busulfan based on a test dose before the first treatment dose is administered and b) bortezomib with a different dose-intensity (different dose and more frequent in the proposed regimen).

We therefore propose a two-stage phase II single arm study[1] evaluating the safety and efficacy of a combination of PK-directed intravenous Busulfan with Bortezomib and Melphalan (Bu/BTZ/Mel140) in the setting of a first transplant in patients with multiple myeloma. Our hypothesis is that this combination will lead to a 20% improved complete response rate compared with historical controls. It is routine practice in clinical research in oncology to perform a feasibility study of a new untested regimen to better assess safety and efficacy in a smaller number of patients via a phase 2 trial prior to subjecting many more patients to a much larger randomized phase 3 trial. We have reliable historical data on the efficacy of melphalan alone, and the presented study utilizes an interim efficacy analysis after 22 patients have been enrolled (part of the Simon 2 stage design (“minimax”), see statistical section 13.1) to explore whether the efficacy is promising enough to proceed to the total of 42 patients. Only once the efficacy and safety of the proposed regimen has been sufficiently established in 42 patients will we propose to proceed to a larger phase 3 trial involving more patients. Randomized phase 2 trials are not statistically powered to declare superiority of one regimen over the other.

2.6 Correlative Studies

Gene expression studies have revealed signatures that are predictive of prognosis in myeloma and can identify patients with high risk disease[38-41]. Gene expression studies have also revealed sets of genes whose expression can predict response to agents such as lenalidomide and bortezomib in myeloma.[42-44]
Gene expression is a tightly regulated process and is influenced by aberrant epigenetic changes that can lead to carcinogenesis. We have used the HELP (HpaII tiny fragment Enrichment by Ligation-mediated PCR) assay[45,46] to perform an unbiased genome-wide analysis of DNA

methylation and shown that extensive DNA methylation changes can be seen in myeloma. [47,48] We observed that DNA methylation profiles are able to distinguish between subsets of myeloma with adverse prognosis. Additionally, important drug resistance genes are aberrantly hypomethylated in myeloma and can be predictive of chemoresistance. Thus it would be informative to perform methylome analysis on pretreatment samples and correlate it with response.

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Patients must have histologically or cytologically confirmed Multiple Myeloma

3.1.2 Measurable disease must be present at diagnosis as defined by Protein criteria (quantifiable M-component in serum, urine or Serum Free Light Chains) in order to evaluate response as per IMWG[3]. Non-secretory patients are eligible provided the patient has > 20% plasmacytosis OR multiple (>3) focal plasmacytomas or focal lesions on MRI

3.1.3 Age 18 to 72 years. Because no dosing or adverse event data are currently available on the use of Busulfan in combination with Melphalan and Bortezomib in patients <18 years of age, children are excluded from this study but will be eligible for future pediatric phase 2 combination trials.

3.1.4 Patients must have received induction chemotherapy for myeloma, but no more than 12 months of prior chemotherapy for this disease, and must be eligible for the first planned autologous transplant. Lenalidomide or bortezomib maintenance do not count towards the 12 months of prior chemotherapy. In cases of uncertainty, please discuss with the PI, who will be the final arbiter.

3.1.5 A minimum stem cell dose of 2.0 x 10^6 CD34+ cells/kg has been collected.

3.1.6 Life expectancy of greater than 12 months

3.1.7 ECOG performance status $\leq$2 (Karnofsky $\geq$60%; see Appendix A).

3.1.8 Patients must have normal organ and marrow function as defined below:

- leukocytes	$\geq$3,000/mcL (unless myeloma related)
- absolute neutrophil count	$\geq$1,500/mcL (unless myeloma related)
- platelets	$\geq$50,000/mcL (unless myeloma related)
- total bilirubin	$\leq$2 X institutional upper limit of normal unless 2nd to Gilbert's disease

- AST(SGOT)/ALT(SGPT) ≤3 X institutional upper limit of normal
- creatinine ≤1.5 X institutional upper limit of normal

OR

- creatinine clearance ≥60 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal

3.1.9 Ejection fraction by ECHO or MUGA ≥ 40% performed within 90 days prior to registration

3.1.10 Patients must have adequate pulmonary function studies: > 50% of predicted on mechanical aspects (FEV1, FVC) and diffusion capacity (DLCO) > 50% of predicted, within 120 days of registration. If the patient is unable to complete pulmonary function tests due to MM related pain or condition, exception may be granted if the principal investigator (PI) documents that the patient is a candidate for high dose therapy.

3.1.11 The effects of Busulfan, Melphalan and Bortezomib on the developing human fetus at the recommended therapeutic dose are unknown. For this reason and because alkylating agents as well as other therapeutic agents used in this trial are known to be teratogenic, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for at least six months following the stem cell transplantation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.

3.1.12 Ability to understand and the willingness to sign a written informed consent document.

3.2 Exclusion Criteria

3.2.1 Patients who have had chemotherapy or pelvic radiotherapy within 4 weeks prior to entering the study or those who have not recovered from adverse events due to agents administered more than 4 weeks earlier. Palliative radiation to for example the extremities or the vertebrae are allowed. In cases of uncertainty, please discuss with the PI who will make the final arbitration.

3.2.2 Prior treatment history of autologous HSCT or high-dose chemotherapy with stem cell rescue for any medical reason, not limited to myeloma treatment

3.2.3 Patients may not be receiving any other investigational agents.

3.2.4 Patients with known brain metastases should be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurological dysfunction that would confound the evaluation of neurological and other adverse events.

3.2.5 History of allergic reactions attributed to compounds of similar chemical or biologic composition to or other agents used in the study, such as busulfan, melphalan, bortezomib, boron, or mannitol

3.2.6 Grade 2 or greater peripheral neuropathy within 14 days prior to enrollment

3.2.7 Unresolved grade >/= 3 non-hematologic toxicity from previous therapy. Patients with grade 2 toxicity will be eligible at the discretion of the PI.

3.2.8 Prior malignancies except resected basal cell carcinoma or treated cervical carcinoma in situ. Cancer treated with curative intent < 5 years will not be allowed unless approved by the principal investigator (PI). Cancer treated with curative intent > 5 years will be allowed.

3.2.9 Patients must not have significant co-morbid medical condition

3.2.10 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements. Patients must not have suffered recent (< 6 months) myocardial infarction, unstable angina, difficult to control congestive heart failure, uncontrolled hypertension, or difficult to control cardiac arrhythmias.

3.2.11 Pregnant women are excluded from this study because Busulfan, melphalan and bortezomib Class D agents with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with the study agent breastfeeding should be discontinued if the mother is treated with Busulfan.

3.2.12 HIV-positive patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with Busulfan. In addition, these patients are at increased risk of lethal infections when treated with marrow-suppressive therapy. Appropriate studies will be undertaken in patients receiving combination antiretroviral therapy when indicated.

3.2.13 Patients found to have an active hepatitis B infection (hepatitis B surface antigen +) are not eligible unless they meet ONE of the following criteria:

- Patient is able to start dual anti-Hep B therapy prior to enrollment with adefovir and telbivudine
- Patient is already on dual anti-hepatitis B therapy
- Consultation and co-management with a hepatitis expert regarding hepatitis B treatment is strongly encouraged before and during the trial.

3.2.14 Patients, who are positive for Hepatits B core antibody, but negative for the Hepatitis B surface antigen, should be started on lamivudine 100mg daily until at least 3 months post stem cell transplant

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. REGISTRATION PROCEDURES

4.1 General Guidelines

The **coordinating center** is: **Montefiore-Einstein Cancer Center**
Montefiore Medical Center-Moses Division
Clinical Trials Office
Department of Oncology, Hofheimer100
111 East 210th Street
Bronx, New York 10467
Phone 718-920-2006
Fax 718-405-4712

The eligible patients will be entered on study centrally at the Montefiore-Einstein Cancer Center Coordinating Center by the Study Coordinator. All sites should call the Clinical Trials Office, phone 718- 920-2006 to verify eligibility. The required forms (eligibility and registration form; form 1) can be found in the Appendix (Appendix D).

Following registration, patients should begin protocol treatment within 5 business days. Issues that would cause treatment delays should be discussed with the Principal Investigator (PI). If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Study Coordinator should be notified of cancellations as soon as possible.

4.2 Registration Process

To register a patient, the following documents should be completed by the research nurse or data manager and faxed or e-mailed to the Study Coordinator at the Montefiore-Einstein Cancer Center Clinical Trials Office, phone 718- 920-2006, fax 718-405-7412 (see also cover page):

- Form 1 (Appendix D)
- Copy of required laboratory tests
- Copies of other supporting documents to determine eligibility (e.g. Echo report, PFT report)
- Signed patient consent form
- HIPAA authorization form

The research nurse or data manager at the participating site will then call the Study Coordinator at the Montefiore-Einstein Cancer Center Clinical Trials Office, phone 718-920-2006, fax 718-405-7412 (see also cover page) to verify eligibility. To complete the registration process, the Coordinator will - after discussion with the PI Ira Braunschweig:

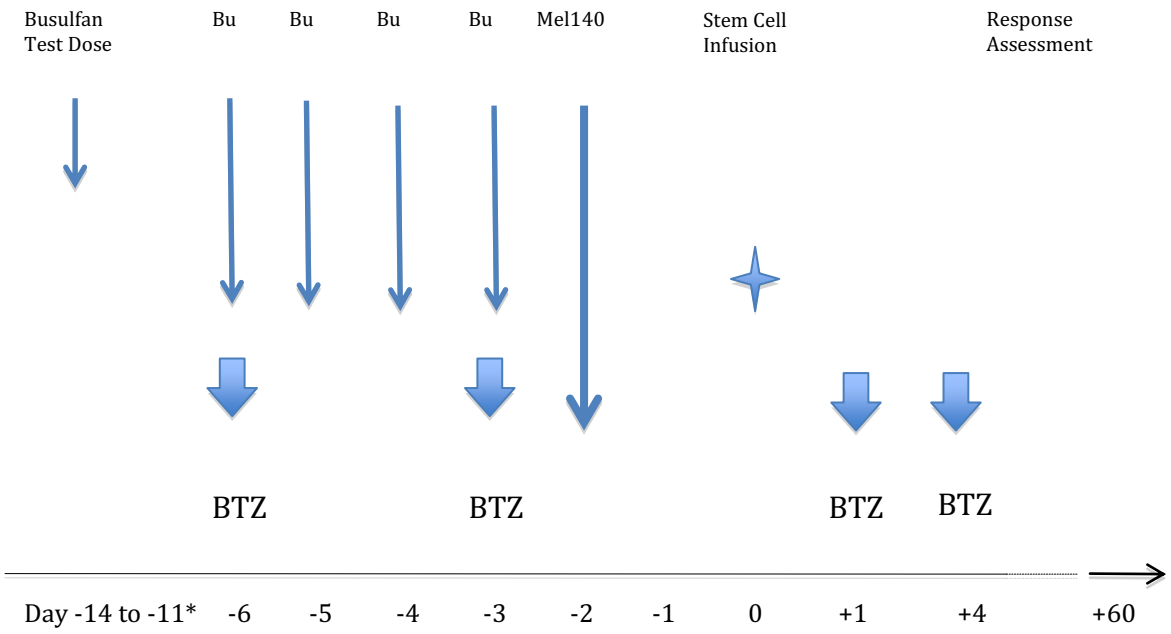
- assign a patient study number
- register the patient on the study
- fax or e-mail the patient study number to the participating site
- call the research nurse or data manager at the participating site and verbally confirm registration.

Once patients are found eligible for the study, they will be registered with the Montefiore-Einstein Cancer Center Coordinating Center. A patient number will be assigned, and a copy of appendix D including the patient number will be faxed back to the participating center/investigator.

5. TREATMENT PLAN

Treatment will be administered on an inpatient basis. **Reported adverse events and potential risks** for busulfan, melphalan and bortezomib are described in **Section 7**. Appropriate **dose modifications** are described in **Section 6**. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

Treatment Schema:



** The baseline period can be extended to -21 to -9 days*

Abbreviations: Bu, busulfan – PK directed; Mel140; melphalan at 140mg/m^2; BTZ, bortezomib at 1mg/m2

Patients will receive an initial test dose of iv busulfan of 0.8 mg/kg IV busulfan as a continuous infusion over 2 hours at any time between Day −14 to −11. Results of the pharmacokinetic (PK) profile following this test dose will inform the dosing for the subsequent administrations of IV busulfan. On Days -6 to -3 patients will be receiving consecutive once daily PK-directed dosing for 4 days of IV busulfan administered as a single daily 3-hour infusion. The overall target integrated AUC (area under the curve) exposure is 20,000 µM-min including the test dose or approximately 5,000µMol-min daily for 4 days (see 5.1.1). This will be followed by IV Melphalan 140mg/m2 on Day -2 (section 5.1.2). IV Bortezomib will be given at a dose of 1mg/m2 on Days -6, -3, +1 and +4 (section 5.1.3) Peripheral blood stem cells (≥2x10^6CD34+ cells/kg) will be infused on day 0 (section 5.1.4).

5.1 Agent Administration

5.1.1 PK directed Busulfan Dosing and Administration

In general busulfan should be administered intravenously via a central venous catheter. DO NOT infuse concomitantly with another intravenous solution of unknown compatibility. Busulfan will be diluted in 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP and is stable at room temperature (25°C) for up to 8 hours but the infusion must be completed within that time. Busulfan diluted in 0.9% Sodium Chloride Injection, USP is stable at refrigerated conditions (2°-8°C) for up to 12 hours but the infusion must be completed within that time.
Busulfan is predominantly metabolized by conjugation with glutathione. Co-administration of busulfan with certain agents can influence clearance and the AUC. A list of restricted

medications is provided in section 5.3.
High dose busulfan can cause seizures and prophylactic use of seizure prophylaxis is mandated (see section 5.2). Antiemetics for a moderately high emetogenic agent (emetogenic score 4) should be used prior as per institutional guidelines (see also section 5.2.3) or as outlined for example by the NATIONAL COMPREHENSIVE CANCER NETWORK® (NCCN) or American Society for Oncology (ASCO) guidelines for supportive care.

Busulfan will be administered with PK directed dosing as outlined below. It is preferred that the Seattle Cancer Care Alliance laboratory is used for the PK measurements. Other centers may use other laboratories for the PK measurements and follow different guidelines for test dose administration, PK assessment and final dose determination. This is allowed as long as the total integrated AUC target for busulfan will be 20,000 µM·min, including the test dose AUC.

5.1.1.1 Test Dose Administration and PK Assessment

A test dose of 0.8 mg/kg of IV busulfan will be administered as a 2-hour continuous infusion (110 minute busulfan infusion + 10 minute flush) in an outpatient setting between Days −21and −9 for PK analysis to inform subsequent PK-directed dosing. The test dose administration day will be chosen to allow for the time required to complete the PK evaluation before the start of the daily consecutive QD IV busulfan dosing on Day −6. For the baseline test dose (recommended Day -14 to Day -11, but can be given between day -21 to -9), a total of six PK samples will be drawn at the following time points: the end of infusion (EOI, upon completion of the 120 minute infusion [110 minute busulfan infusion + 10 minute flush]) (+5minutes), EOI + 15 minutes (± 2 minutes), EOI + 30 minutes (± 2 minutes), and 240, 300 and 360 minutes (±10 minutes) after the start of the infusion. In exceptional circumstances where the is delay from the time from test dose administration to start of the conditioning regimen (e.g. secondary to intercurrent infection, line complication etc.) the window between test dose administration and start of the conditioning regimen can be longer than 1 week. This will need to be discussed on a case-to-case basis with the PI Dr. Braunschweig, who will make the final arbitration after deliberation with the treating physician.

The test dose method assumes the PK of busulfan will be the same on the test dose administration day and the consecutive daily dosing days; as a consequence, medication changes will be restricted from the screening period (or adequate wash-out period for medications discontinued with treatment) through the end of IV busulfan dosing. One exception will be prophylactic anti-seizure treatment; anti-seizure medication is not expected to be initiated until Day −7 as discussed in section 5.2. Refer to section 5.3 for prohibited medications and section 5.2 for supportive care information.

The PK samples will be shipped to the busulfan clinical laboratory in Seattle **(shipping information see Appendix B)** or - if unable to use Seattle Cancer Care Alliance because of institutional guidelines - any other CLIA accredited laboratory that is certified to perform the busulfan PK testing. **If another laboratory than Seattle Cancer Care Alliance is used, please send samples also to Seattle Cancer Care Alliance** under the Montefiore account in order to ensure concordance of the results between the labs. The PK report including busulfan dosing

time and concentration data, estimated PK parameters, and dosing recommendations will be sent to the site after review by the busulfan PK clinical laboratory director or designee. The investigator may contact the Medical Monitor for consultation. **In case of discrepancies of the laboratory results and recommendations, the results of the laboratory required by institutional policy will be used to make the final dose determination after discussion with the study PI** (Ira Braunschweig, MD; email: IBRAUNSC@montefiore.org) or one of his designees.
The total integrated AUC target for busulfan is 20,000 µM·min, including the test dose AUC. The remaining doses will be equally distributed into four doses from Day −6 to Day −3 administered as a continuous 3-hour daily infusion. For subjects whose pharmacokinetic samples were not assayed or missing, the dose of IV busulfan will be 3.2 mg/kg (total daily dose). This dose is consistent with the total daily dose recommended in the US label (0.8 mg/kg x 4 doses per day for 4 days).

5.1.1.2 First PK-Directed Dose Administration and Confirmatory PK

5.1.1.2.1 First PK-Directed Dose Administration

The PK-directed dose recommendation based on the test dose will be provided by the clinical laboratory, available at the start of the consecutive daily dosing regimen (Day -6). The dose will be administered as a single daily 3-hour continuous infusion (170 minute busulfan infusion + 10 minute flush).

5.1.1.2.2 Confirmatory PK

For confirmatory PK assessment on Day -6, a total of six PK samples will be drawn at the following time points: the end of infusion (EOI, upon completion of the 180 minute infusion [170 minute busulfan infusion + 10 minute flush]) (+5 minutes), EOI + 15 minutes (± 2 minutes), EOI + 30 minutes (± 2 minutes), and 270, 360, and 480 minutes (± 10 minutes) after the start of the infusion. The PK report should be available before the third and fourth dose administrations (Days −4 and −3) to confirm the test dose accuracy.

5.1.1.2.3 Further PK-Directed Dose Administration

Results from the confirmatory PK assessment taken on Day −6 will be provided to the investigator. On Day −5 IV busulfan will be administered as a single daily 3-hour continuous infusion (170 minute infusion + 10 minute flush) based on the PK-directed dose recommendation from the test dose as the confirmatory PK results will not yet be available. The investigator may modify subsequent IV busulfan doses according to results from the confirmatory PK sample. Daily exposure to busulfan should be targeted not to exceed 6000 µM/min.

5.1.2 Melphalan

Melphalan will be given as a single dose of 140mg/m² given intravenously once on day -2 over at least 20 minutes. Local oncology pharmacy protocols for the administration of melphalan will be followed.
It is recommended that IV administration of melphalan diluted in 0.9% solutions be completed within one hour of preparation due to the instability of the diluted melphalan.
Care should be taken to avoid possible extravasation of melphalan and it should ONLY be given via a central venous line.
Hypersensitivity reactions including anaphylaxis have occurred in approximately 2% of patients who received the IV formulation. These reactions usually occur after multiple courses of treatment. Treatment is symptomatic.
High dose Melphalan has moderately high emetogenic potential. Therefore antiemetic regimens recommended for agents with emetogenic score 4 should be used as outlined for example by the NATIONAL COMPREHENSIVE CANCER NETWORK® (NCCN) or American Society for Oncology (ASCO) guidelines for supportive care.

5.1.3 Bortezomib

Bortezomib will be administered at a dose of 1mg/m2 as IV push over 3 to 5 seconds followed by a standard saline flush or through a running IV line on Days -6, -3, +1 and +4. On Days -6 and -3 it should be given 120 minutes AFTER the busulfan infusion was completed..
Premedication with antiemetics (e.g. ondansetron* 16 mg PO or IV and dexamethasone 10 mg PO or IV) should be given 60 minutes prior to all Bortezomib doses.
Bortezomib is cytotoxic. As with all cytotoxic drugs, caution is required when preparing and handling bortezomib solutions. Reconstituted Bortezomib should be administered promptly and in no case more than 8 hours after reconstitution.

5.1.4 Peripheral Blood Stem Cell Reinfusion

Peripheral blood stem cell reinfusion may be done according to institutional guidelines. However, the following procedure is suggested:

- On Day 0, the frozen cells are thawed at the bedside (in close proximity to the patient room to minimize transit time) in a 37 - 40°C water bath and reinfused without additional filtering or washing steps. Only one bag of stem cells should be thawed at a time, and not until the preceding bag has been completely infused. The osmolality of the cell suspension requires the use of a flowing central venous catheter. Twelve hours before reinfusion of autologous stem cells, hydration must be initiated with 0.9% NS to ensure urine output of at least 3 ml/kg/hr. Emergency drugs (benadryl, epinephrine and solumedrol) in appropriate doses must be at the bedside. Baseline vital signs are recorded.

- The patient is closely monitored during the infusion. These will be infused IV over 3 - 10 minutes each. If the patient develops chest tightness or other symptoms, a short rest

period may be required. Usually a 30 - 60 minute rest may be required.

• Generally, no additional premedication is needed after the short rest period. It is recommended that a physician be present during the stem cell infusion and for one hour afterwards. A nurse familiar with the adverse signs of blood transfusion should monitor signs between each stem cell infusion and every 15 minutes until one hour after the stem cell infusion is completed. The patient's urine may be red for 12 hours after the infusion because of red cell hemolysis and the pH indicator in the tissue culture media. Orange scent may help mask the DMSO odor which will be present for about 24 hours after the stem cell reinfusion.

5.2 General Concomitant Medication and Supportive Care Guidelines

In general, institutional guidelines can be followed for the following provided the medications show little potential to affect the metabolism of busulfan (see section 5.3).

- Infection prophylaxis
- Prophylactic drugs for blood product infusion
- Antiemetics
- Post-Auto HSCT growth factors.
- Given the high risk for mucositis, the use of Palifermin, a human recombinant keratinocyte growth factor, is encouraged as per institutional guidelines

5.2.1 Seizure prophylaxis

Seizure prophylaxis should start on the evening prior to busulfan infusion (Day -7). Benzodiazepines are the recommended seizure prophylaxis method in this study because they are not eliminated by the same metabolic pathways as busulfan.
Lorazepam can be given in a per os (PO) dose of 0.5 mg every 6 hours beginning the evening on Day −7 before the conditioning regimen of busulfan until the morning of Day −2, one day after the last day of busulfan administration. Lorazepam 0.05 mg/kg IV (maximum 2 mg per dose) may be used as an alternative prophylaxis method. Intravenous lorazepam is given 30 min before each dose of busulfan and continued every 6 h for four additional doses after the last dose of busulfan. The dose may be reduced by 25 to 50% (rounded to the nearest 0.1 mg) if excessive sedation occurs.

Levetiracetam is also an acceptable choice and should be administered as 500 mg PO BID beginning the evening on Day −7 before the conditioning regimen of busulfan until the morning of Day −2, one day after the last day of busulfan administration.

Phenytoin is discouraged for use as prophylaxis for busulfan-induced seizures as phenytoin is known as a potent inducer of hepatic drug-metabolizing enzymes causing increased clearance of busulfan. Subjects with seizures being stable on phenytoin should continue taking it with no change in dosing during the test-dose and conditioning phase of the study.

5.2.2 Infection Prophylaxis

Prophylaxis against Herpes Simplex Virus, Varicellus Zoster Virus, Pneumocystis Jirovecchii Pneumonia, and Candida should be administered to all patients. Institutional guidelines for post-transplant infection prophylaxis should be followed. For example ciprofloxacin 500 mg twice daily (bid) for bacterial prophylaxis, acyclovir 400mg bid or valacyclovir 500mg daily for VZV prophylaxis, fluconazole 400mg daily as fungal prophylaxis, and Bactrim DS 1 tablet three times per week for Pneumocystis Jirovecchii prophylaxis. Fluconazole or other moderate to strong CYP3A4 inhibitors, except as allowed elsewhere in the protocol, may not be given within 3 days of busulfan and bortezomib to prevent interfering with busulfan and bortezomib metabolism (see also list of restricted medications section 5.3)

5.2.3 Antiemetics

Antiemetics can be used as per institutional protocol.
As both busulfan and melphalan at high doses have moderately high emetogenic potential (emetogenic score 4) the use of, a 5-HT_3 antagonist, corticosteroids, and aprepitant or fosaprepitant is recommended.
For details please follow published recommendations by professional societies, such as the NATIONAL COMPREHENSIVE CANCER NETWORK® (NCCN) or American Society for Oncology (ASCO) guidelines for supportive care.

5.2.4 Growth Factors

Post-Auto HSCT growth factors can be used as per institutional guidelines. Use of GCSF (filgrastim) at a dose of 5mcg/kg daily starting by Day +5 is recommended.

5.3 **Restricted Medication**

The following medications listed below are **restricted 72 hours prior to IV busulfan treatment through 48 hours post treatment**:

1. Filgrastim, G-CSF
2. Sargramostim, GM-CSF
3. Vaccines
4. Alkylating Agents (except for protocol specified regimen)
5. Digoxin
6. Herbal supplements
7. Acetaminophen
8. Voriconazole
9. Metronidazole
10. Itraconazole

The following medications listed below are **discouraged 72 hours prior to IV busulfan treatment through 48 hours post treatment:**.

1. Nonsteroidal antiinflammatory drugs
2 Salicylates
3. Thioguanine, 6-TG
4. Anticoagulants
5. Ethotoin
6. Fosphenytoin
7. Immunosuppressives (except for protocol specified regimen)
8. Phenytoin
9. Platelet Inhibitors
10. Strontium-89 Chloride
11. Thrombolytic Agents

Because there is a potential for interaction of busulfan and bortezomib with other concomitantly administered drugs through the cytochrome P450 system, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The Principal Investigator (PI) should be alerted if the patient is taking any agent known to affect or with the potential to affect selected CYP450 isoenzymes.

5.4 Duration of Follow Up

Patients will be followed for up to 5 years, until removal from study or until death, whichever occurs first. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

5.5 Criteria for removal from Study

The following are criteria for removal from the study:

- Disease progression,
- Intercurrent illness that prevents further administration of treatment,
- occurrence of any adverse event (AE), intercurrent illness or abnormality in laboratory assessment results which, in the opinion of the investigator, warrants the subject's permanent withdrawal from the trial
- treatment with a prohibited concomitant medication other than the use of appropriate medications for the treatment of AEs under direction of the investigator
- Participant becomes pregnant
- Participant is lost to follow-up

- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

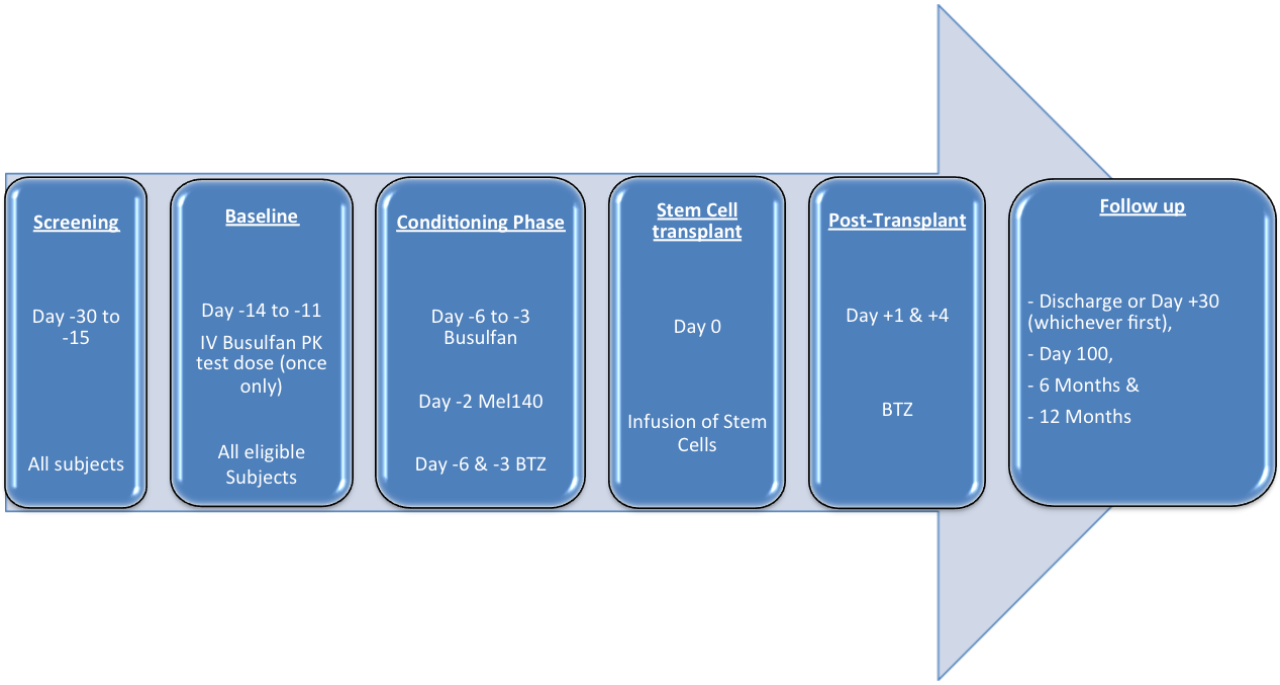
If a participant discontinues the trial prematurely, the reason given must be fully evaluated and recorded appropriately in source documents and the CRF. If the subject is being withdrawn because of an adverse event (AE), that AE should be indicated as the reason for withdrawal. Participants who receive radiation therapy or chemotherapy after transplantation are considered to have treatment failure, unless the radiation was pre-planned consolidation and that decision was made before transplantation. All participants have the right to withdraw at any time during treatment without prejudice. The investigator can discontinue a subject's participation in the trial at any time if medically necessary.

The Principal Investigator should be notified promptly when a subject is withdrawn.

5.6 Consolidation/Maintenance therapy post-transplant

Prospective studies have demonstrated at least a progression-free survival benefit of post-transplant consolidation and/or maintenance therapy.[49-51] More mature results are needed though at present to recommend a unified approach.[52] Therefore the risks and benefits of consolidation and/or maintenance therapy post ASCT need to be discussed with the patient individually and a decision regarding post-transplant consolidation and/or maintenance therapy will be made by the treating physician at their discretion.
NO CONSOLIDATION/MAINTENANCE THERAPY though should be started BEFORE the DAY +100 response evaluation.

6. SCHEDULE OF EVENTS



6.1. Screening Period (Day −30 to Day −15)

Subjects will be screened within 30 to 15 days prior to stem cell transplant. Study procedures and information regarding the nature of the study will be reviewed with potential subjects and written informed consent will be obtained prior to any study related procedures. After signing the informed consent, subjects will be assigned a screening number. Screening procedures will consist of the following:

1) Informed consent will be obtained.
2) Inclusion and exclusion criteria will be reviewed.
3) Demographic information will be collected.
4) Medical history, including previous treatment history (disease characteristics), will be collected.
5) A physical examination, including weight and height, will be performed.
6) Vital signs (blood pressure, heart rate and temperature) will be assessed after the subject has been supine for at least 3 minutes.
7) Hematology and serum chemistry laboratory samples will be collected.
8) INR will be measured.
9) Pulmonary function tests will be performed to determine FEV_1, FVC, or DL_{CO}. If performed within 120 days of the planned IV busulfan test dose, those test results can be used. These data will be reported in the site progress notes only.
10) ECOG and Karnofsky performance status will be assessed. Data from ECOG and Karnofsky status at initial diagnosis will also be collected if available.
11) Myeloma staging and prognostic factors will be performed; this includes at least SPEP, serum IFE, 24h urine UPEP (unless prior urine IFE shows no monoclonal protein), bone marrow biopsy (only if needed to determine complete remission) and serum free light chain assay. Additional tests, such as, skeletal survey, imaging for plasmacytomas might be added if indicated by the individual patient's disease manifestation (see International Myeloma working Group (IMWG) recommendations[3])

- International Staging System (ISS) score[53]
- Cytogenetics at last bone marrow biopsy
- Protein criteria will be determined (quantifiable M-component of IgG, IgA, IgD, or IgE and/or Urinary kappa or lambda Light chain, Bence-Jones protein, or Free Kappa Light Chain or Free Lambda Light Chain)

12) An echocardiogram (ECHO) or MUGA scan will be performed (if done within 90 days of the planned IV busulfan test dose, that result can be used). If cyclophosphamide was used in stem cell harvesting, the ECHO or MUGA must be after the harvest date.
13) A urine or serum pregnancy test will be performed on all women of child bearing potential. If the urine pregnancy test is positive, a serum test to confirm the result should be performed. In the event of a confirmed positive result, the subject will be discontinued from the study.
14) Concomitant medications will be recorded.
15) Adverse events will be assessed
16) Subjects will be instructed to avoid any restricted medications
17) 10ml of Bone marrow Aspirate (taken at time of myeloma staging exam – see 11.) will be sent to Amit Verma's laboratory (for shipping and handling instruction see Appendix E). The patient can refuse participating in this correlative study, but participation is strongly encouraged to maintain scientific validity of the study.
18) At the same time and in the same shipment as the bone marrow aspirate, 10ml of peripheral blood in a green top (sodium heparin) tube with the bone marrow sample clearly labeled "Peripheral Blood pretreatment" as well as patient initials, medical record number and study ID will be sent to Amit Verma's lab. For shipping and handling instructions see Appendix E. The patient can refuse participating in this correlative study, but participation is strongly encouraged to maintain scientific validity of the study.

6.2. Baseline (Day −14 to −11)*

*** The baseline period can be extended from Day -21 to -9**

Prior to IV busulfan test dose administration:
1) Inclusion/exclusion criteria will be re-reviewed to confirm that the subject is still eligible for participation in the study.
2) Vital signs (blood pressure, heart rate and temperature) will be assessed after the subject has been supine for at least 3 minutes.
3) A physical examination, including weight, will be performed. If a physical exam was performed within 72 hours of this visit, the exam does not need to be repeated.
4) A urine or serum pregnancy test will be performed on all women of childbearing potential (WOCBP). If the urine pregnancy test is positive, a serum test to confirm the result should be performed. In the event of a confirmed positive result, the subject will be discontinued from the study.
5) Hematology and serum chemistry laboratory samples will be collected.
6) IV Busulfan test-dose 0.8 mg/kg will be administered as a 2-hour continuous infusion (110 minute busulfan infusion + 10 minute flush).
7) Peripheral blood will be collected at baseline and stored for possible future correlative studies.

Following IV busulfan test dose administration:
8) PK blood samples will be drawn at EOI (upon completion of the 120 minute infusion [110 minute busulfan infusion + 10 minute flush]) (+5 minutes), EOI + 15 minutes (±2 minutes), EOI + 30 minutes (±2 minutes), and 240, 300, and 360 minutes (±10 minutes) after the start of the infusion.
9) Concomitant medications will be recorded.
10) Adverse events will be assessed.
11) Subjects will be instructed to avoid any restricted medications.

6.3. Conditioning Phase

6.3.1. Day −7
1) Body weight will be measured.
2) Vital signs (blood pressure, heart rate and temperature) will be assessed after the subject has been supine for at least 3 minutes.
3) Anti-seizure prophylaxis will be administered.
4) Concomitant medications will be recorded.
5) Adverse events will be assessed.
6) Subjects will be instructed to avoid any restricted medications.
7) Physical exam

6.3.2. Day −6
Prior to IV busulfan PK-directed dose administration:
1) Body weight will be measured.
2) Review IV busulfan dosing recommendation based on PK results following test dose administration. For subjects whose pharmacokinetic samples were not assayed or were missing, the dose of IV busulfan will be 3.2 mg/kg (total daily dose).
3) Vital signs (blood pressure, heart rate and temperature) will be assessed after the subject has been supine for at least 3 minutes.
4) Anti-seizure prophylaxis will be administered.
5) Administration of the first IV busulfan dose per the PK-directed dosing adjustment.
6) Physical exam

Following IV busulfan PK-directed dose administration:
7) PK blood samples will be drawn at EOI (upon completion of the 180 minute infusion [170 minute busulfan infusion + 10 minute flush]) (+5 minutes), EOI + 15 minutes (±2 minutes), EOI + 30 minutes (±2 minutes), and 270, 360, and 480 minutes (±10 minutes) after the start of the infusion
8) Concomitant medications will be recorded.
9) Adverse events will be assessed.
10) Subjects will be instructed to avoid any restricted medications.
11) Bortezomib 1mg/m2 will be given intravenously starting 120 minutes after the Busulfan infusion has been completed

6.3.3. Day −5

Prior to IV busulfan PK-directed dose administration:
1) Body weight will be measured.
2) Vital signs (blood pressure, heart rate and temperature) will be assessed after the subject has been supine for at least 3 minutes.
3) Anti-seizure prophylaxis will be administered.
4) Administration of the second IV busulfan dose as a 3-hour continuous infusion per the PK-directed dosing recommendation. For subjects whose pharmacokinetic samples were not assayed or were missing, the dose of IV busulfan will be 3.2 mg/kg (total daily dose).

Following IV busulfan PK-directed dose administration:
5) Concomitant medications will be recorded.
6) Adverse events will be assessed.
7) Subjects will be instructed to avoid any restricted medications.
8) Physical exam

6.3.4 Day −4
Prior to IV busulfan PK-directed dose administration:
1) Body weight will be measured.
2) Vital signs (blood pressure, heart rate and temperature) will be assessed after the subject has been supine for at least 3 minutes.
3) Anti-seizure prophylaxis will be administered.
4) Review IV busulfan dosing recommendation based on confirmatory PK report and adjust dosing appropriately, if available.
5) Administration of the third IV busulfan dose per the PK-directed dosing recommendation. For subjects whose pharmacokinetic samples were not assayed or were missing, the dose of IV busulfan will be 3.2 mg/kg (total daily dose).
6) Physical exam

Following IV busulfan PK-directed dose administration:
7) Concomitant medications will be recorded
8) Adverse events will be assessed.
9) Subjects will be instructed to avoid any restricted medications
10) PK blood samples might be drawn at EOI (upon completion of the 180 minute infusion [170 minute busulfan infusion + 10 minute flush]) (+5 minutes), EOI + 15 minutes (±2 minutes), EOI + 30 minutes (±2 minutes), and 270, 360, and 480 minutes (±10 minutes) after the start of the infusion

6.3.5. Day −3
Prior to IV busulfan PK-directed dose administration:
1) Body weight will be measured.
2) Vital signs (blood pressure, heart rate and temperature) will be assessed after the subject has been supine for at least 3 minutes.
3) Anti-seizure prophylaxis will be administered.

4) If the confirmatory PK report is received after the Day −4 IV busulfan dose, review IV busulfan dosing recommendation based on confirmatory PK report and adjust dosing appropriately.
5) Administration of the fourth IV busulfan dose per the PK-directed dosing adjustment. For subjects whose pharmacokinetic samples were not assayed or were missing, the dose of IV busulfan will be 3.2 mg/kg (total daily dose).
6) Physical exam
7) Blood work as per calendar

Following IV busulfan PK-directed dose administration:
8) Concomitant medications will be recorded.
9) Adverse events will be assessed.
10) Subjects will be instructed to avoid any restricted medications.
11) Bortezomib 1mg/m^2 will be given intravenously starting 120 minutes after the Busulfan infusion has been completed
12) PK blood samples might be drawn at EOI (upon completion of the 180 minute infusion [170 minute busulfan infusion + 10 minute flush]) (+5 minutes), EOI + 15 minutes (±2 minutes), EOI + 30 minutes (±2 minutes), and 270, 360, and 480 minutes (±10 minutes) after the start of the infusion

6.3.6. Day −2
1) Body weight will be measured.
2) Vital signs (blood pressure, heart rate and temperature) will be assessed after the subject has been supine for at least 3 minutes.
3) Anti-seizure prophylaxis will be administered.
4) Administration of Melphalan 140mg/m_2 as a per institutional protocol
5) Concomitant medications will be recorded.
6) Adverse events will be assessed.
7) Subjects will be instructed to avoid any restricted medications.
8) Physical exam

6.3.7. Day -1 (Rest Day)
No study procedures are scheduled for Day −1. Concomitant medications will be recorded and adverse events will be assessed.

6.3.8. Day 0 (Stem cell re-infusion)
1) Vital signs (blood pressure, heart rate and temperature) will be assessed after the subject has been supine for at least 3 minutes.
2) A physical examination, including weight, will be performed before HSCT.
3) ANC and platelet count recovery information will be collected from Day 0 until day of discharge
4) Stem cell re-infusion will occur according to institutional standard.
5) Concomitant medications will be recorded.

6) Adverse events will be assessed.

6.4. Post-transplant Phase

6.4.1. Day +1

1) Body weight will be measured.
2) Vital signs (blood pressure, heart rate and temperature) will be assessed after the subject has been supine for at least 3 minutes.
3) Concomitant medications will be recorded.
4) Adverse events will be assessed.
5) Bortezomib 1mg/m^2 will be given intravenously
6) Physical exam

6.4.2. Day +4

1) Body weight will be measured.
2) Vital signs (blood pressure, heart rate and temperature) will be assessed after the subject has been supine for at least 3 minutes.
3) Concomitant medications will be recorded.
4) Adverse events will be assessed.
5) Bortezomib 1mg/m^2 will be given intravenously
6) Physical exam

6.5 Follow-Up

6.5.1. Discharge or Day 30 (whichever is earlier)

1) Vital signs (blood pressure, heart rate and temperature) will be assessed after the subject has been supine for at least 3 minutes.
2) Karnofsky performance status will be assessed.
3) A physical examination, including weight, will be performed.
4) ANC and platelet count recovery information will be collected.
5) Full hematology and full clinical chemistry laboratory samples will be collected.
6) Concomitant medications will be recorded.
7) Adverse events will be assessed.

6.5.2. Day 100 Follow-up

1) Vital signs (blood pressure, heart rate and temperature) will be assessed after the subject has been supine for at least 3 minutes.
2) Karnofsky performance status will be assessed.
3) A physical examination, including weight, will be performed.
4) Full hematology and full clinical chemistry laboratory samples will be collected
5) Concomitant medications will be recorded.
6) Adverse events will be assessed.
7) Veno-occlusive disease (VOD) and pulmonary toxicities information will be collected.
8) Disease progression and survival status will be determined (myeloma re-staging – see section 11)
9) Should bone marrow be collected as part of the routine disease restaging at this time, 10ml of bone marrow aspirate and 10ml of peripheral blood in a green top (sodium heparin) tube

will also be sent to Amit Verma's lab. For shipping and handling instructions see Appendix E.
10) Should bone marrow be collected as part of the routine disease restaging at this time, 10ml of bone marrow aspirate will also be sent to Amit Verma's lab. For shipping and handling instructions see Appendix E. The peripheral blood will be sent for storage and possible future research.

6.5.3. Day 180 Follow-up
1) VOD and pulmonary toxicities information will be collected.
2) Disease progression and survival status will be determined (myeloma re-staging – see section 11)
3) Concomitant medications will be recorded.
4) Adverse events will be assessed.

6.5.4. Day 360 Follow-up and every 12 Months thereafter until year 5 post-transplant
(if applicable – can be done via telephone interview)
Follow up visits will be performed every 12 months following transplant
1) VOD and pulmonary toxicities information will be collected.
2) Disease progression and survival status will be determined (myeloma re-staging – see section 11)
3) Adverse events will be assessed.
4) Concomitant medications will be recorded.

6.5.5. End of Study/Early Termination
The End of Study or Early Termination visit will be done via telephone unless the visit occurs prior to Day 100, when it should be done in clinic/inpatient
1) Concomitant medications will be recorded if prior to Discharge.
2) Adverse events will be assessed.
3) Disease progression and survival status will be determined (myeloma re-staging – see section

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited (via AdEERS) reporting **in addition** to routine reporting.

7.1 Adverse Events and Potential Risks Lists

7.1.1 Adverse Events List for iv Busulfan (Busulfex™)

Busulfan is a marketed drug with a well known safety profile which is described in the Investigator's Brochure.[10] The adverse events are below. Adverse events

that are of particular interest are of VOD and pulmonary toxicity. The diagnosis of hepatic veno-occlusive disease (VOD) will be according to the Baltimore criteria[54]. (See Appendix C).

Adverse Events of Busulfan 1/2		
Common (greater than 30%)	**Less common (5%~30%)**	**Rare (less than 5%)**
BLOOD & LYMPHATIC DISORDERS		
Neutropenia	Bruising or bleeding	
Anemia	Thrombosis	
Thrombocytopenia	Neutropenic fever	
	Epistaxis	
EYE DISORDERS		
GASTROINTESTINAL DISORDERS		
Nausea	Rectal discomfort	Hematemesis
Vomiting	Ileus	Gastrointestinal bleeding
Constipation	Ascites	Pancreatitis
Diarrhea	Hepatomegaly	
Abdominal pain	Abnormal liver function	
Sores on mucous membranes and mouth	Jaundice	
Decrease in liver function indicated by abnormal liver function tests	Hepatic Veno-Occlusive Disease (VOD)	
Poor digestion	Hiccups	
Hyperbilirubinemia		
GENERAL DISORDERS & ADMINISTRATIONS SITE CONDITIONS		
Loss of appetite	Inflammation at injection site	Drowsiness
Fever/Chills	Dry Mouth	Secondary Malignancies
Runny nose	Chest pain	
Pain	Pain at injection site	
Allergic reaction	Dizziness	
	Hot flushes	
	Back pain	
INFECTIONS		
Infections	Pharyngitis	Sinusitis
INVESTIGATIONS		
METABOLISM 7 NUTRITION DISORDERS		
Hyperglycemia	Hypohosphosphatemia	Hyponatremia
Low blood level of electrolytes (potassium/calcium/magnesium)		
MUSKULOSKELETAL & CONNECTIVE TISSUE DISORDERS		
	Joint pain	Panniculitis
	Muscle pain	

Adverse Events of Busulfan 2/2		
Common (greater than 30%)	**Less common (5%~30%)**	**Rare (less than 5%)**
Headaches		Tinnitus
		Drowsiness
		Coma
		Cerebral hemorrhage
		Seizure
PSYCHIATRIC DISORDERS		
Anxiety	Depression	Mental disturbances such as severe nervousness, confusion, delusion, agitation, hallucination.
Fatigue		
Insomnia		
RENAL & URINARY DISORDERS		
	Abnormal kidney function tests	
	Decreased urine production	
	Hematuria	
	Dysuria	
RESPIRATORY, THORACIC & MEDIASTINAL DISORDERS		
	Shortness of breath	Pleural effusion
Abnormal breath sounds	Inflammation of the lung	Scar tissue formation in the lungs
Cough	Asthma	Hypoxia
		Respiratory failure
		Collapse of the lung tissue
		Hemoptysis
		Bleeding in the lung
SKIN & SUBCUTANEOUS TISSUE DISORDERS		
Rash	Acne	Redness & scaling of the entire skin
	Itch	
	Change in skin color	
	Alopecia	
VASCULAR DISORDERS		
Arrhthmias	Cardiomegaly	Bradycardia
Tachycardia	Hypotension	Pericarditis/pericardial effusion
Edema	Hypertension	Cardiogenic shock
		Heart failure

7.1.2 Adverse Events List for Bortezomib

Adverse Events with Possible Relationship to Bortezomib (CTCAE v4.0 Term) – 1/2			EXPECTED AEs FOR ADEERS REPORTING For Bortezomib
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	**Expected**
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
Anemia			Anemia
	Febrile neutropenia		
EYE DISORDERS			
	Blurred vision		
GASTROINTESTINAL DISORDERS			
	Abdominal pain		Abdominal pain
Constipation			Constipation
Diarrhea			Diarrhea
	Dyspepsia		Dyspepsia
	Gastrointestinal hemorrhage		
		Gastrointestinal perforation	
	Ileus		Ileus
Nausea			Nausea
Vomiting			Vomiting
GENERAL DISORDERS & ADMINISTRATION SITE CONDITIONS			
	Chills		Chills
Edema limbs			Edema limbs
INFECTIONS & INFESTATIONS			
Infection			Infection
	Infections and infestations - Other (Opportunistic infection associated with >= Grade 2 Lymphopenia))		
INVESTIGATIONS			
Platelet count decreased			Platelet count decreased
	Neutrophil count decreased		Neutrophil count decreased
	Lymphocyte count decreased		
METABOLISM & NUTRITION DISORDERS			
Anorexia			Anorexia
	Dehydration		

Adverse Events with Possible Relationship to Bortezomib (CTCAE v4.0 Term) – 2/2			EXPECTED AEs FOR ADEERS REPORTING For Bortezomib
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	**Expected**
MUSCULOSKELETAL & CONNECTIVE TISSUE DISORDERS			
	Arthralgia		Arthralgia
	Back pain		Back pain
	Bone pain		Bone pain
	Generalized muscle weakness		
	Myalgia		Myalgia
	Pain in extremity		Pain in extremity
NERVOUS SYSTEM DISORDERS			
	Dizziness		Dizziness
	Headache		Headache
		Leukoencephalopathy	
	Neuralgia		Neuralgia
Peripheral motor neuropathy			Peripheral motor neuropathy
Peripheral sensory neuropathy			Peripheral sensory neuropathy
		Reversible posterior leukoencephalopathy syndrome	
	Syncope		
PSYCHIATRIC DISORDERS			
	Anxiety		
	Insomnia		Insomnia
RENAL & URINARY DISORDERS			
		Acute kidney injury	
RESPIRATORY, THORACIC & MEDIASTINAL DISORDERS			
	Cough		Cough
	Dyspnea		
	Epistaxis		
	Pharyngeal mucositis		Pharyngeal mucositis
	Pleural effusion		
SKIN & SUBCUTANEOUS TISSUE DISORDERS			
	Rash maculo-papular		Rash maculo-papular
VASCULAR DISORDERS			
	Hypotension		Hypotension

Also reported on bortezomib trials but with the relationship to bortezomib still undetermined:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (lymphadenopathy); Disseminated intravascular coagulation

CARDIAC DISORDERS - Asystole; Atrial fibrillation; Atrial flutter; Cardiac disorders - Other (cardiac amyloidosis); Heart failure; Left ventricular systolic dysfunction; Myocardial infarction; Pericardial effusion; Right ventricular dysfunction; Sinus bradycardia; Ventricular tachycardia

EAR AND LABYRINTH DISORDERS – Hearing impaired

EYE DISORDERS - Conjunctivitis; Dry eye; Extraocular muscle paresis; Eye disorders - Other (conjunctival hemorrhage); Watering eyes

GASTROINTESTINAL DISORDERS - Abdominal distension; Ascites; Dry mouth; Dysphagia; Esophagitis; Flatulence; Gastritis; Gastrointestinal disorders - Other (ischemic bowel); Gastrointestinal disorders - Other (eructation); Mucositis oral; Oral pain; Pancreatitis; Small intestinal obstruction

GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Edema face; Gait disturbance; General disorders and administration site conditions - Other (hepato-renal syndrome); Injection site reaction; Non-cardiac chest pain; Sudden death NOS

HEPATOBILIARY DISORDERS - Hepatobiliary disorders - Other (portal vein thrombosis)

IMMUNE SYSTEM DISORDERS - Allergic reaction

INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Bruising; Fracture

INVESTIGATIONS - Activated partial thromboplastin time prolonged; Alanine aminotransferase increased; Alkaline phosphatase increased; Aspartate aminotransferase increased; Blood bilirubin increased; CPK increased; Creatinine increased; GGT increased; INR increased; Serum amylase increased; Weight gain; Weight loss

METABOLISM AND NUTRITION DISORDERS - Hypercalcemia; Hyperglycemia; Hyperkalemia; Hyperuricemia; Hypocalcemia; Hypoglycemia; Hypokalemia; Hypomagnesemia; Hyponatremia; Hypophosphatemia; Metabolism and nutrition disorders - Other (hypoproteinemia); Metabolism and nutrition disorders - Other (failure to thrive); Tumor lysis syndrome

MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Buttock pain

NERVOUS SYSTEM DISORDERS - Ataxia; Cognitive disturbance; Depressed level of consciousness; Dysgeusia; Dysphasia; Intracranial hemorrhage; Ischemia cerebrovascular; Memory impairment; Nervous system disorders - Other (spinal cord compression); Nervous

system disorders - Other (cranial palsy); Nervous system disorders - Other (dysautonomia); Seizure

PSYCHIATRIC DISORDERS - Agitation; Confusion; Depression; Psychosis

RENAL AND URINARY DISORDERS - Bladder spasm; Hematuria; Proteinuria; Renal and urinary disorders - Other (calculus renal); Renal and urinary disorders - Other (bilateral hydronephrosis); Renal and urinary disorders - Other (glomerular nephritis proliferative); Urinary frequency; Urinary incontinence; Urinary retention; Urinary tract pain

RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Adult respiratory distress syndrome; Allergic rhinitis; Atelectasis; Bronchopulmonary hemorrhage; Bronchospasm; Hiccups; Hypoxia; Pharyngolaryngeal pain; Pneumonitis; Pulmonary hypertension; Respiratory, thoracic and mediastinal disorders - Other (obstructive airways disease); Voice alteration

SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Dry skin; Hyperhidrosis; Pruritus; Purpura; Skin and subcutaneous tissue disorders - Other (leukoclastic vasculitis); Urticaria

VASCULAR DISORDERS - Capillary leak syndrome; Flushing; Hematoma; Thromboembolic event

Note: Bortezomib (PS-341) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

7.1.3 Adverse Event List for Melphalan

Likely (>10%):
Gastrointestinal: Nausea, vomiting, oral ulceration diarrhea
Hematologic: Myelosuppression, leucopenia, thrombocytopenia, anemia, neutropenia, febrile neutropenia, bleeding
Miscellaneous: Secondary malignancy (<2% to 20%; cumulative dose and duration dependent, includes acute myeloid leukemia, myeloproliferative syndrome, carcinoma), oral ulceration

Less Likely (< 10%):
Miscellaneous: Hypersensitivity, dyspnea, edema, hypotension, pruritus, rash, tachycardia, urticaria), alopecia, amenorrhea, BUN increased, , injection site reactions (ulceration, necrosis), interstitial pneumonitis, jaundice, ovarian suppression, pruritus, pulmonary fibrosis, rash (maculopapular), seizure, SIADH, skin hypersensitivity, skin vesiculation, sterility, stomatitis, testicular suppression, tingling sensation, transaminases increased, vasculitis, warmth sensation, hepatitis, infection, acute renal failure

Rare, but Serious (<1%):
Agranulocytosis, anaphylaxis (rare), bladder irritation, bone marrow failure (irreversible), cardiac arrest, diarrhea, encephalopathy, hemolytic anemia, hemorrhagic cystitis, hemorrhagic

necrotic enterocolitis, hepatic veno-occlusive disease, death

Please refer to the package insert for a complete list of adverse effects.

7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

- **'Expectedness'**: AEs can be 'Unexpected' or 'Expected' (see Section 7.1 above) for expedited reporting purposes only.

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

7.3 Expedited Adverse Event Reporting

7.3.1. Purpose of Adverse Event Reporting and Definition of Serious Adverse Events

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during a trial (please refer to the case report forms). Additionally, certain adverse events must be reported in an expedited manner for timelier monitoring of patient safety and care. The following sections provide information about expedited reporting.

In studies involving human subjects, serious adverse experience means any experience that suggest a significant hazard, contraindication, side effect or precaution. A serious adverse experience includes any experience that is fatal or immediately life threatening, results in a persistent or significant disability/incapacity, requires or prolongs in-patient hospitalization, or is a congenital anomaly, cancer, or overdose. Other important medical events that may not result in death, not be life-threatening, or not require hospitalization may be considered a serious adverse experience when, based upon appropriate medical judgment, the event may jeopardize the subject/patient and may require medical or surgical intervention to prevent one of the outcomes listed previously.

7.3.2. Determination of reporting requirements

Reporting requirements may include the following considerations: 1) whether the patient has received busulfan, melphalan or bortezomib; 2) the characteristics of the adverse event including the grade (severity), the relationship to the study therapy (attribution), and the prior experience (expectedness) of the adverse event; and 3) whether or not hospitalization or prolongation of hospitalization was associated with the event.

Given that high dose chemotherapy by itself is associated with numerous adverse events and an expected prolonged hospitalization of 21-28 days, only certain adverse events of particular concern need to be reported in an expedited manner (see below sections 7.3.6, 7.3.7. and 7.3.8.).

7.3.3. Steps to determine if an adverse event is to be reported in an expedited manner:

Step 1: *Identify the type of event using the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0*. The CTCAE provides descriptive terminology and a grading scale for each adverse event listed. A copy of the CTCAE can be downloaded from the CTEP home page (http://ctep.cancer.gov). Additionally, if assistance is needed, the NCl has an Index to the CTCAE that provides help for classifying and locating terms. All appropriate treatment locations should have access to a copy of the CTCAE.

Step 2: *Grade the event using the NCI CTCAE v4.0*.

Step 3: *Determine whether the adverse event is related to the protocol therapy.* Attribution categories are as follows: Unrelated, Unlikely, Possible, Probable, and Definite.

Step 4: *Determine the prior experience of the adverse event*. *Expected* events are those that have been previously identified as resulting from administration of the agent. An adverse event is considered *unexpected*, for expedited reporting purposes only, when either the type of event or the severity of the event is **NOT** listed in section 7.1.1, 7.1.2 and 7.1.3.

Step 5: *Review the Protocol-Specific Expedited Adverse Event Reporting Exclusions (section 7.3.8).*

Step 6: *Determine if the protocol treatment given prior to the adverse event included busulfan, melphalan or bortezomib, or a combination of these agents.*

7.3.4. Reporting methods

An FDA Medwatch form (available online: http://www.fda.gov/downloads/Safety/MedWatch/HowToReport/DownloadForms/ucm082725.pdf) should be faxed to the Montefiore-Einstein Cancer Center Coordinating Center and to the local institutional IRB**,** according to the guidelines outlined in section 7.3.5-7.3.7.

7.3.5. When to report an event in an expedited manner

This trial will be monitored by the Albert Einstein Cancer Center Data Safety Monitoring Committee (AECC DSMC). Follow guidelines in section 7.3.7 regarding criteria that required reporting of adverse events that required require 24-hour notification. A copy of the adverse event report using MEDWATCH form should be faxed the Albert-Einstein Cancer Center Coordinating Center (see cover page for contact information).
For any adverse events that occur more than 30 days after the last dose of treatment, only those that have an attribution of possibly, probably, or definitely AND meet the reporting requirements in Sections 7.3.6 must be reported on an expedited adverse event report form as per guidelines in section 7.3.7.

7.3.6. Expedited reporting for the conditioning regimen Bu/Btz/Mel140

Adverse events that require expedited reporting are outlined below in Table 1.

NOTE: All deaths on study require both routine and expedited reporting regardless of causality. Attribution to treatment or other cause should be provided. All grade 3 and 4 pulmonary toxicity and neuropathy as well as moderate to severe VOD (see below, section 7.3.7 *and Appendix C)* require expedited adverse events reporting.

Any medical event equivalent to **CTCAE v4.0[2] grade 3, 4, or 5 that precipitates hospitalization (**hospitalizations are defined as lasting 24 hours or longer) **or prolongation of existing hospitalization (>28 days from admission)** must be reported regardless of attribution and designation as expected or unexpected with the exception of any events identified as protocol-specific expedited adverse event reporting exclusions.
Any event that results in **persistent or significant disability/incapacity, congenital anomaly, or birth defect** must be reported via an expedited AE mechanism.

Expedited AE reporting timelines:

- **24 Hours; 5 calendar days** – The investigator must initially report the AE within 24 hours of learning of the event followed by a complete the AE report within 5 calendar days of the initial 24-hour report.
- **10 calendar days** – A complete AE report on the AE must be submitted within ten calendar days of the investigator learning of the event.

Additional instructions, requirements and exceptions for this protocol:

With respect to determining the specific day by which the event must be reported, the day the reporter learns of the adverse event constitutes “Day 0”

Table 1: Expedited Reporting Requirements for Adverse Events that occur within 30 days of the stem cell infusion

CTCAE v4.0 grade	1	2	2	3		3		4 & 5	4 & 5 ¶
Expected-ness	Unexpected and Expected	Unexpected	Expected	Unexpected with Hospitalization	Unexpected Without Hospitalization	Expected With Hospitalization	Expected without Hospitalization	Unexpected	Expected
Unrelated Unlikely	Not Required	Not Required	Not Required	Not Required	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days
Possible Probable Definite	Not Required	10 Calendar Days	Not Required	Not Required	Not Required	Not Required*	Not Required*	24-Hour; 5 Calendar Days	10 Calendar Days**

Adverse events with attribution of possible, probable, or definite that occur greater than 30 days after the last dose of treatment require reporting as follows:

- 24-hour notification followed by complete report within 5 calendar days for:
  - Grade 4 and Grade 5 unexpected events
- Complete SAE report within 10 calendar days:
  - Grade 3 unexpected events with hospitalization or prolongation of hospitalization
  - Grade 5 expected events

¶Although 24-hour notification is not required for death clearly related to progressive disease, a full report is required as outlined in the table.

*except grade 3 pulmonary toxicities and neuropathy

** except grade 4 hematological toxicities

7.3.7. Protocol Specific expedited reporting requirements:

Contact Ira Braunschweig by telephone (718-920-4826) or email (IBRAUNSC@montefiore.org) to report the occurrence of an unexpected grade II or higher adverse drug reaction, any adverse event that requires hospitalizations or toxic death (see section 7.3.6).
Of particular concern are grade 3 and 4 pulmonary toxicity, neuropathy, and moderate to severe VOD (see below, section 7.3.7 and Appendix C). These require expedited adverse events reporting as per the Expedited Reporting Guidelines and immediate contact of the PI Ira Braunschweig.

For grade 2 and 3 unexpected events, reporting is only required if the event is related to the investigational conditioning regimen. It is not required if the event is related only to other agents used in the post-transplant period.

NOTE: For grade 3 unexpected events with hospitalization lasting ≥24 hours following discharge (or prolonged hospitalization of>28 days), an AE report is required even if the event is unrelated to the investigational conditioning regimen (Bu/Mel140/Btz). Specifically, the following should be reported within 24 to 48 hours:

- All life threatening events (grade 3 or 4) which may be due to drug administration.
- All fatal events.
- The first occurrence of any previously unknown clinical event (regardless of grade).
- A permanently or severely disabling event
- Prolonged or unexpected inpatient hospitalization (>28 days)
- Overdose of drug
- Discontinuation of dosing due to an adverse experience
- **Hospitalizations:** Any grade 1 or 2 adverse event which precipitates a hospitalization lasting > 24 hours (or prolongs hospitalization) must be reported via AE report within 10 calendar days of learning of the event regardless of the attribution and designation as expected or unexpected
- **Grade 3 or 4 pulmonary toxicities**
- Grade 3 or 4 neuropathy
- **Veno-occlusive Disease (VOD):** Any grade VOD must be submitted via expedited AE within 10 calendar days of learning of the event, regardless of the attribution.
- For **VOD, severity** will be graded according to the following:[55]
  - **MILD DISEASE:** If patients showed no apparent adverse effect from liver disease; required no medications for diuresis of excessive fluid or for hepatic pain; and had completely reversible signs, symptoms, and laboratory abnormalities.
  - **MODERATE DISEASE:** If patients had an adverse effect from liver disease; required sodium restriction and diuretics to minimize signs of fluid excess (edema, ascites, cardiopulmonary congestion) or medication to alleviate pain from hepatomegaly; and eventually showed a complete resolution of all signs of liver damage (a return of weight to baseline, a decrease in liver size, and a decrease in total serum bilirubin to < 34.2 µmol/L [2 mg/dL]).
  - **SEVERE DISEASE:** If patients showed an effect from liver disease, and signs, symptoms, and laboratory values did not resolve before day 100 or the patient died, whichever occurred first.

An Adverse Reaction Form (Form 1639) will be completed by Dr. Braunschweig, or his staff for timely submission to the FDA, and the other investigators.

The Adverse Drug Reaction needs to be reported to the institutional IRBs of all participating institutions and to the FDA by the IND holder. Dr. Braunschweig or his staff will report any adverse drug reaction or toxic death to all investigators within 48 hours of notification, or sooner should there be another patient being considered for enrollment.

7.3.8. **Protocol-Specific Expedited Adverse Event Reporting Exclusions**

For this protocol only, certain AEs/grades are exceptions to the Expedited Reporting Guidelines and do not require reporting in an expedited manner (*i.e.*, AdEERS). **Grade 3-4 hematological toxicities and Grade 3 non-hematological toxicities** are expected with high dose chemotherapy and **do not need to be reported in an expedited manner except** grade 3 and 4

pulmonary toxicity, neuropathy, and moderate to severe VOD (see above, section 7.3.7.*)*.

7.4 Data Safety and Monitoring Plan

During the recruitment and follow up phases of the trial, the Albert Einstein Cancer Center Data and Safety Monitoring Board (DSMB) will monitor data and oversee patient safety. The principal investigator will participate in the meetings as a non-voting member. The DSMB will meet every 6 months, or after enrollment of every 5 patients whichever comes first. They will review adverse affects, adherence to protocol and study progress. All adverse events will be reported, as per policy, to the CCI/IRB. The DSMB will make recommendations regarding study performance and continuation. Ira Braunschweig will provide requested data to the DSMB to assess progress toward resolving research questions.

In addition, Dr Ira Bruanschweig (PI) will review case report forms (CRFs) and any other toxicity data from all patients enrolled in this trial at regular frequent intervals. Biweekly conference calls will be conducted between the participating institutions to discuss trial patient enrolment, adverse events and protocol adherence.
Each participating investigator will have primary responsibility for the safety of individual participants under his/her care and will review the data and safety on an ongoing basis. In addition, the independent DSMB will have primary responsibility for monitoring the accumulating study data for sign of adverse trends in morbidity / mortality and drug toxicity.

7.5 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions. **AEs reported through AdEERS must also be reported in routine study data submissions.**

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational or commercial agents administered in this study can be found in Section 7.1.

8.1.1 IV Busulfan (Busulfex™)

Supplied Form

BUSULFEX™ is commercially available and is distributed as a unit carton of eight vials. BUSULFEX™ is packaged as a sterile solution in 10 ml single-use clear glass vials each containing 60 mg of busulfan at a concentration of 6 mg/ml for intravenous use, Unopened vials of BUSULFEX™ must be stored under refrigerated conditions between 2°-8°C (36°-46°F).

Storage and Stability

BUSULFEX™ diluted in 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP is stable at room temperature (25°C) for up to 8 hours but the infusion must be completed within that time. BUSULFEX™ diluted in 0.9% Sodium Chloride Injection, USP is stable at refrigerated conditions (2°-8°C) for up to 12 hours but the infusion must be completed within that time

Guidelines for Administration

BUSULFEX™ must be diluted prior to use with either 0.9% Sodium Chloride Injection, USP (normal saline) or 5% Dextrose Injection, USP (D5W). The diluent quantity should be 10 times the volume of BUSULFEX™ , so that the final concentration of busulfan is approximately 0.5 mg/ml.. Parenteral drug products should be visually inspected for particulate matter and discoloration prior to administration whenever the solution and container permit. If particulate matter is seen in the BUSULFEX™ vial the drug should not be used.
An administration set with minimal residual hold-up volume (2-5 cc) should be used for product administration. BUSULFEX™ should be administered intravenously via a central venous catheter.
DO NOT infuse concomitantly with another intravenous solution of unknown compatibility.

As with other cytotoxic compounds, caution should be exercised in handling and preparing the solution of BUSULFEX™ . Skin reactions may occur with accidental exposure. The use of gloves is recommended. Procedures for proper handling and disposal of anticancer drugs should be considered.

8.1.2 Melphalan

Supplied Form

Melphalan for Injection is commercially available in a carton containing one single-use clear

glass vial of a sterile, non-pyrogenic, freeze-dried powder. Each single-use vial contains melphalan hydrochloride equivalent to 50 mg melphalan and 20 mg povidone. Melphalan for Injection is reconstituted using the sterile diluent provided. Each vial of sterile diluent contains sodium citrate 0.2 g, propylene glycol 6.0 ml, ethanol (96%) 0.52 ml, and Water for Injection to a total of 10 ml.

Storage and Stability

Store at controlled room temperature 15° to 30°C (59° to 86°F) and protect from light. Melphalan solution, diluted to final concentration in normal saline, should be administered within 1 hour. Nearly 1% of melphalan hydrolyzes every 10 minutes. The reconstituted solution should not be refrigerated, since a precipitate forms.

Guidelines for Administration

Melphalan is practically insoluble in water and has a pKa1 of ~2.5. Reconstitute the 50 mg vial with 10 ml of the provided diluent to yield a 5 mg/ml solution. Further dilute the solution to a concentration no greater than 0.45 mg/ml in normal saline. Inject 10 ml of the supplied diluent directly into the vial of lyophilized powder using a 20-gauge or larger needle and syringe. Immediately shake vigorously until a clear solution is obtained. This provides 5 mg/ml of melphalan. Immediately dilute dose to be administered in sodium chloride 0.9% injection to a concentration of no more than 0.45 mg/ml..
Complete administration within 60 min of reconstitution. Administer by injecting slowly into a fast-running IV infusion via an injection port of central venous line over at least 15 minutes. Do not administer by direct injection into a peripheral vein.

The time between reconstitution/dilution and administration of Melphalan should be kept to a minimum because reconstituted and diluted solutions are unstable. Over as short a time as 30 minutes, a citrate derivative of melphalan has been detected in reconstituted material from the reaction of melphalan with Sterile Diluent for melphalan. Upon further dilution with saline, nearly 1% label strength of melphalan hydrolyzes every 10 minutes. In order to ensure the administration of IV melphalan doses within one hour, the preparation of the dose on an “on call” basis is a reasonable consideration.

A precipitate forms if the reconstituted solution is stored at 5°C. DO NOT REFRIGERATE THE RECONSTITUTED PRODUCT.

As with other toxic compounds, caution should be exercised in handling and preparing the solution of ALKERAN. Skin reactions associated with accidental exposure may occur. The use of gloves is recommended. If the solution of melphalan contacts the skin or mucosa, immediately wash the skin or mucosa thoroughly with soap and water. Procedures for proper handling and disposal of anticancer drugs should be considered.

Parenteral drug products should be visually inspected for particulate matter and discoloration prior to administration whenever solution and container permit. If either occurs, do not use

this product.

Hypersensitivity reactions including anaphylaxis have occurred in approximately 2% of patients who received the IV formulation. These reactions usually occur after multiple courses of treatment. Treatment is symptomatic. The infusion should be terminated immediately, followed by the administration of volume expanders, pressor agents, corticosteroids, or antihistamines at the discretion of the physician. If a hypersensitivity reaction occurs, IV or oral melphalan should not be re-administered since hypersensitivity reactions have also been reported with oral melphalan.

8.1.3 Bortezomib

Supplied Form
Bortezomib for Injection is a sterile, lyophilized powder for reconstitution and is supplied in vials containing Bortezomib and mannitol at a 1:10 ratio. Drug is available in sterile, single-use vials containing 3.5 mg of bortezomib. Vials containing 3.5 mg of bortezomib contain 35 mg of mannitol.

Storage and Stability
Vials containing lyophilized Bortezomib for Injection should be stored according to the label requirements. For the United States, store at USP Controlled Room Temperature which is 25ºC (77ºF); excursions permitted from 15 to 30ºC (59 to 86ºF). For Europe, do not store above 30ºC (86ºF). To date, stability data indicate that the lyophilized drug product is stable for at least 18 months when stored under the recommended conditions.

Guidelines for Administration
The appropriate amount of Bortezomib will be drawn from the injection vial and administered as IV push over 3 to 5 seconds followed by a standard saline flush or through a running IV line. Vials are for single-use administration.
On days Bortezomib is given with Busulfan, bortezomib should be given 120 minutes AFTER the busulfan infusion has finished.

Bortezomib is cytotoxic. As with all cytotoxic drugs, caution is required when preparing and handling bortezomib solutions. The use of gloves and other appropriate protective clothing is recommended. In case of skin contact, wash the affected area immediately and thoroughly with soap and water for at least 15 minutes. If product contacts eye, immediately flush eye thoroughly with water for at least 15 minutes. Always contact a physician after any form of body contact. All materials that have been used for preparation should be disposed of according to standard practices. A log must be kept of all disposed materials.

Each vial of bortezomib for Injection should be reconstituted under a laminar flow biological cabinet (hood) within 8 hours before dosing with 3.5 ml of NS (0.9%), Sodium Chloride Injection USP, so that the reconstituted solution contains bortezomib at a concentration of 1 mg/ml. Prior to reconstitution the vials should remain in the cartons to protect them from light. Dissolution is completed in approximately 10 seconds. The reconstituted solution is clear and

colorless, with a final pH of 5 to 6. Reconstituted bortezomib should be administered promptly and in no case more than 8 hours after reconstitution. All materials that have been used for preparation should be disposed of according to standard practices.

9. CORRELATIVE/SPECIAL STUDIES

A bone marrow (BM) aspirate will be performed within the framework of the routine staging bone marrow punctures for each patient 1 to 28 days prior to the start of therapy. An extra tube of 10cc will be sent within 24 hours to the Albert-Einstein College of Medicine Department of Molecular Biology Laboratory where they will be processed and centrally analyzed. We will use a multi-parameter high-speed FACS for analysis and sorting of CD138 positive plasma cells. A detailed description of specimen collection, handling and shipping can be found in **Appendix E.**

Methylation studies of myeloma cells from pretreatment samples on CD138+ sorted plasma cells will be performed in Amit Verma's laboratory. The MyPRS Plus™ commercially available gene expression profile will be used as gene expression study.

Gene expression signatures will be correlated with response to therapy in order to develop biomarkers of response and prognosis.
Aberrant epigenetic changes will be determined with a HELP (HpaII tiny fragment Enrichment by Ligation-mediated PCR) assay to perform an unbiased genome-wide analysis of DNA methylation. Methylome analysis on pretreatment samples will be correlated with response.

Peripheral blood will be collected at baseline and at the Day 100 assessment. The blood will be stored for possible future correlative studies, such as SNP arrays or plasma cell enriched FISH studies.

10. STUDY CALENDAR

STUDY CALENDAR																	
Procedures	**Screening**	**Base line**	**Conditioning Phase**							**Stem Cell Trans-plant**	**Post-Transplant**		**Follow-up**				**End of Study**
Day	**-30 to -15**	**-14 to -11^**	**-7**	**-6**	**-5**	**-4**	**-3**	**-2**	**-1**	**0**	**+1**	**+4**	**+30* or Discharge**	**+100 ****	**+180 ****	**+360 ****	
Informed Consent	x																
Inclusion/Exclusion Criteria	x	x															
Demographic Information	x																
Medical & Previous Treatment History	x																
Physical Exam (incl. Height & Weight)	x	x	x	x	x	x	x	x	x	x			x				
Vital Signs	x	x	x	x	x	x	x	x	x	x			x				
Hematology, Serum Chemistry	x	x					x						x	x			
Peripheral blood for correlative studies	x	X												x			
INR	x																
Pulmonary Function Tests	x																
Performance Status (ECOG/KPS)	x												x	x			
Myeloma Staging,***	x													x	x	x	x
Echo/MUGA	x																
Pregnancy Test	x	x															
Anti-seizure Prophylaxis			x	x	x	x	x	x^^									
Test Dose Busulfan (IV 0.8mg/kg)		x															
PK Sampling		x		x													
Review Busulfan Dosing				x		x	x										
PK directed Busulfan dose				x	x	x	x										
Bortezomib				x			x				x	x					
Melphalan								x									
Rest Day									x								
Stem Cell Re-infusion										x							
Assess disease progression/ survival status****														x	x	x	x
ANC & Platelet recovery																	
Concomitant medications	x	x	x	x	x	x	x	x	x	x			x	x	x	x	x
Adverse Events	x	x	x	x	x	x	x	x	x	x			x	x	x	x	x

^ The baseline period can be extended from day -21 to day -9 if required. Day -14 to -9 is the recommended time period
^^ dose only in the morning
* Plus/Minus 5 days around Day +30 is permissible
** Plus/Minus 14 days around Day +100, Day +180 and Day +360 is permissible
*** Myeloma Staging includes at least SPEP, Serum IFE, 24 hour Urine UPEP, Urine IFE, Serum free light chain measurements (SFLC), bone marrow biopsy and aspirate (see 11.1)

**** Disease response determination based on measurable disease at study entry as per the IMWG citeria[3]

Baseline evaluations are to be conducted within 2 weeks prior to start of protocol therapy. Myeloma staging must be done within 4 weeks prior to the start of therapy. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of therapy.

11. MEASUREMENT OF EFFECT

11.1 Response Assessment

For the purposes of this study, patients should be reevaluated for response on Day +100, Day +180 and Day +360 post autologous stem cell transplantation. Additionally, patients should be evaluated earlier at any time that there is a clinical indication for disease progression.

Response and progression will be evaluated in this study using the criteria recommended by the International Myeloma Working Group (IMWG).[3]

11.1.1. Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with BUSULFEX™ .

Evaluable for objective response. Only those patients who have measurable disease present at baseline, have received therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below.

11.1.2 Disease Parameters

Measurable disease. Measurable disease is defined by Protein criteria (quantifiable M-component in serum or Serum Free Light Chain Assay) in order to evaluate response as per IMWG[3]. Non-secretory patients are eligible provided the patient has > 20% plasmacytosis OR multiple (>3) focal plasmacytomas or focal lesions on MRI

11.1.3 Methods for Evaluation of Measurable Disease

All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported measurement at baseline and during follow-up. If a measure was negative during baseline it does not need to be

repeated at the time of follow up (e.g. UPEP)

11.1.4 **Response Criteria**

11.1.4.1 **Definitions of Response**[3]

All response categories require two consecutive assessments made at anytime before the institution of any new therapy; all categories also require no known evidence of progressive or new bone lesions if radiographic studies were performed. Radiographic studies are not required to satisfy these response requirements.

Stringent Complete Response (sCR):

CR as defined below plus

- Normal FLC ratio and
- Absence of clonal cells in bone marrow[a] by immunohistochemistry or immunofluorescence[b]

Complete Response (CR):

- Negative immunofixation on the serum and urine and
- Disappearance of any soft tissue plasmacytomas and
- ≤5% plasma cells in bone marrow[a]

Very Good Partial Response (VGPR):

- Serum and urine M-protein detectable by immunofixation but not on electrophoresis or
- 90% or greater reduction in serum M-protein
- plus urine M-protein level <100mg per 24 h

Partial Response (PR):

- ≥50% reduction of serum M-protein and
- reduction in 24-h urinary M-protein by ≥90% or to <200mg per 24 h

- If the serum and urine M-protein are unmeasurable,[d] a ≥50% decrease in the difference between involved and uninvolved FLC levels is required in place of the M-protein criteria

- If serum and urine M-protein are unmeasurable, and serum free light assay is also unmeasurable, ≥50% reduction in plasma cells is required in place of M-protein, provided baseline bone marrow plasma cell percentage was ≥30%

- In addition to the above listed criteria, if present at baseline, a ≥50% reduction in the size of soft tissue plasmacytomas is also required

Stable Disease (SD):

- Not meeting criteria for CR, VGPR, PR or progressive disease

Abbreviations: CR, complete response; FLC, free light chain; PR, partial response; SD, stable disease; sCR, stringent complete response; VGPR, very good partial response.

[a] Confirmation with repeat bone marrow biopsy not needed.
[b] Presence/absence of clonal cells is based upon the kappa/lambda (k/l) ratio. An abnormal k/l ratio by immunohistochemistry and/or immunofluorescencec requires a minimum of 100 plasma cells for analysis. An abnormal ratio reflecting presence of an abnormal clone is k/l of >4:1 or <1:2.

The rate of Overall Response (OR) is defined as the percentage of participants that achieve either sCR, CR, VGPR and PR.

11.1.4.2 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence. The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria

11.1.5 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented.

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are me.

11.1.6 Survival End Points

Progression-Free Survival

Progression free survival (PFS) is defined as the duration of time from transplant (Day 0) to time of progression.

Progressive disease[a] is defined by any one or more of the following:
Increase of ≥25% from baseline in

- Serum M-component and/or (the absolute increase must be ≥0.5 g/dl) [b]
- Urine M-component and/or (the absolute increase must be ≥200 mg/24 h)

- Only in patients without measurable serum and urine M-protein levels: the difference between involved and uninvolved FLC levels. The absolute increase must be >10 mg/dl.
- Bone marrow plasma cell percentage: the absolute % must be ≥10% [c]
- Definite development of new bone lesions or soft tissue plasmacytomas or
- Definite increase in the size of existing bone lesions or soft tissue plasmacytomas
- Development of hypercalcemia (corrected serum calcium >11.5 mg/dl or 2.65 mmol/l) that can be attributed solely to the plasma cell proliferative disorder

Clinical relapse: [a]
Clinical relapse requires one or more of:
Direct indicators of increasing disease and/or end organ dysfunction (CRAB features).[b] It is not used in calculation of time to progression or progression-free survival but is listed here as something that can be reported optionally or for use in clinical practice

- Development of new soft tissue plasmacytomas or bone lesions
- Definite increase in the size of existing plasmacytomas or bone lesions. A definite increase is defined as a 50% (and at least 1 cm) increase as measured serially by the sum of the products of the cross-diameters of the measurable lesion
- Hypercalcemia (>11.5 mg/dl) [2.65 mmol/l]
- Decrease in hemoglobin of ≥2 g/dl [1.25 mmol/l]
- Rise in serum creatinine by 2 mg/dl or more [177 mmol/l or more]

Abbreviations: CR, complete response; DFS, disease-free survival.
[a] All relapse categories require two consecutive assessments made at anytime before classification as relapse or disease progression and/or the institution of any new therapy.
[b] For progressive disease, serum M-component increases of >1 gm/dl are sufficient to define relapse if starting M-component is ≥5 g/dl.
[c] Relapse from CR has the 5% cutoff versus 10% for other categories of relapse.
[d] For purposes of calculating time to progression and progression-free survival, CR patients should also be evaluated using criteria listed above for progressive disease.

Event-free survival (EFS): The definition of an event is 1) disease progression or 2) death from any cause. EFS is the time from stem cell transplant until an event as defined above has occurred.

TTP (Time to Progression): This is the time from start of treatment to disease progression with deaths owing to causes other than progression not counted, but censored. This is a helpful method to assess the durability of treatment benefit.

Overall survival (OS) is defined as the time from transplant until death of any cause.

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1 Data Management

Data on toxicities and patient outcomes will be collected at the individual participating institution treating the patient on protocol. Every 4 weeks the collected data will be submitted to the Montefiore-Einstein Cancer Center Research Study Coordinator at the Clinical Trials Office. Data submitted as Case Report Forms (CRF) will be submitted by participating institutions for central data management to the Montefiore-Einstein Cancer Center Research Study Coordination Center on a monthly basis, where they will be merged with the other data available for each study participant into one master data set.

12.2 Patient Protection

This study will be conducted in compliance with the protocol, Good Clinical Practice and the applicable regulatory requirements. All research personnel will receive the required education and training needed for conducting clinical research related to the protection of human subjects and personal health information according to the Institutional Review board and the Health Insurance Portability and Accountability Act of 1996 Public Law 104-191. As mentioned above, each patient will be assigned a study identification number at the time of recruitment. Records will be kept confidential. As this research involves a drug, the U.S. Food and Drug Administration (FDA) may inspect the research records and medical records as may the Office for Human Research Protections (OHRP) and employees from Otsuka Pharmaceutical Co, Ltd. The research study doctors and research staff will review medical records and will keep the information private. No patient will be identified in any written or verbal report with the following exception: the local human research committees of the participating institutions may inspect the patient records. All hard copies of information will be stored in locked cabinets, to which only senior research personnel has access. The study data files will be stored on password protected computers.

12.3 Recruitment and Informed Consent

Patients who are identified by clinicians at the participating sites as being eligible for the study will be approached by the local PI or co-PI about their potential participation. An informed consent document in English and Spanish will be provided to the patient. The investigational nature of the study, its objectives, the procedures and the potential risks and benefits involved will be explained to the patient in simple and direct lay language avoiding medical terms. The patient and the person taking the consent will sign the consent form and a copy of it will be given to the patient. A copy of the consent and the protocol will be left in the patient's chart.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

The study is a multi-center phase II study.

The primary endpoint will be the rate of complete response (CR) at Day +100 after ASCT as determined by the IMWG criteria.[3] We aim to demonstrate that the complete response rate (CRR) 100 Days after the ASCT is at least 35%. This would demonstrate at least the same complete response rate as the reported complete response rates of the Mel200 conditioning regimen.

Our null hypothesis states that we will have achieved a complete response of less than 35% 100 days after the ASCT. This CR rate is estimated based on a conditioning regimen using melphalan alone at a dose of 200mg/m2 intravenously in this setting. Traditionally, the complete response (CR) rate observed after Mel200 followed by ASCT is between 10-35% depending on the induction regimen used.[6-8]
Our alternative hypothesis is that use of our regimen will have at least the same complete response rate of 35% or higher than melphalan alone (Mel200) by intention-to-treat analysis.

Secondary endpoints will be the overall response rate (sCR, CR, VGPR plus PR, see section 11.1.4), as well as toxicity assessment using CTCAE v4.0[2] and the Baltimore criteria for VOD[54], time-to-progression and survival (progression-free survival, event-free survival and overall survival).
The association between gender, race and pharmacokinetic data will be explored as another secondary endpoint. Methylation and gene expression profiles will be associated with outcome.

13.2 **Sample Size/Accrual Rate**

If the true response rate for the new regimen (Bu/Mel/Btz) is 55%, with 28 patients in the study, we will be able to construct a 95% confidence interval for the true response rate with the lower bound no less than 35%. In other words, with 28 patients in the study, if the true response rate is 55%, we will be able to conclude with 95% confidence that the response rate for the new regimen is at least 35%.

The primary and coordinating site will be the Montefiore-Einstein Cancer Center (MECC). The Bone Marrow Transplantation team at MECC sees on average 40 to 50 patients per year that would meet the eligibility criteria for the study.

We anticipate an annual accrual of ≥20 patients. The projected time for completion of the study is 24 months. Should the accrual be less than 8 patients per six month period from the start of activation of the protocol, we will be actively approaching other sites to participate in the study.
The New York Cancer Consortium mechanisms® will be utilized to conduct and coordinate the study and sites.

13.3 **Analysis of Secondary Endpoints**

The analysis of the secondary endpoints is exploratory.
The secondary endpoints to be explored will be the overall response (stringent complete response,

complete response, very good partial & partial response, stable disease and progression), toxicity as assessed by the Common Terminology Criteria for Adverse Events (CTCAE) v4.0,[2] mortality at days 30, 100 and 360 post transplant, time to progression, median progression, event free and overall survival, and possibly associations of the pharmacokinetic profile of iv busulfan as determined by the test dose and the variables gender and race.
We will use descriptive statistics and tabular representations to describe and evaluate response rates and toxicities.
Time-to-event analysis for PFS, EFS, and OS (defined in section 11.1.6) will be performed using Kaplan-Meier methods. The log-rank test will be used to compare survival among patients based on their response (as defined in section 11.1.4.1). Bivariate analysis will be used to asses the association of variables such as gender and age with response as the outcome. Categorical variables will be analyzed using the chi-square test or the non-parametric Fisher's exact test; continuous variables will be analyzed using the t-test if normally distributed, or the Wilcoxon rank sum test. We will use logistic regression to analyze association of variables with response if they are biologically significant or were found on bivariate analysis to be significantly associated with the outcome.
Epigenetic and genomic data (methylation and gene expression profiles of CD138 positive bone marrow plasma cells) will be examined in an exploratory manner to assess association with the clinical outcomes response rate and survival.

13.4 Reporting and Exclusions

13.4.1 Evaluation of toxicity.

All patients will be evaluable for toxicity from the time of their first treatment with Busulfex™

13.4.2 Evaluation of response.

All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: 1) stringent complete response, 2) complete response, 3) very good partial response, 4) partial response, 5) stable disease, 6) progressive disease, 7) early death from malignant disease, 8) early death from toxicity, 9) early death because of other cause, or 10) unknown (not assessable, insufficient data).

All of the patients who met the eligibility criteria (with the possible exception of those who received no study medication) should be included in the main analysis of the response rate. Patients in response categories 5-10 should be considered to have a treatment failure (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate.

13.5 Interim Analysis Decision Rules

An interim analysis will be performed after the first 20 patients have enrolled to asses the safety of the proposed treatment. Stopping rules will be based on the 30-day mortality.

The anticipated treatment-related mortality with the proposed regimen is less than 5% based on previous studies using high dose chemotherapy followed by autologous stem cell transplantation in patients with multiple myeloma (ranging from 0 to 5%).[5,6,36,56,57] We will consider a 30-day mortality of more than 5% as unacceptable. The 30-day mortality will be evaluated after the first 20 patients have been enrolled. If no more than 1 patient suffers a mortality within 30 days that is attributable to the therapy and not the underlying disease or unrelated to therapy, we will continue to accrue until the accrual goal is met. If 2 or more patients suffer a mortality that is directly attributable to therapy within 30 days of transplant, we will suspend accrual and investigate the causes of the higher mortality.

Once the root of the high mortality has been determined, the study team will confer with the DSMC and the IRB to make a determination whether accrual can be resumed.

APPENDIX A

Performance Status Criteria

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
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).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
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.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX B - Busulfan PK Testing information

Laboratory Hours and Contact Information:
Tuesday to Saturday: 8:00am to 5:00pm
Sundays, Mondays, and holidays: On call
Phone: (206) 288-7389
Fax: (206) 288-7397
Email: pklab@seattlecca.org
Please send samples to:
Pharmacokinetics Laboratory
Seattle Cancer Care Alliance
825 Eastlake Ave E., Room G7-405
Seattle, WA 98109-1023
http://www.seattlecca.org/busulfan-lab-samples.cfm

Sending Samples to the Busulfan Laboratory
If the institution has already an account with Seattle Cancer Care Alliance, please download the Busulfan Electronic Order Form (http://www.seattlecca.org/client/documents/doctors-providers/Busulfan%20E-order%20Form_8582_0.pdf) to your computer and follow its instructions to order busulfan testing. (You need Adobe Acrobat Reader 7.0 or above; go to http://www.adobe.com for a free download.) If the institution is a new client, please contact pklab@seattlecca.org for account set-up.
This information is also available on the company's website:
http://www.seattlecca.org/busulfan-lab-samples.cfm

Shipment:
Advance notice is required for all sample shipments:
Tuesday to Saturday arrival: At least 48 hours in advance.
Sunday, Monday, or holiday arrival: At least 72 hours in advance (Sample acceptance is only on pre-arranged basis).
Please call or email us to provide package tracking information.

Sample Collection and Processing:
Draw minimum 4 ml of blood into 4-mL Sodium Heparin green top blood tubes.
Busulfan degrades quickly at room temperature. Each sample should be kept in wet ice slurry or refrigerated at all time. Centrifuge them as soon as possible at 4 °C. Separate the plasma from each sample and transfer it to individual 4mL plastic tubes. Immediately freeze these plasma samples at -20 °C.
All samples have to be labeled with patient name, Medical Record #, date and actual clock time of the blood draw. Two unique identifiers and clock times are REQUIRED or samples can be rejected.
Samples must be shipped on a minimum of 3 kg of dry ice using an overnight carrier. Samples that arrived thawed will not be analyzed.

A REQUISITION SHEET for regimen of Q24hr is required to be sent with the samples

(http://www.seattlecca.org/client/documents/Req_Q6-IV_Q24-IV_Busulfex_v2.pdf). Patient identifiers must be matched with those on the collected samples mentioned above. Also, our requisition serves as physician's order and must be signed by the attending physician or designee/caregiver according to the College American Pathologists (CAP) requirement.
Results

We usually release pharmacokinetics results on the same day as receipt of the samples.
Average sample run-time is approximately 3 hours.
Sample processing is prioritized by time zone differences or next dose due time to maximize the advantage of dose adjustments.
All results must be verbally delivered to attending physician, pharmacist, or caregiver who is a certified MD or PharmD. No exceptions.
After the verbal report, a hardcopy can be faxed to designated number(s). CPT Code and Cost of the Services
The CPT code for our GC-MS analysis of busulfan is 82542.
The cost of the analysis for each sample is USD $224.25. This includes the analysis and dose recommendations.

APPENDIX C

Baltimore Criteria[54] for diagnosis of VOD

Development of hyperbilirubinemia with serum bilirubin > 2 mg/dl within 21 days after transplantation and at least 2 of the following clinical signs and symptoms:

• Hepatomegaly, usually painful,

• > 5% weight gain,

• Ascites.

Source: Jones RJ, Lee KS, Beschorner WE, et al. Venoocclusive disease of the liver following bone marrow transplantation. Transplantation. 1987;44:778-783

For **VOD, severity** will be graded according to the following:[55]

• **MILD DISEASE:** If patients showed no apparent adverse effect from liver disease; required no medications for diuresis of excessive fluid or for hepatic pain; and had completely reversible signs, symptoms, and laboratory abnormalities.

• **MODERATE DISEASE:** If patients had an adverse effect from liver disease; required sodium restriction and diuretics to minimize signs of fluid excess (edema, ascites, cardiopulmonary congestion) or medication to alleviate pain from hepatomegaly; and eventually showed a complete resolution of all signs of liver damage (a return of weight to baseline, a decrease in liver size, and a decrease in total serum bilirubin to < 34.2 µmol/L [2 mg/dL]).

• **SEVERE DISEASE:** If patients showed an effect from liver disease, and signs, symptoms, and laboratory values did not resolve before day 100 or the patientdied, whichever occurred first.

APPENDIX D – FORM 1 (REGISTRATION AND ELIGIBLITY)

Initials: _____ MR# _____ DOB: _____ Center:_____Date: _____
Treating Physician: _____

"A Phase II study Assessing the Efficacy and Toxicity of PK-directed Intravenous Busulfan in Combination with High-Dose Melphalan and Bortezomib as Conditioning Regimen for First-line Autologous Hematopoietic Stem Cell Transplantation in Patients with Multiple Myeloma"

Inclusion Criteria: **The answers to the following questions must be YES in order for the patient to be eligible.**	**YES**	**NO**
3.1.1. Histologically or cytologically confirmed Multiple Myeloma		
3.1.2. Measurable disease must be present at diagnosis as defined by Protein criteria (quantifiable M-component in serum, urine or Serum Free Light Chains) in order to evaluate response as per IMWG. Non-secretory patients are eligible provided the patient has > 20% plasmacytosis OR multiple (>3) focal plasmacytomas or focal lesions on MRI		
3.1.3. Age ≥18 years and ≤72 years		
3.1.4. Must have received induction chemotherapy for myeloma, but no more than a total of 12 months of chemotherapy, and must be eligible for the first planned autologous transplant		
3.1.5. A minimum stem cell dose of 2.0 x 10^6 CD34+ cells/kg has been collected.		
3.1.6. Life expectancy of greater than 12 months		
3.1.7. ECOG performance status ≤2 (Karnofsky ≥60%; see Appendix A		
3.1.8. Patients must have normal organ and marrow function as defined below: - leukocytes ≥3,000/mcL (unless myeloma related) - absolute neutrophil count ≥1,500/mcL (unless myeloma related) - platelets ≥50,000/mcL (unless myeloma related) - total bilirubin ≤2 X institutional upper limit of normal unless 2nd to Gilbert's disease - AST(SGOT)/ALT(SGPT) ≤3 X institutional upper limit of normal - creatinine ≤1.5 X institutional upper limit of normal OR - creatinine clearance ≥60 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal		
3.1.9. Ejection fraction by ECHO or MUGA ≥ 40% performed within 90 days prior to registration		
3.1.10. Adequate pulmonary function studies: > 50% of predicted on mechanical aspects (FEV1, FVC) and diffusion capacity (DLCO) > 50% of predicted, within 120 days of registration. If the patient is unable to complete pulmonary function tests due to MM related pain or condition, exception may be granted if the principal investigator (PI) documents that the patient is a candidate for high dose therapy.		
3.1.11. Women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for at least six month following the stem cell transplantation		

3.1.12. Ability to understand and the willingness to sign a written informed consent document.		
Exclusion Criteria: **The answers to the following questions must be NO in order for the patient to be eligible.**	**YES**	**NO**
3.2.1. Patient received chemotherapy or pelvic radiotherapy within 4 weeks prior to entering the study or has not recovered from adverse events due to agents administered more than 4 weeks earlier.		
3.2.2 Prior treatment history of autologous HSCT or high-dose chemotherapy with stem cell rescue for any medical reason		
3.2.3 Patient is receiving another investigational agent.		
3.2.4 Patient has brain metastases		
3.2.5 History of allergic reactions attributed to compounds of similar chemical or biologic composition to or other agents used in the study, such as busulfan, melphalan, bortezomib, boron, or mannitol		
3.2.6 Grade 2 or greater peripheral neuropathy within 14 days prior to enrollment		
3.2.7 Unresolved grade >/= 3 non-hematologic toxicity from previous therapy.		
3.2.8 Prior malignancies except resected basal cell carcinoma or treated cervical carcinoma in situ. Cancer treated with curative intent < 5 years		
3.2.9 Significant co-morbid medical condition		
3.2.10 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements. Patients must not have suffered recent (< 6 months) myocardial infarction, unstable angina, difficult to control congestive heart failure, uncontrolled hypertension, or difficult to control cardiac arrhythmias		
3.2.11 Positive pregnancy test if woman of child bearing potential		
3.2.12 Patient is HIV positive		
3.2.13 Active Hepatitis B as defined by Hepatitis B surface antigen positivity, unless able to start dual anti-HepB therapy, or already on dual anti-HepB therapy		

Fax Registration Form to: Montefiore-Einstein Coordinating Center (see cover page)
To be completed by the Montefiore-Einstein Cancer Center Clinical Trials Office and faxed back to Site:
Patient Eligible: Yes _____ No_____
Patient ID :_____(this should be used as patient identifier)

___ _____ _____
Coordinating Center Staff Signature Date

APPENDIX E

HANDLING AND SHIPPING OF BONE MARROW SPECIMENS:

1. Collect (3) Green top tubes of marrow aspirate that contain sodium heparin
2. Invert the tubes thoroughly to prevent clotting

SPECIMEN TRANSPORT AND DELIVERY TO DEPARTMENT OF MOLECULAR BIOLOGY LABORATORY:

1. Identify all tubes with the patient name, medical record number, and laboratory I.D. number, then seal in a biohazard bag for transportation.
2. YOU MUST NOTIFY EITHER DR. IRA BRAUNSCHWEIG* OR DR. AMIT VERMA PRIOR TO SENDING A SAMPLE
3. Place sealed specimens onto wet ice, and ship container to the following address overnight via FEDEX:

Amit K. Verma, M.B.B.S.
Albert Einstein College of Medicine
Jack and Pearl Resnick Campus,
Chanin Building, Room 302B
1300 Morris Park Avenue
Bronx, New York 10461
Phone 718-930-8761
Email: amit.verma@einstein.yu.edu

3. It is absolutely imperative that all specimens arrive in this laboratory within 24 hr of collection; overnight rush delivery is therefore required.

4. Any specimen demonstrating presence of clots cannot be processed.

** Please call Dr. Ira Braunschweig (phone: 718-920-4826) or Amit Verma to notify that a shipment has been sent and for any questions.*

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