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MD Anderson Cancer Center

Protocol Page

High-dose Gemcitabine, Busulfan and Melphalan with Autologous Hematopoietic-Cell Support for Patients with Poor-Risk Myeloma 2010-0506

Core Protocol Information

Short Title	High-Dose Gemcitabine, Busulfan and Melphalan with Hematopoietic-Cell Support for Patients with Poor-Risk Myeloma
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Which Committee will review this protocol?

◉ The Clinical Research Committee - (CRC)

Protocol Body

1.0 Objectives

Primary Endpoint

1. To evaluate the complete remission (CR) rate of Gemcitabine/Busulfan/Melphalan (GemBuMel) in patients with poor-risk myeloma

Secondary endpoints

1. To determine the progression-free survival (PFS)
2. To determine the overall survival (OS)
3. To describe the toxicity profile of GemBuMel in this population.

2.0 Background

Current Status and Shortcomings of HDC for Myeloma

High-dose chemotherapy (HDC) with autologous peripheral blood progenitor cell (PBPC) support results in significant benefit as part of first-line treatment. The French IFM group randomized 200 patients to receive induction followed by either melphalan-based HDC or conventional-dose treatment. Patients randomized to the HDC arm had superior complete response (CR) rates (22% *v* 5%, p<0.001), progression-free survival (PFS) (median 28 *v* 18 months, p=0.01), and overall survival (OS) (median 56 *v* 44 months) (p<0.05).[1] These results, later confirmed in a separate randomized trial,[2] established HDC as the standard of care for myeloma responding to first-line therapy. However, no plateaus of the PFS curves have been observed in those trials. Tumor relapse, largely caused by insufficient eradication of the myeloma cells, constitutes the single most important cause of failure of high-dose melphalan. In addition, high-dose melphalan induces only 5-10% of CR with a median PFS of around 12 months in patients with primary refractory disease or in refractory relapse.[3-5] These data underscore the need to develop more active HDC regimens than single-agent melphalan. Tandem cycles of high-dose melphalan have been reported to improved outcome in randomized trials,[6, 7] at least in patients with a poor response to the first cycle, but are limited by patients' frequent refusal to undergo a repeat procedure and the often lack of coverage by third-party payors. While long-term post-transplant treatment with thalidomide or lenalidomide improves PFS,[8-10] it is conceivable that ultimate success of maintenance strategies, i.e., long-term OS benefit, will still largely depend on the depth of response achieved by the preceding high-dose treatment. Thus, further exploration of novel preparative regimens for single transplantations is necessary with the short-term goal of improving CR rate.

Complete response is a major surrogate for long-term OS in myeloma. Achievement of a CR with HDC, particularly as defined by modern criteria including negative immunofixation, is a crucial step for long-lasting PFS and OS.[11-13] The response to the pre-transplant therapy, measured by the M paraprotein value, is the most important predictor of CR after ASCT. In patients with M protein less than 1 g/dL at the time of HDC the likelihood of post-transplant CR

is greater than 50%, whereas in those with a serum M protein higher than 1 g/dL or 2 g/dL the probability of CR with high-dose melphalan is 15% and 5%, respectively.[14-16] Achieving a CR with HDC is particularly critical for patients with refractory or high-risk disease, whereas it does not appear to be as essential for patients with more smoldering tumors, who can still enjoy prolonged outcomes despite never achieving a CR.[17, 18]

As with most other tumors in which HDC plays a role, it is possible that an active drug combination will prove more effective for myeloma as the transplant regimen than the current standard of single-agent melphalan. A retrospective registry analysis of patients transplanted with oral busulfan and melphalan (BuMel), melphalan (200 mg/m2) or melphalan (140 mg/m2)/total body irradiation showed significantly better overall response rates (RR) (97% *v* 89% *v* 92%, P=0.003) and PFS (median 32 months *v* 22 *v* 20 months, P=0.01) for those receiving BuMel despite slightly worse prognostic features.[19] Oral busulfan is seriously limited by its unpredictable absorption and substantial risk of hepatic toxicity, the development of an intravenous busulfan formulation by Andersson and collegues has expanded the applicability of this drug. In a phase II study of intravenous (IV) Busulfan/Melphalan in 55 patients, most transplanted to consolidate a response to first-line therapy, this regimen showed good tolerability, a CR/near CR rate of 49%, a RR of 82% and 1-year PFS and OS of 87% and 96%, respectively.[20] Kebriaei from our group has analyzed our recently completed phase II study of IV BuMel for patients with lymphoid tumors (protocol 2004-0190).[21] This regimen was well tolerated and resulted in a 58% RR, 17% CR and 2-year PFS of 44% in the subset of 41 myeloma patients enrolled, treated with a median 2 prior lines of therapy, 34 of whom had chemosensitive disease and 7 chemorefractory tumor at transplant.

We believe these results warrant further study of busulfan and melphalan in myeloma. We have completed a phase I trial of infusional gemcitabine combined with BuMel, with the goal of exploiting their synergistic interaction (protocol 2006-0803). Gemcitabine has very potent activity against myeloma cell lines in vitro, including cell types resistant to other agents.[22, 23] As in all other types of cell lines tested, the cytotoxicity of gemcitabine against myeloma cells correlated with the intracellular accumulation of its active triphosphate metabolite. In spite of its promising preclinical profile, the clinical experience of gemcitabine in myeloma is very limited. When tested as single agent in short 30-minute infusions this drug showed little activity in 29 patients with resistant disease.[24] Importantly, the schedule of gemcitabine employed in this small study results in suboptimal intracellular activation as described below.

Gemcitabine/Busulfan/Melphalan

Our recently completed phase 1 study of gemcitabine, busulfan and melphalan (GemBuMel) for refractory lymphoid malignancies (protocol 2006-0803) was a dose- and schedule-finding trial testing the escalation of the infusion length of gemcitabine, administered at fixed dose rate (FDR) of 10 mg/m2/min, combined with Bu/Mel as previously studied in protocol 2004-0190. The design of GemBuMel was based on the following principles:

1- Individual activity of the three drugs against lymphoid tumors.
2- Synergy between gemcitabine and alkylating agents, based on its inhibition of DNA

damage repair.

3- Gemcitabine infusions at FDR of 10 mg/m2/min avoid saturation of its intracellular metabolic activation, resulting in improved antitumor activity and increased myelotoxicity compared to shorter infusions. The increased myelotoxicity of FDR infusions is overcome by stem-cell support.

4- Minimal overlapping dose-limiting toxicity of the three agents when administered at high doses (busulfan: hepatic injury, melphalan: stomatitis, gemcitabine: diarrhea).

5- Optimization of intravenous busulfan therapy by therapeutic drug monitoring, as shown by Andersson.[25]

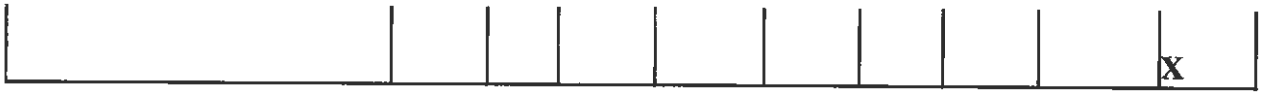
Gemcitabine is dependent for activity on intracellular phosphorylation, a rate-limited process. Previous studies have shown that an extracellular gemcitabine concentration below 20 micromolar produces optimal phosphorylation by deoxycitidine kinase (DCK) and incorporation of its triphosphate metabolite into DNA, whereas higher concentrations saturate DCK and exceed its the capacity for drug activation. An infusion rate of 10 mg/m2/min results in a 15 micromolar steady-state concentration (Css) optimizing drug activation.[26]

Several unique features of gemcitabine may also account for its higher level of antitumor activity than other nucleoside analogs, such as the "masked chain termination" effect, which consists of the addition of one deoxynucleotide to the end of the elongating DNA strand right after the Gemcitabine-nucleotide position, preventing its removal by exonucleases and ultimately locking the drug into DNA. In addition, through several self-potentiation metabolic mechanisms, gemcitabine increases the formation and decreases the elimination of its active metabolites.[27]

We have enrolled in 2006-0803 74 patients with refractory and heavily pretreated lymphoid tumors (45 with Hodgkin's lymphoma, 19 non-Hodgkin's lymphoma and 10 with myeloma). Gemcitabine was administered as a loading dose of 75 mg/m2, calculated to reach a Css of 15 micromolar, followed by a continuous infusion at 10 mg/m2/min. We found that the daily x 6 and the every other day (3 doses) schedules of gemcitabine were associated with excessive skin toxicity. In contrast, we did not observe significant skin toxicity with the 2-dose schedule of gemcitabine. In this schedule gemcitabine is administered on the first treatment day of busulfan (day -8) and melphalan (day -3) (Table 1). The optimal length of infusion of gemcitabine was established at 3 hours on each of its two treatment days.

Table 1. GemBuMel schedule

Day	-8	-7	-6	-5	-4	-3	-2	-1	0
Gemcitabine (loading dose of 75 mg/m2 followed by 3-hour infusion at 10 mg/m2/min)	X				X				
Busulfan (target AUC of 4,000 AUC microM.min/day)	X	X	X	X					X
Melphalan 60 mg/m2/day					X	X			
PBPC infusion									



Toxicity Profile of GemBuMel

At the final dose defined for future testing, GemBuMel produced in this phase I study a reversible side-effect profile of mucositis (60% grade 2, 13% G3), skin toxicity (13% G2) and self-limited elevation of the transaminases (21% G2, 7% G3), with no G4 or 5 toxicities.

Antitumor Activity of GemBuMel

The regimen had high level of activity across all diagnoses. Seven of the 10 myeloma patients had primary refractory disease and 3 had refractory relapse, and had received a median 4 prior regimens. Their disease status at transplant was progressive disease in 6 patients and stable disease or partial response in 4. In this very poor prognosis group, the RR and stringent CR rates were 86% and 43%, respectively, with 43% PFS and 71% OS rates at median follow-up of 27 months. Similarly encouraging results were observed in the refractory Hodgkin's and NHL subsets, warranting further testing of GemBuMel further in disease-specific phase II studies. We intend to study GemBuMel in patients with multiple myeloma candidates for HDC who either have an estimated likelihood of CR with high-dose melphalan of less than 15% or have already failed high-dose melphalan. The dose of gemcitabine for this study will be the one determined as optimal in the prior phase 1 study.

3.0 Patient Eligibility

3.1 Inclusion:

3.1.1. Age 18 to 70 years.
3.1.2. Patients with myeloma treated with first-line therapy including lenalidomide, bortezomib or thalidomide, and one or more of the following:
  3.1.2.1. M paraprotein greater than 1 g/dL at HDC.
  3.1.2.2. Less than partial response to first-line therapy.
  3.1.2.3. Relapse after first-line therapy.
  3.1.2.4. Relapse after a prior autologous stem-cell transplant.
3.1.3. Adequate renal function, as defined by serum creatinine </=1.8 mg/dL and/or estimated serum creatinine clearance >/=50 ml/min
3.1.4. Adequate hepatic function, as defined by SGOT and/or SGPT </=3 x upper limit of normal; serum bilirubin and alkaline phosphatase </=2 x upper limit of normal, unless proven to be due to disease involvement.
3.1.5. Adequate pulmonary function with FEV1, FVC and DLCO >/=50% of expected corrected for hemoglobin and/or volumen.
3.1.6. Adequate cardiac function with left ventricular ejection fraction >/=40%. No uncontrolled arrhythmias or symptomatic cardiac disease.
3.1.7. Zubrod performance status <2.
3.1.8. Negative Beta HCG text in a woman with child-bearing potential, defined as not

post-menopausal for 12 months or no previous surgical sterilization

3.2 Exclusion:

3.2.1. Patients with grade >/= 3 non-hematologic toxicity from previous therapy that has not resolved to </= grade 1.
3.2.2. Patients with prior whole brain irradiation
3.2.3. Patients with active hepatitis B, either active carrier (HBsAg +) or viremic (HBV DNA >/=10,000 copies/mL, or >/= 2,000 IU/mL).
3.2.4. Evidence of either cirrhosis or stage 3-4 liver fibrosis in patients with chronic hepatitis C or positive hepatitis C serology.
3.2.5. Active infection requiring parenteral antibiotics.
3.2.6. HIV infection, unless the patient is receiving effective antiretroviral therapy with undetectable viral load and normal CD4 counts
3.2.7. Patients having received radiation therapy to head and neck (excluding eyes), and internal organs of chest, abdomen or pelvis in the month prior to enrollment.

4.0 Pretreatment evaluation

4.1. Within 30 days of study treatment:

Complete history and physical examination.
Bone marrow aspirate and biopsies for morphology, flow cytometry, cytogenetic and FISH studies.
Bone survey (within 6 months of study entry unless clinically indicated.)
SPEP, UPEP, serum and urine immunofixation,
Serum free kappa and lambda light chain assay.
Beta-2 microglobulin.
Chest X-ray.
Echocardiogram or MUGA scan
Pulmonary function tests

4.2. Within 14 days of study entry:

CBC with differential, electrolytes, BUN, creatinine, glucose, total protein, albumin, calcium, phosphorus, uric acid, total bilirubin, alkaline phosphatase, LDH, AST, ALT, and magnesium.

4.3. On admission:

C-reactive protein, haptoglobin, BNP and ferritin in serum.

5.0 Study Registration

Each patient will be evaluated and approved for enrollment by the primary attending physician, or the Study Chairman, or his/her designee. Designated research nurse will register each patient on protocol. All protocol participants will be registered in the institutional PDMS/CORe system.

6.0 Treatment Plan

6.1. High-Dose Chemotherapy

Treatment will not commence until resolution of prior toxicities to grade 1 or less. Acetaminophen (Tylenol) shall not be administered for 72 hr before and on the day of administration of Busulfan or Melphalan. Voriconazole, posaconazole, fluconazole, itraconazole and metronidazole will be avoided from 7 days before start of chemotherapy to day -1.

For patients whose actual body weight is <= 20% above ideal body weight (defined by the MD Anderson dosing calculator), 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", defined as the midpoint between the actual and ideal body weight, is used to calculate an "adjusted body surface area" for dosing calculation purposes of gemcitabine, busulfan and melphalan.

Day	Date	Treatment
	_____	Busulfan 32 mg/m^2 test dose with PKs (outpatient)
-12	_____	Palifermin 60 microgram/kg (outpatient). Do not start on Monday/Tuesday/Wednesday
-11	_____	Palifermin 60 microgram/kg (outpatient)
-10	_____	Palifermin 60 microgram/kg(outpatient)
-9	_____	Admit
-8	_____	Gemcitabine 1875 mg/m2 (*)/ Busulfan AUC 4,000
-7	_____	Busulfan AUC 4,000
-6	_____	Busulfan AUC 4,000
-5	_____	Busulfan AUC 4,000
-4	_____	Rest
-3	_____	Gemcitabine 1875 mg/m2 (*)/ Melphalan 60 mg/m2
-2	_____	Melphalan 60 mg/m2
-1	_____	Rest
0	_____	Autologous Stem Cell Transplant/ Palifermin 60 microgram/kg
+1	_____	Palifermin 60 microgram/kg
+2	_____	Palifermin 60 microgram/kg

(*) Gemcitabine 75 mg/m2 IV over 1 minute followed by 1800 mg/m2 IV over 180 min (total dose on each gemcitabine day: 1875 mg/m2)

Busulfan pharmacokinetic-guided treatment (PK-guided). The Busulfan test dose can be administered either as an outpatient before Day -12 or as an inpatient on Day -10. Outpatient test dose is done before outpatient palifermin. If outpatient test dose is not possible, test dose would be given as inpatient after outpatient palifermin on Day - 10. In this case, first dose of palifermin will start on Day-13. The "test dose" of 32 mg/m^2 will be based on actual body weight to be administrated over 60 minutes. Busulfan pharmacokinetics will be performed with the test

dose and the first dose on day-8. The doses of days -6 and -5 will be subsequently adjusted to target an AUC of 4,000 microMol.min^{-1}.

In the event that PK adjusting were not possible a dose of busulfan of 105 mg/m2 will be administered on days -6 and -5.

Palifermin will be infused as an IV push over 15-30 seconds. Busulfan will be infused over 3 hours. Melphalan will be infused over 30 minutes.

6.2. Supportive Treatment

Patients will receive standard supportive treatment including:

1. Dexamethasone 8 mg IV BID from day -9 PM to day -2 PM
2. G-CSF at 5 mcg/kg/day (rounded up the nearest vial) subcutaneously beginning on day +5 and continuing until neutrophil recovery is documented.
3. Mucositis supportive care:
    3.1. Patients will receive a total of 6 doses of palifermin 60 mcg/kg IV daily. Three doses administrated prior to start chemo (24 hours must elapse between the last dose and first therapeutic dose of chemo) and three doses after the last chemo starting on day 0.
    3.2. Caphosol oral rinses 30 mL four times a day will be used from day -9.
    3.3. Oral glutamine, 15 g four times a day, swished, gargled and swallowed will be started on day -9.

Other supportive treatment such as antiemetics or infection prophylaxis as per departmental standard of care.

6.3. Post-Transplant Therapies

Post-transplant therapies will be left at the discretion of the primary physician. Common options include maintenance treatment with lenalidomide or thalidomide, bisphosphonates for 1-2 years for those with prior lytic bone disease, or a second autologous procedure with high-dose melphalan if less than a VGPR is achieved with this first transplant.

7.0 Post-treatment evaluation

7.1. Toxicity Monitoring:

During the treatment administration and until day +30 all patients will be monitored for toxicity, specifically for grade 3 or greater side effects, according to CTCAE v3.0. While admitted in hospital, patients will be monitored on a regular basis. Once discharged patient will return to clinic once a week or as determined by the primary physician until day +30.

7.2. Disease restaging:

About 30 to 100 days after transplant:

Pulmonary function with FEV1, FVC and DLCO.

At 1, 3, 6 months and 1 year, and every 3-6 months thereafter for at least 2 years:

Complete history and physical examination.
SPEP, UPEP, serum and urine immunofixation, serum free kappa and lambda light chain assay.
Serum albumin, LDH, beta-2 microglobulin and C-reactive protein.
Bone marrow aspirate and biopsy for morphology, flow cytometry, cytogenetic and FISH studies at 3 months. Afterwards, once a year.
Bone survey: Only once a year.

8.0 Criteria for Response

We will use the International Myeloma Working Group uniform response criteria.[28] All response categories require two consecutive assessments made at any time. All response categories require no known evidence of progressive or new bone lesions.

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.

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

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 24 h

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

Progressive disease (PD) (any one or more of the following):
1. Increase of >= 25% from baseline in:
   - 1.1. Serum M protein (absolute increase must be >= 0.5 g/dL).
   - 1.2. Urine M component (absolute increase must be >= 200 mg/24h).

1.3. (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).

1.4. 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 mmol/L) that can be solely attributed to the myeloma.

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

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's withdrawal 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 PI.
4. Disease progression or relapse.
5. After two years of treatment completion.

10.0 Reporting Requirements

Patients will be monitored for toxicity until day +30 or until documentation of reversal of toxicities related to this treatment. The intensity of adverse events (AE) will be assessed according to the Common Terminology Criteria v3.0 (CTCAE). Adverse events and protocol deviations will be reported accordingly to MDACC policy and procedures. 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 is 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. Co-morbid events will not be scored separately.

10.1 Adverse events (toxicities) known to be produced by the chemotherapy regimen:

Gastrointestinal: Nausea and vomiting, diarrhea, oral mucositis
Hepatic: Self-limited elevations of liver function enzymes; veno-occlusive disease
Hypersensitivity: Acute hypersensitivity reactions characterized by urticaria, pruritus, edema, and in some patients, tachycardia, hypotension and bronchospasm (rare)
Pulmonary: Pulmonary fibrosis and interstitial pneumonitis.

Skin: Rash.

10.2. Adverse events (toxicities) known to be produced by other treatment components:

The following events are not considered to be significant in relationship with the study treatment, will not be considered adverse events and will not be collected in the study database.
Myelosuppression-related: neutropenia, anemia, thrombocytopenia, platelet and RBC transfusions.
Flu-like symptoms: low grade fever, headache, chills, cough, rhinitis, myalgia, fatigue, sweating and insomnia.
Mood alteration: depression, anxiety, and agitation
Readmissions (lasting <10 days)
Low blood pressure due to dehydration requiring fluid replacement
Fluid overload.
Fatigue.
Laboratory serum metabolic values not reflecting end-organ (hepatic, renal) function and or those considered associated to the original disease
Events that are identified to be related to the supportive treatment, e.g., steroids, palifermin, antibiotics.

10.3 Adverse Events Considered Serious (SAEs):

1. Graft failure/rejection
2. Prolonged hospitalization due to infections and/or organ failure requiring extensive supportive care (i.e. dialysis, mechanical ventilation)
3. Readmissions from any cause resulting in a prolonged hospitalization (>10 days).
4. Any expected or unexpected event resulting in an irreversible condition and/ or leading to death.

Serious adverse events (SAE) will be reported to the PI or his designate, who in turn will notify the IRB following institutional policy.

11.0 Correlative studies

Two correlative studies will be part of this protocol:

1) Pharmacokinetic (PK) evaluation and dose adjustment of busulfan

Therapeutic drug monitoring of busulfan is standard practice when high doses are used. It has been shown that PK exposure after the first therapeutic dose is predictive of the PK for subsequent therapeutic doses.[29] We will perform PK evaluation of busulfan exposure and elimination in all available patients and will not be optional. Drug levels will be obtained after the first dose on day -8 from a peripheral IV dedicated to this purpose and the line removed after PK monitoring is complete. Ten 8-cc samples will be obtained at intervals between the start of infusion and 4 hr after the end of infusion (total phlebotomy/infusion 80 cc; or 5-6 tablespoons of

blood). The PK exposure after the first dose of busulfan will be used for PK-adjusted dosing of the drug on its third and fourth treatment days (-6 and -5, respectively), targeting an AUC of 4,000 microM.min/day.

2) Study of single nucleotide polymorphisms (SNPs) of gemcitabine-related genes

This correlative study will be optional for patients participating in this trial. Prior work by Li et al has identified relevant polymorphic variants of key enzymes involved in gemcitabine intracellular metabolism, DNA mismatch repair (MMR), base excision repair (BER), homologous recombination repair (HHR), and multidrug resistance pathways, with a major impact on the activity and toxicity of standard-dose gemcitabine in patients with pancreatic cancer.[30-33] We will study SNPs of the following genes:

a) *Cytidine deaminase (CDA), deoxycytidine kinase (dCK),* and *human concentrative nucleoside transporter-3 (hCNT3)* genes, involved in intracellular metabolism of gemcitabine.
b) *RecQ1*, *RAD54L* and *ATM* (involved in HRR), *XRCC1* (involved in BER) and *MLH1, MSH2, MSH3, TREX, TP73*, and *EXO1* (involved in MMR)
c) *MRP2*, involved in multidrug resistance.

A single 10-cc blood sample will be drawn either before or at any time after treatment. Normal and tumor tissues are expected to have the same genotype for these common germline polymorphic variants. DNA extracted from peripheral blood lymphocytes will be genotyped for the common SNPs of these enzymes.

12.0 Statistical Considerations

This is a Phase II clinical trial examining the effect of Gemcitabine/Busulfan/Melphalan (GemBuMel) with hematopoietic cell support in patients with poor-risk myeloma. The maximum total sample size is 75 patients, of which we expect 60 will be evaluable for response.

Primary Efficacy Endpoints

The primary endpoint of this study is to evaluate the complete remission (CR) rate of GemBuMel in patients with poor-risk myeloma on day 100. It is defined as percentage of number of complete responses in total number of patients treated. A 20% CR rate is considered as clinically significant.

Secondary Efficacy Endpoints

1. To determine the progression-free survival (PFS)
2. To determine the overall survival (OS)
3. To describe the toxicity profile of GemBulMel in this patient population

Toxicity Endpoints

Toxicity is defined as the treatment related mortality (TRM) rate, and this rate will be compared with the 5% maximum rate. The method of Thall et al will be employed to perform interim safety monitoring.[34]

Efficacy Monitoring Rule

For the primary efficacy endpoint of CR rate on day 100, when this study was first designed, a Simon's optimal 2-stage design was used.[35] It was assumed that the GemBuMel combination regimen would have a target CR rate of 30%. A complete response rate of 15% or lower would have been considered a failure and the combination regimen would have been rejected under this circumstance. A total of 19 patients were to enter the study in the first stage and 20 in the second stage. Based upon additional research recently reported, as of August 2012 we realized that a more realistic clinical target was a 20% CR rate. We also wished to accrue additional patients in order to provide more information on the CR rate. A total of 34 of the planned 39 patients had been accrued by August 2012, of which 29 had been evaluated for response. Of these 29, 7 (24%) had experienced a CR. We will not evaluate the study based upon the original rule which would require at least 9/39 patients with CR to declare success. In August 2012, the study was amended to allow for an additional 20 evaluable patients in order to provide additional data on response, survival, and toxicity in this patient population. Because of the 24% CR rate seen at that time, no formal efficacy monitoring was to be performed in the new patient cohort. As of May 2013, 44 patients had been evaluated for response, of which 9 (20%) had experienced a CR. In order to provide additional information on the CR rate, we will enroll an additional 16 patients into the trial. We expect 60 of the total 75 patients to be evaluable for response.

Safety Monitoring Rule

The protocol was originally designed to accrue a maximum of 39 patients (see above), and the following safety monitoring rule was designed to monitor patient safety with respect to treatment-related mortality (TRM):

To monitor the TRM rate, a binary outcome, there are two possible elementary outcomes. They are 1 = [treatment-related death], 2 = [alive or death due to other causes]. We denote the probability vector with the experimental treatment by qE. We assume Beta (3.3, 62.7) prior on qE, which corresponds to a mean TRM rate of 5%.

The following decision criteria will be applied after a minimum of 5 patients has been evaluated, up to the last patient. Targeting a 5% TRM rate as a trade-off, the trial will be stopped early if

$$\Pr[qE(\text{TRM rate}) > 5\% \mid \text{data}] > .85$$

That is, if at any time during the trial we determine that we have greater than 85% posterior probability that the experimental TRM rate is higher than 5%, we will stop the study.

As of August 2012, a total of 2 patients had experienced TRM, and the safety monitoring rule was modified to account for an additional 20 patients, for a total of 59. In May 2013, a total of 3

of the 59 total patients in the study had experienced TRM. The safety monitoring rule is further modified to account for an additional 16 patients, for a total of 75. Stopping boundaries corresponding to this probability criterion are to terminate the trial if

(# of patients died due to treatment) / (# patients evaluated) >/= 3/5, 4/12, 5/28, 6/44, or 7/61.

Note that given the study data evaluated in May 2013, the first four boundaries have not been crossed, but these are listed for completeness. This stopping rule leads to the new operating characteristics found in the table below.

Design Operating Characteristics

Table 2. Operating Characteristics for Monitoring Rule

True Pr(TRM rate)	Early Stopping Probability	Achieved Sample Size 25th, 50th, 75th percentiles		
0.02	0.005	75	75	75
0.03	0.020	75	75	75
0.04	0.058	75	75	75
0.05	0.129	75	75	75
0.06	0.219	75	75	75
0.07	0.353	53	75	75
0.08	0.461	40	75	75
0.09	0.571	32	62	75

Analyses Methods

Summary statistics will be provided for continuous variables. Frequency tables will be used to summarize categorical variables. For the primary endpoint of complete response rate on day 100, it will be calculated and reported, together with its 95% confidence interval. Wilcoxon rank sum test or Fisher's exact test will be used to test the association between the response and the prognostic factors. Patients' overall survival (OS) and progression free survival (PFS) will be monitored. The survival rate for time to event outcomes will be estimated by Kaplan-Meier method. Comparison of time to event endpoints by important subgroups will be made using the log-rank test. Cox proportional hazard regression will be employed for multivariate analysis on time-to-event outcomes. Treatment related morality rate will be computed and presented with 95% confidence interval. Adverse events will be tabulated for all the patients.

13.0 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 2-8oC (35-46oF). Stable at 4oC 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.

Break off the top of the ampoule and use a syringe needle to remove the calculated volume of busulfan from the primary container. Remove the needle, replace with a new needle which has been fitted with a 5.0 micron nylon filter (provided with packaged drug) and transfer the contents of the syringe into the calculated amount of either normal saline or D5W making sure that the drug flows into and through the solution. Do not put the busulfan solution into a syringe or IV bag, which does not contain the normal saline or D5W. Mix by inverting the container numerous times to ensure a homogenous solution. Place an appropriate label on the container with an expiration time of eight (8) hours from the time of preparation with directions to store at room temperature. Do not use if solution contains visible particulates. Record the actual volume on the label.

Place a suitable (non-vented or universal) intravenous administration set (gravity flow) into the outflow port of the container of the infusion solution.

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, controlled-rate pump.

The 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 (20-25oC), but administration must be completed within eight (8) hours of preparation including the three (3) hour drug infusion.

Side effects: 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.

Animal Toxicology: Busulfan fed to rats in an amount equivalent to about 0.5 mg/kg of final body weight per day slowed weight gain and produced bone marrow depression, pancytopenia and cataracts after about 10 weeks. In rats, LD5O was found to be 34 mg/kg intraperitoneally. When the drug was administered on day 13, 14, or 15 of gestation at a dose of 10 mg/kg to rats, the progeny were prematurely sterile.

Human Pharmacology: Limited pharmacology data are available for the parenteral formulation to be used in this study and is detailed in Attachment II, Preliminary Pharmacokinetic Evaluation of Busulfan in a Phase II human Trial. The pharmacokinetic data suggests that the plasma decay of the formulation fits a two-compartment model. The oral formulation is absorbed from the gastrointestinal tract, and measurable blood levels are obtained within one-half to two (0.5-2.0) hours after ingestion. Within three (3) minutes after IV administration in rats, 90% of the drug disappears from the blood; similar rapid decreases in blood concentrations have been reported in man. Busulfan 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.

Melphalan (Alkeran®)

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 associated with melphalan: 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.

Gemcitabine

Synonym(S): Gemcitabine hydrochloride, difluorodeoxycytidine, 2',2'-difluorodeoxycytidine, dFdC, LY 188011

Common Trade Name(S): Gemzar® (notice of compliance, December 1996; patent expires March 2004)

Classification: Antimetabolite, cytotoxic

Special pediatric considerations are noted when applicable, otherwise adult provisions apply.

Mechanism of Action:

Gemcitabine, a pyrimidine analog, is structurally similar to cytarabine, but has a wider spectrum of antitumour activity due to its different cellular pharmacology and mechanism of action. Gemcitabine is metabolized intracellularly to two active metabolites, Gemcitabine diphosphate (dFdCDP) and Gemcitabine triphosphate (dFdCTP). The cytotoxic effects of Gemcitabine are exerted through incorporation of dFdCTP into DNA with the assistance of dFdCDP, resulting in inhibition of DNA synthesis and induction of apoptosis. Gemcitabine is a radiation-sensitizing agent.5 It is cell-cycle phase specific (S and G1/S-phases).

Pharmacokinetics:

Interpatient variability	3- to 4-fold interpatient and intrapatient variability
Oral absorption	no information found
Distribution	widely distributed into tissues; also present in ascitic fluid.
	cross blood brain barrier?no information found
	volume of distributionIV infusion < 70 min: 50 L/m^2; IV infusion 70-285 min: 370 L/m^2
	plasma protein binding< 10%
Metabolism	Metabolized intracellularly by nucleoside kinases to active metabolites dFdCDP and dFdCTP; also metabolized intracellularly and extracellularly by cytidine deaminase to inactive metabolite difluorodeoxyuridine (dFdU)
	active metabolite(s)dFdCDP, dFdCTP
	inactive metabolite(s)dFdU
Excretion	mainly renal excretion
	Urine92-98% over one week (89% as dFdU, < 10% as Gemcitabine) after a single dose of 1000 mg/m2 given over 30 minutes.
	terminal half lifeIV infusion < 70 min: 0.7-1.6 h IV infusion 70-285 min: 4.1-10.6 h
	ClearanceIV infusion < 70 min: 41-92 $L/h/m^2$ (male), 31-69 $L/h/m^2$ (female)
Gender	decreased volume of distribution and clearance in women
Elderly	decreased clearance and increased half-life with increasing age
Children	no information found
Ethnicity	no information found

Special Precautions:

Carcinogenicity: No information found.

Mutagenicity: Not mutagenic in Ames test but mutagenic in mammalian *in vitro* mutation test. Gemcitabine is clastogenic in mammalian *in vitro* and *in vivo* chromosome tests.

Fertility: Decreased spermatogenesis and fertility in male mice.

Pregnancy: FDA Pregnancy Category D. There is positive evidence of human fetal risk, but the benefits from use in pregnant women may be acceptable despite the risk (eg, if the drug is needed in a life-threatening situation or for a serious disease for which safer drugs cannot be used or are ineffective).

Breastfeeding is not recommended due to the potential secretion into breast milk

Side Effects:

ORGAN SITE	SIDE EFFECT	ONSET
	Dose-limiting side effects are in ***bold, italics***	I = immediate (onset in hours to days); E = early (days to weeks); D = delayed (weeks to months); L = late (months to years)
allergy/immunology	allergic reaction (4%, severe 0.2%)	I
blood/bone marrow febrile neutropenia	***anemia*** (68%, severe 8%)	E
	Leucopenia (62%, severe 9%)	E
	neutropenia (63%, severe 25%) nadir 7-10 days, recovery within 7 days	E
	thrombocytopenia (24%, severe 5%) nadir 7-10 days, recovery within 7 days	E
cardiovascular (arrhythmia)	cardiac arrhythmia (2%, severe 0.2%)	E
cardiovascular (general)	edema/peripheral edema (28%, severe 3%)	ED
Coagulation	hemolytic uremic syndrome (0.3%)	D
constitutional symptoms	asthenia (42%, severe 2%)	E
	fever (37%, severe < 1%)	IE
dermatology/skin	*extravasation hazard:* none	
	alopecia (14%)	D

	skin rash (25%, severe < 1%)	IE
Gastrointestinal	*emetogenic potential:* low moderate	
	constipation (8%, severe < 1%)	E
	diarrhea (12%, severe < 1%)	E
	nausea and vomiting (64%, severe 18%)	I
	stomatitis (8%, severe < 1%)	E
Hemorrhage	hematuria (31%, severe < 1%)	E
Hepatic	elevated alkaline phosphatase (55%, severe 9%)	E
	elevated AST (67%, severe 9%)	E
	elevated ALT (68%, severe 10%)	E
	elevated bilirubin (13%, severe 2%)	E
Infection	infection (9%, severe 1%)	E
Neurology	decreased level of consciousness (9%, severe < 1%)	E
	peripheral neuropathy (3%)	ED
Pain	pain (16%, severe 1%)	ED
Pulmonary	dyspnea (8%, severe 1%)	IE
renal/genitourinary	elevated BUN (16%, severe 0%)	E
	elevated creatinine (7%, severe < 1%)	E
	Proteinuria (36%, severe < 1%)	E
Syndromes	flu-like symptoms (19%, severe 1%)	E

Hemolytic uremic syndrome has been infrequently reported and is characterized by microangiopathic hemolytic anemia, thrombocytopenia and renal failure. The syndrome can present either acutely with severe hemolysis, thrombocytopenia and rapidly progressive renal failure, or more insidiously with mild or no thrombocytopenia and slowly progressive renal failure. The etiology of hemolytic uremic syndrome is unknown.The onset of the syndrome has been reported to occur during and shortly after Gemcitabine therapy. If not treated promptly, the syndrome may result in irreversible renal failure requiring dialysis. Therefore, patients with impaired renal function should be monitored closely while being treated with Gemcitabine.

Elevated liver enzymes: Gemcitabine causes transient and reversible elevations of liver function enzymes in about two-thirds of patients. However, these increases are rarely of clinical significance and there is no evidence of increasing hepatic toxicity with either longer duration of Gemcitabine treatment or cumulative dose.

Fever/Flu-like symptoms: Fever of any severity was reported in 37% of patients. It is frequently associated with other flu-like symptoms such as headache, chills, cough, rhinitis, myalgia, fatigue, sweating and insomnia. These symptoms are usually mild and transient, and rarely dose-limiting. The use of acetaminophen may provide symptomatic relief.

Severe pulmonary toxicity: Acute dyspnea may sometimes occur with Gemcitabine therapy, but is usually self-limiting. However, severe pulmonary toxicities such as pulmonary edema,

interstitial pneumonitis and adult respiratory distress syndrome have rarely been reported. The symptoms are manifested as progressive dyspnea, tachypnea, hypoxemia and pulmonary infiltrates on chest radiograph that are sometimes accompanied by fever and cough. Pulmonary toxicities usually occur after several cycles of Gemcitabine, but have also been seen as early as the first cycle. Risk factors for pulmonary toxicities include prior radiation to the mediastinum. Because of its structural similarities to cytarabine, Gemcitabine is thought to cause lung injury by the same mechanism by inducing pulmonary capillary leakage. ManaGement of pulmonary toxicities consists of discontinuation of Gemcitabine and early supportive care with bronchodilators, corticosteroids, diuretics, and/or oxygen. Although pulmonary toxicities may be reversible with treatment, fatal recurrence of severe pulmonary symptoms was reported in one patient upon rechallenge with Gemcitabine.

Skin rash: Typically mild to moderate in severity, with macular or finely granular maculopapular pruritic eruption on the trunk and extremities. It is not dose-limiting and usually responds to topical corticosteroidsIf needed, antihistamines such as diphenhydramine can be used.

INTERACTIONS:

AGENT	EFFECT	MECHANISM	MANAGEMENT
Warfarin	increased anticoagulant effect of warfarin	possibly decreased metabolism of warfarin and decreased hepatic synthesis of clotting factors	monitor INR carefully during and for 1-2 months after Gemcitabine therapy; adjust warfarin dose as needed

SOLUTION PREPARATION AND COMPATIBILITY:

Injection: 200 mg and 1000 mg vials (as the hydrochloride salt). Store at room temperature.

Reconstitute 200 mg vial with 5 mL of NS without preservative and 1000 mg vial with 25 mL of NS without preservative to yield a Gemcitabine concentration of 38 mg/mL. Reconstitution of concentrations greater than 40 mg/mL may result in incomplete dissolution and should be avoided. Reconstituted solution is stable for 24 hours at room temperature and should not be days at room temperature and under refrigeration. However, the manufacturer recommends that the admixture be used within 24 hours since the solution does not contain preservatives.

Bacterial challenge: Gemcitabine 2.4 mg/mL diluted in NS did not exhibit a substantial antimicrobial effect on the growth of four organisms inoculated into the solution. Diluted solutions should be stored under refrigeration whenever possible and that the potential for microbiological growth should be considered when assigning expiration periods.

Compatibility: The following are ***compatible*** via Y-site injection: amifostine, bleomycin, carboplatin, carmustine, cisplatin, cyclophosphamide, cytarabine, dactinomycin, daunorubicin, dexamethasone, dexrazoxane, diphenhydramine, docetaxel, dopamine, doxorubicin, etoposide,

fludarabine, fluorouracil, granisetron, heparin, hydrocortisone, hydromorphone, idarubicin, ifosfamide, leucovorin, lorazepam, mannitol, meperidine, mesna, metoclopramide, mitoxantrone, morphine, ondansetron, paclitaxel, plicamycin, potassium chloride, ranitidine, sodium bicarbonate, streptozocin, teniposide, thiotepa, topotecan, vinblastine, vincristine, vinorelbine.

Incompatibility: The following are ***incompatible*** via Y-site injection: furosemide, irinotecan, methotrexate, methylprednisolone, mitomycin, prochlorperazine.

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