Bortezomib and dexamethasone consolidation following risk-adapted melphalan and stem cell transplantation for patients with newly diagnosed light chain amyloidosis

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Bortezomib and Dexamethasone Consolidation Following Risk-Adapted Melphalan and Stem Cell Transplantation for Patients with Newly Diagnosed Light Chain Amyloidosis

Running title: Bortezomib after transplant in amyloidosis

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ABSTRACT

To improve the efficacy of risk-adapted melphalan in patients with AL amyloidosis, we conducted a phase II trial using bortezomib and dexamethasone (BD) as consolidation. Forty untreated patients with renal (70%), cardiac (65%), liver/GI (15%) or nervous system (13%) AL were assigned melphalan 100, 140 or 200mg/m2 based on age, renal function and cardiac involvement. Hematologic response was assessed at 3 months post-SCT; patients with less than CR received BD consolidation. Four patients with advanced cardiac AL died within 100 days of SCT (10% TRM). Survival at 12 and 24 months post treatment start was 88% and 82% overall and was 81% and 72% in patients with cardiac AL. At 3 months post-SCT, 45% had $\geq$ PR including 27% CR. Twenty-three patients received consolidation and in 86% response improved; all patients responded in one cycle. At 12 and 24 months, 79% and 60% had $\geq$ PR, 58% and 40% CR. Organ responses occurred in 55% and 70% at 12 and 24 months. Eight patients relapsed/progressed. One patient with serologic progression had organ impairment at time of progression. In newly diagnosed AL, BD following SCT rapidly and effectively improves responses resulting in high CR rates and maintained organ improvement.

KEYWORDS: AL Amyloidosis, stem cell transplant, bortezomib

INTRODUCTION

Light chain amyloidosis (AL) is a clonal plasma cell disorder and organ disease associated with the production of pathologic free light chains (FLC).(1) Misfolded FLCs deposit in the form of amyloid in affected organs such as the heart, kidney, liver or gastrointestinal (GI) tract and the peripheral or autonomic nervous systems. Amyloid causes morbidity by impairing organ function and patients with cardiac involvement have shortened survival.(2) Organ dysfunction can be reversed if the synthesis of the amyloidogenic protein is shut down.(3) Treatment of AL is aimed at eradicating the pathologic plasma cells and reducing the circulating FLC so that organs can improve and survival can be extended.(2, 4)

High-dose melphalan and autologous stem cell transplant (SCT) induce responses in patients with AL.(2, 4, 5) Despite being effective, this strategy can be toxic in AL patients who have compromised organ function.(5) To deliver high-dose melphalan safely, risk adapted dosing has been explored but may be associated with lower response rates.(6) We have incorporated consolidation with novel agents following SCT in AL patients who have persistent clonal plasma cell disease in an effort to increase response rates. In our prior phase II study, thalidomide and dexamethasone were administered following risk adapted melphalan and SCT to patients who did not achieve a complete hematologic response (CR).(7) At 12 months after SCT, 78% of patients responded including 39% with CR; 42% of patients who received thalidomide and dexamethasone had improved hematologic responses.(7, 8) Median progression free survival (PFS) was 40 months; at a median of 52 months of follow up 69% of patients were alive.(7, 8)

The reversible proteasome inhibitor bortezomib is active and well-tolerated in relapsed AL amyloidosis, resulting in durable hematologic responses in over two-thirds of patients.(9) In the current trial, we treated patients who had not achieved CR following risk adapted melphalan and SCT with bortezomib and dexamethasone (BD) as consolidation. We found that BD effectively improves hematologic responses post transplantation and results in high hematologic response rates, durable organ improvement and promising overall survival (OS) for newly diagnosed transplant-eligible patients with AL amyloidosis.

Subjects and Methods

Patient eligibility

Patients with untreated AL amyloidosis were eligible for enrollment on this Institutional Review Board approved phase II clinical trial (NCT00458822). Patients required a histologic diagnosis of amyloid, clonal plasma cell disease and symptomatic involvement of no more than two major organ systems.(10) Hereditary amyloidosis was excluded by gene sequencing of commonly inherited amyloidogenic proteins in patients who met previously defined clinical criteria.(11) Adequate organ function was required including serum bilirubin $\leq$ 2.0 mg/dl; pulmonary diffusion capacity $\geq$50% and left ventricular ejection fraction $\geq$45%. Patients were excluded for uncompensated New York Heart Association (NYHA) class 3 or greater congestive heart failure, symptomatic cardiac arrhythmia, or cardiac syncope within 60 days of enrollment. Patients with symptomatic multiple myeloma (> 30% plasma cells or lytic lesions on skeletal survey) or soft tissue amyloid as the only organ involvement were ineligible.

Study design

Stem cells were mobilized with granulocyte colony stimulating (G-CSF) alone. Patients with cardiac arrhythmias or orthostatic hypotension were admitted and monitored on telemetry during stem cell mobilization and collection. Patients were assigned to one of three melphalan (MEL) dose levels (MEL 200mg/m2, 140mg/m2 or 100mg/m2) based on age, cardiac involvement and renal function as defined by 24-hour creatinine clearance (Cr Cl) $\leq$ 50ml/min (**Figure 1**).(7) At 2-3 months following SCT, patients with less than CR were eligible to receive 6 cycles of BD that included bortezomib 1.3mg/m2 IV days 1,4,8,11 and dexamethasone 20mg po on days 1, 2, 4, 5, 8, 9, 11, 12 every 21 days for 2 cycles and then bortezomib 1.3mg/m2 IV days 1, 8, 15 and 22 and dexamethasone 20mg po on days 1, 2, 8, 9, 15, 16 and 22, 23 for 4 cycles. Patients with grade > 2 sensory neuropathy received dexamethasone 20mg/m2 (only) as a 4 day pulse, up to 3 pulses each month for 6 months. Patients were assessed monthly following SCT through 12 months post-SCT, every 2 months for the subsequent year and then every 3 months until progression. Toxicity was scored using National Cancer Institute common toxicity criteria (CTCAEv3.0).

Response assessment

Hematologic response was assessed at 2-3, 12 and 24 months following SCT in accordance with the guidelines established by the 10th Annual International Symposium on Amyloid and Amyloidosis.(10) A

CR required a negative serum and urine immunofixation electrophoresis, normal serum FLC ratio and < 5% clonal plasma cells on bone marrow studies; partial response (PR), stable disease (SD) and disease progression (PD) was defined as previously described.(10)

Organ involvement was defined for each patient by standard and updated criteria.(10, 12) Response was scored at 12 and 24 months following SCT as improved, stable or worsened.(10, 12, 13) Brain natriuretic peptide (BNP) levels were obtained over the course of treatment and changes with therapy were noted. Stable or worsening organ function was defined as previously described but also updated to include cardiac BNP data.(10, 12-14)

Biostatistics

The primary endpoint of this phase II study was the rate of response improvement in patients receiving BD consolidation. Response improvement was defined as any improvement in the response (i.e., SD to PR/CR or PR to CR) at 12 months compared to post-SCT re-staging. A single-stage design was used to differentiate between response improvement rates of $\leq$45% and $\geq$68% using 10% type I and type II errors rates. We planned on accruing 31 patients who received BD and, if at least 18 patients had response improvement, then the treatment was to be declared a success. Response rates were calculated along with exact 95% confidence intervals (CI). Complete restaging studies were performed and overall hematologic and organ response rates determined, at 2-3, 12 and 24 months post-SCT. Overall survival (OS) was defined as time from treatment start until date of death or last follow-up. PFS was defined as time from treatment start until date of progression, death or last follow-up. PFS and OS were estimated using the method of Kaplan-Meier. Differences between categorical variables were assessed using Fisher's exact test. Differences between continuous variables were assessed using the Wilcoxon rank sum test. The associations of BNP and troponin on OS were evaluated using the Cox proportional hazards model. Hazard ratios (HR) for these associations are reported.

RESULTS

Patient Characteristics

Between March 2007 and May 2011, 40 patients (**Table 1**) with untreated AL amyloidosis who provided informed consent were enrolled and treated on this clinical trial. The median time from diagnosis to transplant was 2.3 months (range 0.6 – 16.3). The median age was 57 (38-67) and 58% were female. Patients had kidney (N=28), heart (N=26), liver/GI (N=6) and autonomic or peripheral nervous system

involvement (N=5), and 55% (N=22) had more than one organ involved. By biomarker cardiac staging criteria, 35%, 37% and 28% of patients were stage I, II and III respectively.(15, 16)

Treatment

Of forty patients who initiated treatment, 14 received MEL 200mg/m2, 17 MEL 140mg/m2 and 8 MEL 100mg/m2 (**Figure 1**). Four of 11 patients with stage 3 cardiac involvement died within 100 days of SCT including 1 who died during G-CSF mobilization resulting in a treatment related mortality (TRM) of 10%.

Twenty-five patients with persistent clonal plasma cell disease were eligible to receive consolidation with BD; one patient declined and another relocated. The median time from transplant to consolidation was 2.7 months (range 2.0 – 6.8). Twenty-two patients received a median of 6 cycles of BD consolidation (range 1-6) and 1 received dexamethasone due to preexisting grade 2 painful neuropathy.

Toxicity

Grade 3-4 adverse events (AEs) possibly related to BD consolidation are shown in **Table 2**. One patient with advanced cardiac disease (BNP > 5000) died during consolidation. Other grade 3 cardiac AEs included supraventricular tachycardia (N=1), hypotension (N=2) and congestive heart failure (N=1). Grade 3 gastrointestinal toxicity was only seen in 1 patient who developed abdominal bloating. Grade 2 neuropathy occurred in ten patients and grade 3 in two. Significant (grade 3-4) hematologic AEs included 43% thrombocytopenia, 13% anemia and 4% neutropenia.

Hematologic Responses

At 2-3 months post transplant, hematologic responses were PR 18% (n=7), CR 27% (n=11) and SD 45% (n=18). By intention-to-treat (ITT), at 12 months post-SCT hematologic responses were seen in 79% (95% CI: 65%-92%) of patients including 58% who achieved CR (95% CI: 42%-75%) (**Table 3**). Of the patients in CR following SCT (N=11), 10%, 45% and 45% received MEL 100, MEL 140 and MEL 200, respectively. No significant difference was seen based on MEL dosing at either 2-3 (P = 0.21) or 12 months post-SCT (P = 0.45) (**Table 4**). Of the 23 patients who received consolidation, 21 were assessable for response improvement at 12 months, the primary endpoint. Eighteen (86%, 95% CI: 64%-97%) of these patients achieved better responses at 12 months post SCT, including eight who improved from SD to CR, and four from PR to CR. Since the number of patients with response improvement met the pre-defined target, the criterion for declaring the regimen effective was met. The maximal FLC response following BD was seen with the first cycle of treatment in 95% of patients and there was no

statistically significant association between the number of cycles of BD received and response (P = 0.15), although patients who achieved CR tended to have received fewer cycles (**Figure 2**). Among patients classified as PR at 12 months post SCT, the very good partial response (VGPR) rate was 100% using updated FLC criteria.(14)

Organ Responses

By ITT, 55% (N=21) of patients had improvement in at least one involved organ by 12 months and 70% (N=21) by 24 months (**Table 3**). When assessed by individual organ, at 12 months post SCT improvement was seen in 9/17 surviving patients with cardiac involvement, 12/23 with renal, 3/5 with hepatic/GI and 4/4 with nervous system involvement. By 24 months post-SCT, 21 of 22 evaluable patients (7 deceased, 10 ongoing and 1 off study) had achieved organ responses including 5/9(56%) patients with cardiac and 13/15 (87%) with renal involvement. In patients with Cr Cl $\geq$45ml/min, the median percent reduction of the BNP at 12 and 24 months post transplant was 50% and 77%, respectively.(12)

Progression free and overall survival

Kaplan-Meier curves of PFS and OS are shown in **Figure 3**. The median follow up of surviving patients is 45 months (range 10-60 months) and the median PFS and OS have not been reached. At 24 months following treatment initiation, 82% of patients are alive and 69% are progression free. Only 1 patient who met criteria for hematologic progression (PD) developed worsening organ function at the time of progression.

Survival of patients with cardiac involvement was 81% at 12 months and 72% at 24 months following initiation of treatment (**Figure 3**). The cardiac patients who died on this trial had baseline median BNP of 638 pg/ml (120-1720 pg/ml) and troponin-I of 0.12 ng/ml (0-0.3ng/ml) while those who survived had baseline BNP and troponin-I of 105 pg/ml (0-713 pg/ml) and 0 ng/ml (0-0.2ng/ml), respectively. Higher values of BNP (HR 1.3; 95% CI: 1.1-1.4, P= 0.0001) and troponin-I (HR 4.0; 95% CI 1.9-8.3, P= 0.0002) were independently associated with inferior survival. The OS of patients with stage III cardiac involvement was 50% at 12 months and 36% at 24 months, while all stage I and II patients were alive at 24 months following SCT (**Figure 3**).

DISCUSSION

Risk-adapted melphalan and SCT followed by bortezomib and dexamethasone is an effective strategy for treating newly diagnosed patients with AL amyloidosis. The majority of patients (79%) achieved hematologic responses including over half (58%) who achieved strictly defined CR. While one-third of patients achieved CR with high dose melphalan and SCT alone, 86% of patients with persistent disease improved their response with additional BD consolidation, supporting the activity of this treatment program. While patients undergoing SCT may achieve maximal hematologic responses beyond 2-3 months post-SCT, the rapidity of FLC reduction with consolidation suggests that BD accounted for the up-graded responses.

The combination of an alkylator and bortezomib acts synergistically against plasma cells in multiple myeloma, at least in part by down regulation of DNA repair mechanisms after genotoxic chemotherapy.(17) Bortezomib administered following rather than prior to high dose melphalan results in increased apoptotic plasma cells,(18) and may account for the rapid and high complete response rate seen in 57% (12/21) of our patients who received consolidation following SCT (**Figure 2**). High dose melphalan (on days -2 and -1) and bortezomib (1mg/m2 days -6, -3, +1, +4) administered in combination has been studied in 10 patients with AL amyloidosis.(19) Responses were seen in 80% of these selected patients including 67% who achieved CR,(19) which also compares favorably with high dose melphalan alone.(2, 7, 20) In contrast, responders to cyclophosphamide, bortezomib and dexamethasone (CyBorD) eligible for SCT did not achieve deeper responses after high dose melphalan.(21) At present, the optimal combinations and sequencing of alkylators and proteasome inhibitors in AL require larger phase III studies.

Encouraging response rates have recently been reported in patient with AL treated with cyclophosphamide, bortezomib and dexamethasone (CyBorD) without high dose therapy.(21, 22) In one series hematologic responses were achieved in 16/17 (94%) patients who were either transplant ineligible (N=10) or relapsed (N=17).(21) In a second larger series, responses were achieved in 90% of treatment naïve (N=20) and 74% of relapsed (N=23) patients.(22) With short follow up in both studies (21 and 14 months) the durability of these responses cannot yet be determined.(21, 22) On our trial, 85% and 69% of patients had not progressed at 12 and 24 months following treatment initiation (**Figure 3**).

High- dose therapy and SCT has been challenged by phase III data showing inferior survival for patients who received high-dose melphalan compared to oral melphalan and dexamethasone (22.2 vs 56.9 months, $P = 0.04$).(20) However, 9/37 (24%) patients on their study died within 100 days of transplant

highlighting the importance of appropriate patient selection, risk-adapted melphalan dosing and supportive measures instituted at centers experienced at caring for AL patients.(7, 20) On the current study, the treatment related mortality was low (10%), similar to other large single institution studies.(2, 7, 23)

Patients with cardiac disease are frequently excluded from stem cell transplant studies. While early mortality remains a challenge especially for patients with stage III cardiac involvement, 55% of stage III patients in this phase II trial were alive at 12 months which compares favorably to the median that has been reported, 4-7 months.(15, 24) Recognizing the heterogeneity of this group, we excluded only patients with NYHA stage 3 or 4 heart failure, symptomatic arrhythmias or cardiac syncope. Among 11 patients with stage III cardiac disease there were 4 toxic deaths. Yet, 36% of patients remain alive at 2 years post SCT. Despite the definite value of cardiac biomarker staging,(15) a more discriminatory approach to risk stratification may increase access to clinical trials and help define populations who benefit from consolidation and/or maintenance therapies.(24, 25)

On this study BD consolidation was tolerated without unexpected toxicity (**Table 2**). Fifty-seven percent of patients experienced $\geq$ grade 2 neuropathy. The propensity of light chain amyloid to affect peripheral nerves may predispose patients to neuropathy especially because reliable methods to define peripheral nervous system involvement in AL are lacking.(10) In addition, twice weekly bortezomib likely contributed,(9, 26) and we were specifically focused on detecting this toxicity.(27) While subcutaneous bortezomib has been shown to reduce the incidence of peripheral neuropathy in patients with multiple myeloma,(28) the bioavailability and pharmacokinetics of subcutaneous administration in patients with AL has not been established. In patients with AL who may have heart failure and/or nephrosis, it is not our practice to administer bortezomib subcutaneously. Proteasome inhibitors with different toxicity profiles such as carfilzomib(29) and MLN-9708(30) may be important for patients with AL and studies using these drugs are ongoing. We currently employ weekly administration of bortezomib following alkylator therapy.

Durable hematologic responses are necessary for restoration of organ function over time and three quarters of patients treated on our study had organ improvement at 2 years following transplant. Interestingly, only half of patients met criteria for organ response in the first year following SCT. Thus resolution of amyloid deposition and/or compensation of involved organs occur very gradually when the free light chains are controlled.(4) Pre-clinical efforts to speed organ recovery have focused on immune-

based therapies.(31-33) Direct targeting of amyloid deposition in combination with cytotoxic therapy may ultimately lead to faster organ and functional improvement as well as better outcomes.

In summary, this phase II study demonstrates that bortezomib and dexamethasone administered as consolidation following SCT was an effective therapeutic strategy for patients with newly diagnosed AL amyloidosis. Careful patient selection, risk-adapted melphalan dosing and supportive measures rendered treatment safe and increased patient access to SCT and the novel agent bortezomib. With 45 months of follow up, responses are durable; however, several questions are raised. With routine FLC assessments we observed that hematologic relapse or progression occurs most often in the absence of organ progression. We know that patients who relapse and progress following high dose melphalan alone can be salvaged with bortezomib-based therapy.(9) Yet, we do not know the response rates to bortezomib or other proteasome inhibitors in AL patients who receive bortezomib post-SCT or as part of initial therapy. Therefore, research focusing on new drugs for this disease remains essential. Moreover, we do not know whether the PFS in patients who achieve a CR to BD consolidation following SCT is equivalent to CR achieved with alkylator therapy alone. It is possible that BD consolidation results in longer PFS and perhaps consolidation should be considered for all patients following SCT, regardless of response. On the other hand, if PFS is similar, reserving the proteasome inhibitor for the time of relapse for patients who achieve a CR to SCT makes sense. Furthermore, response duration after BD consolidation may be prolonged with further bortezomib treatment and evaluating maintenance therapy in this setting is warranted. Finally, it is also worth studying whether there is a benefit of high-dose therapy when patients respond to initial proteasome inhibitor therapy. We are currently conducting a phase II study using bortezomib in initial therapy, and in consolidation and maintenance in the context of risk-adapted melphalan and SCT in order to assess these issues.

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Authorship contributions: HL and RLC designed and performed research, collected data, analyzed and interpreted data and wrote the manuscript; HH performed research, analyzed and interpreted data; MR, MM, JL and CF performed research; CB and EH collected data; ER performed statistical analysis; SG edited the manuscript with critical review.

Conflict if interest disclosure: HL and RLC received research support and served on the advisory board for Millenium Pharmaceuticals. HH and SG served on the advisory board for Millenium Pharmaceuticals.

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TABLES:

Table 1. Patient characteristics

Table 2. Adverse events possibly related to BD consolidation

Table 3. Hematologic and organ responses

Table 4. Association of melphalan dose on response at 2-3 and at 12 months

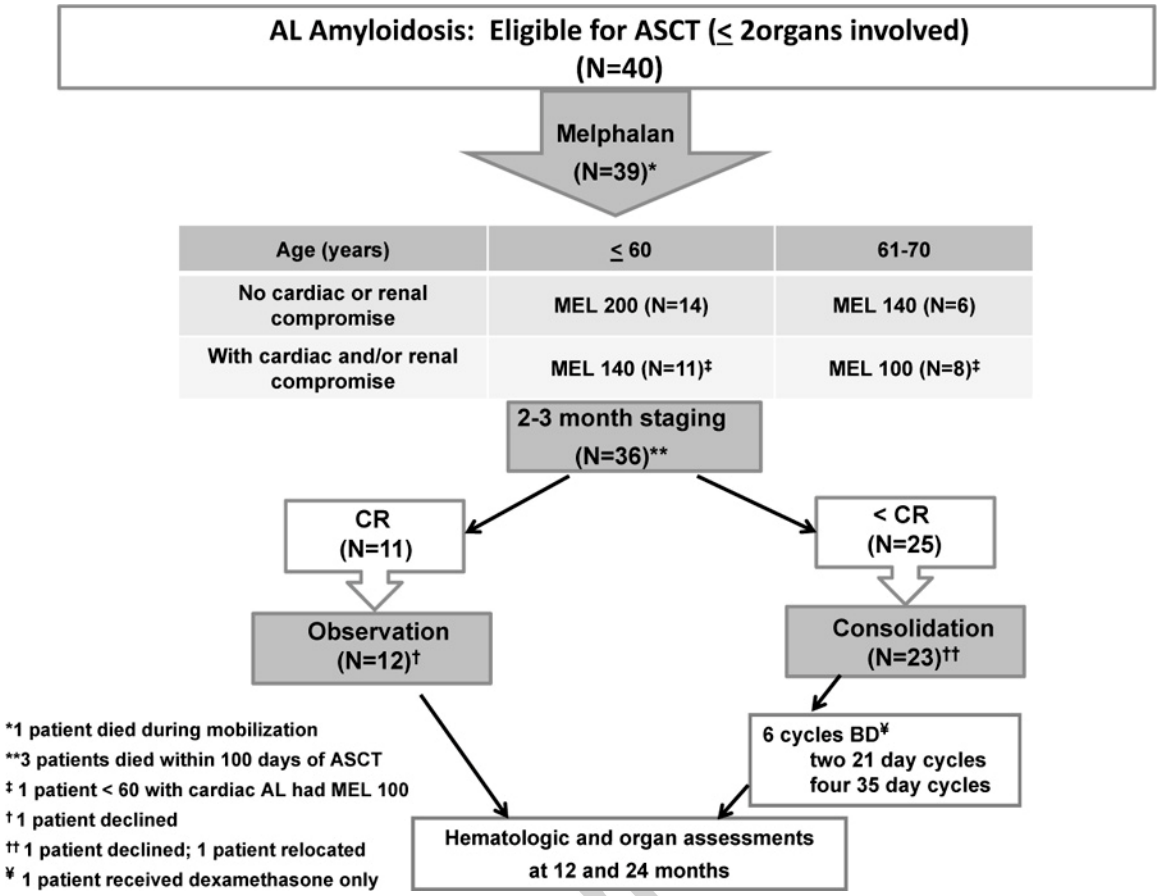
FIGURE LEGENDS:

Figure 1. Study schema. Patients with untreated AL amyloidosis and ≤ 2 major organs involved were treated with melphalan (MEL) based on age (≤ 60, 61-70), impaired renal function (creatinine clearance ≤ 50ml/min) and cardiac involvement. Disease was assessed at 2-3 months post-SCT. Patients with <CR were eligible for consolidation with up to 6 cycles of BD. *Patients with grade > 2 sensory neuropathy received dexamethasone only.

Figure 2. Responses to BD and cycles administered. In this plot, each horizontal bar represents a patient who received BD consolidation post-SCT. The responses post-SCT and pre-BD are shown along the Y axis and the number of cycles of BD each patient received is indicated by the length of each bar. Patients who achieved CR tended to receive fewer cycles. 3 patients who received consolidation have not been evaluated at 12 months (1 died, 2 ongoing).

Figure 3. Progression free survival (PFS) and overall survival (OS). Kaplan-Meier estimates are shown for PFS (**A**) and OS (**B**) for all patients (N=40), and for OS survival for patients with and without cardiac involvement (**C**) and by Mayo cardiac stage (**D**).

FIGURES:



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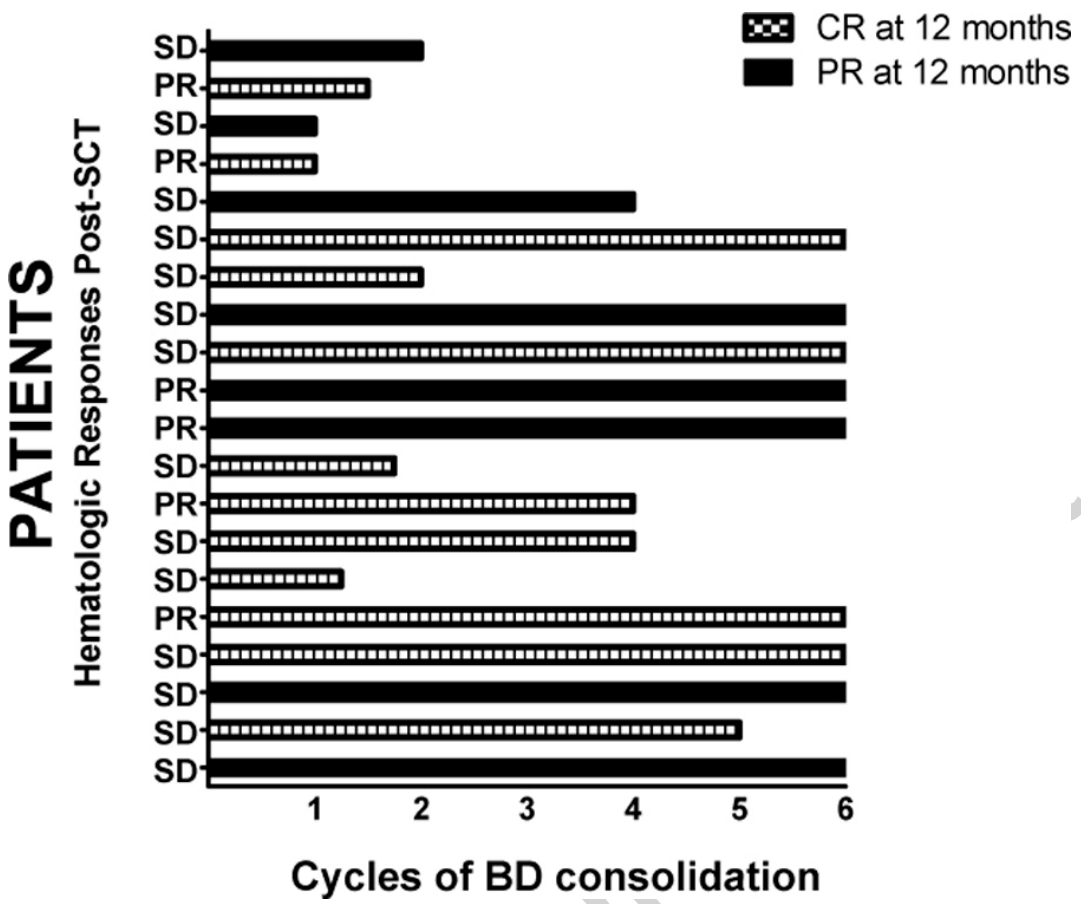

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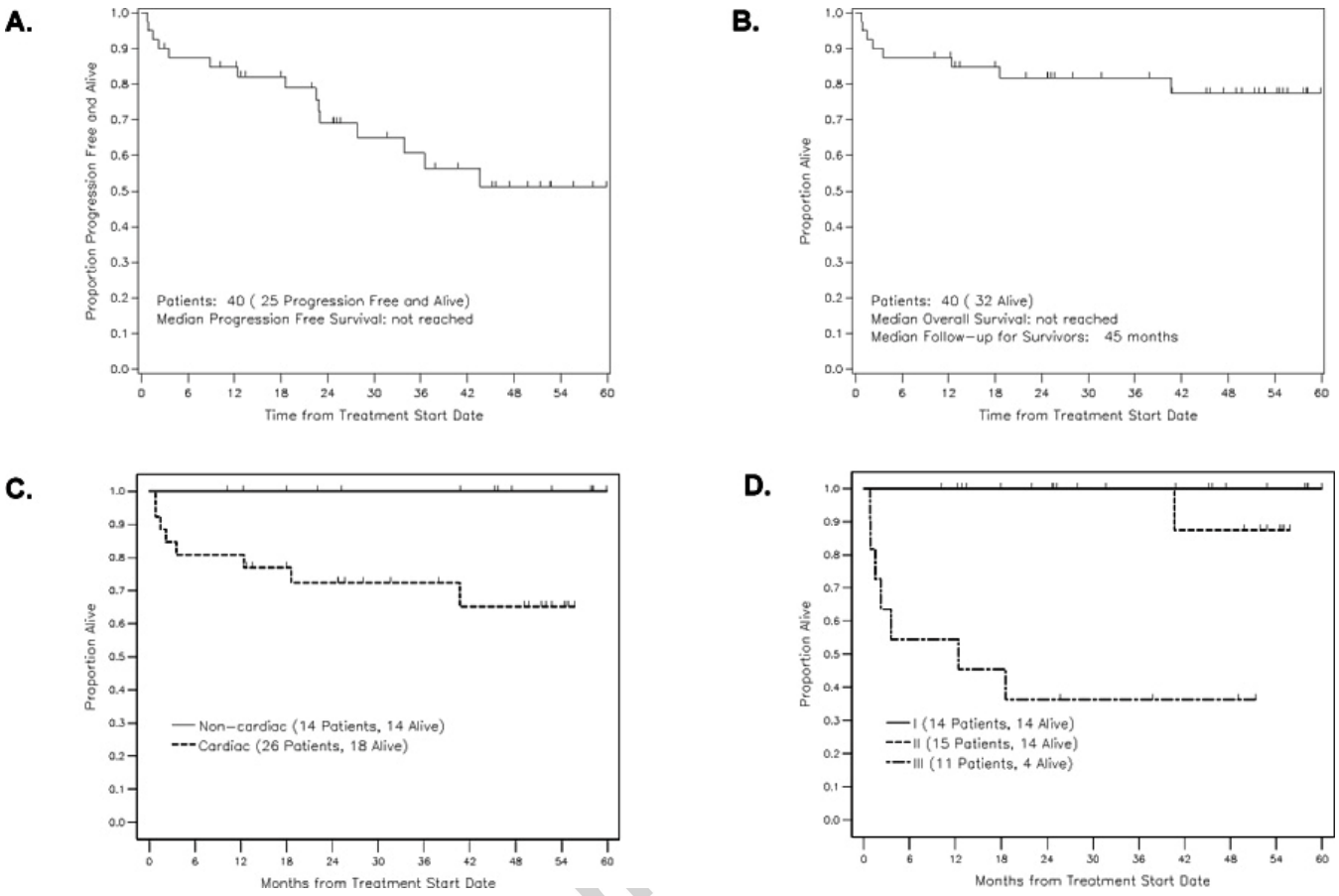

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Table 1. Patient characteristics

	N = 40
Median age, years, median (range)	57 (38–67)
No. male/female	17/23
ECOG PS (0/1/2), n (%)	8/21/11 (20/52/28)
Organ involvement, n (%)	
> 1 organ involved	22 (55)
Kidney	28 (70)
Heart	26 (65)
Liver/GI	6 (15)
Nervous system	5 (13)
*Cardiac stage, n (%)	
I	14 (35)
II	15 (37)
III	11 (28)
Brain natriuretic peptide (BNP) (pg/mL) (range)	128 (0–1720)
Troponin-I (ng/mL) (range)	0.0 (0–0.3)
Proteinuria (g/24hr) (range)	2.9 (0–34.9)
Involved free light chains (FLC), n (%)	
κ	4 (10)
λ	36 (90)
Abnormal FLC κ-to-λ ratio	37 (93)
M-spike on SPEP (>0.5g/dl)	9 (23)
M-spike on UPEP (>100mg/24hrs)	14 (35)

The institutional normal for BNP and Troponin I are 0-100pg/ml and 0-0.62ng/ml, respectively.
Conversion between BNP and NT-proBNP is: log BNP = 0.28 + 0.66 * log NT-ProBNP (Dispenzieri et al. BBMT 2008).(15)

Table 2. Adverse events possibly related to BD consolidation

	Grade 3	Grade 4	Grade 5
Any AE			1 (4%)
Thrombocytopenia	9 (39%)	1 (4%)	0
Neutropenia	1 (4%)	0	0
Anemia	3 (13%)	0	0
Bleeding	1 (4%)	0	0
Hyperglycemia	2 (9%)	0	0
Neuropathy	2 (9%)	0	0
Cardiac	4 (17%)	0	1 (4%)
Gastrointestinal	1 (4%)	0	0
Infection	2 (9%)	0	0

Table 3. Hematologic and organ responses

	Months post-SCT		
	2-3	**12**	**24**
ITT	N = 40	N = 38*	N = 30**
CR	11 (27%)	22 (58%)	12 (40%)
PR	7 (18%)	8 (21%)	6 (20%)
SD	18 (45%)	--	--
PD	--	1 (3%)	4 (13%)
% with ≥1 OR (ITT)		21 (55%)	21 (70%)
†Heart		53% (9/17)	56% (5/9)
†Kidney		52% (12/23)	87% (13/15)
†Liver/GI		60% (3/5)	60% (3/5)
†NS		100% (4/4)	100% (4/4)

OR = organ response; NS response was based on clinical parameters (14).

*** 2 ongoing**

**** 10 ongoing**

† Evaluable patients

Table 4. Association of melphalan dose on response at 2-3 and at 12 months

	Melphalan dose			
	100	**140**	**200**	*P* value
2-3 month response (N=39*)				
CR	1 (13%)	5 (29%)	5 (36%)	0.21
PR	4 (50%)	2 (12%)	1 (7%)	
Other	3 (37%)	10 (59%)	8 (57%)	
12 month response (N=37)**				
CR	6 (75%)	9 (53%)	7 (58%)	0.45
PR	0	4 (24%)	4 (33%)	
Other	2 (25%)	4 (24%)	1 (8%)	

Other includes SD, PD or death

* **1 died during mobilization**

** **1 died during mobilization, 2 ongoing**

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