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ORIGINAL ARTICLE

Development of a method for clinical pharmacokinetic testing to allow for targeted Melphalan dosing in multiple myeloma patients undergoing autologous transplant

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Aims: High dose melphalan (HDM) and autologous stem cell transplant (ASCT) is standard of care for multiple myeloma (MM), but there is significant variability in melphalan exposure (area under the plasma drug concentration–time curve, AUC) when using body surface area-based dosing. Our aim was to establish a method of pharmacokinetic (PK) testing for real-time melphalan dose adjustments.

Methods: We performed a prospective PK study of melphalan 140 or 200 mg/m^2 in MM patients undergoing ASCT. Twenty MM patients were administered HDM on days −2 and − 1, with PK sampling at 8–10 time points. PK testing was performed on day −2 in all patients, and on day −1 in 5 patients.

Results: Less than 20% interpatient variation in the day −2 and − 1 AUC was observed. The day −2 range in AUC (4.95–11.28 mg h/L) confirmed significant interpatient variability. The hypothetical total dose ranged from 133–302 mg/m^2 to achieve the total median AUC. A 4-time point AUC (0, 30, 150 and 240 min) highly correlated with the AUC from the 8-time point schedule. A higher AUC correlated with increased risk of febrile neutropenia (P = .05).

Conclusion: Here we outline the methods to establish novel melphalan dosing using PK testing in MM patients undergoing ASCT to target a desired melphalan AUC.

KEYWORDS

chemotherapy, mass spectrometry, pharmacokinetics, therapeutic drug monitoring, transplantation

1 | INTRODUCTION

High-dose melphalan (HDM) followed by autologous stem cell transplantation (ASCT) remains a central component of upfront treatment for multiple myeloma (MM) based on results from randomized trials demonstrating significant improvement in progression-free survival compared to the use of novel agents alone.[1–4] However, even with advances in treatment, the majority of patients relapse.[5–8] Although the use of ASCT increases each year, outcomes can be highly variable, with up to 40% of patients failing to achieve a complete

The authors confirm that the PI for this paper is Karen Sweiss and that she had direct clinical responsibility for patients.

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remission (CR), and 10–15% of patients becoming functionally cured with remissions lasting more than 10 years. In addition to this, up to 30% of patients experience severe oral mucositis.[1,5,9] This is in part attributed to significant interpatient variability (~10 fold) in melphalan pharmacokinetics (PK) and exposure (area under the plasma drug concentration–time curve, AUC).[10–15]

The standard body surface area (BSA)-based method of HDM dosing fails to account for variables that alter melphalan exposure (AUC), and thus, a proportion of patients will experience suboptimal clinical response as a result of subtherapeutic melphalan exposure, or conversely, severe toxicity due to supratherapeutic exposure.[16–18] Melphalan is a DNA-alkylating drug that is highly protein bound, in particular to proteins in red cell membranes, and undergoes ~40% renal elimination.[9,11–15,19–21] Low creatinine clearance and haemoglobin, are mediators of melphalan PK, and are in turn strong clinical predictors of improved survival and increased toxicity.[15–17] Additionally, PK studies have demonstrated that higher melphalan exposure (above the median of 12.84 mg h/L) is associated with improved survival.[17] Given that wide interpatient variability in PK exists and that melphalan exposure correlates with clinical outcomes as well as toxicities, the development of a method by which real time melphalan PK testing can be easily performed is necessary to perform targeted HDM dosing in the future. A 2-day HDM regimen, administered at a dose of 70–100 mg/m^2 on days −2 and −1, permits for potential modification of the day −1 dose if PK testing with rapid turnaround can be performed on day −2. Here we describe a clinically feasible, reproducible method of measuring melphalan PK that allows for real-time dose adjustments in clinical trials and practice.

What is already known about this subject

- Large interpatient variability in melphalan exposure exists among multiple myeloma patients undergoing autologous stem cell transplant.
- Higher melphalan exposure improves survival but also results in increased toxicities.
- Methods outlining real-time melphalan pharmacokinetic testing and dose adjustment have not been well established in clinical practice.

What this study adds

- We sought to develop methods for performing real-time melphalan pharmacokinetic testing that allows for rapid turnaround of results and dose-adjustment to achieve the desired therapeutic range.
- We demonstrate similar pharmacokinetics between days −2 and −1, suggesting that a linear dose-proportional adjustment can be used to achieve the desired therapeutic range.
- We demonstrate that a 4-time point blood sampling schedule (0, 30, 150 and 240 min of infusion) accurately predicts the melphalan area under the plasma drug concentration–time curve and allows for a feasible method of testing in real-time clinical practice.
- Using our methods of blood sampling, pharmacokinetic analysis and dose calculation, real-time melphalan pharmacokinetic testing can be applied in future clinical trials to examine the impact of individualized dosing on clinical outcomes after autologous transplant.

2 | MATERIALS AND METHODS

2.1 | Study design and patients

We performed a prospective, single-centre PK study of HDM in MM patients undergoing ASCT. Prior to commencing any study procedures, approval was obtained from the Institutional Review Board of the University of Illinois at Chicago. Our primary objective was to establish feasibility of a real-time method to test melphalan PK within the clinical setting. Our secondary objectives were to determine if intrapatient variability between the day −2 and day −1 melphalan doses exists, and to correlate clinical outcomes with melphalan exposure. Patients aged ≥18 years with symptomatic MM (either newly diagnosed or relapsed) who met criteria to receive HDM at either 140 or 200 mg/m^2 followed by ASCT were eligible. Patients who had received prior ASCT were eligible, while receiving a prior allogeneic stem cell transplant represented as an exclusion criterion. Patients received a reduced melphalan dose of 140 mg/m^2 if they were age ≥70 years with significant comorbidities or have a history of severe renal insufficiency, defined by a creatinine clearance <30 mL/min or requiring haemodialysis.

2.2 | Melphalan administration and supportive care

Patients were admitted to the hospital either 1 day prior to or the day of HDM initiation and administered melphalan at a total dose of 140 or 200 mg/m^2, based upon renal function and age (Figure 1). All doses were administered based upon actual body weight, regardless of body mass index. Melphalan was supplied as melphalan hydrochloride 50 mg vials (Mylan Institutional LLC, Rockford, IL, USA). Melphalan was reconstituted to a final concentration of 0.45 mg/mL and was divided equally over days −2 and − 1 (approximately 24 hours apart), followed by ASCT on day 0. Infusion times ranged from 30 to 40 minutes, depending on the final dose. As part of our institutional standard operating procedures for high dose melphalan, all doses were administered within 30 minutes of admixture, and therefore there were no expected delays from admixture to administration time that would result in spontaneous degradation and compromised melphalan stability.[22] A 14-mL normal saline flush was delivered through

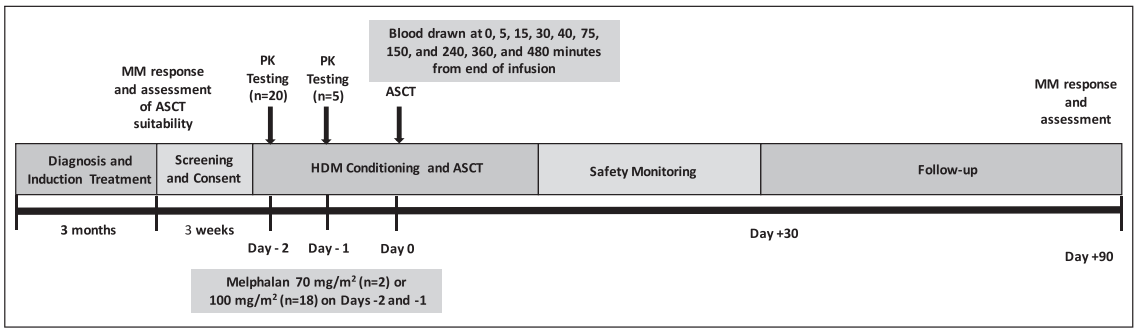

FIGURE 1 Study schema. Patients enrolled in the pharmacokinetic study received melphalan 140 or 200 mg/m^2 as 2 divided doses on days −2 and − 1 followed by autologous stem cell transplant. Patients were evaluated for safety until day +30 and response at day +90. Blood was drawn at different time points for pharmacokinetic analysis as shown

the primary tubing immediately prior to infusion of HDM. Infusion of this flush was noted as the start time for the melphalan infusion (start of infusion time). Following the completion of the melphalan infusion, a second 14-mL normal saline flush was administered, with its completion considered as the end of the melphalan infusion (end of infusion, EOI). As part of our standard operating procedures, cryotherapy was recommended during the melphalan infusion in all patients. In addition, all patients received antibacterial and antifungal prophylaxis with levofloxacin and fluconazole, respectively, beginning day +4 of ASCT and continuing until neutrophil engraftment, antiviral prophylaxis with acyclovir beginning day +4 and continuing indefinitely, and filgrastim beginning on day +5 and continuing until the absolute neutrophil count was >500 cells/μL for 3 consecutive days.

2.3 | PK blood sampling and analysis

Prior to initiation of PK blood sampling and analysis, we developed and validated a melphalan assay that could be used for real time PK testing. Our methods of measuring melphalan concentrations and assay validation parameters including limits of detection, quantification, accuracy, precision and stability are outlined in detail in the Supplementary Methods. We first developed standard operating procedures for transportation and storage of blood samples. Aside from the first blood sample which was collected at the EOI from a peripheral intravenous line to avoid contamination, all subsequent samples for PK were collected from a quarantined port of the central venous indwelling catheter. Eight 5-mL blood samples were originally planned to be drawn at 0 (EOI), 5, 15, 30, 40, 75, 150 and 240 minutes after completion of the infusion. After analysis of the first 8 samples, melphalan concentrations were detectable at 240 minutes, and therefore, the blood sampling window was extended to include 360 and 480 minutes' postinfusion. PK was performed on day −2 in all 20 patients and on day −1 in 5 patients.

Samples were immediately collected in prechilled and prelabelled heparinized tubes and placed in prelabelled specimen bags, placed on ice, and delivered to our in-house PK laboratory adjacent to the University of Illinois Hospital within 5 minutes of each blood draw. The first 2 PK samples were delivered together, while all other samples were delivered individually after each blood to the PK laboratory. Blood was immediately centrifuged at 3000 *g* for 10 minutes at 4°C and supernatant plasma was removed and stored at −80°C until liquid chromatography–tandem mass spectrometry analysis. Noncompartmental analyses (NCA; Phoenix WinNonlin, version 7; Pharsight Corporation, Cary, NC, USA) were used to generate PK parameters for each subject for melphalan in plasma. The patient-specific duration of infusion was included in the NCA. Reported parameters following intravenous administration of HDM included peak plasma concentration (C_{max}), time to maximum concentration (T_{max}), apparent volume of distribution (Vd), apparent clearance (CL_T) and elimination half-life ($t_{1/2}$). The AUC was calculated by use of the linear-up log-down trapezoidal method. To demonstrate reproducible PK between days −2 and − 1, we performed a paired *t*-test for the 5 paired samples. To determine a limited sampling window, the mean AUC calculated from all 8 sampling points via NCA was sequentially compared to the AUC obtained from fewer sampling points in a descending manner while maintaining an R^2 value >90%. In addition to this correlation analysis, we performed 1-way ANOVA (i.e. within-subjects ANOVA) to determine any statistically significant differences between the means of 3 or more levels of a within-subject related factor.

2.4 | Clinical outcomes measurements and statistical analysis

Toxicities directly related to melphalan such as mucositis, diarrhoea, nausea, vomiting and infection were assessed up to 30 days after ASCT using NCI-CTCAE, version 5.0 grading.[23] Treatment-emergent adverse events were defined as adverse events that occurred or worsened on or after first study treatment up to 30 days after last study treatment and/or any treatment-related adverse events regardless of the onset date. Other markers of toxicity were also evaluated such as duration of total parenteral nutrition, use of antimotility drugs, use of antiemetics, days of diarrhoea and use of opioids. Patients were followed for Day 90 disease response rates, using standard

International Myeloma Working Group (IMWG) criteria.[24] Neutrophil engraftment was defined as ANC ≥ 0.5 × 10^9/L for 3 consecutive laboratory values obtained on different days. Platelet engraftment was defined as un-transfused platelet counts ≥20 × 10^9/L for 3 consecutive daily measurements.

The median, range and interquartile range for continuous data and frequencies with percentages for categorical data were assessed using descriptive statistics. Time-to-event endpoints were evaluated using the Kaplan–Meier method including time to myeloablation and time to neutrophil and platelet engraftment. The rates of myeloablation, engraftment, and nonengraftment as defined below were summarized by the proportions of patients. The proportions of patients meeting response levels based on International Myeloma Working Group uniform response criteria (stringent CR, CR, very good partial response, partial response, stable disease and progressive disease) were also summarized. Adverse events were summarized by severity and relatedness to study treatment as assessed by the investigators. Clinically relevant NCI-CTAE, version 5.0[23] grade 3 and 4 laboratory abnormalities were summarized. Associations between efficacy and toxicity with median AUC were analysed using modified Poisson regression.[25] To estimate age- and sex-adjusted rate ratios (RR) we followed the approach of Zou[25] with robust standard errors given that: (i) the toxicity outcomes of interest were nonrare and inconsistently approximated relative risk using odds ratios; (ii) the logarithm link in this sandwich estimator was relatively robust to omitted covariates; and (iii) we sought to draw identify associations by analysing clustered data with multiple measures performed on the same subject. Multivariable models were fit for calculation of adjusted RR and 95% confidence intervals (CI) with robust standard errors for duration of hospitalization, number of rescue antiemetics, days using antimotility drugs, 30-day readmission and febrile neutropenia.

3 | RESULTS

3.1 | Baseline characteristics

A total of 20 MM patients were enrolled with 18 receiving melphalan 200 mg/m^2 (refer to Figure 1 for study schema). Two patients received reduced-dose melphalan (140 mg/m^2): 1 with end-stage renal disease receiving intermittent dialysis and 1 patient whose age was 74 years. Table 1 summarizes the baseline characteristics. Median age was 59.5 years (range: 50–74) and most patients were male (*n* = 12; 60%), African-American (*n* = 11, 55%), IgG subtype (*n* = 13, 65%) and R-ISS stage 3 (*n* = 9, 52.9%). Three (15%) of patients had high risk cytogenetic abnormalities at diagnosis. The majority of patients received prior immunomodulator- (*n* = 16; 80%) or proteasome-inhibitor-based treatment (*n* = 19; 95%). Patients achieved a complete response (*n* = 3), partial response (*n* = 7), very good partial response (*n* = 9) or stable disease (*n* = 1) pre-ASCT. The median time to ASCT was 295.5 days (range: 79–3523). In our cohort, 1 patient had a history of end stage renal disease and was undergoing haemodialysis, while among the remaining 19 patients, the mean creatinine clearance was 81.1 (±27.33) mL/min. Melphalan was administered within 30 minutes of admixture in all patients. There was little variability across infusion times. The mean infusion time was 31 (±2) minutes, and the median infusion time was 30 (range: 29–40) minutes. There were 6 subjects whose infusion time was not exactly 30 minutes.

TABLE 1 Patient characteristics

	***n* = 20**
Sex	
Male, *n* (%)	12 (60)
Female, *n*, %)	8 (40)
Age (y), median (range)	59.5 (50–74)
Time to transplant (d), median (range)	295.5 (79–3523)
Protein subtype	
A	3 (15)
G	13 (65)
FLC	4 (20)
Patients receiving > 1 prior treatment, *n* (%)	4 (20)
Prior IMiD, *n* (%)	16 (80)
Prior PI, *n* (%)	19 (95)
Prior ASCT, *n* (%)	2 (10)
Race, *n* (%)	
Black	11 (55)
White	5 (25)
Hispanic	3 (15)
Other	1 (5)
ISS	
1	6 (35)
2	2 (12)
3	9 (53)
High risk FISH/karyotype, *n* (%)	3 (16)
Patients receiving maintenance therapy, *n* (%)	55 (41)
Disease response pre-ASCT	
CR	3 (15)
VGPR	9 (45)
PR	7 (35)
SD	1 (5)

IMiD, immunomodulator drug; PI, proteasome inhibitor; ASCT, autologous stem cell transplantation; ISS, International Staging System; FISH, fluorescent in situ hybridization; CR, complete response; VGPR, very good partial response; PR, partial response; SD, stable disease

3.2 | PK and hypothetical dose adjustment

In the first 5 patients, we performed PK analysis on days −2 and −1. The percent difference in the median AUC between days −2 and −1 melphalan doses is summarized for the 5 patients in Table 2. The mean percent difference in the median AUC between days −2 and −1 was

TABLE 2 Mean percent difference in area under the plasma drug concentration–time curve (AUC) between day −2 and day −1 melphalan dose

	AUC day −2	AUC day −1	% difference
Patient 1	6.8	5.9	−15.1%
Patient 2	5.0	6.0	16.2%
Patient 3	8.7	6.4	−37.3%
Patient 4	9.0	7.2	−24.4%
Patient 5	10.6	11.0	4.3%

19.46% (range: −37.3-16.2%). Using a paired *t*-test for the 5 paired samples, the overall mean difference was 0.72 ± 1.41 (95% CI 1.03–2.47, P = .316). The median AUC on day −2 in all 20 patients was 7.49 (range: 4.95–11.28) mg h/L with an interquartile range of 2.66 mg h/L (Figure 2). A 2.3-fold variation in melphalan exposure was observed within our patient cohort.

Given prior data demonstrating linear PK for melphalan[26,27] and our findings that demonstrate reproducible PK between the days −2 and −1 dose, we proceeded to use linear, dose-proportional adjustments and performed dose simulations for each patient to estimate the day −1 dose in order to target the total median melphalan AUC (14.98 mg h/L) and compared it to the BSA-based dose received. The total median melphalan AUC was derived by multiplying the median day −2 AUC (7.49 mg h/L) by a factor of 2. The equations used to determine the day −1 dose required to target the total median are outlined in the Supplementary Methods. In patients whose day −2 AUC fell below the median, a day −1 dose increase to target the total target AUC would be required. In patients whose day −2 AUC fell above the median, a day −1 decrease to target the total target AUC would be required. The change in the day −1 melphalan dose necessary to achieve the total median AUC ranged from −67 to +102 mg/m^2, or a percent change from −33.6 to +51.2% (Figure 3A). The hypothetical total melphalan dose to target the median AUC ranged from 133 to 302 mg/m^2 (Figure 3B).

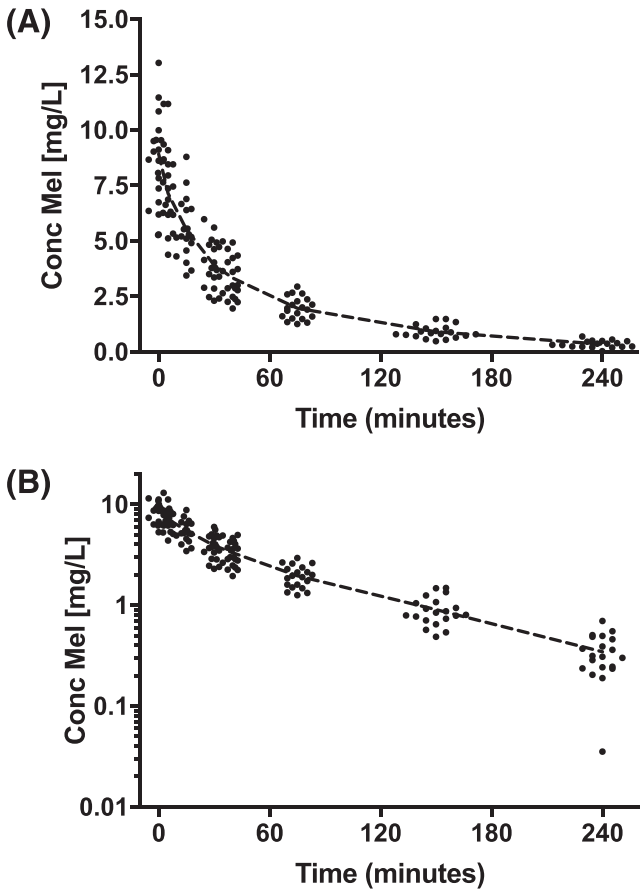

FIGURE 2 Median day −2 melphalan (Mel) concentration (mg/L) *vs* time (min) curve (dotted line) with actual data points. Data are presented in (A) linear and (B) log scale

3.3 | Modified PK sampling window

Melphalan AUC was not significantly correlated to either C_{min} (R^2 = .06) or C_{max} (R^2 = .39), suggesting that a single time point post-infusion (i.e. trough or C_{max}) cannot be used to accurately determine melphalan exposure (Supplementary Figure S1A and B). To maximize clinical application, we sought to determine an abbreviated blood sampling schedule. The mean AUC calculated from all 8 sampling points via NCA was sequentially correlated to the AUC obtained from fewer sampling points in a descending manner while attempting to maintain an R^2 value >90% (Supplementary Figure S1C-F). We found that a 2-time point sampling schedule (30, 150 min or 30, 240 min) did not correlate well, with the R^2 values being .75 and .83, respectively (Supplementary Figure S1C and D). A 4-time point schedule including 0, 5, 30 and 150 minutes also did not correlate well with the 8-time point AUC (R^2 = .86; Supplementary Figure S1F). However, both a 4-time point schedule consisting of 0, 30, 150 and 240 minutes (R^2 = .93; Figure 4) and a 3-time point schedule consisting of 30, 150 and 240 minutes (R^2 = .92; Supplementary Figure S1E) strongly correlated with the original schedule. To validate these findings, we performed a 1-way repeated measures ANOVA analysis to determine differences between the means (Table 3). There were no outliers or extreme points in any AUC group and the AUCs were normally distributed in each group. Mauchly's assumption of sphericity was violated and therefore, the Greenhouse–Geissier correction was used since ε was <0.75 (ε = 0.599). There was a statistically significant difference between the mean AUC across the 6 groups based on the number of samples used (P < .001). The mean of the 4-point sampling strategy including 0, 30, 150 and 240 minutes was similar to the 8 sampling points (P = .846). The 3-time point schedule of 30, 150 and 240 minutes had a larger mean difference that was borderline statistically significant (P = .06). Therefore, overall, the optimum sampling strategy was 4 time points including 0, 30, 150 and 240 minutes. Figure 5 summarizes the absolute median AUC as well as the percent change in the median AUC for each individual patient when comparing this 4-time point schedule with the original 8-time point schedule.

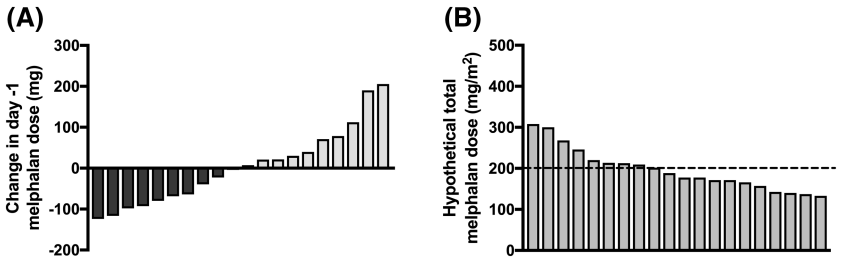

FIGURE 3 (A) Percent change in day −1 melphalan dose necessary to achieve the median area under the plasma drug concentration–time curve among the 20 patients; (B) hypothetical total melphalan dose (mg/m^2) required to target the median area under the curve across the 20 patients

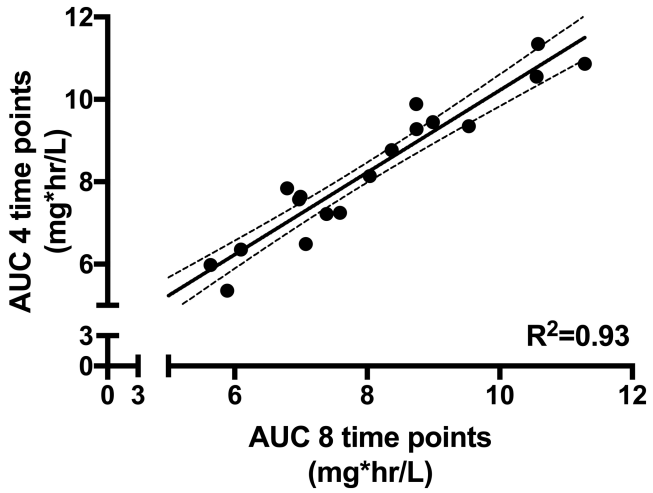

FIGURE 4 Correlation demonstrated between area under the plasma drug concentration–time curve (AUC) using modified 4-time point blood sampling schedule (0, 30, 150 and 240 min) and original 8-time point sampling schedule (R^2 = 0.93)

3.4 | Efficacy and toxicity outcomes

The median duration of hospitalization was 16 days (range: 14–24), with 3 (15%) patients having a hospital re-admission within 30 days of ASCT. The median time to neutrophil and platelet engraftment was 11 days (range: 9–13) and 15 days (range: 10–22) days, respectively. The median number of days of neutropenia (absolute neutrophil count <500 cells/mm^3) was 6 days (4–8). There were no grade 4 toxicities reported. Grade 3 toxicities were observed in 11 patients and the AUC in those patients are as follows: 1 patient with mucositis (median AUC 5.89 mg h/L), 7 with diarrhoea (median AUC 7.59 mg h/L), 1 with nausea (median AUC 8.75 mg h/L) and 2 with vomiting (median AUC 8.56 mg h/L). The average duration of antiemetic treatment use was 2.55 days (range: 1–6) and the number of days that patients used anti-motility drugs was 7.65 days (range: 0–15). Eight (40%) patients required opioids (i.e. morphine, hydromorphone) for pain relief during the pre- and immediate postengraftment phase. Twelve (60%) of patients had febrile neutropenia that resolved with systemic antimicrobial therapy. Twelve of 17 evaluable patients (70.6%) achieved either a stringent CR or CR at day 90 post-ASCT. In modified Poisson regression adjusted for age and sex, higher AUC correlated with increased risk of febrile neutropenia (adjusted RR 3.02, CI 1.00–9.26, P = .05) and a trend towards increased use of antiemetics (adjusted RR 1.86, CI 0.95–3.65, P = .07). No correlation between AUC and efficacy was observed.

4 | DISCUSSION

MM is characterized by a pattern of recurrent remissions and relapses and remains incurable despite numerous drug classes developed specifically for myeloma, e.g. immunomodulator drugs, proteasome inhibitors, CD38 antibodies, nuclear export inhibitors and chimeric antigen receptor T-cells.[28,29] Despite significant pharmaceutical innovations,

TABLE 3 Comparison of melphalan area under the plasma drug concentration–time curve (AUC) up to the last treatment stratified by number and timing of PK samples

Group (n and timing of sample (min))	Estimated marginal mean (95% CI)	Mean difference in marginal mean compared to group 1 (95% CI for difference)	P value for difference[a]	Correlation to group 1 AUC (R^2)
1 (n = all samples; 0, 5, 15, 30, 40, 75, 150, 240 min)	7.8 (6.9–8.6)	Ref	Ref	Ref
2 (n = 4; 0, 5, 30, 150)	7.8 (6.9–8.7)	−0.04 (−0.58–0.51)	>.999	.8562
3 (n = 4; 0, 30, 150, 240)	8.0 (7.1–8.9)	−0.23 (−0.6–0.15)	.846	.9308
4 (n = 3; 30, 150, 240)	7.4 (6.5–8.2)	0.4 (−0.01–0.81)	.06	.9180
5 (n = 2; 30, 150)	5.9 (5.2–6.6)	1.86 (1.17–2.55)	<.001	.7493
6 (n = 2; 30, 240)	7.46 (6.56–8.36)	0.3 (−0.3–0.9)	>.999	.8317
6 (n = 2; 30, 240)	7.46 (6.56–8.36)	0.3 (−0.3–0.9)	>.999	.8317

[a]Bonferroni adjusted for multiple comparisons.

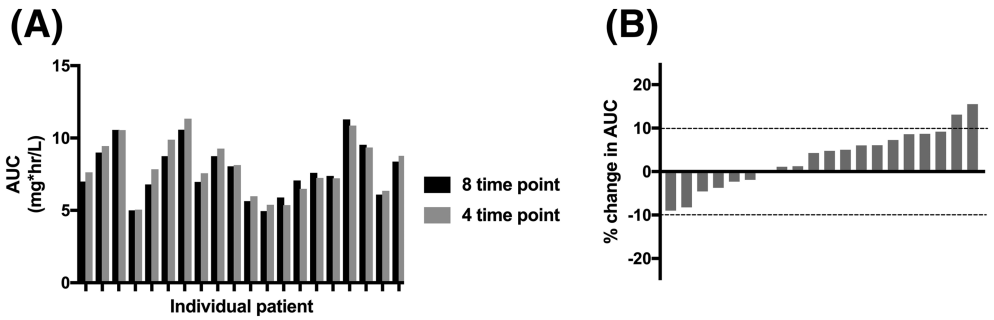

FIGURE 5 (A) Area under the plasma drug concentration–time curve (AUC) for each patient using a 4-time point schedule (0, 30, 150 and 240 min) compared to the original 8-time point schedule; (B) the percent change in the AUC in each patient between a 4-time point schedule (0, 30, 150 and 240 minutes) and the original 8-time point schedule

HDM and ASCT remains standard of care based on randomized trials demonstrating significant improvement in progression-free survival[1] and higher minimal residual disease negativity proportion when compared to induction therapy that did not involve transplant.[30] While it remains a standard of care in fit patients, efforts to improve efficacy to decrease the 20% of patients who relapse within 24 months[1] while decreasing toxicities (5% with significant mucositis despite cryotherapy and 10% with tachyarrhythmia) are needed.

Interpatient variability in melphalan exposure may represent an important mediator of outcomes after ASCT.[10,15,17] The current body surface area-based HDM dosing fails to account for variables that alter AUC (i.e. protein binding, renal function, pharmacogenomics) and, thus, many patients experience suboptimal response due to subtherapeutic HDM exposure.[11,13–17,31–33] Relatively recent data have shown that the standard 200 mg/m^2 melphalan yields a 500% variation in AUC, which coincides with a wide variation in clinical responses and toxicities suffered.[15] While HDM has been evaluated at 100–300 mg/m^2, these studies did not obtain PK data, did not test pharmacodynamic or pharmacogenomic markers, and did not incorporate sensitive assays for minimal residual disease. In fact, melphalan 280 mg/m^2 with amifostine protection has been explored in prior studies and although this regimen has been associated with slightly increased toxicities, variability in responses have also been observed, with 1 study demonstrating improved CR rates and another not observing major improvement in PFS with long-term follow-up.[34,35] Because of the known wide interpatient variability in melphalan exposure after BSA-based dosing, it is possible that there were some patients who would have an already expected higher melphalan exposure after melphalan 200 mg/m^2 and upon further dose escalation experienced an even higher AUC and increased toxicities.

Melphalan is a DNA-alkylator that is highly bound to red cell membrane proteins and undergoes ~40% renal elimination.[9,13–16,36] In addition, a single nucleotide polymorphism (rs4240803) in SLC7A5, a gene that encodes L-type amino acid transporter 1 (LAT1), which is a transporter that mediates melphalan uptake into cells, has been associated with increased mucositis and requirement for total parenteral nutrition but also has been linked to response.[10,31,32,37] Factors modifying melphalan exposure could explain recent studies that suggest that HDM PK significantly impacts ASCT outcomes.[15–18] Several groups have demonstrated that a higher HDM AUC leads to improved overall survival and increased toxicities (e.g. mucositis).[10,13,17] In particular, Nath et al demonstrated that a melphalan AUC above the median was a risk factor for severe mucositis but was also associated with significantly improved overall survival, with an estimated median survival of 8.50 *vs* 5.38 years for high *vs* low AUC groups.[17] As with prior studies, we report significant interpatient variability, and dosing simulations based on patients' PK data demonstrate that large, bidirectional dose adjustments are required to target the median AUC.

Based on these findings, it appears that a clinical approach using therapeutic drug monitoring for HDM is necessary to achieve targeted exposure in all patients. Here we also provide detailed methods for performing rapid melphalan PK testing in a clinical setting. We report methods for melphalan infusion, timing of PK blood draws, assay development and validation, with emphasis on minimizing factors that would decrease melphalan stability in the clinical setting (i.e. delays from admixture to administration, maintaining all samples on ice, etc.).[22] Specifically, we highlight the feasibility of using a 4-time point (0, 30, 150 and 240 min) blood sampling schedule in determining melphalan PK in clinical practice. Our proposed schedule is consistent with the 4-time point schema proposed by the population modelling from the Cincinnati group at 5, 35, 120 and 240 minutes post-melphalan infusion.[12] Although we show that the AUC from a 3-time point schedule also correlates with the AUC from 8 time points, the mean of the AUC diverged from the 8-time point strategy and in the clinical environment many unforeseeable events can occur that may impact the integrity of blood samples. Therefore, we propose that the 4-time point schedule may be preferred in the case that 1 sample cannot be used in the PK analysis. Furthermore, future studies using Bayesian PK methodology which incorporate both population PK modelling (i.e. covariates including creatinine clearance, haematocrit, fat free mass, etc.) and measured blood concentrations will provide more accurate AUC estimations.

We further report, for the first time, similar PK between days −2 and − 1 with <20% interpatient variation in AUCs, important findings that will need further validation in a larger cohort of patients. Factors that could contribute to variability include hydration status, concurrent diuretics, inconsistency in blood sampling

procedures between nurses and variable infusion times. While prior studies have demonstrated a linear association between melphalan dose and AUC[26,27] and test dose strategies for various conditioning regimens (i.e. BEAM, VP16-melphalan) have been successful in determining subsequent doses (24 hours later) under well-controlled conditions,[27] a method for PK testing and dose-adjustment has yet to be implemented in clinical practice. A recent abstract demonstrated in patients administered a melphalan test dose (20 mg/m^2) 1–3 days prior to the remaining full dose that the median percent deviation of the actual from predicted values was −8%, (range: 43–11%), with predictions for 23 patients (70%), being within ±15%.[38]

Our observation showing similar PK between 2 consecutive days, along with the linear PK profile of melphalan, suggest that the use of a linear, dose-proportional method of melphalan day −1 dose adjustment using day −2 PK can be used to achieve a target AUC.

Here we outline in detail our methods for PK testing and individualized dosing of melphalan in MM patients undergoing ASCT that can be applied within the clinical setting across multiple centres. Application of PK-directed HDM dosing at centres where a PK laboratory is not on site may require a novel dosing regimen with melphalan being administered on days −3 and − 1 to allow for timely turnaround of PK results and dose adjustments. Our methods can be used in future prospective studies to examine the safety and efficacy of using a PK-directed approach in dosing melphalan in the autologous transplant setting.

COMPETING INTERESTS

P.P. receives honoraria from Celgene, Janssen and Amgen and consulting fees from Celgene and Amgen.

CONTRIBUTORS

K.S., B.V., P.R.P., J.J.J., G.S.C., E.W. and D.R. contributed to the study design and analysed all data; K.S., P.R.P., C.C.H., J.J.J., G.S.C., C.C.H. and E.W. contributed to the writing of the manuscript; all authors assisted in the critical review of the manuscript and all authors approved the final version of the manuscript for submission.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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