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Differences in engraftment with day-1 compared with day-2 melphalan prior to stem cell infusion in myeloma patients receiving autologous stem cell transplant

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Abstract

We conducted a retrospective study comparing posttransplant outcomes between myeloma patients receiving conditioning melphalan on day-2 vs day-1 for autologous stem cell transplant. Between January 2017 and December 2018, 201 patients received melphalan on day-2 and 166 on day-1 prior to stem cell infusion. Baseline disease and clinical characteristics between the two groups were similar. Although rates of hospitalization were similar between the cohorts, duration of hospital admission was longer for day-1 (median 7 days for day-1 vs 5 days for day-2, $p = 0.003$). Rates of fever were higher in the day-1 cohort (69% vs 49%, $p = 0.0002$). Time to platelet and neutrophil engraftment was significantly longer in the day-1 cohort (platelet engraftment median days 17 for day-1 vs 15 for day-2, $p < 0.0001$, neutrophil engraftment median days 16 for day-1 vs 16 for day-2, $p = 0.025$). Overall response rate was similar between the cohorts (99% for day-1, vs 100% for day-2). Day-2 melphalan infusions should be considered in preference for day-1 protocols, given the clinically significant delay in platelet and neutrophil engraftment and longer duration of hospitalization with day-1 infusions.

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Author contributions ASA, MHS designed the study, analyzed the data, wrote the first draft, and approved the final version of the paper. JL, EJ, CAS, SKW collected the data, revised the paper and approved the final version of the paper. AD, EM, FKB, RW, MQL, DD, NL, WIG, PK, TVK, WJH, RW, and SKK performed patient management, revised the paper critically, and approved the final version of the paper; and MAG designed the study, analyzed the data, wrote the first draft, approved the final version of the paper, and performed patient management.

Conflict of interest The authors declare that they have no conflict of interest.

Introduction

Autologous stem cell transplantation (ASCT) has been standard therapy for multiple myeloma for over two decades with clinical trials showing an improvement in overall survival [1]. Single agent high dose melphalan has been the standard conditioning chemotherapy with studies combining this with other agents or radiation showing greater toxicity without additional benefit [2–4]. The optimal dose of melphalan identified and currently considered standard of care has been 200 mg/m² [5]. Melphalan is a bifunctional non cell cycle specific alkylating agent that binds strands of DNA forming cross links that prevent cell division leading to cell death. The drug is highly protein bound (90%) with a half-life that varies from 17 to 75 min and is primarily eliminated by spontaneous degradation through chemical hydrolysis to inactive metabolites [6, 7]. Although studies support the dose of melphalan used in myeloma patients receiving ASCT, there is little data on the optimal schedule of administration of melphalan prior to stem cell infusion. A variety of regimens have been used including split dosing on two days or single day melphalan administration commencing anywhere from day-3 to day-1 prior to stem cell infusion. Some institutions have advocated for a one day interval between melphalan and stem cell infusion leading to administration of melphalan on day-3 (for split dose infusions) or day-2 (single dose infusions). This is in an effort to avoid cytotoxic effects of melphalan on the infused stem cells. However there is little clinical or pharmacological data to support the necessity of a rest day between melphalan and stem cell infusion. Two studies have been reported in the literature on this topic with mixed results; the first was a small study ($n = 40$) (abstract only) that showed a longer time to neutrophil engraftment in patients receiving melphalan on day-1 compared with day-2 with similar time to platelet engraftment, and the second study ($n = 138$) showed a clinically insignificant delay in neutrophil and platelet engraftment of 24 h in patients receiving melphalan on day-1 compared with day-2 [8, 9]. The second study included a cohort of patients infused with stem cells between 8 and 12 h after melphalan infusion all of whom successfully engrafted without significant delay. Nevertheless there remains inconsistency in clinical practice as well as large multicenter phase III clinical trials on the schedule of melphalan administration prior to ASCT in MM [10, 11]. Given data on the pharmacology of the drug our institution changed protocols for melphalan administration from day-2 to day-1 on February 5th 2018 with the hope of avoiding an unnecessary day of rest in the conditioning regimen. Herein we report on the outcomes of patients with multiple myeloma receiving ASCT with melphalan administered on day-2 compared with those receiving melphalan on day-1 prior to stem cell infusion.

Methods

We conducted a retrospective review of all patients seen at Mayo Clinic Rochester that received ASCT for multiple myeloma, between 1st January 2017 and 31st of December 2018. Prior to February 5th 2018, patients received a single day infusion of high dose melphalan on day-2 with a rest day on day-1 followed by stem cell infusion on day 0. From February 5th 2018 onwards our institutional protocol changed to a single day infusion of high dose melphalan on day-1 followed by stem cell infusion on day 0. Our protocol aimed for a minimum of 18 h from the end of melphalan infusion to the commencement of stem cell infusion. Typically, this is done as outpatient and patients are admitted only if

complications develop. We compared outcomes in patients receiving melphalan on day-2 with those receiving the conditioning chemotherapy on day-1 prior to ASCT. During the study period 425 patients received ASCT for multiple myeloma, of whom 367 were eligible for the study, day-2 cohort ($n = 201$) and day-1 Cohort ($n = 166$), Fig. 1. Of the 58 patients excluded from the study, 28 received an alternative conditioning regimen to single agent melphalan and 30 patients received melphalan as a split infusion (half on day-2 and half on day-1) due to physician preference. The study was approved by the Mayo Clinic Institutional Review Board.

Risk stratification was according to the international staging system (ISS) and the revised international staging system (RISS) for multiple myeloma [12, 13]. High risk cytogenetic abnormalities were categorized in accordance with the International Myeloma Working Group (IMWG) definition and included deletion (17p), t(4;14), and t(14;16) [13]. Response was defined according to consensus criteria published by the IMWG [14]. All patients received granulocyte-colony stimulating factor (G-CSF) for 5 days with peripheral CD34 count checked on day 4 and if adequate, stem cell collection was started on day 5. Plerixafor was added if the peripheral CD34+ count was less than 10 cells/mL. Neutrophil engraftment was defined as the date of the first of three consecutive neutrophil counts $>0.5 \times 10^9/L$ and platelet engraftment was defined as the date of the first of three consecutive platelet counts $>50 \times 10^9/L$. Time to engraftment was calculated from the day of stem cell infusion to day of platelet and neutrophil engraftment respectively. Response at day 100 was determined by assessing individual patient data of serum and urine protein electrophoresis, immunofixation, serum free light chain assay, and bone marrow (BM) aspiration and biopsy obtained ~100 days post ASCT.

Statistical analysis was performed on JMP software (SAS, Cary, NC). Patient and disease related factors were compared using the χ^2 test for categorical variables, and the Wilcoxon rank sum test for continuous variables. Time to engraftment was compared between the two cohorts using Kaplan–Meier analysis and the median with interquartile range (IQR) was reported. Treatment related mortality was defined as death from any cause within 100 days from transplant. All tests were two sided and a P value of <0.05 was considered significant.

Results

Baseline characteristics for the two cohorts are listed Table 1. Baseline demographics and disease characteristics were well matched between the two cohorts. The BM disease burden and disease status at transplant were similar between the two cohorts. Time from stem cell collection to transplant did not differ between day-2 and day-1 groups (median = 8 days in both groups, $P = 0.3$). CD34 progenitor cell dose was not significantly different between the groups. The number of stem cell collections patients underwent was higher in the day-2 cohort (median 2 for day-2 vs 1 for day-1, $p = 0.02$). The median time between end of melphalan infusion and beginning of stem cell infusion for day-1 cohort was 18.6 h with 80% of patients being infused between 18.1 and 19.8 h after melphalan (range 17.1–23.8 h).

Transplant outcomes

Rates of hospital admission were higher in the day-1 cohort although this did not meet statistical significance (48% for day-1 vs 42% for day-2, $p = 0.29$). However duration of hospital admission was longer amongst the day-1 cohort (median 7 days for day-1 vs 5 days for day-2, $p = 0.003$). Furthermore rates of fever were significantly higher in the day-1 cohort (69% vs 49%, $p = 0.0002$). The causes for hospitalization were similar between the two cohorts although admission for fever/infection was higher amongst the day-1 cohort, Table 2.

Neutrophil and platelet engraftment was achieved in all but one patient who died on day 13 post ASCT from hemorrhagic stroke. Three patients required a second dose of stem cell infusion prior to engraftment, two in the day-1 cohort and one in the day-2 cohort. Time to platelet engraftment was significantly longer in the day-1 cohort (median days 17 (16–17) for day-1 vs 15 (15–16) for day-2, $p < 0.0001$), Fig. 2a. Although the median time to neutrophil engraftment was similar between the two cohorts (median days 16 (15–16) for day-1 vs 16 (15–16) for day-2, $p = 0.025$) there were a greater proportion of patients with a longer time to neutrophil engraftment, Fig. 2b. Results did not change when analyzing patients who received full dose melphalan (200 mg/m²) only. Transfusion requirements in the first 100 days post transplant were similar between the two cohorts (median platelet units transfused 1 unit (range 0–27) for day-1 vs 1 unit (range 0–9) for day-2, $p = 0.84$; median red cell units transfused 0 units (range 0–24) for day-1 vs 0 units (range 0–6) for day-2, $p = 0.84$), Fig. 3a,b.

Overall response rate (at least partial response) was similar between the two cohorts (99% for day-1, vs 100% for day-2), Fig. 4. The proportion of patients achieving at least a very good partial response was similar between the two cohorts (79% for day-1, vs 73.5% for day-2, $p = 0.22$). Three patients died within 100 days of transplant, all in the day-2 cohort (100 day mortality 0 for day-1 vs 1.5% for day-2, $p = 0.25$). Two patients died from aggressive myeloma relapse and one died from bilateral subdural hemorrhage secondary to a fall with very low platelet count.

Discussion

The lack of consistency in timing of intravenous melphalan prior to stem cell infusion in patients receiving autologous stem cell transplant for multiple myeloma is largely driven by the lack of data to support the clinical practice. Two studies have tackled this question, however they showed mixed results and were limited by small numbers and conducted over a decade ago. Our study presents data on a large cohort of patients treated for myeloma in the contemporary era, where ASCT is done mainly in the outpatient sitting, showing a clinically significant difference in early posttransplant outcomes when melphalan is infused on day-1 compared with day-2 prior to stem cell infusion. Engraftment of platelets and neutrophils was delayed in the day-1 cohort, although platelets were more significantly affected than neutrophils. This also translated into significantly more episodes of fever amongst the day-1 cohort and longer duration of hospital admission. Overall these data suggest that day-1 melphalan administration should be reconsidered in patients undergoing

autologous stem cell transplant for multiple myeloma. In the study of Talamo et al. [9] the outcomes of patients receiving day-1 ($n = 91$) vs. day-2 ($n = 47$) melphalan were described. Infusion of stem cells was between 8 and 24 h for day-1 melphalan, similar to our patients. The median neutrophil and platelet engraftment was significantly increased by 1 day in patients with day-1 melphalan (if analyzed from day of stem cell infusion to engraftment, $P < 0.05$). However, the conclusion of non-significance between both arms was based on the analysis done from the day of melphalan infusion to engraftment. In our opinion, this is less accurate, as patients with day-2 melphalan will have an additional day added for the analysis of days to engraftment. Also, our sample size is substantially larger and the definition of platelet engraftment differs as in their study they used $PLT > 20$ compared with our definition of $PLT > 50$. Patients in their study were admitted for 2–3 days for the melphalan, but in our cohort, we discharged the patients on the same day of melphalan infusion. In addition, we did not use G-CSF post transplant in contrast to their study where G-CSF was administered on day 6 after transplant until neutrophil engraftment.

The question of why a delay in engraftment was seen remains unanswered. One reason may be that our understanding of the pharmacology of melphalan, specifically with regards to its short half-life rendering it inactive within hours of infusion, may be limited. Even after 18 h post melphalan infusion there may be some melphalan remaining causing stem cell toxicity and leading to delayed engraftment. It is important to note that in our study the target time for stem cell infusion post melphalan was 18 h. In many institutions where a day-1 melphalan protocol is followed, a minimum 24 h delay between melphalan and stem cell infusions is mandated. It is unclear whether a similar delay in engraftment would be seen in these patients compared with a day-2 melphalan infusion protocol. It should also be noted that the definition of platelet engraftment in our study differs from the standard definition of a platelet count of $>20 \times 10^9/L$ at least 7 days from the last platelet transfusion. The reason we picked a platelet count is twofold. Firstly it allows for more reliable exclusion of the confounding due to platelet increments seen with platelet transfusion. Secondly an unsupported platelet count $>50 \times 10^9/L$ is a clinically relevant endpoint in our institution as we use this cutoff to decide on removal of central venous catheters and discharge from Mayo Clinic. It is unknown if we would get similar results if we used a definition of $PLT > 20$, as this information is not in our database. Our study evaluated ASCT being done mainly in the outpatient setting and while the difference in the duration of hospital stay does not apply to centers where ASCT is done as inpatient, the delayed engraftment is still a critical outcome that might decrease the total duration of hospital stay, as these patients are typically discharged after engraftment.

Although our study is retrospective, it is the largest of its kind addressing the question of optimal timing of melphalan administration prior to stem cell infusion in patients receiving ASCT for MM. Our study highlights a clinically significant delay in both platelet and neutrophil engraftment when following a day-1 melphalan conditioning protocol. We strongly recommend a day of rest between melphalan and stem cell infusion in these patients and our protocol at Mayo Clinic has been revised to discontinue melphalan administration on day-1.

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Fig. 1. Study population.
ASCT autologous stem cell transplant.

Fig. 2. Engraftment post transplant.

Platelet (a) and neutrophil (b) engraftment. TTE time to engraftment.

Fig. 3. Transfusion requirements.
Platelet (a) and red cell (b) transfusion requirements.

Fig. 4. Hematologic response at day 100.

sCR stringent CR, CR complete response, VGPR very good partial response, PR partial response, NR no response.

Table 1

Baseline characteristics.

Variable	Melphalan day-2 (<i>n</i> = 201)	Melphalan day-1 (<i>n</i> = 166)	<i>P</i> value
Age, median years (range)	63 (31–76)	62 (38–76)	0.81
Male, <i>n</i> (%)	123 (61)	108 (65)	0.45
Creatinine clearance, median (IQR)	86 (71–100)	87 (70–105)	0.64
Diagnosis BMPC's, % median (IQR)	50 (28–70)	55 (25–80)	0.31
ISS stage, <i>n</i> (%)			0.4
I	50 (35)	38 (29)	
II	47 (33)	53 (40)	
III	45 (32)	41 (31)	
Missing	59	34	
Revised ISS stage, <i>n</i> (%)			0.17
I	25 (22)	17 (15)	
II	65 (56)	75 (68)	
III	26 (22)	18 (16)	
Missing	85	56	
Prior therapy			0.65
I regimen	139 (69)	119 (72)	
> I regimen	62 (31)	47 (28)	
Conditioning dose			0.29
Melphalan 200 mg/m ²	178 (89)	153 (92)	
Melphalan <200 mg/m ²	23(11)	13(8)	
Pretransplant BMPC's, <i>n</i> (%)			0.58
<5%	137 (70)	122 (74)	
5–9%	24 (12)	16 (10)	
10%	36 (18)	26 (16)	
Disease status at transplant, <i>n</i> (%)			0.25
sCR	28 (14)	33 (20)	
CR	12(6)	15(9)	
VGPR	83 (41)	53 (32)	

Variable	Melphalan day-2 (<i>n</i> = 201)	Melphalan day-1 (<i>n</i> = 166)	<i>P</i> value
PR	62 (31)	53 (32)	
PD	16(8)	12(7)	
Time stem cell collection to ASCT, <i>n</i> (%)			0.6
<6 months	179 (89)	150 (91)	
6 months	22(11)	15(9)	
Number of collections, median (range)	2 (1–8)	1 (1–6)	0.02
Total CD34 cells collected, median ×10 ⁶ /kg (range)	9.02 (3.06–29.8)	9.27 (3.1–31.42)	0.51
CD34 stem cell dose infused, median ×10 ⁶ /kg (range)	3.96 (1.79–14.9)	3.94 (1.99–13.1)	0.82
Hours melphalan to stem cell infusion, median (range)	42.2 (35.7–18.3)	18.6 (17.1–23.8)	–

BMPC: bone marrow plasma cells, *IQR* interquartile range, *ISS* international staging system, *ASCT* autologous stem cell transplant, *sCR* stringent CR, *CR* complete response, *VGPR* very good partial response, *PR* partial response, *NR* no response.

Table 2

Transplant outcomes.

Transplant outcomes	Melphalan day-2 (<i>n</i> = 201)	Melphalan day-1 (<i>n</i> = 166)	<i>P</i> value
Fever >38 Celsius, <i>n</i> (%)	99 (49)	114 (69)	0.0002
Bacteremia, <i>n</i> (%)	30 (15)	29 (17)	0.57
Hospital admission, <i>n</i> (%)	84 (42)	79 (48)	0.29
Days in hospital, median days (range)	5 (1–31)	7 (1–80)	0.003
Cause for hospitalization, <i>n</i> (%)			0.48
Fever/Infection	26 (31)	32 (40)	
Mucositis	20 (24)	20 (23)	
AF/CVS	8(10)	3(4)	
Orthostasis	4(5)	3(4)	
Caregiver	5(6)	8(10)	
Other	20 (24)	15 (19)	
TRM, <i>n</i> (%)	3 (1.5%)	0 (0%)	0.25

BMPC's bone marrow plasma cells, *IQR* interquartile range, *ISS* international staging system, *ASCT* autologous stem cell transplant, *sCR* stringent CR, *CR* complete response, *VGPR* very good partial response, *PR* partial response, *NR* no response, *TRM* transplant related mortality.