Induction Therapy with Bortezomib and Dexamethasone and Conditioning with High-Dose Melphalan and Bortezomib Followed by Autologous Stem Cell Transplantation for AL Amyloidosis: Long Term Follow-Up Analysis

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Running title: Long term outcome of SCT in AL amyloidosis

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ABSTRACT:

In light-chain (AL) amyloidosis, the depth of hematologic response to treatment is associated with improved survival and organ responses. We conducted a clinical trial utilizing bortezomib in induction and in conditioning with melphalan prior to stem cell transplantation (SCT) for AL amyloidosis. The results of this clinical trial with a median follow-up of 36 months have been reported previously. Here we report the long-term results of this clinical trial with a median follow-up of 77 months. The objectives of this follow-up report are to describe survival, durability of hematologic and organ responses, and relapse rates. Thirty-five patients were enrolled from 2010 to 2013. Hematologic complete responses (CR) and very good partial responses (VGPR) were noted in 27/27 (100%) of assessable patients at 6 months following SCT. Four patients (15%) had hematologic relapse at a median of 42 months and 1 patient (3.7%) had organ progression despite maintaining a VGPR at 37 months. The median overall and progression-free survivals have not yet been reached. Renal and cardiac responses occurred in 65% and 88% at 5 years post SCT, respectively. Median time to renal and cardiac response was 12 months and 6 months, respectively. In conclusion, incorporating bortezomib into induction and conditioning yielded durable hematologic responses of AL amyloidosis with corresponding organ responses and prolonged survival.

Key words: bortezomib; autologous stem cell transplantation; AL amyloidosis; melphalan; relapse; long term outcome

INTRODUCTION

Immunoglobulin light chain (AL) amyloidosis is a rare disease caused by monoclonal light chains secreted by clonal plasma cells. These light chains misofold and form insoluble fibrils which can deposit into the extracellular space of tissues and organs, leading to progressive organ impairment and organ failure if left untreated.

Current treatments for AL amyloidosis target the underlying plasma cell dyscrasia in an effort to stop production and hence the deposition of amyloidogenic proteins, as this has been shown to improve survival.[1] Among a highly selected group of patients at a tertiary referral center, high dose intravenous melphalan followed by autologous stem cell transplantation (HDM/SCT) results in a hematologic complete response rate of 34% and median overall survival of up to 6.3 years.[2] For intermediate to high-risk patients who are not candidates for HDM/SCT, melphalan in conjunction with dexamethasone (MelDex) is considered standard therapy, and has been shown to result in a high rate of hematologic response (67%) and median overall survival of 5.1 years.[3] More recently, the proteasome inhibitor bortezomib, either as a single-agent[4] or as part of a multi-drug regimen (cyclophosphamide, bortezomib, dexamethasone (CyBorD)), has been shown to improve hematologic response rates and overall survival.[5]

In an earlier report in 2015, we published the results of a clinical trial (clinicaltrial.gov identifier NCT01083316) utilizing bortezomib and dexamethasone followed by high dose melphalan and stem cell transplantation for AL amyloidosis.[6] This prospective single-arm trial aimed to assess the efficacy of induction therapy with bortezomib and

dexamethasone for 2 cycles followed by conditioning with bortezomib in addition to high or modified doses of the melphalan and autologous stem cell transplantation for 35 patients with AL amyloidosis. We found hematologic responses to be unprecedentedly high, with hematologic very good partial response (VGPR) or complete response (CR) at 6 months after SCT to be 77% among all enrolled participants and 100% for the 27 evaluable patients. Additionally, organ-specific responses were reported, noting a renal response and cardiac response in 56% and 87% at 24 months respectively.

Now we report on the long-term outcomes of these patients treated on this clinical trial with a median follow-up of 77.3 months (range, 55.4-100.1). This longer follow-up allows us to address the issues of durability of hematologic and organ response and probabilities of relapse and survival.

METHODS

Patient Eligibility and Treatment Design

This clinical trial was approved by the Institutional Review Board at Boston Medical Center in accordance with federal regulations and the Declaration of Helsinki (ClinicalTrials.gov Identifier: NCT01083316). Criteria for undergoing HDM/SCT with bortezomib induction and patient eligibility were previously reported.[6] The treatment design consisted of 2 cycles of induction chemotherapy with bortezomib 1.3 mg/m^2 I.V. and dexamethasone 20 mg I.V. on D1, 4, 8, 11 in a 21-day cycle. This was followed by stem cell mobilization using granulocyte colony-stimulating factor at 16 µg/kg/day. Patients then underwent conditioning with bortezomib at 1.0 mg/m^2 I.V. on days -6, -3,

+1, and +4, and high-dose melphalan at 200mg/m$_2$ or at a modified dose of 140mg/m$_2$, administered in divided doses on days -2 and -1, followed by reinfusion of collected stem cells.

Hematologic and Organ Response Criteria

Hematologic responses were evaluated according to the consensus criteria of the International Society of Amyloidosis published in 2010.[7] Cardiac response was measured as a 30% reduction in BNP from baseline.[7] Renal response was measured as decrease in proteinuria by 30% (or below 0.5 g/24h) compared to baseline, in the absence of reduction in eGFR by >25%.[8]

Outcomes

The primary outcome was hematologic CR rate at 2, 3, 4, and 5 years following HDM/SCT. Secondary outcomes included overall survival (OS) as measured from the time of enrollment; progression-free survival (PFS) as measured from time of enrollment to initiation of second-line treatment; and organ response rate. All patients were censored at the time of last contact (May 8th, 2018).

Statistical Analysis

This follow-up analysis was conducted using MatLab. OS and PFS were performed using the Kaplan-Meier method, with a start date at time of enrollment and a data cutoff date of May 8th, 2018.

RESULTS

Patient Characteristics

A total of 35 patients with newly diagnosed AL amyloidosis were enrolled from January 2010 to August 2013. Demographics and baseline disease characteristics of the study cohort were previously reported.[6] In summary, the median age at time of enrollment was 56 years (range, 36-70) and 22 (63%) were women. As expected, 30 (86%) patients had a lambda light chain isotype, 20 (57%) had multi-organ involvement, 30 (86%) had renal involvement and 18 (51%) had cardiac involvement. Of the 35 enrolled patients, 32 proceeded to stem cell mobilization and collection, and 30 proceeded to SCT. Of 5 patients who did not undergo SCT, 3 (8.6%) did not proceed to stem cell mobilization due to worsening performance status and organ function during induction and 2 (5.7%) after stem cell collection due to the development of heparin-induced thrombocytopenia in one, and worsening of performance status due to lumbar radiculopathy in the second complications rendering them not eligible to receive SCT. Five patients (14%) required dose modifications and/or discontinuation of bortezomib during induction due to grade 3 or 4 adverse events: skin rash (n=1); syncope due to orthostatic hypotension (n=2); and worsening renal function progressing to ESRD (n=2). One patient (3%) required discontinuation of dexamethasone due to grade 3 peripheral edema.

Hematologic Responses

Hematologic CR and VGPR were achieved by 100% (n=27/27) of evaluable patients at 6 months and 12 months post SCT. Hematologic CR was achieved by 20 patients (77%) and hematologic VGPR by 6 patients (23%) at 12 months following SCT. By intention-to-treat analysis (ITT), hematologic CR and VGPR were achieved by 77% (n=27/35) and 74% (n=26/35) at 6 months and 12 months post SCT, respectively. Two patients

converted from VGPR at 1 year to CR at 2 years post SCT without receiving additional therapy.

Hematologic Relapse and Progression

Of the 27 patients who achieved a hematologic CR or VGPR, four patients (14.8%) had hematologic relapse at a median of 42.3 months (range, 34.5-63.0), and one patient (3.7%) required second-line treatment due to worsening proteinuria and renal function despite maintaining a hematologic VGPR. Of the 4 patients who experienced hematologic relapse, two had achieved a VGPR and 2 had achieved a CR at 6 months. Immunomodulatory agents were used for treatment in 2 patients (1 treated with lenalidomide and 1 with pomalidomide) and ixazomib in other 2 patients. The patient with organ progression and maintenance of VGPR received treatment with bortezomib followed by daratumumab.

In addition to the 5 patients requiring 2nd line treatment, four patients (14.8%) also had biochemical relapse, defined as reappearance of monoclonal light chain in serum or urine immunofixation electrophoresis without associated dFLC progression or organ progression, and did not need second-line treatment. Median time to biochemical relapse was 52.7 months (range, 49-78.6). Median time since biochemical relapse without need for additional treatment was 12.5 months (range, 7-44). All four had initially achieved a hematologic CR at 1 year following SCT.

OS and PFS

The median follow-up for surviving patients was 77.3 months from enrollment (range, 55.4-100.1). The Kaplan-Meier survival curves for OS and PFS are shown in Figure 1. The median OS and PFS have not yet been reached.

Eight deaths have occurred in the follow up period. Three deaths occurred within 100 days of SCT, an overall early mortality (EM) of 8.5%. The causes of death were multiorgan failure due to sepsis, invasive aspergillosis, and influenza A infection complicated by multilobar bronchopneumonia and respiratory failure. Other five deaths occurred due to fluid overload due to end-stage renal disease, two from congestive heart failure, bacterial pneumonia, and *Klebsiella pneumoniae* bacteremia with multi-organ failure.

Organ Response

Renal response was achieved by 65% of patients at 5 years and cardiac response by 88% at 5 years. The median time for renal response was 12 months (range, 6-24) after SCT. The median time for cardiac responses was 6 months after SCT (range, 6-24). Renal responses deepened from 40% at 1 year to 50% at 2, 3 and 4 years (Figure 2) and similarly cardiac responses deepened from 75% at 1 year to 92% in the subsequent years following SCT (Table 1). Of the 6 patients who did not achieve a renal response at 5 years, 3 have needed renal replacement therapy for end stage renal disease (ESRD) at a median of 26.3 months (range, 2.5-44.8). One patient who did not achieve a cardiac response at 5 years is doing well, although cardiac response assessment by biomarkers was difficult to access due to development of ESRD.

Toxicity

Treatment-related morbidity was described in the prior report and was notable for two cases of autologous graft-versus-host disease (GVHD). Both of these patients with possible autologous GVHD are alive at 6.1 and 8 years after SCT, and remain in hematologic CR without signs of GVHD at this time. One patient developed spontaneous splenic rupture on Day + 16 that was successfully treated with splenic artery embolization.[9] She is alive without any associated complications or recurrence at 58 months from enrollment.

DISCUSSION

The results of long term follow up of this clinical trial provide support for the use of novel agents for induction and conditioning prior to SCT in AL amyloidosis to achieve deep and durable hematologic and organ responses, low hematologic relapse and prolonged overall survival and progression-free survival.

This is among the first studies to show benefit of bortezomib in the induction and conditioning regimen in AL amyloidosis. AL amyloidosis is characterized by a small tumor mass and therefore induction chemotherapy is often not necessary prior to HDM/SCT. Increasingly however, induction therapy with novel agents (proteasome inhibitors, immunomodulatory "IMiD" drugs) has been more frequently studied and has shown higher hematologic response rates (87% partial response or better) and overall survival (87% 2-year overall survival) compared to induction with conventional chemotherapy or no induction.[10] Induction therapy can produce rapid hematologic

response and prevent further decline in organ damage while stem cell transplantation is being arranged.

In addition to achieving unprecedentedly high hematologic response rates at 1 year (63% hematologic CR with 74% overall hematologic response rate on ITT analysis), bortezomib induction and bortezomib-melphalan conditioning SCT resulted in a durable hematologic response, with 57% of patients with continued hematologic CR and 64% overall hematologic response at 5 years post SCT respectively. These hematologic response rates compare favorably to response rates with HDM/SCT without incorporation of novel agents into induction and conditioning. Our previous experience of 647 patients with AL amyloidosis treated with HDM/SCT reported a hematologic CR rate of 32.9% by intention-to–treat analysis.[11] The results of the current clinical trial are favorable with hematologic CR rate of 63% by intention-to-treat analysis, and this is partly attributed to incorporation of bortezomib into induction and conditioning regimens. Hematologic relapse rate after initial achievement of CR on this trial was quite low at 15% at a median of 3.8 years. This again compares favorably to our prior report of hematologic relapse of 32.3% in a cohort of 647 patients.[11] Biochemical relapse, defined as reappearance of monoclonal light chain in serum or urine immunofixation electrophoresis without associated dFLC progression or organ progression, and no need for second-line treatment, was noted in 15% of patients on this trial. This group warrants careful and comprehensive evaluation to appropriately determine the need for further anti–plasma cell therapy vs ongoing active surveillance. It is important to note that early treatment for this group of patients may not be warranted.

Organ responses were also evident in a high proportion of patients with renal response in 65% and cardiac response in 88% of patients at 5 years on this trial. Median time to organ response was 12 months for renal response and 6 months for cardiac response. Organ responses were higher than reported in prior series from our center[12] (cardiac response 21% and renal response 32%) as well as from the Mayo clinic (cardiac response 5% and renal response 23%).[13] We attribute this to higher hematologic response rates in the current clinical trial leading to higher organ responses.

The organ responses notably occurred within 1 year after SCT. Early organ response, defined as any organ response within 1 year following normalization of FLC ratio, has been associated with improved overall survival among those who achieve a hematologic response from treatment.[14] Even though some did not show organ response until 2 years following HDM/SCT in our trial, 86% (18/21) of patients who ultimately achieved an organ response met criteria for early organ response. Organ responses were similar to those reported by Huang *et al* from a randomized controlled trial of 56 patients with AL amyloidosis comparing bortezomib induction with HDM/SCT to HDM/SCT alone.[15] Organ responses deepened from 12 months to 24 months.

Overall and progression free survivals are significantly better on this trial even with a longer median follow-up of 77 months than prior reports from our center and others. In studies utilizing HDM/SCT, Landau *et al* described a cohort of 143 patients with a median OS of 10.4 years[16], Cibeira *et al* reported a median OS 6.3 years in 421 patients[2]

and Warsame *et al* a median OS of 7.4 years[14]. Improved OS could be attributable to better patient selection as well as incorporation of novel agents in the current trial.

There are a few limitations to the study. First, this was a highly selected group of patients with AL amyloidosis, as only 25% of presenting patients are eligible for SCT due to age, comorbidities, or extent of cardiac damage.[17] Second, the small sample size precludes accurate and direct comparison from many larger studies that employed HDM/SCT alone, however we believe our report may function as a proof-of-concept to be replicated on a larger scale given the number and durability of responses. Third, cardiac response was evaluated based on modified biomarker response criteria with the use of BNP rather than NT-proBNP, which is the gold standard and validated biomarker.[18] Fourth, the small sample size in our study did not allow for analysis of subgroups or predictive value of risk factors. Further research is needed to better evaluate these conditions.

In conclusion, the addition of bortezomib to induction and conditioning with HDM prior to SCT results in deep and durable hematologic and organ responses, with a median OS and PFS greater than 6 years. More research is needed to evaluate the role of incorporating other novel therapies into induction or conditioning prior to SCT, and to assess if there is a role for novel agents in reducing amyloidogenic light chains such that patients who were previously ineligible for SCT may become candidates. Of note, five patients (14%), who were eligible for SCT at enrollment, did not proceed to SCT due to clinical deterioration during induction treatment, suggesting careful selection of patients and type of induction therapy are crucial prior to SCT.

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Figure legends:

Figure 1

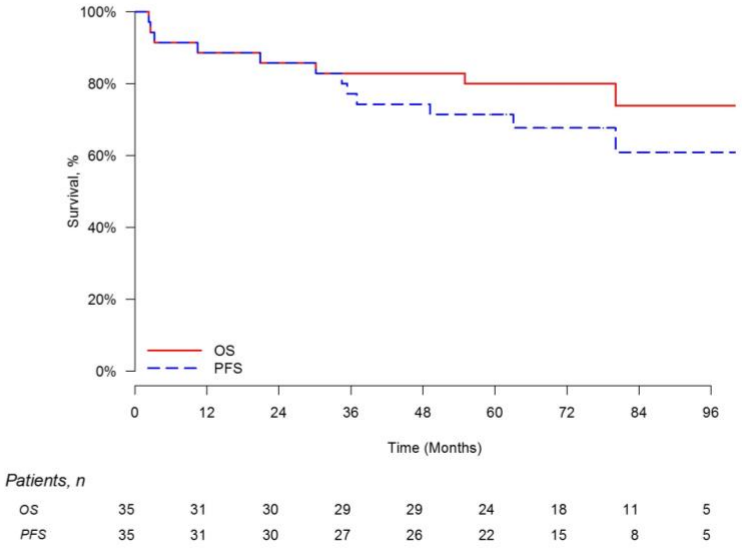

Figure 1: Overall Survival and Progression-Free Survival

Figure 2

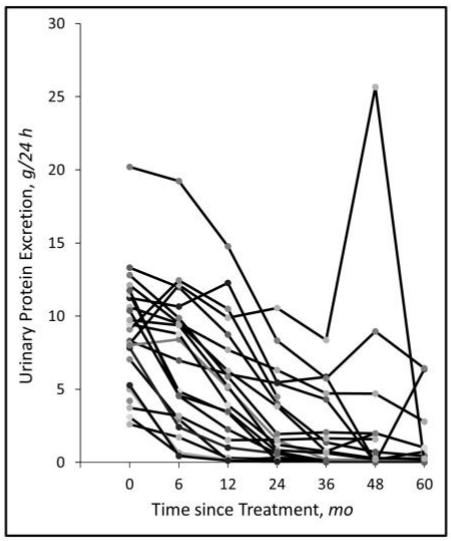

Figure 2: Change in 24-hour urinary protein excretion in 23 patients with renal involvement after enrollment