ORIGINAL ARTICLE

A phase II, single-arm, prospective study of bendamustine plus melphalan conditioning for second autologous stem cell transplantation in *de novo* multiple myeloma patients through a tandem transplant strategy

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This phase II trial evaluates, for the first time, the safety and efficacy of bendamustine plus high-dose melphalan (HDM) as a conditioning regimen before the second autologous stem cell transplantation (ASCT) in previously untreated multiple myeloma (MM) patients. In total, 32 ASCT patients received HDM (200 mg/m^2) as conditioning for the first ASCT. After 3–6 months from the first ASCT, responding patients underwent a second ASCT following bendamustine (200 mg/m^2) and HDM (140 mg/m^2). High-dose chemotherapy and ASCT were performed with complete neutrophil and platelet recovery in all patients. The median number of days to neutrophil and platelet engraftment was 11 (range 9–15) and 12 (range 10–19), respectively. Only one subject experienced grade 3 diarrhea; the rate of mucositis and vomiting was significantly lower with the bendamustine plus HDM regimen compared with the HDM-only regimen (81.2 vs 96.9%, $P = 0.025$ and 78.1 vs 100%, $P = 0.008$). Overall response rate (ORR) was 81.2% after the first transplant, and 90.6% after the second, while complete response rates were 46.8 and 62.5%, respectively ($P = 0.016$). Actuarial 2-year PFS and OS were 79% (95% confidence interval (CI), 60–98) and 97% (95% CI, 91–100), respectively. Bendamustine+HDM is feasible as the conditioning regimen for second ASCT in MM patients. The present study may pave the way for phase III studies specifically aimed at further investigating this combination strategy. The role of this combination in MM for conditioning regimen in a first or single ASCT setting should be also investigated.

INTRODUCTION

With the advent of modern therapies, as well as their integration into existing treatment approaches and the improved management of complications, the outcome of patients with multiple myeloma (MM) has dramatically improved in the past decade.[1] Thanks to the unprecedented higher and deeper responses and the increasing number of treatment options, questions regarding existing treatment paradigms and optimal treatment have been raised.[2] For almost 25 years, therapeutic intensification with high-dose chemotherapy (HDC) and autologous stem cell transplantation (ASCT) has been considered as the standard of care in MM patients without significant comorbidities.[2,3] Although this approach is currently being debated by some investigators in the modern therapy era,[4] it still represents the standard treatment strategy;[5] and the incorporation of novel agents into different phases of the transplant procedure, including induction,[6–9] consolidation,[10,11] and maintenance[12,13] has substantially improved outcomes.

Although many studies have been performed in the induction, consolidation and maintenance settings, few trials have been dedicated to conditioning regimens before ASCT;[14–22] and the current standard conditioning regimen is considered high-dose melphalan (HDM).[14]

Bendamustine (BENDA) (Ribomustin/Levact; Mundipharma International Ltd, and Treanda; Cephalon) is a synthetic chemotherapeutic agent that combines the alkylating properties of a mustard group with the antimetabolic activity of a purine analog. It can induce responses in disease resistant to other alkylating agents via direct induction of apoptosis, inhibition of mitosis, and activation of an alternative DNA repair pathway distinct from standard alkylator mechanisms of action.[23] BENDA has proven activity in both newly diagnosed and relapsed/refractory MM;[24] and it has already been shown to be a feasible part of a modified BeEAM (bendamustine, etoposide, cytarabine, melphalan) ASCT conditioning regimen for non-Hodgkin lymphoma with acceptable safety and promising efficacy.[25] Recently, Mark *et al.*[19] published a phase I trial on escalating doses of BENDA added to the current standard conditioning of HDM at 200 mg/m^2 in patients with MM at their first transplant. We report herein the results of a phase II trial evaluating the safety and efficacy of BENDA plus HDM as the conditioning regimen for ASCT after induction therapy in MM patients. This is the first study in transplant-eligible patients with newly diagnosed MM to use a

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BENDA-based conditioning regimen before the second ASCT in a tandem treatment strategy.

PATIENTS AND METHODS

Setting and design

This was a single-arm, prospective phase II study. Patients were recruited from three hematological centers in the South of Italy (Calabria Region); all patients were referred to the Stem Cell Transplantation Unit of the Azienda Ospedaliera BMM, Reggio Calabria (Italy) after response to induction therapy for PBSC collection and transplantation. The study was approved by the institutional review board of Azienda Ospedaliera BMM of Reggio Calabria (Italy) and was conducted according to the Declaration of Helsinki and the International Conference on Harmonisation Guidelines for Good Clinical Practice. All patients provided written informed consent before inclusion.

Patients

Inclusion criteria were as follows: transplant-eligible patients, aged 18–70 years, with *de novo* MM who after induction achieved a favorable response (International Myeloma Working Group (IMWG) criteria) and who presented International Staging System (ISS) stage 1–3 and World Health Organization (WHO) performance status 0–3 could be enrolled. A WHO performance of 3 was allowed only if it was caused by MM rather than a comorbid condition. Exclusion criteria were as follows: New York Heart Association class II to IV heart failure; abnormal pulmonary-function findings; systematic amyloid light-chain amyloidosis; non-secretory MM; Waldenstrom macroglobulinemia or IgM MM; history of active malignancy during the past 5 years with the exception of basal cell carcinoma or stage 0 cervical cancer. Laboratory exclusion criteria were creatinine clearance < 15 mL/min; absolute neutrophil count $< 1.0 \times 10^9$/L and platelet count $< 75 \times 10^9$/L. Patients who had a refractory disease (progression or no response) to induction chemotherapy were also excluded.

Treatment

All patients received a bortezomib-based induction therapy (in association with corticosteroids with or without thalidomide). High-dose CY (3–4 g/m^2) plus granulocyte CSF (G-CSF) were used to mobilize PBSCs. The minimum target dose of CD34+ cells to safely support two sequential courses of high-dose conditioning regimen was 5×10^6/kg (2.5×10^6/kg per transplant).

All patients received HDM (200 mg/m^2) as conditioning regimen before the first ASCT. After 3–6 months from the first ASCT, patients who achieved a stable disease or a more favorable response (as defined below) underwent a second ASCT following BENDA (100 mg/m^2 days -3 and -2) and HDM (140 mg/m^2 day -1) as a conditioning regimen. No consolidation or maintenance therapies were permitted.

Supportive care

During the aplastic phase of both ASCT procedures, all patients received oral prophylaxis with ciprofloxacin at 500 mg twice-daily or levofloxacin at 500 mg/day from day 0 until neutrophil recovery, and with acyclovir at 800 mg twice-daily from day +3 post transplantation until approximately day +90. *Pneumocystis jiroveci* prophylaxis with trimethoprim/sulfamethoxazole (1 double-strength tablet 2 or 3 times weekly) was started after hematological recovery and was continued for 3 months. G-CSF (filgrastim or lenograstim) at 5 mcg/kg/day was started at day +5 and continued until neutrophil recovery. Red blood cell and platelet transfusions were administered in order to maintain hemoglobin levels $\geqslant 8$ g/dL and platelet counts $\geqslant 10 \times 10^9$/L, or in case of symptomatic anemia and/or minimal mucocutaneous hemorrhagic syndrome. Patients also received i.v. hydration and electrolyte support.

Evaluations

The primary objective of this study was to evaluate: (i) treatment-related toxicity and TRM; (ii) time to neutrophil and platelet engraftment after BENDA plus HDM; and (iii) cumulative incidence of neutrophil and platelet recovery. Secondary objectives were to evaluate the complete response (CR) rate, the overall response rate (ORR; defined as the CR+very good partial response (VGPR)), PFS and OS. The evaluation of response was performed at enrollment and at day +30 and day +100 after both ASCTs.

Moreover, toxicities and engraftment rates between the first and second transplants were compared.

Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 4.0). TRM was defined as death from any cause other than disease progression within 100 days from transplantation. Time to neutrophil engraftment was defined as the duration between day 0 and the first of 3 consecutive days of ANC $> 0.5 \times 10^9$/L after transplant. Time to platelet engraftment was defined as the duration between day 0 and the first day of platelet count sustained at $> 20 \times 10^9$/L without any transfusion in the previous 7 days. Response and progression were reported by investigators according to revised uniform response criteria by the International Myeloma Working Group.[26,27] In particular, we considered as a CR a negative immunofixation of serum and urine, disappearance of any soft tissue plasmacytomas and $< 5\%$ plasma cells in bone marrow. In patients for whom only measurable disease was by serum free light chains level, the normal free light chains ratio of 0.26–1.65 in addition to CR criteria was required. Two consecutive assessments were needed. Bone marrow biopsy and aspirate samples were collected at baseline and as needed to confirm CR. Patients with CR who lacked confirmation from bone marrow biopsy samples were downgraded to VGPR.

Statistical analysis

Data were summarized as median and range (continuous variables) or as absolute frequencies and percentages (binary variables), as appropriate. Within-patient comparisons were made by the Wilcoxon rank test (for continuous variables) and the McNemar test (binary data), as appropriate. Response rates, survival and toxicity were summarized by descriptive statistics. TRM was evaluated as cumulative incidence. PFS and OS were investigated by the Kaplan–Meier method. The response rate after BENDA plus HDM was calculated and compared with HDM (200 mg/m^2) response rates. The degree of uncertainty (precision) around PFS and OS was expressed as point estimate and the corresponding 95% confidence interval (CI). Data analysis was performed using SPSS for Windows (version 20.0.0; IBM, Armonk, NY, USA).

RESULTS

Study population

A total of 32 patients (median age 56 years; 56% males) were consecutively enrolled in the study between January 2013 and December 2014. Baseline demographic and disease characteristics are summarized in Table 1. Patients ranged in age from 40 to 66 years. Data on cytogenetic abnormalities, del(13q), t(4;14) and del (17p), detected by FISH on highly purified bone marrow plasma cells, were available for 25 patients. The isotype distribution of M-proteins reflected the typical MM population (IgG, 75.0%; IgA, 21.9%; free light chain only, 3.1%). All 32 registered patients started induction therapy and 16 (50%) received VTD (velcade, thalidomide and dexamethasone) (Table 1). All patients continued to receive CY priming and the tandem HDC because of responsive disease.

Stem cell engraftment

After the BENDA plus HDM conditioning regimen, a median of 5.0 (range 3.2–7.0) CD34+ cells $\times 10^6$/kg was infused. HDC and ASCT were performed with complete neutrophil and platelet recovery in all patients. The median number of days to neutrophil and platelet engraftment was 11 (range 9–15) and 12 (range 10–19), respectively. The median number of days with ANC $\leqslant 0.1$, $\leqslant 0.5$ and $\leqslant 1 \times 10^9$/L was 3 (range 2–8), 5 (range 3–10) and 6 (range 3–13), respectively. The median duration of hospitalization after stem cell infusion was 16 days (range 13–27). The engraftment kinetics observed for BENDA plus HDM conditioning at second transplant were similar to those reported previously with the first HDM (200 mg/m^2) conditioning regimen (Table 2).

Treatment-related toxicity and TRM

Overall, the BENDA plus HDM regimen was well tolerated (Table 3). The majority of patients ($n = 26$; 81.2%) experienced mucositis, but it was of grade 1–2 in all cases. Similarly, most patients reported nausea ($n = 25$; 78.1%) and diarrhea ($n = 24$; 75.0%), but only one subject experienced grade 3 diarrhea. The rate of mucositis and vomiting was significantly lower with the BENDA plus HDM regimen compared with the HDM-only regimen administered for the first ASCT (81.2 vs 96.9%, $P = 0.025$ and 78.1 vs 100%, $P = 0.008$). After complete hematologic recovery after the second transplant, one patient showed a skin rash after 40 days and two subjects experienced CMV infections, documented 30 days after the first transplant and 40 days after the second transplant, respectively. Both CMV infections were successfully eradicated with valacyclovir treatment (900 mg/day for 15 days).

Fever $\geqslant 38.2\,°C$ was reported in 15 patients (46.9%) during neutropenia. It was clinically documented in two patients (6.2%). In one case of E. coli infection was microbiologically documented. The median duration of fever was 3 days (range: 2–7).

No cases of TRM were reported. One patient died of brain hemorrhage 150 days after the second ASCT.

Response assessment

Clinical responses to treatment are shown in Figure 1. At the end of induction therapy, 25 and 37.5% of the patients had achieved a CR and a VGPR, respectively, with an ORR of 62.5%. After the first and second transplantation, the CR improved to 46.8 and 62.5%, respectively ($P = 0.016$ and $P < 0.0001$). ORR was 81.2% after the first transplant ($P = 0.031$), and 90.6% after the second transplant ($P = 0.004$). At a median follow-up of 16 months (range, 9–32) after the second ASCT, four patients (12.5%) had progressed and one (3.1%) died. The median PFS and OS were not reached. Actuarial 2-year PFS and OS were 79% (95% CI, 60–98) and 97% (95% CI, 91–100), respectively (Figures 2 and 3).

DISCUSSION

In 1992, the IFM group published the first study where HDC combined with ASCT improved the response rate, PFS and OS of patients with MM.[28] Several prospective randomized trials confirmed a benefit with HDC when compared with conventional chemotherapy,[29–32] and this procedure has been part of the standard of care for over two decades.[33] In the 2000s, an improvement of clinical outcomes was observed with the advent of immunomodulatory drugs and proteasome inhibitors.[34] The subsequent years have been characterized by studying the optimal combination of new agents, and questions regarding existing treatment paradigm and optimal treatment have been raised.

In the new era for MM treatment, transplant trials support the use of upfront ASCT in the context of novel agents.[4,6–9,29] The transplant may be single or tandem (that is, second course of HDC within 6 months of the first) and the optimal strategy that routinely incorporates a tandem transplant remains an open question.[4,35,36] The IMWG suggests considering timely second ASCT in those patients who fail to achieve a VGPR or better after

Table 1. Summary of patients' characteristics

Characteristics	No. 32
Age, years[a]	56 (40–66)
Male gender	18 (56.2%)
Durie-Salmon stage of myeloma on diagnosis	
II	3 (9.4%)
III	29 (90.6%)
ISS stage of myeloma on diagnosis	
I	13 (40.6%)
II	11 (34.4%)
III	8 (25%)
FISH analysis for cytogenetic abnormalities[b]	
Negative	33 (66%)
del(13q)	12 (24%)
t(4;14) and del(17p)	5 (10%)
Monoclonal protein type	
IgG	24 (75%)
IgA	7 (21.9%)
Light chain only	1 (3.1%)
Induction treatment	
VTD	16 (50%)
VCD	12 (37.5%)
PAD	3 (9.4%)
VD	1 (3.1%)
Response to induction treatment (%)	
CR	25.0
VGPR	37.5
PR	37.5
Interval between first and second transplants, months[a]	5 (3–6)

Abbreviations: CR = complete response; ISS = International Staging System; PAD = velcade, adriamycin, dexamethasone; PR = partial response; VCD = velcade, cyclophosphamide, dexamethasone; VD = velcade, dexamethasone; VGPR = very good partial response; VTD = velcade, thalidomide, dexamethasone. [a]Value expressed as median and range. [b]25 patients were available for assessment.

Table 2. Engraftment and transplant data

	Second transplant (MEL+BENDA conditioning regimen)	First transplant (MEL conditioning regimen)	P-value
Number of transplants	32	32	
CD34+ cells infused, $\times10^6$/kg (range)	5.0 (3.2–7)	5.0 (3.6–7.2)	NS
Time to neutrophil engraftment[a]	11 (9–15)	11 (10–13)	NS
Time to platelet engraftment[b]	12 (10–19)	13 (11–20)	0.005
Days with ANC $\leqslant 0.1\times10^9$/L	3 (2–8)	3 (2–6)	NS
Days with ANC $\leqslant 0.5\times10^9$/L	5 (3–10)	5 (3–10)	NS
Days with ANC $\leqslant 1.0\times10^9$/L	6 (3–13)	6 (4–11)	NS
Duration of hospitalization[c]	16 (13–27)	16 (13–28)	NS

Abbreviations: Benda = bendamustine; MEL = melphalan. P-value: Wilcoxon Ranks test. [a]Time to neutrophil engraftment was defined as the duration between day 0 and the first of 3 consecutive days of ANC $> 0.5\times10^9$/L. [b]Time to platelet engraftment was defined as the duration between day 0 and the first day of platelet count sustained at $> 20\times10^9$/L. [c]Days after stem cell infusion. Value are expressed as median (and range).

Table 3. Treatment-related toxicity

	Second transplant (MEL+BENDA Conditioning regimen)	First transplant (MEL conditioning regimen)	P-value
Number of transplants	32	32	
No. (%) of patients with fever ⩾ 38.2 °C	15 (46.9%)	10 (31.2%)	NS
Fever origin, n (%)			NS
FUO	13 (40.6%)	8 (25%)	
CVC related	0	0	
Clinically documented[a]	2 (6.2%)	1 (3.1%)	
Only microbiologically documented infection	0	1 (3.1%)	
No. of days of fever ⩾ 38.2 °C[a]	3 (2–7)	3 (2–9)	NS
No. of days antibiotic therapy	6 (5–10)	6 (5–12)	NS
No. (%) of patients with mucositis	26 (81.2%)	31 (96.9%)	0.025
WHO 1–2 vs WHO 3–4	23 (71.9%) vs 3 (9.4%)	28 (87.5%) vs 3 (9.4%)	NS
No. (%) of patients with Nausea/vomit	25 (78.1%)	32 (100%)	0.008
Grade 1 vs grade 2 vs grade 3	13 (40.6%) vs 12 (37.5%) vs 0	21 (65.6%) vs 10 (31.2%) vs 1 (3.2%)	NS
No. (%) of patients with diarrhea	24 (75%)	28 (87.5%)	NS
Grade 1 vs grade 2 vs grade 3	15 (46.9% vs 8 (25%) vs 1 (3.1%)	20 (62.5%) vs 7 (21.9%) vs 1 (3.1%)	NS
Number of RBC transfusions	0 (0–3)	0 (0–2)	NS
Number of PLT transfusions	1 (0–5)	1 (0–5)	NS

Abbreviations: BENDA = bendamustine; MEL = melphalan; RBC = red blood cell; PLT = platelet. P-value: Wilcoxon ranks test. [a]Clinically and microbiologically documented infection in one case. Values are expressed as median (range).
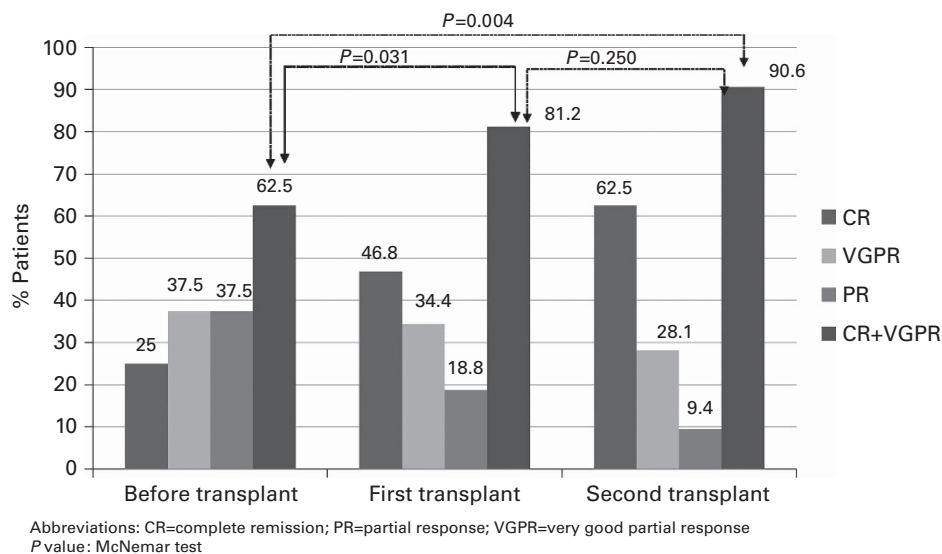

Abbreviations: CR=complete remission; PR=partial response; VGPR=very good partial response
P value: McNemar test

Figure 1. Response status pre-transplant and on day +100 after first and second transplants.

the first ASCT;[37] this clinical outcome is often used in some centers as the criterion for planning the tandem procedure. Recent studies with the new induction regimens indicate that there is a role for tandem ASCT in MM in the presence of adverse cytogenetic abnormalities.[38,39] Results from an upcoming integrated analysis of four phase III European trials,[40] in which one-third of patients have been randomized to a tandem ASCT procedure, could alter future transplantation algorithms.

Nowadays, there is no consensus regarding the best conditioning regimen.[3,14] HDM at a dose of 200 mg/m² is considered to be the standard conditioning regimen.[14,17,18,30] However, a variety of strategies has been explored with the aim of improving the results of HDM 200 mg/m², including dose escalation,[22] addition of other agents such as busulfan, thiotepa or TBI[14,15,17–22] or, conversely, dose reduction as part of a tandem auto-SCT procedure,[20] with overall mixed results. Further evidence is however necessary on the best conditioning regimen.

BENDA combines the alkylating activity of the mustard group with the antimetabolite activity of the purine analog structure and has activity in multiresistant cell lines that do not respond to treatment with other alkylating agents.[23] Recently, Mark et al.[19] conducted a phase I trial adding escalating doses of BENDA to HDM (200 mg/m²) as conditioning regimen in MM patients. In this study, a maximum tolerated dose was not achieved and the highest dose cohort of BENDA 225 mg/m² plus HDM was expanded in order to further evaluate the safety. Overall, this combination regimen did not increase transplantation risk or toxicity, and was not associated with any TRM. Although preliminary, the efficacy results show an ORR of 80% at day +100 and a CR or improved rate of approximately 45% at 1 year after ASCT.

For the first time, in the present phase II study we evaluated the safety and feasibility of adding BENDA (200 mg/m²) to HDM (140 mg/m²) as a conditioning regimen before the second ASCT in
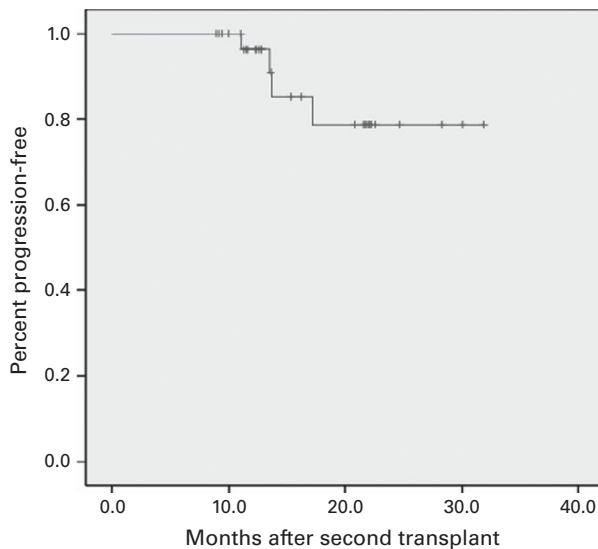
N =32 patients, 4 with progression of disease. Median progression-free survival not reached.
Actuarial 2-year PFS=79% (95% CI, 60 to 98)

Figure 2. Progression-free survival.
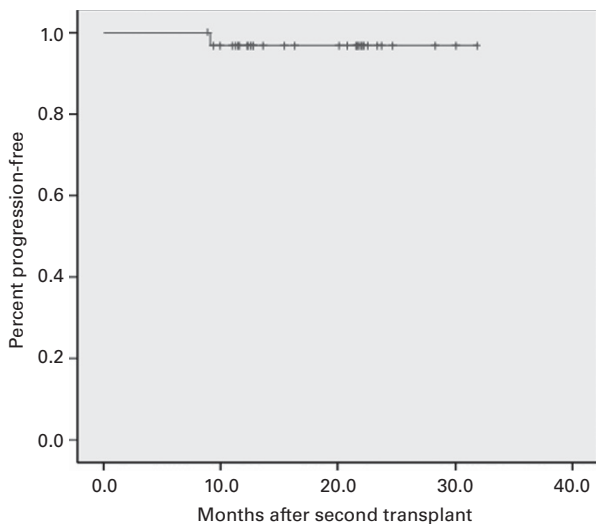
N =32 patients, 1 death. Median overall not reached.
Actuarial 2-year OS=97% (95% CI, 91 to 100)

Figure 3. Overall survival.

de novo MM patients in a tandem transplant strategy. In our study, the choice of BENDA was based on the documented sensitivity of myeloma cells to the drug in the context of resistance to previous treatments;[24,41,42] and the decision was made to explore the association between a pure alkylating agent (melphalan) with an agent (BENDA) that combines the alkylating properties of a mustard group with the antimetabolic activity of a purine analog and can induce responses in disease resistant in a distinct way from standard alkylator mechanisms of action.[43]

We believe that the use of a preparative regimen that combines two agents in the second ASCT could have a key role in long-term results. In this regard, when two conditioning regimens with alkylating agents were prospectively compared by a Spanish Group a higher response rate was found with busulfan/melphalan than with melphalan alone.[17,18,44] We decided to use a fixed dose of 200 mg/m^2 because of the reported cardiotoxicity in previous series at higher doses of BENDA[42,43] and to reduce the risk of synergistic toxicity between the BENDA and HDM and keeping in mind that all patients performed, in the previous 6 months, a first ASCT. Moreover, the association between BENDA at 200 mg/m^2+plus HDM at 140 mg/m^2 was investigated in lymphoma patients in the context of BeEAM chemotherapy regimen with an extremely low-toxicity profile.[25] However, a formal phase I study is recommended to address the maximum tolerated dose of bendamustine with fixed-dose melphalan. Overall, our conditioning regimen showed a favorable tolerability profile, and no cases of TRM were reported. Although most patients experienced commonly-occurring adverse events such as mucositis, vomiting and diarrhea, these were mild in severity in almost all cases. Interestingly, a lower incidence of mucositis and vomiting was reported as compared with the first ASCT. Although this study was not specifically aimed at comparing tolerability between the former and the latter conditioning regimen, one may speculate that the use of a lower dose of HDM (140 mg/m^2 instead of 200 mg/m^2) combined with BENDA, a molecule with a different mechanism of action, could cause less adverse events. Importantly, stem cell engraftment was successful in all patients, and the BENDA plus HDM regimen was associated with an overall short hospital stay (16 days), with some potential advantages from a cost-saving perspective.

The evaluation of efficacy was a secondary end point of our study; and therefore, no definite conclusions on this issue can be drawn also due to the limited number of patients enrolled. Overall, the addition of BENDA to HDM determined a high incidence of response, with an ORR of about 91% and more than 60% of patients achieving CR; these rates, although not statistical significantly probably due to the small simple size of the study, are higher than those obtained after the first transplant, lending some further support to the feasibility and soundness of the combination regimen.

It must be acknowledged that our study has several limitations, mainly related to the limited number of recruited subjects and the absence of a true control arm; these issues should be taken into account when evaluating the safety, tolerability and efficacy of the new conditioning regimen. Indeed, although it may be appropriate to use a different conditioning regimen in patients not attaining a CR, it would probably not be appropriate to change the conditioning regimen in patients attaining a CR after the first transplant, as these patients have proven to be very sensitive to HDM. The capacity of the second, different,

conditioning regimen to induce CR in patients attaining a suboptimal response after the first procedure is probably crucial to the efficacy of any strategy based on tandem transplants.

In conclusion, BENDA plus HDM is feasible as the conditioning regimen for second ASCT in patients with MM. The present study, despite its limitations, may pave the way for larger phase III studies specifically aimed at investigating safety, therapeutic activity and also the cost/efficacy ratio of this combination strategy and can give a contribute in the continuous debate about new conditioning regimen for ASCT as double alkylating agents, single alkylating agent or new alkylants that could include new formulation of HDM or adding new drugs different from chemotherapy. The role of this combination in MM for conditioning regimen in a first or single ASCT setting should be also investigated.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

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