HHS Public Access

Author manuscript

*Lancet Haematol*. Author manuscript; available in PMC 2018 June 01.

Published in final edited form as:

*Lancet Haematol*. 2017 June ; 4(6): e283–e292. doi:10.1016/S2352-3026(17)30080-7.

# Phase 2 Clinical Trial of High-Dose Gemcitabine/Busulfan/Melphalan for Autologous Stem-Cell Transplantation in Relapsed/Refractory Myeloma: Matched-Pair Comparison with Melphalan

**Yago Nieto, MD PhD**[1], **Benigno C Valdez, PhD**[1], **Sai R Pingali, MD**[2], **Roland Bassett, MS**[2], **Ruby Delgado, MS**[1], **John Nguyen, MS**[1], **Nina Shah, MD**[1], **Uday Popat, MD**[1], **Roy B Jones, PhD MD**[1], **Borje S Andersson, MD PhD**[1], **Alison Gulbis, Pharm D**[3], **Sairah Ahmed, MD**[1], **Qaiser Bashir, MD**[1], **Simrit Parmar, MD**[1], **Krina Patel, MD**[4], **Alan Myers, Pharm D**[3], **Gabriela Rondon, MD**[1], **Robert Z Orlowski, MD**[4], **Richard Champlin, MD**[1], and **Muzaffar Qazilbash, MD**[1]

[1]Department of Stem-Cell Transplantation and Cellular Therapy, The University of Texas MD Anderson Cancer Center, Houston, TX

[2]Department of Biostatistics, The University of Texas MD Anderson Cancer Center, Houston, TX

[3]Department of Pharmacy, The University of Texas MD Anderson Cancer Center, Houston, TX

[4]Department of Lymphoma and Myeloma, The University of Texas MD Anderson Cancer Center, Houston, TX

## Abstract

**BACKGROUND—**High-dose melphalan is of limited benefit as autologous stem-cell transplantation (ASCT) regimen for relapsed/refractory myeloma. Its poor results in this setting prompted us to study a new high-dose combination of infusional gemcitabine/busulfan/melphalan (Gem/Bu/Mel).

**METHODS—**We conducted a phase 2 trial of Gem/Bu/Mel in patients with primary refractory or relapsed disease after bortezomib and/or an immunomodulatory drug (IMiD), or receiving a



**Corresponding Author**: Yago Nieto, MD, Department of Stem Cell Transplantation and Cellular Therapy, The University of Texas MD Anderson Cancer Center, Houston, TX, Phone: 713-792-2466, fax: 713-794-4902, ynieto@mdanderson.org.
The following authors are full professors: Yago Nieto, Uday Popat, Roy B Jones, Borje S Andersson, Robert Z Orlowski, Richard Champlin and Muzaffar Qazilbash,



**Author Contributions:**
YN designed the research, treated patients, interpreted the data and wrote the paper. RP and RB analyzed the data. BV and AM performed the correlative studies. RB designed the research. RP, RD, JN and GR collected the data. NS, UP, RBJ, BSA, AG, SA, KP, QB, SP, RZO, RC and MQ treated patients and interpreted the data. All authors revised and approved the final version of the manuscript.

**Conflict of Interest Disclosure:**
The remaining authors made no disclosures.

salvage ASCT. Gemcitabine (1,875 mg/m$^2$ over 3 hours × 2 days) was followed by busulfan (target AUC 4,000/day × 4 days) and melphalan (60 mg/m$^2$/day × 2 days). The primary endpoint of this trial was to determine the stringent complete remission (sCR) rate of Gem/Bu/Mel in this population. We then retrospectively compared the study patients with all other concurrent patients eligible for this trial who, instead, received melphalan at 200 mg/m$^2$ IV at our center. For survival outcomes, we used a statistical algorithm to select a subset from the control cohort that matched with the Gem/Bu/Mel patients by gender, age, disease status, double refractoriness to proteasome inhibitors/IMIDs, duration from diagnosis to transplant and cytogenetic risk, in a 1–2:1 ratio. All analyses are per protocol. This is the final analysis of the clinical trial. Trial registered at NCI.gov (NCT01237951).

**FINDINGS—**We enrolled 74 patients on the Gem/Bu/Mel trial, median age 58 (interquartile range [IQR], 11), median 2 prior therapy lines (IQR, 3), 38 high-risk cytogenetics, 17 unresponsive to all prior treatments, and 33 receiving a salvage ASCT. Toxicities of Gem/Bu/Mel included grade 3 mucositis (N=12), grade 3 dermatitis (N=5), grade 3 transaminase elevation (N=7), grade 3 diarrhea (N=2), grade 5 sudden death (N=1) and grade 5 sepsis (N=2). The study patients and the 184 concurrent controls received similar post-ASCT maintenance. Gem/Bu/Mel resulted in more sCR (24.6% *v* 12.6%, *P*=0.040), similar overall responses (73.8% *v* 74.1%, *P*=0.77) and similar transplant-related mortality (4.0% *v* 3.8%, *P*=0.90). The median follow-up times for the Gem/Bu/Mel patients and the matched subset (N=111) were 36 months (IQR, 15.2) and 34 months (IQR, 27), respectively. Gem/Bu/Mel resulted in improved progression-free survival (median 15.1 *v* 9.3 months, P=0.0030; hazard ratio=0.60; *P*=0.021) and overall survival (median 37.5 *v* 23 months, *P*=0.0092; hazard ratio=0.65, *P*=0.0087).

**INTERPRETATION—**Gem/Bu/Mel is a safe and active ASCT regimen for refractory/relapsed myeloma, with better outcomes than a concurrent matched cohort receiving melphalan.

**Funding—**Supported by a grant from Otsuka Pharmaceutical Development & Commercialization Inc. and NCI Grant P30 CA016672.

## INTRODUCTION

High-dose chemotherapy (HDC) with single-agent melphalan and autologous stem-cell transplant (ASCT) results in significant benefit as frontline consolidation of sensitive myeloma.[1,2] In contrast, the efficacy of high-dose melphalan relapsed or refractory (R/R) disease is more limited, with only 5–10% complete remissions (CR) and median post-ASCT progression-free survival (PFS) of approximately 12 months.[3] On the other hand, retrospective registry analyses and single-center studies suggested that salvage ASCT in patients whose myeloma had relapsed at least 1 year after their first ASCT were efficacious (reviewed in [4]). This was subsequently confirmed in a prospective randomized trial in patients relapsing around 2 years after their first ASCT, where salvage high-dose melphalan resulted in superior PFS and overall survival (OS) compared to a control arm receiving weekly cyclophosphamide.[5]

As with most other tumors in which HDC plays a role, it is conceivable that an active combination will prove to be more effective than single-agent melphalan for myeloma. A retrospective registry analysis showed improved outcomes after oral busulfan and melphalan

(Bu/Mel), compared to melphalan or melphalan/total body irradiation, despite worse prognostic features.[6] While oral busulfan was seriously limited by its unpredictable absorption and substantial risk of hepatic toxicity, the development of an intravenous (IV) busulfan formulation by Andersson and colleagues avoids the risk of hepatic venoocclusive disease associated with prior oral formulations of this drug and expanded its applicability, which is further optimized by pharmacokinetic-guided dosing.[7,8]

Building on our prior experience with IV Bu/Mel,[9] we developed gemcitabine/busulfan/melphalan (Gem/Bu/Mel), which showed a strong synergistic interaction based on gemcitabine's inhibition of DNA damage repair.[10] Gemcitabine was infused at a 10 mg/m$^2$/min, a dose rate previously shown to avoid saturation of its intracellular activating enzymes, optimizing the formation of its active intracellular metabolite, gemcitabine-triphosphate.[11] This prolonged infusion mimics the preclinical experiments where prolonged exposure to gemcitabine was very active against resistant myeloma cell lines, in direct correlation with the intracellular accumulation of its triphosphate metabolite.[12,13] This infusion schedule of gemcitabine stands in contrast to its more common short 30-minute infusions, which result in suboptimal intracellular activation, and showed no objective responses in patients with resistant myeloma.[14]

The early signals of activity in myeloma seen in the phase 1 trial of Gem/Bu/Mel led us to test this regimen in a phase 2 study in patients with R/R myeloma. We hypothesized that Gem/Bu/Mel is safe in patients with R/R myeloma with superior outcomes to a concurrent matched cohort of patients transplanted with melphalan alone.

## PATIENTS AND METHODS

### Trial Design

Eligibility criteria included age 18–70 and prior first-line therapy with a proteasome inhibitor (PI) and/or an immunomodulatory drug (IMiD), within one or more of the following settings, as defined by the International Myeloma Working Group (IMWG)[15]: Primary refractory disease (no response to any prior therapy), relapsed and refractory disease (no response to salvage therapy), multiple relapses, or relapse after a prior ASCT. Additional eligibility criteria included an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2, adequate renal (creatinine clearance ≥50 ml/min), hepatic (SGOT/SGPT/bilirubin ≤3 × upper normal limit), pulmonary (FEV1/FVC/cDLCO ≥50%) and cardiac function (left ventricular ejection fraction ≥40%); no prior whole brain irradiation or radiation within one month of enrollment, no active hepatitis B, and no chronic hepatitis C with cirrhosis or stage 3–4 fibrosis. The study protocol was approved by the MD Anderson Cancer Center Clinical Research Committee and the Institutional Review Board (IRB). Patients provided written informed consent. The study was registered at NCI.gov (NCT01237951).

Patients received an intravenous test dose of busulfan of 32 mg/m$^2$ over 60 minutes in the preadmission week (Table 3, in Appendix, page 1). Gemcitabine was administered on days −8 and −3 as a loading bolus of 75 mg/m$^2$, followed by a continuous infusion of 1,800 mg/m$^2$ over 3 hours at 10 mg/m$^2$/minute, followed by busulfan (days −8 to −5) and

melphalan (days −3 and −2). Busulfan was infused daily over 3 hours targeting an average daily area under the curve (AUC) of 4,000 μM-min, with the first two therapeutic doses adjusted from the test dose pharmacokinetics. If necessary, the third and fourth doses were readjusted after the first therapeutic dose analysis, targeting an aggregate course AUC of 16,000 μM-min. The sampling and analytical processes have been described previously.[9] As we have previously shown, this strategy results in uniform busulfan exposure.[10] A fixed busulfan dose of 100 mg/m$^2$/day was to be given in cases where pharmacokinetic dosing was not feasible. Melphalan was administered at 60 mg/m$^2$/day over 30 minutes on days −3 and −2.

The supportive care of patients enrolled in the trial was as follows: Acetaminophen, azoles and metronidazole were avoided from day −10 to −1. Phenytoin 300 to 600 mg/day was given from day −9 to −4. Dexamethasone 8 mg IV was given twice daily from day −9 to −2. IV Hydration started on admission until day −1. Oral care with palifermin, glutamine and supersaturated calcium/phosphate rinses, and oral cryotherapy during melphalan, was performed as previously described.[15] Peripheral blood progenitor cells (PBPCs) were infused on day 0. Institutional guidelines for filgrastim, antiemetics, antimicrobials and blood product transfusions were followed.

Post-ASCT tumor restaging included serum/urine protein electrophoresis, serum/urine immunofixation and serum free light chain assay, at 1, 3, 6 months and every 3–6 months thereafter for at least 2 years. Bone marrow aspirate and biopsy with morphologic, flow cytometry, cytogenetic and fluorescence in situ hybridization (FISH) studies were repeated at 3 months after ASCT and subsequently once a year. Bone survey was done once a year.

## Clinical Trial Endpoints

The primary endpoint of the trial was to determine the stringent complete remission (sCR) rate of Gem/Bu/Mel in a population of patients with R/R myeloma. Secondary endpoints included PFS, OS, and description of the toxicity profile. We used a Simon's 2-stage design with an original total accrual of 39 patients (19 in the 1$^{st}$ phase and 20 in the 2$^{nd}$ phase), targeting a sCR rate of 20% with a null sCR rate of 10%, 10% alpha rate and 80% power.[16] After the prespecified sCR rate was met in the 1$^{st}$ and 2$^{nd}$ phases, accrual was expanded by an additional 35 patients to obtain more data on response, outcomes and toxicity profile.

Overall response (ORR) and sCR rates were calculated among patients with measurable disease at ASCT following the International Myeloma Working Group (IMWG) criteria.[17] Likewise, we followed the IMWG definitions of refractory, primary refractory myeloma, relapsed and refractory myeloma, relapsed myeloma, progressive disease and relapse from sCR.[15] The definition of lines of therapy and high-risk cytogenetics were as proposed by the IMWG.[18,19] Toxicity scoring followed the NCI Common Toxicity Criteria, v3.0.[20]

## Correlative Studies of DNA Damage Response and Apoptosis

The phosphorylation status of histone 2AX (γ-H2AX) and poly-ADP ribose polymerase 1 (PARP1) levels were determined in peripheral blood mononuclear cells (PBMNC) from Gem/Bu/Mel patients. Samples were collected at baseline, day −7 (1 hour post-busulfan) and

day −2 (1 hour post-melphalan). The γ-H2AX flow cytometry and PARP-1 Western blot assays have been previously described.[10]

## Matched Pair Comparison with a Concurrent Control Population

Following a separate IRB-approved protocol, we retrospectively identified all those patients who, during the course of the trial met eligibility criteria but, instead, received single-agent melphalan at 200 mg/m$^2$ IV, either due to patient decision or no financial coverage for ASCT in a clinical trial. Eligibility of all of these control patients for this analysis was determined by four of the coauthors (MQ, YN, RD and JN) after individually reviewing each case. From this control cohort a subset matched with the Gem/Bu/Mel patients was selected by our statistician. The nearest neighbor matching method was applied to correct for potential imbalances between both groups.[21,22] Matching used a distance measure estimated from a logistic regression model (via propensity scores). Matches were chosen for each Gem/Bu/Mel patient one at a time from largest to smallest distance measure value within transplant number (2:1 matching for 1$^{st}$ transplant patients, and 1:1 matching for salvage transplant patients). Variables used in matching were gender, age, disease status, double refractory (proteasome inhibitor + IMiD) status, cytogenetic risk, and duration from diagnosis to ASCT. In addition to the matched group comparison we performed prespecified subgroup comparisons among patients receiving a first ASCT or a salvage ASCT, and those with high-risk cytogenetics.

Categorical variables were assessed using either Fisher's exact test or generalized Fisher's exact test, while the difference in age was determined by Wilcoxon rank sum test. Associations between tumor responses and treatment-related mortality (TRM) and treatment were assessed using Fisher's exact test. PFS was defined as the time from transplant to either progression or death, whichever occurred first, or last contact. OS was defined as the time from transplant to death or last contact. The Kaplan-Meier method was used to estimate unadjusted time-to-event distributions. The log rank test was used to compare PFS and OS distributions between treatments. In addition, the association between PFS and OS and treatment was determined using Cox proportional hazard regression models, accounting for the matched pairs. The Cox proportional hazard assumption was not verified before performing the Cox proportional hazard model.

The statistical analyses for the matched pairs were performed using SAS 9.3 for Windows (SAS Institute Inc., Cary, NC). The nearest neighbor matching was performed using the MatchIt package in R (MatchIt: Nonparametric Preprocessing for Parametric Causal Inference). All statistical tests used a significance level of 5%. No adjustments for multiple testing were made. All analyses are per protocol.

## Role of the funding source

The study sponsors had no role in the study design, collection, analysis or interpretation of the data, writing of the report. The raw data was accessible to YN, SRP, RB, RD, JN, GR and MQ. The corresponding author had full access to all of the data and the final responsibility to submit for publication.

# RESULTS

## Patient Characteristics (Table 2)

We enrolled 74 patients between November 30th, 2010 and December 11th, 2013, at the University of Texas MD Anderson Cancer Center. Their median age was 58 (interquartile range [IQR], 11)) (Table 1). Forty-two patients (57%) received Gem/Bu/Mel for their first ASCT and 32 patients (43%) received it for a salvage ASCT. They were previously treated with a median of 2 lines of therapy (IQR, 3); 68 (92%) and 66 patients (89%) had previously received a PI and an IMiD, respectively, and 49 patients (66%) were double refractory to PI and IMiDs. Among those patients receiving a salvage ASCT, 38 (51%) and 36 patients (49%) had a prior remission post-first ASCT of ≥18 months and <18 months, respectively. Thirty-eight patients (51%) had high-risk cytogenetics at some point in their disease, including del(17p) (N=11), t(4;14) (N=11), t(14;16) (N=7), and 1q+ (N=9). Eight patients (11%) had extramedullary myeloma (2 orbits, 2 epidura, 2 paraspinal soft tissue, 1 testes, 1 scalp).

## Hematologic Recovery and Regimen-Related Toxicities

The stem-cell source was peripheral blood. Neutrophil and platelets engrafted at medians of 10 days (IQR, 1)) and 12 days (IQR, 3)), respectively.

There were 3 treatment-related deaths: The first one was a 64 year-old male patient who died on day +13 from respiratory syncytial virus (RSV) pneumonia; the second case was a 51 year-old male who developed chemotherapy-induced enterocolitis complicated with overwhelming E coli sepsis and died on day +7; the third patient was a 63 year-old male who experienced unexplained sudden death on day +10, after resolution of earlier mild side effects and with unrevealing findings on autopsy. All three were heavily pretreated and the latter two were receiving a salvage transplant.

The toxicity profile of the remaining study patients was the following:

**Mucositis—**Grade 2 and 3 mucositis was observed in 39 (53%) and 12 patients (17%), respectively, starting at median day +5 (IQR, 3)). Grade 3 mucositis lasted at maximal severity for a median of 4 (IQR, 3)) days.

**Dermatitis—**Thirteen (18%) and 5 patients (7%) had a grade 2 and a grade 3 rash, respectively. All cases resolved spontaneously or with topical sunburn remedies or topical steroids. One patient experienced grade 2 hand-foot syndrome.

**Hepatic effects—**Early self-limited transaminase elevation was common: 11 patients (15%) and 7 patients (9%) had grade 2 and 3, respectively, peaking on median day −1 (IQR, 2) at a median value of 121 (IQR, 92)) IU/L and resolving within 1 week. Transient hyperbilirubinemia was seen in 18 patients (24%) in the first week post-transplant (7 patients (9%) grade 2 and 9 patients (12%) grade 3), with no cases of venoocclusive disease.

**Other toxicities—**One patient experienced grade 3 colitis with ileus. Otherwise, diarrhea was mild, with only 3 and 2 cases of grade 2 and grade 3, respectively. No renal, pulmonary, neurological or cardiac toxicities were observed.

## Infections

The following documented infections resolved with antimicrobials: methicillin-resistant *S aureus* pneumonia (N=2), candidemia (N=1), *Stenotrophomonas* bacteremia/urinary tract infection (UTI) (N=1), *S epidermidis* bacteremia (N=1), cytomegalovirus pneumonia (N=1), RSV upper (N=1) and lower (N=1) respiratory infections, herpes simplex esophagitis (N=1), *Enterococcus* UTI (N=1) and *Pseudomonas aeruginosa* UTI (N=1).

## Busulfan Pharmacokinetic Studies

Busulfan pharmacokinetics were calculated in all patients. The overall mean of the variation of the calculated test-to-therapeutic clearance was 6.2% (95% confidence interval (CI), −8.5 to 21%). Only 1 patient showed a busulfan clearance change >20%. For the remaining patients the clearance variation was <20%. The mean (% coefficient of variation) population clearance, volume of distribution and plasma elimination half-life from the first therapeutic dose were 94 mL/min/$m^2$ (19%), 24.6 L/$m^2$ (15%) and 3 hours (16%). These population pharmacokinetics do not differ from those previously estimated with Gem/Bu/Mel in patients with lymphoma (data not shown).[6]

## Tumor Responses

Among patients with measurable disease at ASCT, Gem/Bu/Mel resulted in a higher sCR rate than melphalan: 16 of 65 patients (24.6%), as compared to 22 of 174 patients (12.6%) (P=0.040). Their respective ORR were similar: 48 (73.8%) *v* 129 patients (74.1%) (*P*=0.77)

## Post-ASCT Maintenance

Fifty of 65 Gem/Bu/Mel patients (77%) and 112 of 159 control patients (70%) not progressing within the first 100 posttransplant days received maintenance treatment (*P*=0.32). Gem/Bu/Mel patients received lenalidomide±dexamethasone (N=27), bortezomib ±lenalidomide±dexamethasone (N=15), thalidomide±dexamethasone (N=3), pomalidomide/dexamethasone (Pd) (N=3), carfilzomib/lenalidomide/dexamethasone (KRd) (N=1) and bendamustine/lenalidomide (N=1). Similarly, patients in the control cohort received lenalidomide±dexamethasone (N=81), bortezomib±lenalidomide±dexamethasone (N=17), thalidomide ± dexamethasone (N=2), KRd (N=4), carfilzomib/pomalidomide (N=1), cyclophosphamide/dexamethasone (N=2), and Pd (N=5).

## Patient Outcomes

The concurrent control cohort treated with melphalan at our center included all patients meeting trial eligibility criteria (N=184) (Table 2). The Gem/Bu/Mel group had significantly more patients with high-risk cytogenetics, double refractoriness to PI/IMiDs, refractory disease at ASCT and receiving a salvage ASCT.

A subset of 111 patients (60%) was selected from the control cohort that matched with the Gem/Bu/Mel patients in gender, age, disease status, double refractory status, cytogenetic risk, and duration from diagnosis to ASCT (Table 2). While maintenance treatment was not used as a matching variable (in order to maximize the number of matched controls for each study patient), its prevalence was similar between both cohorts.

Within the matched subset of melphalan patients, there were 4 treatment-related deaths (3.6% TRM), 74 responses to HDC (ORR 70.4%) and 14 CRs (14.2% CR rate), all of them similar to the entire control cohort. The median follow-up times for the Gem/Bu/Mel and matched groups were similar at 36 months (IQR, 15.2) and 34 months (IQR, 27), respectively. The Gem/Bu/Mel cohort experienced significantly longer median PFS (15.1 months *v* 9.3 months, hazard ratio [HR]: 0.55; 95% CI: 0.38–0.81; *P* =0.0030) (Figure 1-A) and a significantly reduced risk of progression or death (HR: 0.60; 95% CI: 0.34–0.84; *P* =0.021). Patients in the Gem/Bu/Mel group also experienced significantly longer median OS (37.5 months *v* 23.0 months, *P* =0.0092) (Figure 1-B) and a lower risk of death (HR: 0.65; 95% CI: 0.36–0.89; *P*=0.0087). There was a significant correlation between response status after ASCT (sCR vs. no sCR) and PFS and OS (Figures 4-A and 4-B, Appendix, page 2) in both groups.

Comparing the two treatments in the matched subgroups without prior ASCT, the Gem/Bu/Mel patients had improved PFS (median 19.9 *v* 10.1 months, *P*=0.004; HR 0.48, 95% CI: 0.34–0.82; *P*=0.009) and OS (median 44.8 *v* 24.0 months, *P*=0.006; HR 0.40, 95% CI 0.28–0.83; *P*=0.007) (Figures 2A and 2B). Within the smaller subgroups receiving a salvage ASCT, the differences in PFS (median 12.8 *v* 8.7 months, HR 0.68; 95% CI: 0.40–1.17; *P*=0.28) and OS (median 33.2 *v* 20.5 months, HR 0.77; 95% CI: 0.41–1.45; *P*=0.50) were not statistically significant (Figures 3-A and 3-B).

Finally, for the patients with high-risk cytogenetics, Gem/Bu/Mel resulted in improved OS (median 26.0 *v* 14.2 months, HR 0.53; 95% CI: 0.32–0.89; *P*=0.004) and PFS (median 12.9 *v* 6.5 months, HR 0.43; 95% CI: 0.27–0.69; *P*<0.001) compared to melphalan (Figures 5-A and 5-B, Appendix, page 3).

The toxicities of the study patients and the entire control cohort (N=184) are shown on Table 2. Three Gem/Bu/Mel patients and 7 melphalan patients experienced TRM, with similar TRM rates between the Gem/Bu/Mel and full control cohorts (4.0% *v* 3.8%, *P*=0.90). There were 2 cases of second primary malignancies (both therapy-related myelodysplastic syndrome) in the control group, and none among Gem/Bu/Mel patients.

## DNA Damage Response (DDR) and Apoptosis Studies

We studied markers of DDR and apoptosis in 17 patients receiving Gem/Bu/Mel. γ-H2AX increased by a median 2-fold (IRQ, 1.2) and 6-fold (IQR, 1.3) on days −7 and −2, respectively, in PBMNC. PARP1 levels dropped on day −7 (median, 0.5 of baseline levels; IQR, 0.5) and further on day −2 (median, 0 of baseline levels; IQR, 0.3) (Figure 6, Appendix, page 4). These results were indicative of activation of DDR and apoptosis, respectively.

## DISCUSSION

Our study shows that Gem/Bu/Mel is safe and effective in R/R myeloma, with superior outcomes to a concurrent matched cohort of patients transplanted with melphalan.

We elected to study Gem/Bu/Mel in R/R myeloma, a challenging scenario where ASCT with melphalan offers limited benefit to patients.[23] The poor results of single-agent melphalan has prompted many investigators to combine this drug with other DNA-targeting agents, such as cyclophosphamide, idarubicin, topotecan, carmustine or bendamustine (reviewed in [24]). These studies have largely shown increased toxicity without improved outcomes. More promising results were observed when combining melphalan with IV busulfan,[9] bortezomib (when given after melphalan)[25] or lenalidomide.[26]

In contrast to those efforts, we attempted to augment the effect of melphalan through inhibition of DNA damage repair by gemcitabine, an effect first described by Plunkett and coworkers.[27] While gemcitabine, used in prolonged exposure, has shown potent *in vitro* activity against resistant myeloma cell lines,[12,13] this drug is rarely used in myeloma given its minimal activity in resistant disease when administered in short 30-minute infusions.[14] In contrast, prolonged infusions of gemcitabine, as in Gem/Bu/Mel, avoid saturation of its intracellular activating enzymes and optimizes the incorporation of its triphosphate metabolite into the DNA.[10] The correlative studies in samples from patients enrolled in our trial indicate marked activation of DNA damage response and apoptosis after exposure to Gem/Bu/Mel, consistent with our previous *in vitro* data.[10] The use of PBPC circumvents the increased myelotoxicity of infusional gemcitabine compared to shorter infusions of this drug. Similar to our experience in lymphomas,[10] Gem/Bu/Mel was shown to be safe, with manageable mucositis as the most relevant side effect.

Several new drugs have recently shown efficacy and have received approval for R/R myeloma, such as carfilzomib, pomalidomide, panobinostat, ixazomib, elotuzumab and daratumumab (reviewed in [28]). It remains to be elucidated how these new drugs and ASCT can be strategically best combined to the patients' advantage. Better results in the R/R setting with these new drugs may result in more patients who subsequently benefit from more effective HDC.

We designed our Gem/Bu/Mel trial with sCR as the primary endpoint, given that sCR is broadly considered a major surrogate for long-term outcomes in myeloma, including after HDC.[29] This effect seems particularly important in refractory or high-risk disease, whereas it may be less important in patients with more indolent tumors, who can still enjoy prolonged outcomes despite never achieving a sCR.[30] Consistent with these prior data, Gem/Bu/Mel induced twice as many sCR as melphalan, even with a similar ORR, which correlated with improved outcomes of the Gem/Bu/Mel group compared to the Melphalan cohort in our matched-pair analysis Further, we confirmed the prognostic effect of sCR after ASCT within each of the two matched groups. During the timespan of this trial we did not apply the new techniques to evaluate the depth of sCR and detect minimal residual disease (MRD), such as multiparametric flow cytometry or gene sequencing.[31,32,33] Their emerging

role and the important prognostic effect of detection of minimal residual disease have generated considerable interest.[34,35]

The superiority of Gem/Bu/Mel was of similar magnitude across all patient categories, although it did not reach statistical significance in the smaller subgroups receiving a salvage ASCT. The TRM rates were similar between the Gem/Bu/Mel and control cohorts (4% and 3.8%, respectively), and were consistent with the 3–5% TRM in previous reports in heavily pretreated patients.[4]

At the time we conducted this phase 2 trial of Gem/Bu/Mel we opted for a concurrent matched pair comparison analysis before launching a larger randomized trial. Thus, a limitation of our analysis is the nonrandomized nature of the comparison between both groups. Treatment assignment was determined in most cases by the type of insurance coverage but in some others by patient preference, raising the possibility of bias. While the concurrent control group was rigorously selected following the trial's inclusion criteria and a matching algorithm accounted for treatment imbalances, definitive proof of superiority of Gem/Bu/Mel over melphalan will ultimately require a randomized phase 3 study.

In conclusion, mature results of our phase II trial of Gem/Bu/Mel show that this regimen is safe and active for ASCT for R/R myeloma, with better outcomes than a concurrent matched cohort of patients receiving melphalan.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

YN reports research support from Otsuka Pharmaceuticals, Novartis Pharmaceuticals and Celgene Pharmaceuticals outside the submitted work. QB reports research funding from Celgene Pharmaceuticals and Takeda Pharmaceuticals and personal fees from Takeda Pharmaceuticals and Spectrum Pharmaceuticals outside the submitted work. RC reports research support from Sanofi and Celgene Pharmaceuticals outside the submitted work. BSA reports personal fees from Otsuka America Pharmaceutical, Inc, outside the submitted work.

## References

1. Attal M, Harousseau JL, Stoppa AM, et al. A prospective randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. N Engl J Med. 1996; 335:91–7. [PubMed: 8649495]
2. Child JA, Morgan GJ, Davies FE, et al. High-dose chemotherapy with hematopoietic stem-cell rescue for multiple myeloma. N Engl J Med. 2003; 348:1875–83. [PubMed: 12736280]
3. Vesole DH, Crowley JJ, Catchatourian R, et al. High-dose melphalan with autotransplantation for refractory multiple myeloma; Results of a Southwest Oncology Group phase II trial. J Clin Oncol. 1999; 17:2173–9. [PubMed: 10561273]
4. Atanackovic D, Schilling G. Second autologous transplant as salvage therapy in multiple myeloma. Br J Haematol. 2013; 163:565–72. [PubMed: 24111632]
5. Cook G, Ashcroft AJ, Cairns DA, et al. The effect of salvage autologous stem-cell transplantation on overall survival in patients with relapsed multiple myeloma (final results from BSBMT/UKMF Myeloma X Relapse [Intensive]): a randomised, open-label, phase 3 trial. Lancet Haematol. 2016; 3:e340–e351. [PubMed: 27374467]

6. Lahuerta JJ, Martinez-Lopez J, Grande C, et al. Conditioning regimens in autologous stem cell transplantation for multiple myeloma: a comparative study of efficacy and toxicity from the Spanish Registry for Transplantation in Multiple Myeloma. Br J Haematol. 2000; 109:138–47. [PubMed: 10848793]
7. Andersson BS, Kashyap A, Gian V, et al. Conditioning therapy with intravenous busulfan and cyclophosphamide (IV BuCy2) for hematologic malignancies prior to allogeneic stem cell transplantation: A phase II study. Biol Blood Marrow Transplant. 2002; 8:145–154. [PubMed: 11939604]
8. Madden T, de Lima M, Thapar N, et al. Pharmacokinetics of once-daily IV busulfan as part of pretransplant preparative regimens: A comparison with an every 6–hour dosing schedule. Biol Blood Marrow Transplant. 2007; 13:56–64. [PubMed: 17222753]
9. Kebriaei P, Madden T, Kazerooni R, et al. Intravenous busulfan plus melphalan is a highly effective, well-tolerated preparative regimen for autologous stem cell transplantation in patients with advanced lymphoid malignancies. Biol Blood Marrow Transplant. 2011; 17:412–20. [PubMed: 20674757]
10. Nieto Y, Thall P, Valdez B, et al. High-dose infusional gemcitabine combined with busulfan and melphalan with autologous stem-cell transplantation in patients with refractory lymphoid malignancies. Biol Blood Marrow Transplant. 2012; 18:1677–1686. [PubMed: 22643322]
11. Gandhi V, Plunkett W, Du M, Ayres M, Estey EH. Prolonged infusion of gemcitabine: Clinical and pharmacodynamic studies during a phase I trial in relapsed acute myelogenous leukemia. J Clin Oncol. 2002; 20:665–73. [PubMed: 11821446]
12. Gruber J, Geisen F, Sgonc R, et al. 2',2'-Difluorodeoxycytidine (gemcitabine) induces apoptosis in myeloma cell lines resistant to steroids and 2-chlorodeoxyadenosine (2-CdA). Stem Cells. 1996; 14:351–62. [PubMed: 8724701]
13. Krett NL, Ayres M, Nabhan C, et al. In vitro assessment of nucleoside analogs in multiple myeloma. Cancer Chemother Pharmacol. 2004; 54:113–21. [PubMed: 15133625]
14. Weick JK, Crowley JJ, Hussein MA, Moore DF, Barlogie B. Southwest Oncology Group. The evaluation of gemcitabine in resistant or relapsing multiple myeloma, phase II: a Southwest Oncology Group study. Invest New Drugs. 2002; 20:117–21. [PubMed: 12003187]
15. Rajkumar SV, Harousseau J-L, Durie B, et al. Consensus recommendations for the uniform reporting of clinical trials: report of the International Myeloma Workshop Consensus Panel 1. Blood. 2011; 117:4691–5. [PubMed: 21292775]
16. Simon R. Optimal two-stage designs for phase II clinical trials. Control Clin Trials. 1989; 10:1–10. [PubMed: 2702835]
17. Durie BGM, Harousseau J-L, San Miguel J, et al. International uniform response criteria for multiple myeloma. Leukemia. 2006; 20:1467–73. [PubMed: 16855634]
18. Rajkumar SV, Richardson P, San Miguel JF. Guidelines for determination of the number of prior lines of therapy in multiple myeloma. Blood. 2015; 126:921–2. [PubMed: 26272048]
19. Sonneveld P, Avet-Loiseau H, Lonial S, et al. Treatment of multiple myeloma with high-risk cytogenetics: a consensus of the International Myeloma Working Group. Blood. 2016; 127:2955–62. [PubMed: 27002115]
20. [last accessed on March 8th, 2017] https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcaev3.pdf
21. Rosenbaum PR, Rubin DB. Constructing a control group using multivariate matched sampling methods that incorporate the propensity score. American Statistician. 1985; 39:33–8.
22. Ho D, Imai K, King G, Stuart E. Matching as Nonparametric Preprocessing for Reducing Model Dependence in Parametric Causal Inference. Political Analysis. 2007; 15:199–236.
23. Laubach J, Garderet L, Mahindra A, et al. Management of relapsed multiple myeloma: recommendations of the International Myeloma Working Group. Leukemia. 2016; 30:1005–17. [PubMed: 26710887]
24. Maybury B, Cook G, Pratt G, Yong K, Ramasamy K. Augmenting autologous stem cell transplantation to improve outcomes in myeloma. Biol Blood Marrow Transplant. 2016:1926–37. [PubMed: 27288955]

25. Lonial S, Kaufman J, Tighiouart M, et al. A phase I/II trial combining high-dose melphalan and autologous transplant with bortezomib for multiple myeloma: a dose- and schedule-finding study. Clin Cancer Res. 2010; 16:5079–86. [PubMed: 20739431]

26. Shah N, Thall PF, Fox PS, et al. Phase I/II trial of lenalidomide and high-dose melphalan with autologous stem cell transplantation for relapsed myeloma. Leukemia. 2015; 29:1945–8. [PubMed: 25721897]

27. Plunkett W, Huang P, Searcy CE, et al. Gemcitabine: preclinical pharmacology and mechanisms of action. Semin Oncol. 1996; 23(5 Suppl 10):3–15.

28. Rajan AM, Kumar S. New investigational drugs with single-agent activity in multiple myeloma. Blood Cancer J. 2016; 6:e451. [PubMed: 27471867]

29. Van de Velde HJK, Liu X, Chen G, et al. Complete response correlates with long-term survival and progression-free survival in high-dose therapy in multiple myeloma. Haematologica. 2007; 92:1399–40. [PubMed: 18024376]

30. Harousseau J-L, Attal M, Avet-Loiseau H. The role of complete response in multiple myeloma. Blood. 2009; 114:3139–46. [PubMed: 19638622]

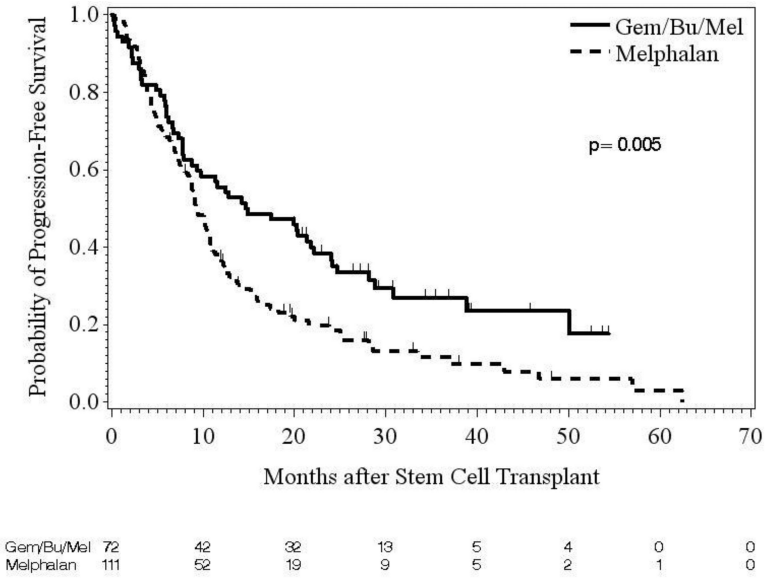
31. Paiva B, Chandia M, Puig N, et al. The prognostic value of multiparameter flow cytometry minimal residual disease assessment in relapsed multiple myeloma. Haematologica. 2015; 100:e53–5. [PubMed: 25381128]

32. Silvennoinen R, Kairisto V, Pelliniemi TT, et al. Assessment of molecular remission rate after bortezomib plus dexamethasone induction treatment and autologous stem cell transplantation in newly diagnosed multiple myeloma patients. Br J Haematol. 2013; 160:561–4. [PubMed: 23206270]

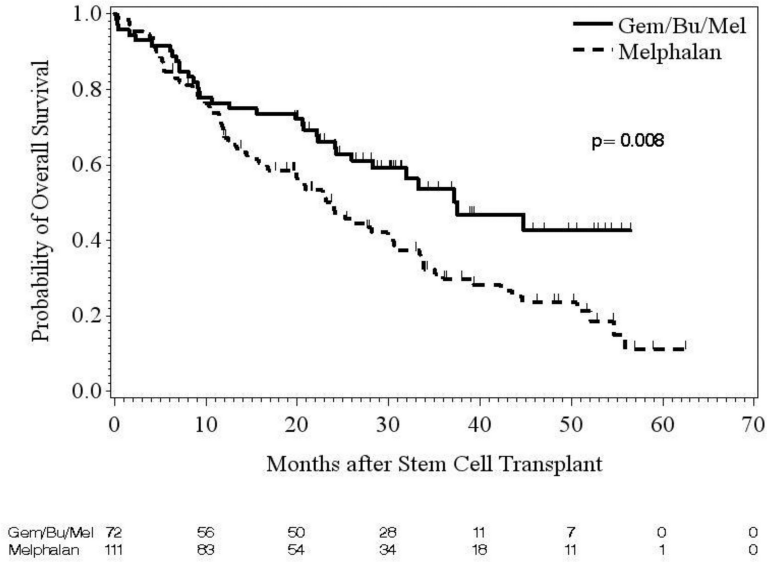
33. Avet-Loisseau H, Corre J, Lauwers-Cances V, et al. Evaluation of minimal residual disease (MRD) by next generation sequencing (NGS) is highly predictive of progression free survival in the IFM.DFCI 2009 trial. Blood. 2015; 126:191.

34. Munshi NC, Avet-Loiseau H, Rawstron AC, et al. Association of Minimal Residual Disease With Superior Survival Outcomes in Patients With Multiple Myeloma: A Meta-analysis. JAMA Oncol. 2016; 3:28–35.

35. Landgren O, Devlin S, Boulad M, Mailankody S. Role of MRD status in relation to clinical outcomes in newly diagnosed multiple myeloma patients: a meta-analysis. Bone Marrow Transplant. 2016; 51:1565–8. [PubMed: 27595280]



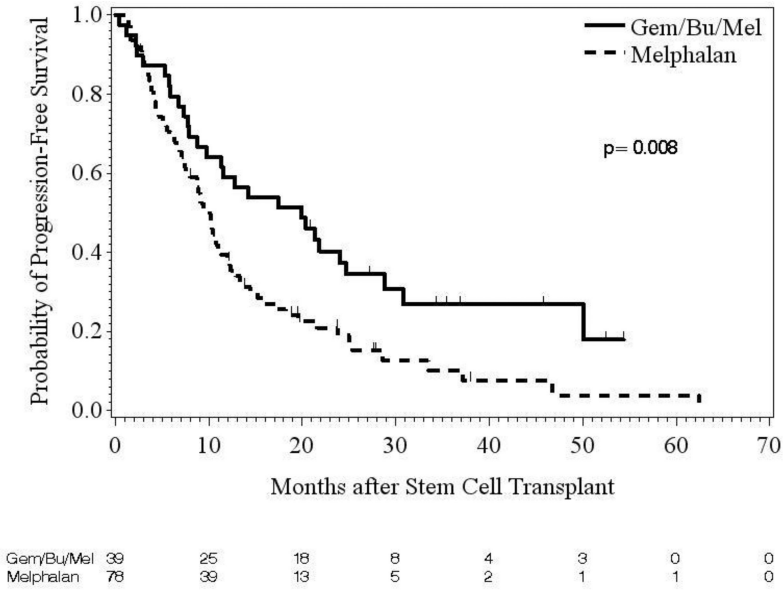

**A**



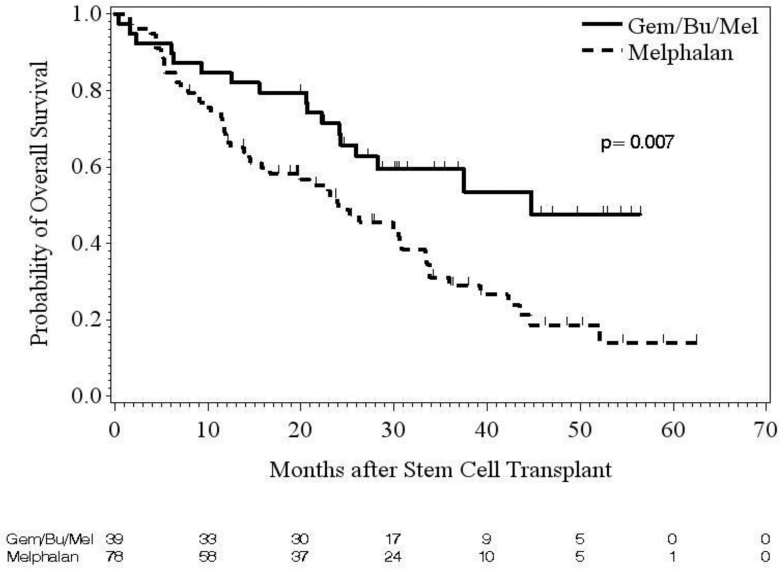

**B**

**Figure 1.**

Outcomes of all matched patients. Fig. 1-A, progression-free survival. Fig. 1-B, overall survival.



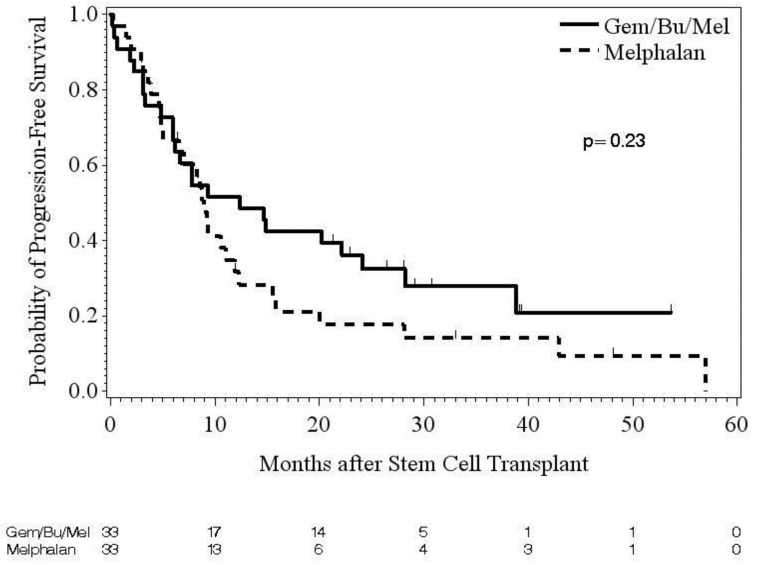

**A**



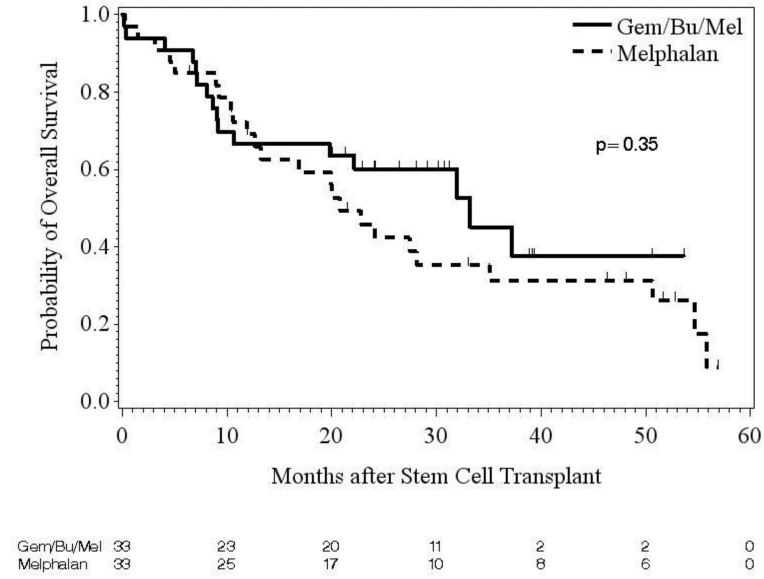

**B**

**Figure 2.**

Outcomes after first transplant, matched patients. Fig. 2-A, progression-free survival. Fig. 2-B, overall survival.




**A**




**B**

**Figure 3.**

Outcomes after salvage transplant, matched patients. Fig. 3-A, progression-free survival. Fig. 3-B, overall survival.

**Table 1**

Patient characteristics of the Gem/Bu/Mel study file, the entire concurrent melphalan-treated control cohort and the matched melphalan subset.

| | Gem/Bu/Mel (N=74) | Melphalan control cohort (N=184) | P value(1) | Matched melphalan subset (N=111) | P value(2) |
|---|---|---|---|---|---|
| Age, median (IQR) | 58 () | 60 ( | 0.03 | 59 () | 0.38 |
| Male / female | 57 (77%) / 17 (23%) | 105 (57%) / 79 (43%) | 0.003 | 76 (69%) / 35 (31%) | 0.32 |
| ISS stage at diagnosis | | | | 47 (42%) | 0.36 |
| I | 28 (38%) | 59 (32%) | 0.4 | | |
| II | 22 (30%) | 46 (25%) | | | |
| III | 22 (30%) | 68 (37%) | | | |
| Unavailable | 2 (2%) | 11 (6%) | | | |
| Poor-risk cytogenetics | 38 (51%) | 66 (36%) | <0.001 | 44 (40%) | 0.35 |
| del(17p) | 11 (15%) | 9 (5%) | | | |
| t(4;14) | 11 (15%) | 20 (11%) | | | |
| amp(1q) / del(1p) | 9 (12%) | 17 (9%) | | | |
| t(4;16) | 7 (9%) | 20 (11%) | | | |
| Extramedullary disease | 8 (11%) | 9 (5%) | 0.02 | | |
| Double refractory (IMiDs + proteasome inhibitors) | 49 (66%) | 63 (34%) | <0.001 | | |
| Setting | | | | | |
| First ASCT | 41 (55%) | 136 (74%) | 0.01 | | |
| Salvage ASCT | 33 (45%) | 48 (26%) | | | |
| Disease status: | | | | | |
| Primary refractory | 7 (9%) | 9 (5%) | <0.001 | | |
| Refractory relapse | 24 (32%) | 40 (22%) | | | |
| Sensitive relapse | 42 (57%) | 97 (53%) | | | |
| Untreated relapse | 1 (1%) | 38 (20%) | | | |
| Response at ASCT: | | | | | |

*Lancet Haematol*. Author manuscript; available in PMC 2018 June 01.

| | Gem/Bu/Mel (N=74) | Melphalan control cohort (N=184) | P value(1) | Matched melphalan subset (N=111) | P value(2) |
|---|---|---|---|---|---|
| Responsive | 42 (57%) | 98 (53%) | <0.001 | 65 (59%) | 0.88 |
| Refractory | 30 (41%) | 50 (27%) | | 46 (41%) | |
| Untreated | 2 (2%) | 36 (20%) | | | |
| No. prior lines of treatment: | | | | | |
| 2 | 39 (53%) | 105 (57%) | 0.58 | 51 (46%) | 0.55 |
| >2 | 35 (47%) | 79 (43%) | | 60 (54%) | |
| No. prior relapses: | | | | | |
| 1 | 45 (61%) | 110 (60%) | 0.95 | 57 (51%) | 0.29 |
| >1 | 29 (39%) | 74 (40%) | | 54 (49%) | |
| Months from diagnosis to ASCT, median (range) | 20 (5–143) | 20 (4–143) | 0.92 | | |
| Duration of prior remission post first ASCT: | | | | | |
| ≥18 | 38 (51%) | 108 (58%) | 0.6 | 56 (50%) | 1 |
| <18 months | 36 (49%) | 76 (42%) | | 55 (50%) | |
| Post-ASCT maintenance | 50/65 (77%) | 112/155 (72%) | 0.32 | 64/90 (71%) | 0.46 |

(1)Comparisons between Gem/Bu/Mel patients and the entire melphalan cohort.

(2)Comparisons between Gem/Bu/Mel patients and matched melphalan subset.

**Table 2**

Toxicities ≥ Grade 2 of the Gem/Bu/Mel Study Patients and All Concurrent Melphalan Controls

| | Mucositis | | Dermatitis | | Hepatic (transaminitis) | | Diarrhea | | Pulmonary | | Cardiac | | | Infection | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Grade (G) | G 2 | G 3 | G 2 | G 3 | G 2 | G 3 | G 2 | G 3 | G 3 | G 4 | G 3 | G 4 | G 5 | G 3 | G 5 |
| Gem/Bu/Mel (N=74) | 39 (53%) | 12 (17%) | 13 (18%) | 5 (7%) | 11 (15%) | 7 (9%) | 3 (4%) | 2 (3%) | 0 | 0 | 0 | 0 | 1 (1%) | 63 (85%) | 2 (2%) |
| Melphalan (N=184) | 55 (30%) | 30 (16%) | 0 | 0 | 9 (5%) | 0 | 9 (5%) | 4 (2%) | 7 (4%) | 14 (8%) | 37 (20%) | 7 (4%) | 1 0.05%) | 59 (70%) | 2 1%) |