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Protocol Page

A Randomized Trial to Compare Busulfan + Melphalan 140 mg/m2 with Melphalan 200 mg/m2 as Preparative Regimen for Autologous Hematopoietic Stem Cell Transplantation for Multiple Myeloma
2010-0071

Core Protocol Information

Short Title	Bu-Mel vs. Mel 200
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Which Committee will review this protocol?

(•) The Clinical Research Committee - (CRC)

Protocol Body

1.0 Objectives

Primary Objective

To compare the progression-free survival (PFS) rates of Busulfan + Melphalan (Bu-Mel) with Melphalan alone in patients with multiple myeloma (MM).

Secondary Objectives

1. To compare rates of complete responses at day 90 (CR) between the two arms.
2. To compare rates of complete responses + very good partial responses (CR+VGPR) between the two arms.
3. To compare overall survival (OS) between the two treatment arms.
4. To compare the outcome (CR, PFS and OS) between the 2 arms in high-risk patients.
5. To compare Grade 3-4 toxicities, graded according to CTCAE between the 2 arms.
6. To compare treatment-related mortality (TRM) between the 2 arms.
7. To compare quality of life between the 2 arms by measuring the symptom burden using the MD Anderson Symptom Inventory (MDASI).

2.0 Background

The Role for Busulfan in Marrow Transplantation for Advanced Hematologic Malignancies

Busulfan [1, 4-bis-(methanesolfonoxyl) butane] is a bifunctional alkylating agent, and its use for a long period (~1954-1975) was limited to low dose oral therapy with palliative intent, requiring frequent blood count monitoring (Canellos 1985, Hughes 1991). In 1974, however, Santos and Tutschka investigated the use of busulfan to create a murine model of aplastic anemia (Santos 1974). Subsequently, the experience gained in this model system was used to introduce high-dose combination chemotherapy based on oral busulfan for pretransplant conditioning of non-human primates and, thereafter, for patients undergoing both autologous and allogeneic marrow transplantation (Santos 1983, Lu 1984, Yeager 1986, Tutschka 1987, Peters 1987, Geller 1989, Grochow 1989, Dix 1996, Sheridan 1989, Clift 1994, Schwertfeger 1992, Vaughan 1991). Since then, high-dose busulfan, most commonly in combination with cyclophosphamide (BuCY), has proven to be an effective antileukemic regimen when used in conjunction with autologous or allogeneic hematopoietic stem cell support.

Rationale for the Combination of Busulfan and Melphalan

Several series have demonstrated the potent antitumor effects of melphalan in advanced hematologic malignancies (Maraninchi, 1986). Furthermore, because it has tolerable non-overlapping, non-hematologic toxicities with busulfan, it has been combined with busulfan to provide an effective, myelosuppressive

preparative regimen prior to autologous and allogeneic HSCT (Vey 1996, Cony-Makhoul 1995, Meloni 2001). However, the majority of these studies used oral busulfan, and extensive mucositis and veno-occlusive disease (VOD) were documented. Trials from our insitution and others (Andersson 2003, De Lima 2004, Shimoni 2003, Callander 2003, Schuler 2001) have shown that the i.v. preparation of busulfan is more effective and less toxic.

However, some experience has already accumulated from a phase I study of escalating doses of i.v. busulfan with fixed dose melphalan in multiple myeloma patients receiving autologous HSCT at the University of Texas Health Science Center in San Antonio. Engraftment and toxicity data were recorded for patients receiving busulfan, with doses ranging from 10-13.5 mg/kg i.v., combined with melphalan at 140 mg/m^2. Thus far, 25 patients have received this regimen: neutrophil engraftment occurred at 9.5 $\pm$ 0.9 days, platelet engraftent occurred at 11.3 $\pm$ 1.5 days, no grade IV mucositis or diarrhea occurred, and notably, no patients developed VOD (Callander 2003).

We will use fixed doses of i.v. busulfan, derived from the pharamcokinetic (PK) data from a previous phase II study (see below), and melphalan in this phase II study.

The main cytotoxic actions of busulfan and melphalan are through DNA alkylation. To investigate whether these biochemical events can be correlated with treatment outcome, we intend to collect cells from consenting patients with circulating myeloma cells (>2,000 cells/μL), or bone marrow cells and study the *in vitro* alkylating effects of busulfan and melphalan and, if possible, in relation to cytokine activation. Correlative samples will also be collected after the i.v. administration of busulfan and melphalan during the conditioning treatment. All of these correlative studies will be conducted under separate IRB-approved studies and will require separate consent.

2.2 Rationale for Busulfan Dose and Schedule

The IV dose to be administered in this study was derived from the PK data from the previous phase II study of i.v. high-dose busulfan with cyclophosphamide as pretransplant conditioning therapy, and from information obtained from Dr J. Russell (Calgary), who has used i.v. busulfan with fludarabine, as pretransplant conditioning therapy for approximately 250 patients undergoing allogeneic hematopoietic stem cell transplant (HSCT) for hematologic malignancies (J Russell, MD, personal communication, December, 2000). His dose of 3.2 mg/kg body weight was extrapolated from our previous experience of using 0.8 mg/kg IV every six hours for a total of 16 doses. The 0.8 mg/kg dose has previously been demonstrated to be pharmacokinetically similar to what was achieved with an oral dose of 1.0 mg/kg. The 3.2 mg/kg body weight is equivalent to a dose of 130 mg/m2 dose. This dose can be anticipated to yield a median target AUC of 5 – 6,500 μM-min, and it should be considered safe, affording a low risk for hepatic VOD. The careful PK analysis of i.v. busulfan in relationship to dosing

weight, actual weight vs. adjusted ideal weight vs. ideal weight, yielded the information that dosing based on body surface area (BSA) derived from actual weight would give the most consistent dosing of i.v. busulfan for adults (Vaughan 1999). Therefore, in this protocol, BSA will be derived from actual body weight for all patients, including those who are greater than 20% above their ideal body weight. This is a shift in dosing strategy from our previous standard, in which we used ideal body weight to derive BSA to determine dosage.

Rationale for Melphalan Dose and Schedule

The melphalan total dose of 140 mg/M2 has been well-studied in a variety of combination regimens both at MD Anderson and elsewhere and is tolerable. In subsequent studies, we propose to test the addition of a third agent, e.g a nucleoside analogue, to busulfan plus melphalan to effect controlled inhibition of DNA repair which is produced by busulfan/melphalan, and thus wish to allow a safety margin for any additional organ toxicity which might result. We have provided for a 24 hr "rest day" between busulfan and melphalan for two purposes: 1) to allow repletion of hepatic glutathione stores which will be depleted by busulfan (Meister 1988) and which can exascerbate melphalan toxicity (Awasthi 1996, Troyano 2001) and 2) to allow for pilot dose melphalan administration to consenting patients. Ultimately, we propose to deliver both busulfan and melphalan in pharmacokinetically(PK)-directed doses to minimize interpatient variability in toxic and therapeutic effects. To this end, we have shown that when melphalan is administered as a fractionated dose over 2-3 days, the PK of the first day's dose predicts well for the PK of the subsequent doses (Jones, unpublished data). Administering melphalan over two days not only coincides with a pattern established here in multiple other studies, but would allow an alternate method of PK-directed dosing if the pilot studies do not prove useful.

Rationale for the Combination of Busulfan and Melphalan for Myeloma

At least two randomized trials have shown that compared to conventional agents, high dose chemotherapy followed by autologous bone marrow transplantation results in significantly prolonged survival inpatients with newly diagnosed multiple myeloma (MM). However, relapse remains the most important cause of treatment failure in these patients. Based on the results of randomized phase III trials, high-dose melphalan at 200 mg/m2 is considered the standard preparative regimen for MM. The median duration of remission after this regimen is only about 24 months, which warrants exploration of more effective regimens.

Earlier attempts at improving results with more intensive regimens have not resulted in improved outcomes. Based on at least two recent single arm clinical trials, a combination of busulfan and melphalan was found to be safe and effective in patients with myeloma. in the first trial, conditioning with oral busulfan 12 mg/kg plus melphalan 140 mg/m(2) was associated with longer progression-free survival but equivalent survival to that achieved with melphalan 200 mg/m(2). There was a higher frequency of veno-occlusive

disease-related deaths in the busulfan arm. This latter fact together with the limited access to novel salvage therapies in patients conditioned with oral busulfan 12 mg/kg plus melphalan 140 mg/m(2) may explain the absence of a survival difference. Oral busulfan was used in that study; and the authors concluded that the use of intravenous busulfan may reduce toxicity and result in greater efficacy, and warrants further investigation in myeloma patients.

In the second trial from our institution, intravenous (i.v.) busulfan (Bu) was combined with melphalan (Mel) in patients with advanced lymphoid malignancies undergoing autologous stem cell transplantation. Bu 130 mg/m^2 was infused daily for 4 days, either as a fixed dose per BSA, or to target an average daily area under the curve of 5000 µmol-min, determined by a test dose of i.v. Bu at 32 mg/m^2 given 48 hours prior to the high-dose regimen, followed by a rest day, followed by 2 daily doses of Mel at 70 mg/m^2. Eighty patients had i.v. Bu delivered per test dose guidance. The median daily systemic Bu exposure was 4867 µmol-min. One hundred two patients (Hodgkin lymphoma n = 49, non-Hodgkin lymphoma n = 12, multiple myeloma = 41) with a median age of 44 years (range: 19-65 years) were treated. The 2-year overall survival and progression-free survival rates were 82% and 42%, respectively, for patients with multiple myeloma. The regimen was very well tolerated with treatment-related mortality at 100 days, 1 year, and 2 years of 1%, 3%, and 3%, respectively. Intravenous Bu-Mel was well tolerated. Disease control was encouraging, and should be explored in larger phase II studies.

We compared the CR rates and PFS in patients who received an auto-hematopoetic stem cell transplantation (HCT) after Bu-Mel with several novel preparative regimens that were used at our institution. We restricted our analyses to patients undergoing auto-HCT for consolidation of first remission. The results are summarized in the Table below.

	CR (%)	Median PFS (months)
Bu-Mel	9/32 (28)	33
TMC	5/37 (13)	20.6
MAC	6/28 (21)	26.8
MAC-V	8/41(19)	28.8

TMC= topotecan, melphalan, cyclophosphamide (Kazmi et al. 2010)

MAC = melphalan, arsenic trioxide, Vitamin C (Qazilbash et al. 2008)

MAC-V = melphalan, arsenic trioxide, Vitamin C, bortezomib (Sharma et al. 2009)

Based on these encouraging preliminary results, we would like to propose this

phase III trial to compare the safety and efficacy of a combination of a combination of IV busulfan and melphalan 140 mg/m2 with melphalan 200 mg/m2 in an autologous SCT setting.

At least two randomized trials have shown that high dose chemotherapy followed by autologous bone marrow transplantation results in significantly prolonged survival of patients with newly diagnosed MM, when compared to conventional agents (Attal et al. 1996, Child et al. 2003) However, relapse remains the most important cause of treatment failure in these patients. Based on the results of randomized phase III trials, high-dose melphalan at 200 mg/m2 is considered the standard-of-care preparative regimen for MM. The median duration of remission after this regimen is only about 24 months. (Attal et al. 1996, Child et al. 2003)That warrants exploration of more effective preparative regimens for MM. Earlier attempts at improving results with more intensive regimens have not resulted in improved outcomes. Based on at least two recent single arm clinical trials, a combination of busulfan and melphalan was found to be safe and effective in patients with myeloma. There is a suggestion from these trials that this combination may result in a superior outcome. (Lahuerta et al. 2010, Kebriei et al)

In this phase III trial we propose to compare the safety and efficacy of a combination of single daily dose IV busulfan and melphalan 140 mg/m2 with melphalan 200 mg/m2 in an autologous stem cell transplant setting.

This was a randomized clinical trial of IV Busulfan + Melphalan (B+M) versus Melphalan (M) as preparative regimens for autologous hematopoietic stem cell transplantation (auto-HCT) for multiple myeloma. Following the prescribed rule in the trial design, this trial was stopped early due to an imbalance in the empirical rates of complete remission at day 90 (CR90) in the two arms. The empirical rates were 35% in the M arm versus 16% in the B+M arm. This result was the opposite of what was anticipated when planning the trial, which was motivated by the hypothesis that adding IV Busulfan to Melphalan would improve patient outcome.

On closer inspection of the data, a very surprising result was seen in terms of progression free survival (PFS) time. For PFS time, the opposite effect of that for CR90 was seen. Specifically, the B+M arm has better PFS than the M arm, as shown in Figure 1, which records PFS time from day 90 when CR90 is evaluated. A similar difference is seen for PFS time recorded from date of transplant. This statistical result may be considered perplexing because, within each treatment arm, for patients for whom CR90 was evaluated, on average the occurrence of CR90 increased the time to failure (progression or death). That is, CR90 improved PFS in both arms.

So the question has arisen of how it can be the case that

1) CR90 is higher in the M arm compared to the B+M arm,
2) CR90 improves PFS in both arms, yet
3) PFS is superior in the B+M arm compared to the M arm.

A computation explaining this somewhat counterintuitive result is given in the Appendix. Essentially, for each treatment arm, the time to failure probability distribution is a mixture (average) of the time to failure probability distributions for the two subgroups of patients for whom (i) treatment achieves CR90 and (ii) treatment does not achieve CR90.

At this point, the trial has been stopped because the rule based on CR90 worked correctly, showing that the M arm is superior the B+M arm in terms of CR90, but the data also show that the B+M arm is superior to the M arm in terms of PFS.

However, the current data are not sufficiently strong, in terms of sample size and magnitude of the difference in PFS, to provide confirmatory evidence that B+M is superior to M for these patients.

Consequently, we feel strongly that this trial should be re-opened, but using a design based on PFS time as the primary endpoint, with CR90 as a secondary endpoint.

3.0 Background Drug Information

Busulfan:
Therapeutic Classification: Antineoplastic Alkylating agent.

Pharmaceutical data: Busulfan injection is a sterile, pyrogen-free solution provided in a mixture of dimethylacetamide (DMA) and polyethyleneglycol 400 (PEG400). It is supplied in 10 ml single use ampoules at a concentration of six (6) mg busulfan per ml. Each ampoule contains 60 mg of busulfan in 3.3 ml of DMA and 6.7 ml of PEG400. When diluted in normal saline or D5W to a concentration of 0.5 mg/ml, the resulting solution must be administered within eight (8) hours of preparation including the three (3) hour infusion of the drug.

Stability and storage: Ampoules should be stored refrigerated at 2-8°C (35-46°F). Stable at 4°C for at least twelve (12) months. Additional stability studies are in progress. DO NOT use beyond the expiration date. DO NOT use if the solution is cloudy or if particulates are present.

Solution Preparation: Prepare the busulfan solution as follows (The patient is to receive a dose of 130 mg/m^2 of busulfan): mix into normal saline to a final concentration of 0.5 mg/mL.

In each bag 6.0 mg busulfan (1.0 ml at 6 mg/ml and 11 ml saline) should be added to compensate for drug lost in the tubing with each infusion (approximately 12 ml at 0.5 mg/ml is lost in the tubing when using the controlled rate infusion pump).

Route of Administration: It is to be noted, that a sufficient amount of diluted busulfan should be added to compensate for the amount needed to prime the IV tubing; when hanging the infusate, the tubing should be primed with the busulfan solution and connected as close to the patient as possible, i.e. by a 3-way connector at the level of the central venous catheter. After completed infusion, the tubing with remaining busulfan (approximately 12 mL) should be disconnected and discarded. All busulfan infusions should be performed by programmable pump.

The high-dose busulfan will be given by slow intravenous infusion over three (3) hours into a central venous catheter.

CAUTION: DO NOT ADMINISTER AS AN INTRAVENOUS PUSH OR BOLUS. An infusion pump will be used with the busulfan solutions as prepared above. A new infusion set must be used for administration of each dose. Prior to and following each infusion, flush the catheter line with normal saline or (approximately 5 ml). Start the three-hour infusion at the calculated flow rate. DO NOT infuse concomitantly with another intravenous solution of unknown compatibility.

If a delay in administration occurs after the infusion solution is prepared, the properly identified container should be kept at room temperature (20-25°C), but administration must be completed within eight (8) hours of preparation including the three (3) hour drug infusion.

Adverse Events: Dose limiting toxicity is expected to be hematological when used without stem cell support. Other toxicities seen frequently following high-dose busulfan in preparative regimens for bone marrow transplantation include: VOD, nausea, vomiting, pulmonary fibrosis, seizures, rash, and an Addison's-like syndrome.

Mechanism of action: Interferes with DNA replication and transcription of RNA through DNA alkylation, and ultimately results in the disruption of nucleic acid function.

Animal Tumor Data: Busulfan has been shown to be active against a variety of animal neoplasm *in vivo*, including mouse sarcoma 180 and Ehrlich's mouse ascites tumor.

Human Pharmacology: Limited pharmacology data are available for the parenteral formulation to be used in this study and is detailed in the evaluation of IV Bu in a Phase II Trial using IV Bu at 0.8 mg/kg BW given over 2 hr every 6 hr for a total of 16 doses (Andersson et al, 2002) and when administered once daily for 4 days at a dose of 130 mg/m2 in combination with Flu (Madden et al, AHS 2003, de Lima et al, BLOOD 2004). The pharmacokinetic data suggests that the plasma decay of the formulation fits an open one-compartment model with linear pharmacokinetics in the dose range of 12 mg-130 mg/m2. Based on studies of oral Bu, the drug is reported

to be extensively metabolized; twelve (12) metabolites have been isolated, but most have not been identified. The drug is slowly excreted in the urine, chiefly as methanesulfonic acid. Ten to fifty percent (10-50%) of a dose is excreted as metabolites within twenty-four (24) hours (Nadkarni 1959).

Melphalan

Therapeutic Classification: Melphalan is an alkylating agent of the bischloroethylamine type. As a result, its cytotoxicity appears to be related to the extent of its interstrand cross-linking with DNA, probably by binding at the N^7 position of guanine. Like other bifunctional alkylating agents, it is active against both resting and rapidly dividing tumor cells.

FORMULATION: Melphalan for injection is supplied as a sterile, nonpyrogenic, freeze-dried powder. Each single-use vial contains melphalan hydrochloride equivalent to 50 mg melphalan and 20 mg povidone.

PREPARATION: Melphalan for injection must be reconstituted by rapidly injecting 10 mL of the supplied diluent directly into the vial of lyophilized powder using a sterile needle and syringe. This provides a 5 mg/mL solution of melphalan. Immediately dilute the dose to be administered in 0.9% Sodium Chloride Injection, USP, to a concentration of 1.5 mg/mL. Administer the diluted product over a minimum of 15 minutes. Complete the administration within 60 minutes of reconstitution.

STORAGE AND STABILITY: Melphalan for injection vials should be stored at controlled room temperature 15° to 30° C (59° to 86° F) and protected from light. The time between reconstitution/dilution and administration of melphalan should be kept to a minimum because reconstituted and diluted solutions of melphalan 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 the diluent. Upon further dilution with saline, nearly 1% label strength of melphalan hydrolizes every 10 minutes. A precipitate forms if the reconstituted solution is stored at 5° C. Do not refrigerate the reconstituted product.

ADVERSE EVENTS: The following information on adverse reactions is based on data from both oral and IV administration of melphalan as a single agent, using several different dose schedules for treatment of a wide variety of malignancies. Please refer to the Adverse Reactions and Warnings sections of the product package insert.

Hematologic: The most common side effect is bone marrow suppression. White blood cell count and platelet count nadirs usually occur 2 to 3 weeks after treatment, with recovery in 4 to 5 weeks after treatment. Irreversible bone marrow failure has been reported.

Gastrointestinal: Gastrointestinal disturbances such as nausea and vomiting,

diarrhea, and oral ulceration occur infrequently. Hepatic toxicity, including veno-occlusive disease, has been reported.Hypersensitivity: Acute hypersensitivity reactions including anaphylaxis were reported in 2.4% of 425 patients receiving melphalan for myeloma. These reactions were characterized by urticaria, pruritus, edema, and in some patients, tachycardia, hypotension and bronchospasm. These patients appeared to respond to antihistamine and corticosteroid therapy. If a hypersensitivity reaction occurs, IV or oral melphalan should not be readministered since hypersensitivity reactions have also been reported with oral melphalan.

Carcinogenesis: Secondary malignancies, including acute nonlymphocytic leukemia, myeloproliferative syndrome, and carcinoma, have been reported in patients with cancer treated with alkylating agents (including melphalan).

Other: Other reported adverse reactions include skin hypersensitivity, skin ulceration at injection site, skin necrosis rarely requiring skin grafting, vasculitis, alopecia, hemolytic anemia, pulmonary fibrosis and interstitial pneumonitis.

4.0 Patient Eligibility

Inclusion Criteria:

1. Patients with multiple myeloma in complete remission (CR), partial remission (PR), or very good partial remission (VGPR), or symptomatic stable disease (no evidence of progression) including patients with light chain MM detected in the serum by free light chain assay.
2. Patients with non-secretory multiple myeloma [absence of a monoclonal protein (M protein) in serum as measured by electrophoresis (SPEP) and immunofixation (SIFE) and the absence of Bence Jones protein in the urine (UPEP) defined by use of conventional electrophoresis and immunofixation (UIFE) techniques] but with measurable disease on imaging studies like MRI, CT scan or PET scan.
3. Who have received at least two cycles of initial systemic therapy and are within 2 to 12 months of the first dose. Mobilization therapy is not considered initial therapy.
4. 70 years of age or younger.
5. Karnofsky performance score 70% or higher.
6. Cardiac function: left ventricular ejection fraction at rest > 40% within 3 months of registration.
7. Hepatic function: bilirubin < 2x the upper limit of normal and ALT and AST < 2.5x the upper limit of normal.
8. Renal function: creatinine clearance of >/= 40 mL/min, estimated or calculated.
9. Pulmonary function: DLCO, FEV1, FVC >/= 50% of predicted value (corrected for hemoglobin) within 3 months of registration.
10. Signed informed consent form.

Exclusion Criteria:

1. Patients with uncontrolled bacterial, viral or fungal infections (currently taking medication and progression of clinical symptoms).
2. Patients seropositive for the human immunodeficiency virus (HIV).
3. Patients with history of myocardial infarction within 6 months prior to enrollment or

has New York Heart Association (NYHA) Class III or IV heart failure, uncontrolled angina, severe uncontrolled ventricular arrhythmias, or electrocardiographic evidence of acute ischemia or active conduction system abnormalities.
4. Patients participating in an investigational new drug protocol within 14 days before enrollment.
5. Female patients who are pregnant (positive b-HCG) or breastfeeding.
6. Prior stem cell transplantation allogeneic or autologous.
7. Prior organ transplant requiring immunosuppressive therapy.

5.0 Treatment Plan

Mobilization and Collection of Peripheral Blood Progenitor Cells (PBPCs).
The mobilization and collection of the PBPCs is a standard procedure for which patients sign a separate informed consent. This procedure does not constitute part of the proposed research therefore can occur prior or after protocol registration.

After registration patients will be selected to receive one of the two preparative regimens. The randomization will be 1:1, using a computer to assign each patient to either arm 1 (Busulfan-Melphalan) or 2 (Melphalan alone).

Chemotherapy agents doses and administration.
Chemotherapy agents used in the proposed treatment plans will be dosed and administered as outlined below.

When possible, Busulfan pharmacokinetics will be performed. In the event that dose adjustment is not possible Busulfan will be dosed at 130 mg/m^2 starting on day minus 7 to be administrated over 3 hours. The test dose administration time is 45 minutes.

Pharmacokinetic-guided (PK-guided) treatment arm: The Busulfan “testdose” of 32 mg/m^2 will be based on actual body weight, no arbitrary dose adjustment(s) are allowed for this busulfan dose on this protocol.

The busulfan dose in the “fixed-dose” treatment arm will be calculated as follows: For patients whose actual body weight is $\leq$ 20% above ideal body weight, the actual body weight isused to calculate the body surface area (BSA). For patients whose actual body weight is >20% above ideal body weight, an “adjusted body weight” is used to calculate the BSA.

Melphalan will be dosed as follows: for patients whose actual body weight is $\leq$ 20% above ideal body weight, the actual body weight is used to calculate the body surface area (BSA). For patients whose actual body weight is >20% above ideal body weight, an “adjusted body weight” is used to calculate the BSA.

Formula to calculate adjusted body weight: Adjusted BW (Kg) = IBW + 0.5 (Actual body weight - IBW).

ARM 1. Busulfan-Melphalan

Busulfan "testdose" of 32 mg/m^2 can be given IV as out-patient days in advance of starting treatment.

Patients who receive the test dose as an outpatient will be admitted on day -8 to start hydration followed by Busulfan 130 mg/m^2 or adjusted dose IV on days -7, -6, -5 and -4. Patients who receive the test dose as in-patient will be admitted on day -10 to start hydration, followed by busulfan test dose (32 mg/m^2) on day -9 then busulfan 130 mg/m2 or adjusted dose on days -7, -6, -5, and -4. After a day of rest Melphalan 70 mg/m^2 IV over 30 minutes minutes will be administrated on days -2 and -1 followed by the PBPCs infusion on D0. Busulfan target daily area under the curve (AUC) is 5000 μ mol-minute.

ARM 2. Melphalan alone.
This preparative regimen can be administrated as in-patient or out-patient. If in-patient, patient will be admitted on day -3 to start hydration. On day -2 the patients will receive high-dose melphalan at 200 mg/m^2/day IV over 30 minutes. There will be a rest day on day -1 followed by the PBPC infusion on D0.

Supportive treatment
All patients will receive supportive care as clinical indicated following standard practice. This includes G-CSF at a dose of 5 mcg/kg/day (round up to the nearest vial) subcutaneously beginning on day +5, and continuing until evidence of an absolute neutrophil count (ANC) of 0.5 x 10^9/L.

6.0 Study Evaluations

Disease assessment prior to start treatment (baseline).
Studies listed below will be done prior to start treatment only if these were not done before study entry either as part of diagnostic or routine pre-transplant workup.

1. Bone marrow aspirate and biopsies with cytogenetics.
2. Bone survey with long bones within 6 months of registration.
3. SPEP and serum IFE,
4. UPEP and urine IFE,
5. Serum free light chain assay, immunoglobulins IgG, IgA, IgM
6. Beta 2 microglobulin

Disease assessment after treatment (during the study)

Around 3 months post transplant.

1. Bone marrow aspirate and biopsy for morphology with cytogenetics only if cytogenetic abnormalities were diagnosed at baseline.

Every three months during the first year post transplant

1. SPEP and serum IFE only if SPEP is negative.
2. UPEP and urine IFE only if UPEP is negative.
3. Serum free light chain assay, immunoglobulins IgG, IgA, IgM. B-type naturietic peptide (BNP) only if the patient has incidental amyloidosis.

Around one year post transplant
1. Bone survey with long bones only if abnormal at baseline.

Quality of Life Assessment (MDASI)
Participants will complete the M. D. Anderson Symptom Inventory (MDASI - MM) at baseline before the start of preparative regimen, and then once weekly for the first 2 weeks and at week 4 (+/- 14 days) after stem cell infusion pending participants' availability.

7.0 Adverse Events Assessment and Reporting

Adverse Event Assessment and Reporting:
Severity of adverse events (AE) will be assessed according to the Common Terminology Criteria v3.0 (CTCAE).

Most common expected AE related to the use of high dose chemotherapy followed by autologous stem cell infusion are:

A. Related to myelosuppression: thrombocytopenia, bleeding, platelets and RBCs transfusions.
B. Fever: Non Neutropenic or Neutropenic without infection
C. Infections in the presence or absence of neutropenia
D. Readmissions (lasting <10 days)
E. Cytopenias post transplant including secondary graft failure
F. Low blood pressure due to dehydration requiring fluid replacement
G. Fluid overload leading to cardiac dysfunction
H. GI related: nausea, vomiting, diarrhea, mucositis
I. Organ dysfunction: cardiac, pulmonary, hepatic, CNS and/ or renal
J. Fatigue
K. Neurologic: seizures, neuropathies
L. Stem Cell Transplant Syndromes: Cytokine Storm, TTP, hemorrhagic cystitis, interstitial pneumonitis (including pulmonary hemorrhage).

Adverse Events Considered Serious:
A. Graft failure/ rejection
B. Prolonged hospitalization due to infections and/or organ failure requiring extensive supportive care (i.e. dialysis, mechanical ventilation)
C. Readmissions from any cause resulting in a prolonged hospitalization (>10 days).
D. Any expected or unexpected event resulting in an irreversible condition and/ or leading to death.

For the purpose of this study, abnormal laboratory findings considered associated to the original disease as well as isolated changes in laboratory parameters such as electrolyte magnesium and metabolic imbalances, uric acid changes, elevations of GPT, GOT, LDH and alkaline phosphatase would not be

considered adverse events and would not be collected in the database.

Data Collection During Active Treatment
The last day of active treatment is the day of the PBPCs infusion. The end of active treatment period is 30 days from the PBPCs infusion. During the active treatment period collection of adverse events will reflect the onset and resolution date and maximum grade. Intermittent events should be labeled as such and followed until resolution. If a patient is taken off study while an event still ongoing, this will be followed until resolution unless another therapy is initiated.
Pre-existing medical conditions will be recorded only if an exacerbation occurs during the active treatment period. Events not considered adverse events will not be collected. Co-morbid events will not be scored separately.

AE and Protocol Deviations Reporting Requirements
Adverse events will be reported accordingly to MDACC (HSRM chapter 15.001) and SCT&CT Department (HSRM chapter 15.053) policy and procedures.
This study will be conducted in compliance however in the event of any protocol deviations or vilolations these will be reported accordingly to MDACC (HSRM chapter 25).

8.0 Criteria for Response

International Myeloma Working Group uniform response criteria.
All response categories require two consecutive assessments made at any time.
All response categories require no known evidence of progressive or new bone lesions.

Stringent complete response (sCR) (all of the following):
1. CR as defined.
2. Normal free light chain ratio
3. Absence of clonal cells in bone marrow by inmmunohistochemistry or immunofluorescence (defined by absence of abnormal ê/ë ratio of >4:1 or <1:2)

Complete response (CR) (all of the following):
1. Negative immunofixation in serum and urine.
2. ≤ 5% plasma cells in the bone marrow.
3. Disappearance of any soft tissue plasmacytomas.

Note: While healing of preexisting bone lesions is not required, no new lytic lesions should appear. Further compression fracture of previously known spine lesion will not be considered as progressive disease.

Very good partial response (VGPR) (one of the following):
1. Serum and urine M protein detectable by immunofixation but not by electrophoresis.
2. 90% or greater reduction in serum M protein plus urine M protein level <100 mg

per 4h.

Partial response (PR) (all of the following):
1. Reduction by > 50% in serum monoclonal protein.
2. Reduction of urinary monoclonal protein to < 200 mg/24h or >90%.

Stable disease:
1. Not meeting criteria for CR, VGPR, PR or PD.

Progressive disease (PD) (any one or more of the following):
1. Increase of >= 25% from baseline in:
   Serum M protein (absolute increase must be >= 0.5 g/dL).
   Urine M component (absolute increase must be >= 200 mg/24h).Only in patients without measurable serum and urine M protein levels.
   Difference between involved and uninvolved FLC levels (absolute increase must be > 10 mg/dL).
   Bone marrow plasma percentage (absolute % must be >=10%).
2. Definite development of new bone lesions or soft tissue plasmacytomas or definite increase in the size of existing bone lesions or soft tissue plasmacytomas.
3. Development of hypercalcemia (corrected serum calcium >11.5 mg/dL or 2.65 mol/L) that can be solely attributed to the myeloma.

Relapse from CR (any one or more of the following):
1. Reappearance of serum or urine M protein by immunofixation or electrophoresis.
2. Development >=5% plasma cells in the bone marrow.
3. Appearance of any other sign of progression (i.e., new plasmacytoma, lytic bone lesion or hypercalcemia).

9.0 Criteria for Removal from the Study

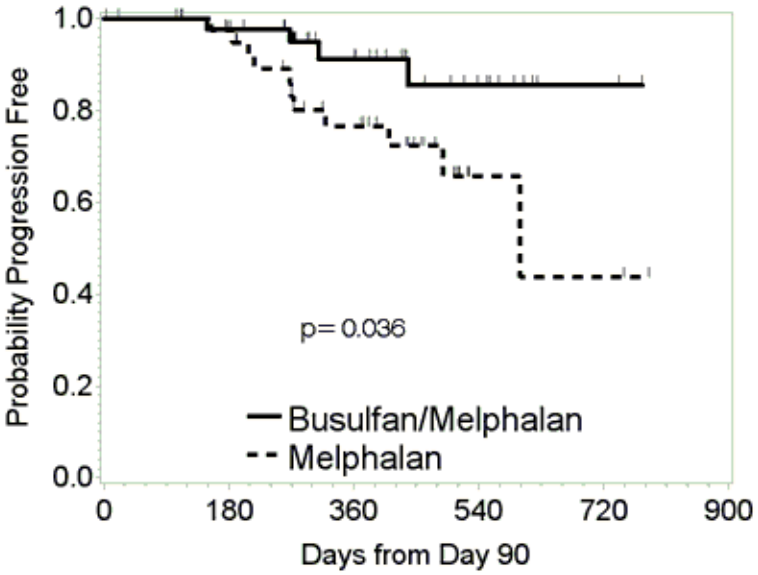
1. Patient withdraw of the informed consent.
2. Patient's inability or unwillingness to have follow-up visits and/or laboratory tests required by this protocol.
3. An unexpected toxicity that is deemed unacceptable by the study chairman.
4. Disease progression.
5. After one year of treatment completion.

If a patient withdraws from the study, or is unable or unwilling to complete follow-up visits and/or laboratory tests required by this protocol, then (s)he will be replaced and will not be counted in regard to complete response and transplant-related mortality assessments. However, patients who experience an unexpected toxicity that is deemed unacceptable by the study chairman will not be replaced and will be counted as non-responders in regard to complete response.

10.0 Statistical Considerations

This is a randomized group sequential design to compare B+M to M based on PFS time, assuming a null median PFS time of 20 months with M, with two-sided tests having nominal overall type I error .029 and power .80 to detect a median PFS of 34 months, which is a 70% increase with B+M, with up to three tests using O'Brien-Fleming decision boundaries. The tests will be conducted when there are a total of 45, 90, and 135 failure events in the two arms, with respective standardized log rank test Z-score test cut-offs +/- 4.0757, 2.7768, and 2.2077. Assuming an accrual rate of 5 patients per month, this design has an expected accrual of 205 patients total, for 205 - 92 = 113 additional patients.

Figure 1. Kaplan-Meier estimates of PFS starting from Day 90 post transplant



In the original design of this randomized trial, an early stopping rule based 90-day complete remission (CR90) was used. Following this rule, the trial was stopped early due to an imbalance in the empirical rates of CR90 in the two arms. The empirical rates were 35% in the M arm versus 16% in the B+M arm, which was the opposite of what was anticipated when planning the trial, motivated by the hypothesis that adding IV Busulfan to Melphalan would improve patient outcome. On closer inspection of the data, a very surprising result was seen in terms of progression free survival (PFS) time. For PFS time, the opposite effect of that for CR90 was seen. Specifically, the B+M arm has better PFS than the M arm, starting from day 90 when CR90 was evaluated. A similar difference was seen for PFS time recorded from date of transplant. This statistical result may be considered perplexing because, within each treatment arm, for patients for whom CR90 was evaluated on average the occurrence of CR90 increased the time to failure (progression or death). That is, CR90 improved PFS in both arms. So the question now arises of how it can be the case that

1) CR90 is higher in the M arm compared to the B+M arm,

2) CR90 improves PFS in both arms, yet
3) PFS is superior in the B+M arm compared to the M arm.

A computation explaining this somewhat counterintuitive result is given in the Appendix.

APPENDIX

This appendix provides a detailed explanation of the counterintuitive results of this randomized clinical trial of IV Busulfan + Melphalanj (B+M) versus Melphalan (M) as preparative regimens for autologous hematopoietic stem cell transplantation (auto-HCT) for multiple myeloma.

For simplicity, the following are landmark analyses starting at day 90 post SCT. They exclude the small number of patients who had disease progression or death prior to day 90, hence did not have CR90 evaluated. The same conclusions for PFS are obtained, however, if time is started at the time of transplant, i.e. if all patients are included.

Notation

CR90 = the event that the patient had a CR when evaluated at day 90

T = Progression free survival (PFS) time = time to progression or death, starting at day 90.

P(T > 12 | M, CR90) = the probability that a patient on the Melphalan arm who achieved CR90 had PFS time of at least 12 months

P(T > 12 | M, NCR90) = the probability that a patient on the Melphalan arm who did NOT achieve CR90 had PFS time of at least 12 months

P(T > 12 | M+B, CR90) = the probability that a patient on the Melphalan + Busulfan arm who achieved CR90 had PFS time of at least 12 months

P(T > 12 | M+B, NCR90) = the probability that a patient on the Melphalan+ Busulfan arm who did NOT achieve CR90 had PFS time of at least 12 months

P(T > 12 | M) = the probability that a patient on the Melphalan arm had PFS time of at least 12 months

P(T > 12 | M+B) = the probability that a patient on the Melphalan+ Busulfan arm had PFS time of at least 12 months

Pr(CR90 | M) = the probability that a patient on the Melphalan arm achieved a CR90

Pr(CR90 | M+B) = the probability that a patient on the Melphalan + Busulfan arm

achieved a CR90

Probability Mixture Computations

For the M arm,

$$P(T > 12 \mid M) = P(T > 12 \mid M, CR90) \times Pr(CR90 \mid M) + P(T > 12 \mid M, NCR90) \times \{1 - Pr(CR90 \mid M)\}$$

For the B+M arm,

$$P(T > 12 \mid M+B) = P(T > 12 \mid M+B, CR90) \times Pr(CR90 \mid M+B) + P(T > 12 \mid M+B, NCR90) \times \{1 - Pr(CR90 \mid M+B)\}$$

In words, these equations say that, in each treatment arm, the overall probability of 12-month PFS (starting at day 90) is the average of two 12-month PFS probabilities: one for the patients who achieved a CR at day 90, and the other for the patients who do not achieve a CR at day 90.

Statistical Estimates from the Trial Data

Using a * to denote a statistical estimate

$Pr(CR90 \mid M)^* = .35$

$Pr(CR90 \mid M+B)^* = .16$

$P(T > 12 \mid M, CR90)^* = .91$

$P(T > 12 \mid M, NCR90)^* = .68$

$P(T > 12 \mid M+B, CR90)^* = 1.00$

$P(T > 12 \mid M+B, NCR90)^* = .89$

Note that the M arm has a much higher CR90 probability, .35, compared to .16 for the B+M arm. This is why the trial was stopped early. Substituting these estimates into the mixture model equations gives

$P(T > 12 \mid M)^* = .91 \times .35 + .68 \times (1 - .35) = .76$ (95% ci .65 - .89)

$P(T > 12 \mid M+B)^* = 1.00 \times .16 + .89 \times (1 - .16) = .91$ (95% ci .83 - .99)

The logrank test to compare PFS has p-value = .047, so the M+B arm had significantly

higher PFS

Conclusions:

1. The M arm had a much higher CR90 rate, more than double that of the M+B arm.

2. In each arm, CR90 increased the probability of 12-month PFS.

3. M+B had higher PFS than M among patients who achieved CR90.

4. M+B had higher PFS than M among patients who did not achieve CR90.

5. M+B had higher overall PFS probability than the M arm

This is because the 12-month PFS probability was very high for both the CR90 and NCR90 groups in the M+B arm, 1.00 and .89, respectively. But the M arm patients who did not achieve CR90 had the much lower 12-month PFS probability .68.

Trial Conduct. The randomization will use the Pocock-Simon method [Pocock 1975] will used to balance dynamically on age, dichotomizing this as [age $\leq$ 65] and [66 $\leq$ age $\leq$ 70] be carried out using the Department of Biostatistics Clinical Trials Conduct Website. The interim and final decision rules will be applied using a specialized computer program written by John Venier in the Biostatistics Department, and will be based on the interim and final CR data provided by the trial's Principal Investigator.

Secondary Endpoints. Overall survival (OS) time, transplant related mortality, NIH toxicities, quality of life, and 90-day complete response (CR) will be evaluated as secondary endpoints. CR is defined as (i) negative immofixation of the MM protein in urine and serum, (ii) disappearance of any soft tissue plasmacytomas, and (iii) less than 5% plasma MM cells in the bone marrow.

Trial Conduct. The randomization will use the Pocock-Simon method [Pocock 1975] will used to balance dynamically on age, dichotomizing this as [age $\leq$ 65] and [66 $\leq$ age $\leq$ 70] be carried out using the Department of Biostatistics Clinical Trials Conduct Website.

Data Analyses. Logistic regression models will be fit to assess the joint effects of treatment arm and patient covariates on the probability of CR [Venables 2002]. Kaplan-Meier plots [Kaplan 1958] will be used to estimate the unadjusted OS and PFS time distributions in the two arms. Time-to-event regression analysis will be used to evaluate the ability of treatment arm and patient covariates to predict OS and PFS, with the model underlying model chosen based on preliminary goodness-of-fit analyses of the data. Additionally, landmark analyses of PFS and OS will be conducted starting at day 90, when CR is evaluated.

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