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Journal Title: CPT: Pharmacometrics and Systems Pharmacology
Volume: Volume 7, Number 11
Publisher: Wiley Open Access: Various Creative Commons Licenses | 2018-11-01, Pages 748-758
Type of Work: Article | Final Publisher PDF
Publisher DOI: 10.1002/psp4.12345
Permanent URL: https://pid.emory.edu/ark:/25593/tpcjj

Final published version: http://dx.doi.org/10.1002/psp4.12345

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ARTICLE

Pharmacokinetic-Pharmacodynamic Model of Neutropenia in Patients With Myeloma Receiving High-Dose Melphalan for Autologous Stem Cell Transplant

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High-dose melphalan (HDM) is part of the conditioning regimen in patients with multiple myeloma (MM) receiving autologous stem cell transplantation (ASCT). However, individual sensitivity to melphalan varies, and many patients experience severe toxicities. Prolonged severe neutropenia is one of the most severe toxicities and contributes to potentially life-threatening infections and failure of ASCT. Granulocyte-colony stimulating factor (G-CSF) is given to stimulate neutrophil proliferation after melphalan administration. The aim of this study was to develop a population pharmacokinetic/pharmacodynamic (PK/PD) model capable of predicting neutrophil kinetics in individual patients with MM undergoing ASCT with high-dose melphalan and G-CSF administration. The extended PK/PD model incorporated several covariates, including G-CSF regimen, stem cell dose, hematocrit, sex, creatinine clearance, *p53* fold change, and race. The resulting model explained portions of interindividual variability in melphalan exposure, therapeutic effect, and feedback regulation of G-CSF on neutrophils, thus enabling simulation of various doses and prediction of neutropenia duration.

CPT Pharmacometrics Syst. Pharmacol. (2018) **7**, 748–758; doi:10.1002/psp4.12345; published online on 20 October 2018.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Currently, all patients with MM undergoing ASCT receive standard HDM at 200 mg/m^2 or 140 mg/m^2 based on renal function. Although population modeling of melphalan PKs was previously completed, PK/PD modeling/simulation has not been attempted to characterize neutropenia in this setting nor individualize dosing regimens to improve outcomes in patients undergoing ASCT.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ The objective was to identify important covariates associated with variability in melphalan exposure and neutropenia in patients with MM undergoing ASCT. Furthermore, we aimed to develop a PK/PD model that could be used prospectively in combination with other outcome models for personalizing melphalan dosing in ASCT to minimize the duration of severe neutropenia.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ This study identified new covariates, presented a PK/PD dataset for neutropenia in ASCT with HDM and two different G-CSF regimens. We also concluded that previously published models of neutropenia with G-CSF could not adequately describe features present in our data, and we present a new model that successfully describes ANC in patients with MM receiving HDM, ASCT, and G-CSF starting on day +1.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

☑ The adverse effects associated with prolonged severe neutropenia can potentially be reduced by application of PK/PD modeling and simulation. The model that was derived in this study can be combined with other outcome models to eventually achieve personalized treatment in patients with MM undergoing ASCT with HDM.

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Received 27 Nov 2017; accepted 24 July 2018; published online on 20 October 2018. doi:10.1002/psp4.12345

Multiple myeloma (MM) is the second most frequent blood malignancy in the United States and causes 1% of all cancer deaths.[1] Autologous stem cell transplant (ASCT) is highly effective in patients with MM with ~40–55% achieving complete response with high-dose chemotherapy.[2,3] Melphalan, a DNA alkylating agent, is given at a high, standard dose of 200 mg/m^2 in most patients as part of the conditioning regimen for ASCT in MM.[4,5] A challenge with high-dose melphalan (HDM) is the high variability in exposures (e.g., a fivefold range in plasma area under the curve (AUC; ~5–24 mg*h/L) among patients who are dosed at the standard 200 mg/m^2[6]. Hence, excessive toxicities experienced by some patients receiving ASCT may be due to overdosing, whereas lack of durable response in other patients may be due to underdosing.

Prolonged severe neutropenia is one of the major adverse effects and dose-limiting toxicities of HDM, which in some patients leads to serious infections and other complications.[6,7] Granulocyte-colony stimulating factor (G-CSF) is given to restore circulating neutrophils after chemotherapy-induced neutropenia in MM, although the timing for starting G-CSF after transplant varies among institutions.[8] Recent studies conducted by our group and prior studies by others have also demonstrated that the G-CSF regimen can significantly influence the duration of neutropenia following ASCT.[9,10]

Since the introduction of HDM in ASCT, only minor adjustments have been made to the overall dosing regimen, and all patients receive either 200 mg/m^2 or 140 mg/m^2 depending on significant medical comorbidities,[11] despite numerous studies reporting the variability in response and adverse outcomes. Although previous, separate studies have been successful at modeling melphalan pharmacokinetics (PKs) and some adverse events caused by ASCT, these approaches have thus far not been combined to evaluate potential improvement in HDM and ASCT regimens.

Among a number of semimechanistic mathematical models proposed to describe neutrophil kinetics after treatment with chemotherapies, the model by Friberg *et al.*[12] is well established and has been applied to various chemotherapeutic agents. Furthermore, several approaches have been proposed to incorporate the impact on neutropenia from G-CSF after primary chemotherapy.[13–16] However, these approaches have not yet been evaluated in HDM/ASCT.

Other groups have also demonstrated *p53* accumulation, and the induction of apoptosis upon melphalan *ex vivo* treatment is associated with clinical response to melphalan.[17,18] This finding suggested that *p53* function and cell proliferation level may serve as predictors for adverse outcomes caused by differential sensitivity to HDM across a population of patients with MM.

The aim of this study was to integrate melphalan PKs and neutrophil kinetics into a single, semimechanistic model capable of describing the neutrophil time course resulting from HDM/ASCT followed by G-CSF in patients with MM. The resulting PK/PD model incorporating covariates that significantly influence interindividual variability (IIV) is proposed as a potentially useful tool for personalizing HDM/ASCT.

METHODS

Population, drug treatment, and data collection

The clinical and PK portions of this study were recently published.[19] Briefly, blood samples and peripheral blood mononuclear cells (PBMCs) were collected from 119 patients (69 men and 50 women) enrolled on OSU11055 (NCT01653106). This study was approved by the Ohio State University Institutional Review Board and conducted in accordance with the Helsinki Declaration of 1975 (as revised in 1983). All patients received melphalan 140 or 200 mg/m^2 2 days prior to ASCT. The first 42 patients treated on study received G-CSF (filgrastim) daily starting the day after ASCT (day +1), and the remaining 77 patients received G-CSF starting day 7 after ASCT (day +7). Patients also received 12 mg dexamethasone orally within 1 hour prior to melphalan administration (day 0) then 8 mg i.v. once on day 1 then every 12 hours on days 2 and 3. The patient characteristics evaluated within this study are summarized in **Table 1**. Creatinine clearance was calculated by the Cockcroft and Gault equation using total body weight.[20] Missing absolute neutrophil count (ANC) values for nadir (when the total white blood cell (WBC) count was <0.5K/μL and differential yielded no neutrophils) were estimated using the equation ANC = 0.894 WBC. Please see the **Supplementary Methods** for additional details on observed and calculated ANC values.

Population PK model and data integration

PK data were available from 118 patients, from whom 6–8 plasma melphalan concentrations were available for analysis.[19] Melphalan PK data were best described using a two-compartment model with first order elimination from the central compartment, as previously described.[19,21] All PK parameter values were fixed prior to pharmacodynamic (PD) neutropenia modeling.

Ex vivo p53 gene expression response to melphalan and *SLC7A5* genotyping

The *p53* gene expression level upon *ex vivo* melphalan treatment was measured by real-time quantitative polymerase chain reaction after exposing 1.0×10^6 PBMCs to 75 μg/mL melphalan for 24 hours. Detailed methods can be found in the online information.

SLC7A5 genotype (coded as 0 or 1 if patients had AA/AG or GG genotype, respectively) data were collected from PBMCs, as described previously.[19]

PD structural model development

ANC was measured every 24 hours starting on the day of melphalan administration and until the patients' ANC recovered from neutropenia. ANC data from the 118 patients who also had PK data included a median of 16 observations per patient (range 13–24) with a median of 5 (range 2–11; 33%; range 13–50%) of these being missing and replaced using our linear regression equation. ANC data was transformed to approximately normally distributed by Box-Cox transformation with lambda = 0.2 (i.e., $ANC_{transformed} = (ANC^{0.2}-1)/0.2$), as previously described.[22] Several variations of the compartmental neutropenia model previously proposed by Friberg *et al.*[12] were evaluated, including those that incorporated direct G-CSF effect on neutrophil proliferation

Table 1 Summary of patient characteristics

Characteristic	G-CSF regimen		All patients
	Day +1	Day +7	
No. (%)	42 (0.35)	77 (0.65)	119
Gender			
Male (%)	64.0	55.0	58.0
Female (%)	36.0	45.0	42.0
Age			
Mean (SD)	57.5 (8.1)	57.9 (8.0)	57.8 (8.0)
Median (min–max)	57 (40–72)	59 (35–70)	59 (35–72)
Race			
White (%)	88.0	87.0	87.0
Other (%)	12.0	13.0	13.0
Height			
Mean (SD)	1.7 (0.1)	1.7 (0.1)	1.7 (0.1)
Median (min–max)	1.7 (1.4–1.9)	1.7 (1.5–1.9)	1.7 (1.4–1.9)
Weight			
Mean (SD)	87.7 (20.9)	84.8 (17.2)	85.8 (18.6)
Median (min–max)	84.5 (45.4–145.3)	84.1 (52.5–120.8)	84.052 (45.4–145.3)
BSA			
Mean (SD)	2.0 (0.2)	1.9 (0.2)	2.0 (0.2)
Median (min–max)	2.0 (1.4–2.5)	2.0 (1.5–2.4)	2.0 (1.4–2.5)
CrCL			
Mean (SD)	89.7 (33.4)	88.34 (34.2)	88.9 (33.8)
Median (min–max)	92.6 (12.1–165.8)	91.3 (5.3–195.8)	91.7 (5.3–195.8)
FFM			
Mean (SD)	58.4 (12.1)	56.0 (12.7)	56.8 (12.5)
Median (min–max)	60.1 (31.3–81.9)	58.7 (33.5–78.1)	60.0 (31.3–81.9)
STEM			
Mean (SD)	5.3 (2.2)	4.4 (2.1)	4.7 (2.2)
Median (min–max)	4.7 (2.5–11.7)	3.9 (1.9–15.7)	4.2 (1.9–15.7)
Baseline HCT			
Mean (SD)	32.7 (4.9)	32.2 (4.9)	32.4 (4.9)
Median (min–max)	33.7 (23.0–40.7)	31.9 (20.6–44.6)	32.5 (20.6–44.6)
Baseline WBC			
Mean (SD)	5.7 (2.3)	5.6 (3.2)	5.6 (2.9)
Median (min–max)	5.0 (2.5–11.4)	4.7 (1.7–18.3)	4.9 (1.7–18.3)
Baseline ANC			
Mean (SD)	4.0 (2.1)	4.0 (2.8)	4.0 (2.6)
Median (min–max)	3.6 (0.8–9.1)	3.1 (0.7–13.8)	3.2 (0.7–13.8)
Baseline hem			
Mean (SD)	11.0 (1.6)	11.0 (2.8)	11.0 (2.5)
Median (min–max)	11.3 (8.0–13.9)	10.8 (7.0–31.1)	10.9 (7.0–31.1)
Baseline platelets			
Mean (SD)	223.5 (80.3)	179.7 (64.3)	195.2 (73.1)
Median (min–max)	217.5 (69.0–420.0)	175.0 (41.0–383.0)	188.0 (41.0–420.0)
Baseline BUN			
Mean (SD)	15.2 (10.2)	16.4 (9.3)	16.0 (9.6)
Median (min–max)	13.0 (5.0–53.0)	15.0 (5.0–59.0)	14.0 (5.0–59.0)
Baseline bicarbonate			
Mean (SD)	26.1 (2.0)	26.7 (2.2)	26.5 (2.1)
Median (min–max)	26.0 (21.0–30.0)	27.0 (21.0–33.0)	27.0 (21.0–33.0)
Baseline SeCR			
Mean (SD)	1.2 (1.0)	1.4 (2.0)	1.3 (1.7)
Median (min–max)	0.9 (0.3–6.2)	0.8 (0.4–14.5)	0.8 (0.3–14.5)

(*Continues*)

Table 1 (Continued)

Characteristic	G-CSF regimen		All patients
	Day +1	Day +7	
Baseline C-reactive protein			
Mean (SD)	5.1 (6.5)	6.1 (10.3)	5.7 (9.1)
Median (min–max)	3.3 (0.0–36.0)	2.8 (0.3–56.3)	3.0 (0.0–56.3)
Baseline albumin			
Mean (SD)	3.5 (0.5)	3.5 (0.5)	3.5 (0.5)
Median (min–max)	3.4 (2.4–4.6)	3.5 (2.2–4.7)	3.5 (2.2–4.7)
Baseline bilirubin (total)			
Mean (SD)	0.5 (0.2)	0.6 (0.3)	0.6 (0.2)
Median (min–max)	0.5 (0.2–1.0)	0.5 (0.2–1.5)	0.5 (0.2–1.5)
ln *p53* fold change[a]			
Mean (SD)	3.0 (1.0)	2.6 (0.8)	2.7 (0.9)
Median (min–max)	2.8 (1.8–5.1)	2.5 (0.8–5.8)	2.6 (0.8–5.8)
SLC7A5 rs_4240803			
m/m or m/M	22.0	33.0	55.0
M/M	13.0	37.0	50.0
Missing	7.0	7.0	14.0

Characteristics of patients and variables evaluated as covariates are presented in the table for all patients and for both groups receiving daily G-CSF beginning on day +1 or day +7 after transplantation. Age (years); body weight (kg); CrCL (mL/min); FFM (kg); STEM ($\times 10^6$ cells); height (m), BSA (m^2); HCT (%); WBC ($\times 10^9$ cells/μL); ANC ($\times 10^9$ cells/μL); hem (g/dL); BUN (mg/dL); SeCR (mg/dL); platelets ($\times 10^3$/μL); bicarbonate (mEq/L); C-reactive protein (mg/dL); albumin (g/dL); and bilirubin (mg/dL). For SLC7A5 genotype, M, major allele and m, minor allele.
ANC, absolute neutrophil count; BSA, body surface-area; BUN, blood urea nitrogen; CrCL, creatinine clearance; FFM, fat-free mass; G-CSF, granulocyte-colony stimulating factor; HCT, hematocrit; hem, hemoglobin; SeCR, serum creatinine; STEM, stem cell dose; WBC, white blood cells.
[a]Quantifiable values for ln *p53* fold change were available from 90 patients total. Therefore, the numbers presented in this table reflect a summary of 23 and 67 values for day +1 and day +7 G-CSF regimens, respectively.

and maturation.[16] The final neutropenia model, depicted in **Figure 1**, comprised PD parameters to describe proliferation of cells, delayed PD effect through transition to the observed neutrophils, elimination of circulating neutrophils, and feedback from circulating neutrophils to proliferation rate constant (k_{prol}). The k_{prol} was assumed equal to the transition rate constant between transit compartments (k_{tr}), which was defined as 4/mean transit time (MTT) in this three-compartment transit model, where MTT is the mean transit time of neutrophils. The elimination rate constant of neutrophils (k_{circ}), in which $k_{circ} = \frac{\ln(2)}{\text{neutrophils half-life}}$ was fixed by the reported neutrophil half-life of 7 hours.[16,23] The feedback mechanism was described by $(Circ_0/Circ)^{\gamma}$, the ratio between estimated baseline ANC ($Circ_0$ or BASE) and circulating ANC (Circ) at a given time with γ parameter by G-CSF regulation. The marginated pool compartment was added to directly flow neutrophils into the circulating ANC compartment without a delay. Input BASE was estimated as the baseline of total neutrophils within the marginated pool compartment. The input rate constant (k_{in}), which was defined as 1/estimated input transit time (ITT), was estimated.

The IIV of PD parameters was defined as $\theta_i = \theta \times \exp(\eta_i)$, in which η_i indicates the deviation between the true PD parameter of individual *i* (θ_i) and the typical population value (θ) with a distribution of mean zero and variance ω^2.

Covariate analysis

Single covariates to explain IIV in PD parameters were considered first. Covariates having significant influence ($P < 0.05$) were then added in a forward stepwise manner,

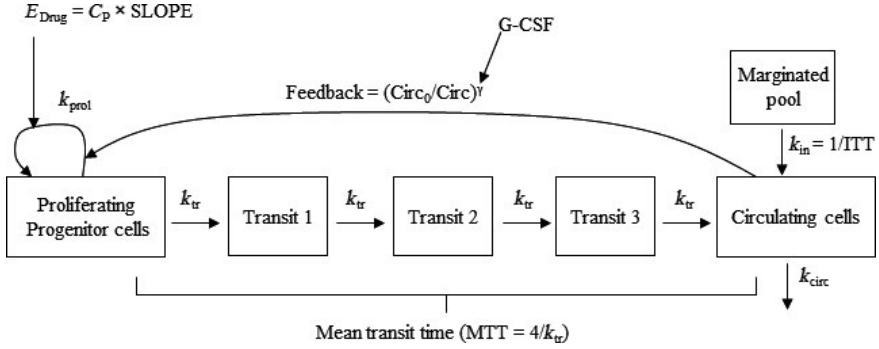

Figure 1 Scheme of semimechanistic pharmacokinetic/pharmacodynamic model for neutropenia after melphalan dosing. G-CSF, granulocyte-colony stimulating factor; ITT, input transit time; MTT, mean transit time; SLOPE, proportionality constant defining the relationship between plasma melphalan concentration (C_p) and drug effect (E_{Drug}).

Table 2 Population parameter estimates from the initial structural model, the final covariate model, and the 200 bootstrap runs.

	Structural model			Final model			Bootstrap	
	Estimate	RSE, %	IIV, CV% (% shrinkage)	Estimate	RSE, %	IIV, CV% (% shrinkage)	Estimate median (95% CI)	IIV, CV% median (95% CI)
BASE (K/μL)	5.69	4.5	35.1 (11.5)	5.61	4.7	34.4 (59.6)	5.62 (5.17–6.01)	33.9 (29.4–39.2)
SLOPE (mL/μg)	11.3	4.4	33.3 (12.1)	7.46	7.4	25.1 (18.3)	7.48 (6.67–8.99)	24.2 (19.0–29.4)
MTT (hours)	106	2.4	10.7 (11.5)	97	2.5	6.6 (22.7)	96.7 (92.56–101.00)	6.3 (4.3–7.7)
γ	0.221	2.3	–	0.218	2.3	–	0.218 (0.206–0.230)	–
ANC half-life (hours)	7 FIX	–	–	7 FIX	–	–	7 FIX	–
Input BASE (K/μL) for group 1	106	12.5	49.5 (47.8)	114	11.6	43.5 (50.0)	115 (95.57–133.95)	40.1 (19.0–67.3)
Input BASE (K/μL) for group 2	0.183	55.7		0.0682	142.7		0.0722 (0.007–0.168)	
ITT (hours)	14	5.5	–	14.6	5.2	–	14.6 (13.95–15.45)	–
ε (additive)	0.24	1.7	–	0.242	1.8	–	0.242 (0.203–0.282)	–

For s, estimates are represented as SDs. Groups 1 and 2 represent patients who received granulocyte-colony stimulating factor beginning day +1 and day +7, respectively.
ANC, absolute neutrophil count; BASE, baseline ANC; CI, confidence interval; CV%, coefficient of variation; FIX, ANC half-life was fixed to 7 hours; IIV, inter-individual variability; ITT, input transit time; MTT, mean transit time; RSE, relative standard error; SLOPE, proportionality constant defining the relationship between plasma melphalan concentration (C_p) and drug effect (E_{Drug}).

until no significant reduction in objective function value (OFV) was observed. Backward elimination from the full PD model was then performed. The *P* values of 0.05 and 0.01 were used in forward addition and backward elimination, respectively. The model was evaluated by the difference in OFV (ΔOFV), goodness of fit plots, and standard error and shrinkage of parameter estimates.

Model evaluation

Evaluation of the final model on ANC prediction and duration of grade 4 neutropenia prediction were performed. Two hundred (200) bootstrap runs were completed to evaluate the accuracy and stability of the final model,[24] and 95% confidence intervals of all parameters from the bootstrap replicates were evaluated in comparison to parameter estimates from the final neutropenia model. Simulation (n = 1,000) was performed to evaluate the prediction performance of the final neutropenia model using visual predictive check (VPC).[25] For observed and simulated durations of severe neutropenia (DOSN), we estimated the times at which ANC fell below and rose above 500 neutrophils per microliter using a straight line to connect the ANC above and below the cutoff threshold. The DOSN was simply determined by the difference in estimated time from when ANC rose above and fell below 0.5 K/uL.

Software and statistical analysis

Population pharmacokinetic/pharmacodynamic (PK/PD) analysis of melphalan was performed using NONMEM 7, version 7.3.0 (ICON Development Solutions; Ellicott City, MD). ADVAN 6 and first-order conditional estimation with interaction were used in the model development. The ΔOFV >3.84 were considered in model development and covariate stepwise selection, indicating statistical significance ($P < 0.05$, degree of freedom = 1) by the log likelihood ratio test for nested models. Graphic analysis was performed using R version 3.3.2 (The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Structural PK/PD model

The characteristics of the 118 patients with MM in this trial are summarized in **Table 1** and in our previous publication.[19] The semimechanistic neutropenia model capable of describing the ANC time course after melphalan and G-CSF dosing was modified from the previous models by Friberg *et al.*,[12] and most closely mimicked the models presented by Ozawa *et al*.[15] and Soto *et al*.,[26] as shown in **Figure 1**. The structural PK/PD model was first developed using first-order conditional estimation with interaction. Estimated population PD base model parameters are listed in **Table 2**. Drug effect (E_{Drug}) was converted by a linear slope model from plasma melphalan concentration (C_P) in the PK central compartment, described as $E_{Drug} = C_P \times$ SLOPE where SLOPE is an estimated parameter.

Regulation of neutrophils was achieved by a feedback loop via endogenous and exogenous G-CSF, which ultimately affected neutrophil dynamics. Despite many attempts to model the observed data with only G-CSF modulation of neutrophil proliferation and transit, a marginated pool compartment was ultimately required to explain both rapidly increasing ANC after transplant and G-CSF administration followed immediately by rapid decline of circulating neutrophils. Differential equations describing melphalan PD effects on ANC are provided in **Supplementary Methods**.

Effect of *ex vivo* melphalan exposure on *p53* mRNA level in untreated PBMCs

The *p53* mRNA level was measured *ex vivo* to test if variability among patients in *p53* gene expression response to melphalan-induced DNA damage correlated with variability in neutropenia or other outcomes. The *p53* relative gene expression level ($2^{-\Delta\Delta CT}$) in 91 patients' PBMCs was 7.9 ± 7.6 (mean ± SD) without melphalan treatment and increased to 19.51 ± 13.3 with 75 μg/mL *ex vivo* melphalan treatment. Because the baseline level of *p53* mRNA varied among patients, *p53* gene expression level was normalized to the

individual baseline level. The fold change of the *p53* gene expression level in 90 patients' PBMCs was 2.70 ± 0.90 (mean ± SD), with 46 patients falling between 0.5 and 2 and 44 patients >2 (a twofold change or greater was considered significant). The log normally distributed *p53* fold change was screened in covariate analysis.

Final PK/PD model

Individual covariates (**Table 1**) were first screened, and significant covariates having $P < 0.05$ were considered for subsequent analysis (**Table S1**). Following step-wise regression, forward addition/backward elimination identified the significant covariates in the final model: hematocrit on BASE, sex, hematocrit, and G-CSF on SLOPE, G-CSF, stem cell dose, race, and creatinine clearance on MTT, and *p53* fold change on Input BASE (**Table 3**). Note the G-CSF covariate was a dichotomous variable for G-CSF regimen (either starting on day +1 or day +7) and was distinct from the way G-CSF effects were incorporated into the structural model both as an estimated exponent, γ, which modulated feedback on neutrophil dynamics, and as a switch to turn on neutrophil flow (via K_{in}) from the marginated pool compartment once the G-CSF dosing was started. The estimated model parameters are displayed in **Table 2**. The final PK/PD model incorporating the nine covariates reduced the IIV of PD parameters: 2%, 25%, 38%, and 12% of BASE, SLOPE, MTT, and input BASE, respectively, in which percent difference was calculated by $\frac{IIV_{Structuralmodel}-IIV_{Finalmodel}}{IIV_{Structuralmodel}}\times 100$. The final model OFV was reduced by 148.407 compared to the base model.

Model evaluation for ANC prediction

General fit of ANC data was assessed using diagnostic plots, and the appropriateness and stability of the final model was evaluated using bootstrap resampling and simulation. Diagnostic plots displayed in **Figure 2a,b** demonstrate individual and population predicted vs. observed Box-Cox transformed ANC data for all patients agree reasonably well. The PD parameters 95% confidence intervals from the 200 bootstrap replicates were comparable to parameter estimates from the final model and did not contain the Null (**Table 2**). One thousand datasets were simulated to evaluate prediction performance of the final model. The 95% confidence interval of observed data was mostly included in the 95% prediction interval of simulated data, and the medians of simulated and observed data were comparable (**Figure 3a**). When separated by G-CSF regimen, predicted ANC vs. time and VPC data seemed to match the distinctly different profiles between the two groups (**Figure 3b,c**). We note that the prediction interval (gray shaded area) around the nadir is broad, which is likely related to the limited observed data in this region along with our replacement of missing data with empirically calculated, simulated data. Additional diagnostic plots (predicted (PRED) and individual predicted (IPRED) vs. observed (OBS)) are shown in **Figure S1** for all patients and those receiving G-CSF on day +1 vs. day +7.

Prediction of duration of severe neutropenia

Durations of severe neutropenia obtained from observed and predicted neutrophil-time profiles were compared. Both individual (median 5.02, range 2.48–7.76 days) and population (5.14, 2.75–6.53 days) predictions (i.e., durations calculated from individual and population ANC profile predictions for each individual) were similar in central tendency to observations in all patients (5.69, 2.88–9.64; with one outlier removed who had 13.23 days of severe neutropenia; see **Figure 2c,d** and **Figure S2**). Both individual and population predictions tended to underpredict DOSN (medians of 12% and 10% underprediction, respectively, across all patients) and range of DOSN (**Table 4**). Notably, observed DOSN was significantly lower in patients receiving G-CSF starting day +1 after transplant (median 4.20, range 2.88–6.48 days) vs. those receiving G-CSF starting day +7 (6.32, 4.41–9.64), and the final model successfully distinguished effects of the two different G-CSF regimens on DOSN. The median in individual and population predicted durations of severe neutropenia were 3.79 and 4.00 days, respectively, vs. 4.20 days observed for patients receiving G-CSF day +1 and 5.52 and 5.45 days, respectively, vs. 6.32 days observed in patients receiving G-CSF day +7 (**Table 4**).

Following evaluation of the final PD model, a series of dose simulations (1,000 replicates) were performed for each individual. Melphalan doses were simulated at five different

Table 3 Stepwise selection of covariates in the neutropenia model

Run		OFV	ΔOFV	*P* value
Forward addition				
1.	Base model	1557.321	–	
2.	1+ G-CSF on MTT	1511.194	46.127	< 0.0001
3.	2+ STEM on MTT	1500.869	10.325	< 0.01
4.	3+ G-CSF on SLOPE	1470.694	30.175	< 0.0001
5.	4+ Race on MTT	1456.228	14.466	< 0.001
6.	5+ Creatinine clearance on MTT	1442.914	13.314	< 0.001
7.	6+ Sex on SLOPE	1432.634	10.28	< 0.01
8.	7+ Hematocrit on SLOPE	1423.338	9.296	< 0.01
9.	8+ *p53* fold change on Input BASE	1416.426	6.912	< 0.01
10.	9+ Hematocrit on BASE	1408.914	7.512	< 0.01
	Full model	1408.914	–	
Backward deletion				
11.	– G-CSF on MTT	1452.489	43.575	< 0.0001
12.	– STEM on MTT	1422.605	13.691	< 0.001
13.	– G-CSF on SLOPE	1446.492	37.578	< 0.0001
14.	– Race on MTT	1423.76	14.846	< 0.001
15.	– Creatinine clearance on MTT	1422.459	13.545	< 0.001
16.	– Sex on SLOPE	1418.855	9.941	< 0.01
17.	– Hematocrit on SLOPE	1420.481	11.567	< 0.001
18.	– *p53* fold change on Input BASE	1415.843	6.929	< 0.01
19.	– Hematocrit on BASE	1416.426	7.512	< 0.01

Covariates that met the cutoff for forward addition ($P < 0.05$) and backward deletion ($P < 0.01$) are shown in the table. Note that in addition to sex and race, G-CSF is a dichotomous categorical variable indicating that patients either started G-CSF on day +1 or day +7.
G-CSF, granulocyte-colony stimulating factor; MTT, mean transit time; OFV, objective function value; ΔOFV, difference of objective function value; SLOPE, the proportionality constant between plasma melphalan concentration and drug effect; STEM, stem cell dose.

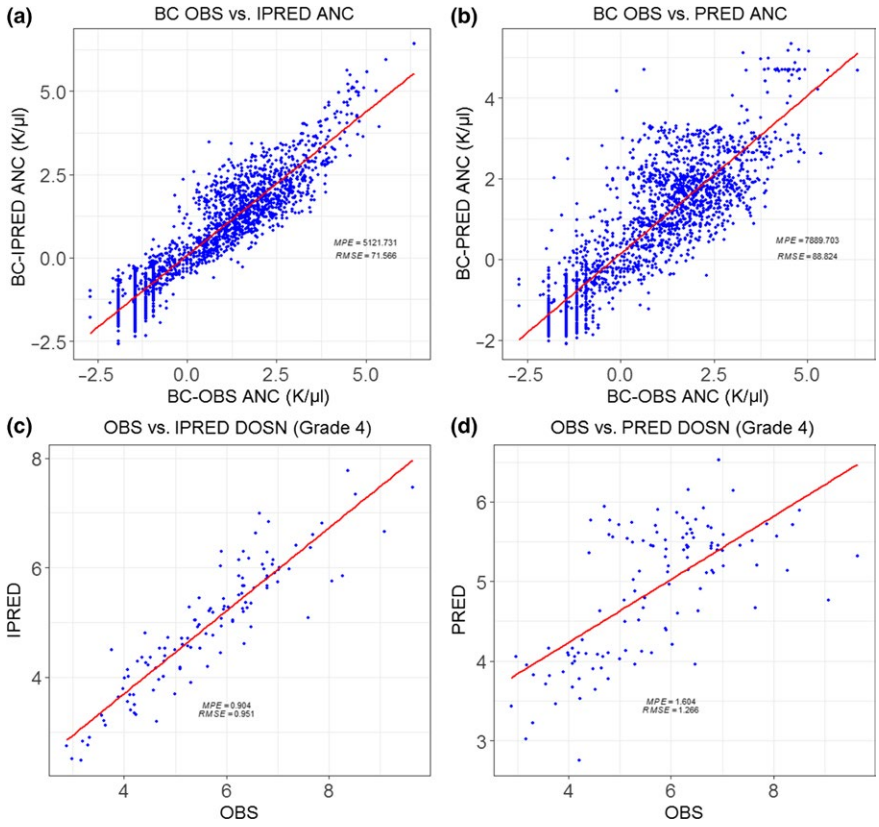

Figure 2 Diagnostic plots of predicted (PRED) vs. observed (OBS) absolute neutrophil count (ANC) and duration of severe neutropenia. The data shown include individual predicted (IPRED, **a**) and population PRED, (**b**) vs. OBS Box-Cox (BC) transformed ANC along with IPRED vs. OBS, (**c**) and PRED vs. OBS, (**d**) duration of severe, grade 4 neutropenia (DOSN). The straight line is a linear regression of the data in each plot. Mean percentage error (MPE) and root mean square error (RMSE) are also displayed.

levels in the range of 100–300 mg/m^2 in four individual patients (two each for G-CSF day +1 and day +7), and the individualized DOSN was calculated. Resulting ANC profiles and corresponding predicted durations of severe neutropenia for these five dose levels are displayed in **Figure S3** for four representative individuals and for all patients broken out into those with G-CSF starting on day +1 vs. day +7. These results demonstrate the range of expected DOSN across patients receiving the same dose and also within each patient who may receive doses in the range of 100–300 mg/m^2, which represents the range of HDM doses previously administered to patients undergoing ASCT.[27–31]

DISCUSSION

Traditionally, drug development has aimed at identifying the single "best" dose, "one size fits all" medicine, based on average responses to care. However, all patients do not respond to drug therapy in an equal and desirable manner.[32] Individual variability in drug response may be attributed to several sources, including genetic variation, environmental factors, and physiological characteristics.[33] Furthermore, response to chemotherapeutic agents can vary among individuals due to tumor heterogeneity.[34] Indeed, although a standard dose of melphalan is an effective chemotherapy in most patients with MM undergoing ASCT,[4,5] toxicities can be severe in some patients, whereas other patients have minimal or short-lived response.[6] Therefore, identifying the factors that influence drug exposure and outcomes, integrating these factors into a PK/PD model, and utilizing this model to identify safe and effective doses may be a viable strategy for personalizing HDM therapy in ASCT.

Our preliminary PD modeling in the setting of HDM, ASCT, and G-CSF was first carried out by adapting the neutropenia model developed by Friberg *et al.*[12] However, the model was insufficient for describing our data, primarily due to the model's inability to capture observed differences in ANC between the two different G-CSF regimens. Perhaps the most distinguishing feature of our ANC data is the spike in circulating neutrophils observed on day 4 in the patients who received G-CSF on day +1 after transplantation (i.e., G-CSF given on day 3 after melphalan dosing), but not in those starting G-CSF on day +7 (see **Figure 3b,c**). This spike represents nearly a fivefold increase in median ANC when comparing day +1 to day +2 (5.3, range 1.8–14, 1×10^9 cells/μL day +1 vs. 24.4, range 0.9–60, 1×10^9 cells/μL), which is similar to what has been observed in other studies with G-CSF administration.[10,35,36] Others have also demonstrated increased ANC resulting from glucocorticoid administration, although the magnitude of the increase was much lower.[15,37] Patients in our study received dexamethasone

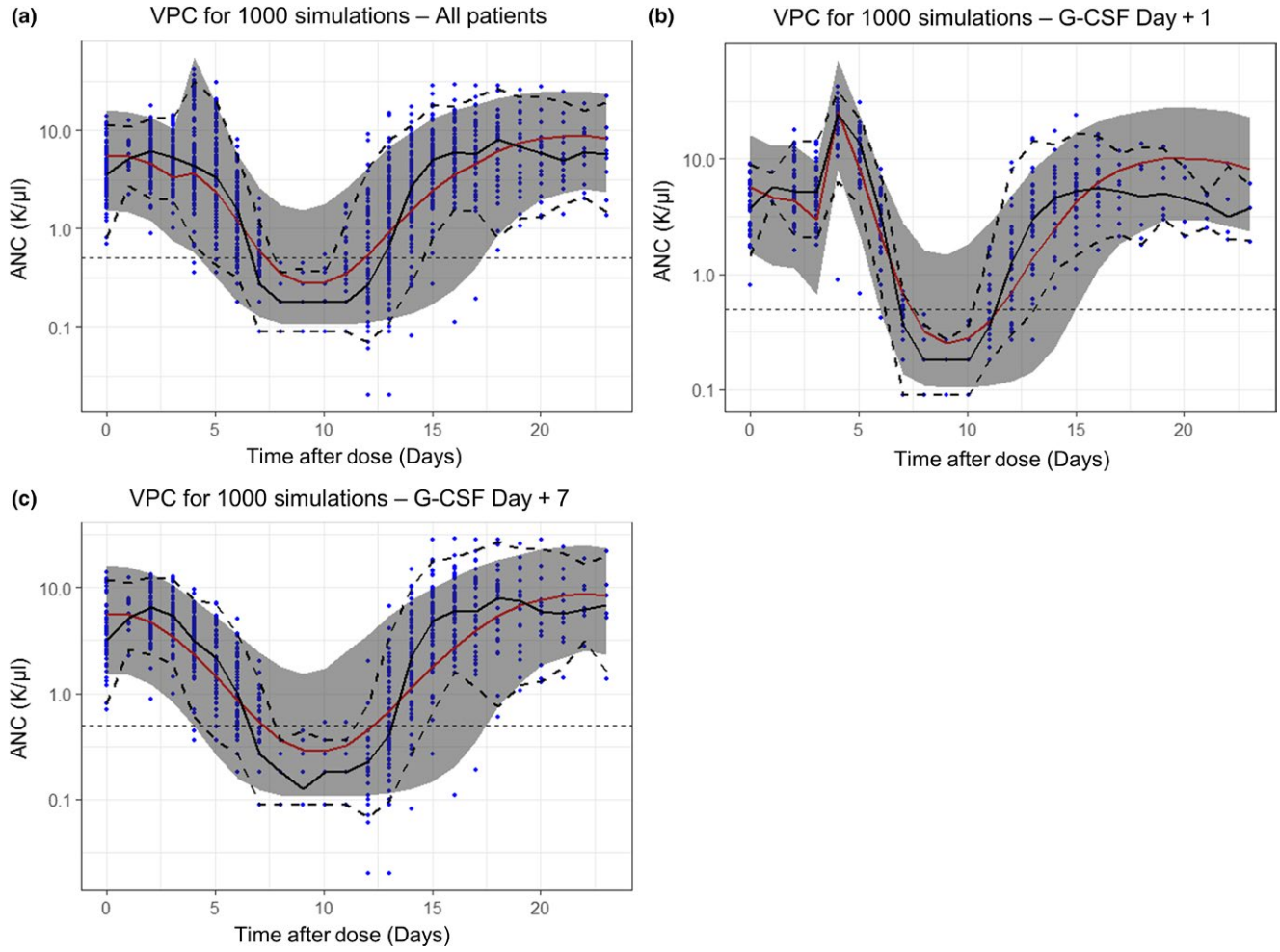

Figure 3 Visual predictive check (VPC) plot of the final model simulated data vs. observed data in (**a**) all patients, (**b**) with granulocyte-colony stimulating factor (G-CSF) regimen starting on day +1, and (**c**) with G-CSF regimen starting on day +7 after transplantation. Blue dots, the observed data; black dashed line, 2.5th and 97.5th percentiles of the observed data; black solid line, the median of the observed data; red solid line, the median of the simulated data; gray area, 95% prediction interval of the simulated data; black dashed straight line, absolute neutrophil count (ANC) = 0.5 K cells/µL. Note the 95% prediction interval was truncated at the lower limit of quantification (LLOQ) for ANC (100 cells/uL) and that within the time region of severe neutropenia (days ~5–15) the observed data includes both measured and calculated values, some of which fall below the LLOQ for ANC.

on days 0–3, and we did notice a modest increase in ANC between day 0 and day 2 followed by a slight decrease on day 3 (**Figure 3**). We did, in fact, attempt to incorporate into the model a dexamethasone effect on both endogenous G-CSF and on ANC. However, given the modest observed effect of dexamethasone relative to G-CSF, the fact that dexamethasone was stopped on day 3, and our lack of measured G-CSF plasma concentrations, we ultimately did not include a corticosteroid effect on ANC within our model.

Modified neutropenia models were previously proposed in order to explain the feedback mechanism incorporating endogenous G-CSF level,[13,16] and we had evaluated different versions of these, including the model by Quartino *et al.*[16] In fact, among other models attempted and despite our lack of measured G-CSF levels, we evaluated use of the model presented by Quartino *et al.*,[16] as it offered the potential to model the day +2 ANC spike observed in our dataset after G-CSF day +1 as a surge through the transit proliferation/maturation compartments. Use of this model required us to integrate their corticosteroid-induced G-CSF production with our exogenous G-CSF dosing to feed into the circulating G-CSF compartment. Because we did not have measured G-CSF levels, we adopted their published parameter values for our model. Not too surprisingly, this model did not perform well with our data (see **Figure S4**). Although the lack of measured G-CSF levels and the need to adopt fixed parameter values from the Quartino *et al.*[16] study to fit our dataset most likely contributed to the relatively poor performance of this model, we did learn from this and other attempted models that the observed spike in ANC on day +2 in patients who received G-CSF on day +1 could not be achieved with this approach. Again, this made sense given that the ~1-day delay in the ANC spike needed to occur immediately prior to the much slower ~5-day delay of HDM-induced neutropenia, even though G-CSF was given

Table 4 Summary of the OBS, population PRED, and IPRED durations of severe neutropenia in all patients, in patients receiving G-CSF starting on day +1, and in patients receiving G-CSF starting on day +7

	OBS (days)	PRED (days)	Diff (PRED-OBS) (days)	% Diff ((PRED-OBS/OBS) *100%)	IPRED (days)	Diff (IPRED-OBS) (days)	% Diff ((IPRED-OBS/OBS) *100%)
All patients							
Mean	5.64	4.89	−0.75	−13%	4.94	−0.70	−12%
SD	1.36	0.81	−0.55	−40%	1.13	−0.23	−17%
Median	5.69	5.14	−0.55	−10%	5.02	−0.67	−12%
Range	2.88–9.64	2.75–6.53	−4.32–1.34	−48–36%	2.48–7.76	−2.50–0.75	−33–20%
G-CSF day +1 only							
Mean	4.35	3.92	−0.43	−10%	3.83	−0.52	−12%
SD	0.83	0.36	−0.47	−57%	0.72	−0.11	−13%
Median	4.20	4.00	−0.21	−5%	3.79	−0.41	−10%
Range	2.88–6.48	2.75–4.63	−2.52–1.08	−39–36%	2.48–5.35	−1.45–0.75	−31–20%
G-CSF day +7 only							
Mean	6.34	5.41	−0.93	−15%	5.55	−0.79	−12%
SD	1.04	0.40	−0.64	−61%	0.81	−0.23	−22%
Median	6.32	5.45	−0.87	−14%	5.52	−0.80	−13%
Range	4.41–9.64	4.41–6.53	−4.32–1.34	−48–30%	3.90–7.76	−2.50–0.40	−33–9%

Note the data in this table do not include one outlier patient with a 13.23-day duration of neutropenia (in the day +7 regimen).
% Diff, percent difference between PRED and OBS ((PRED-OBS/OBS)*100%) or between IPRED and OBS ((IPRED-OBS/OBS)*100%); Abs. Diff, absolute difference between PRED and OBS (PRED-OBS) or between IPRED and OBS (IPRED-OBS); IPRED, individual predicted duration of severe neutropenia; OBS, observed duration of severe neutropenia; PRED, population predicted duration of severe neutropenia.

3 days after HDM; ultimately, we learned this combination could not be achieved with the single transit model for neutrophil proliferation/maturation.

To adequately characterize the ANC spike caused from rapid influx of neutrophils into circulation from exogenous G-CSF dosing, we adopted the model first presented by Ozawa *et al*.[15] and later used by Soto *et al*.[37] who demonstrated the incorporation of an input compartment could achieve the rapid influx of neutrophils to adequately describe the more modest increase in ANC caused by corticosteroid administration. However, in our model, we utilized the input compartment (now termed marginated pool) as a reservoir for neutrophils to rapidly transition into circulation after G-CSF dosing, similar to what was presented by Roskos *et al.*,[38] and more recently by Ho *et al*.[14] and Melhem *et al*.[39] The marginated pool of granulocytes and neutrophils is a well-established "compartment" that represents a reserve of neutrophils known to respond and demarginate rapidly into circulation with increased G-CSF and other stimuli.[40] The marginated pool compartment enabled the rapid increase in neutrophils after G-CSF administration, which was not achievable by assuming that G-CSF only increases the rates of proliferation and maturation of neutrophils through the transit compartments.

Another unique aspect of our dataset and model is the two different G-CSF regimens (starting either on day +1 or day +7 after transplant). Incorporation of a feedback loop in the Friberg *et al.*[12] model was necessary to describe the regulation of neutrophils via the G-CSF level. However, because we did not have G-CSF plasma levels, in our model, the estimated feedback parameter, γ, was used more generically as a single term to modulate both the endogenous and exogenous G-CSF effect on neutrophil proliferation/maturation. In addition, the timing of the start of G-CSF administration was used as a switch to "turn on" K_{in}, which was the rate constant for flow of neutrophils from the marginated pool compartment into circulation. Beyond the feedback mechanism and switch for K_{in}, the influence of different G-CSF regimens on neutropenia after chemotherapy was further highlighted by its significance and inclusion as a covariate on PD parameters, as described in other studies.[41–43] In our model, we evaluated a dichotomous variable that represented the two G-CSF regimens, and this was ultimately chosen as a significant covariate on both MTT and SLOPE and influenced feedback regulation on ANC and chemotherapy-induced neutropenia. It seemed counterintuitive that G-CSF regimen could impact SLOPE because G-CSF was started either 3 or 9 days after HDM. However, the starting time for G-CSF administration clearly has an effect on both the time at which neutrophils fall below 500/uL and also on the duration of severe neutropenia. Therefore, the later timing of G-CSF administration essentially makes the neutrophils seem to be more sensitive to melphalan because there is less of a G-CSF effect to be had in this case (i.e., earlier entry into and longer duration of severe neutropenia resulting from melphalan in the day +7 relative to the day +1 groups). In summary, the timing of the start of G-CSF administration was an important factor that showed up in multiple places within our final model.

We also identified other covariates that significantly influenced ANC profile after melphalan dosing, which were stem cell dose, hematocrit, sex, race, *p53* fold change, and creatinine clearance. Use of hematocrit and *p53* fold change as covariates helped to improve prediction of the BASE and Input BASE parameters, respectively. Low baseline hematocrit value was previously reported as a risk factor for neutropenia after chemotherapy in lung cancer, which was consistent with our model results.[44] Interestingly, hematocrit was also a covariate in our PK model, which was consistent with the melphalan PK model published previously by Nath *et al.*[21]

In response to DNA damage, *p53* tumor suppressor protein, encoded by *TP*53, could be stimulated to induce DNA repair or apoptosis.[45] Due to *p53* abnormalities in MM,[46] the cellular activity in response to stress could vary among patients. Therefore, *p53* expression after melphalan exposure is a potential biomarker corresponding to clinical response to melphalan,[17,47] which is in agreement with our modeling results. Furthermore, previous articles reported gender[48,49] and ethnicity[48,50] as risk factors for neutropenia after other chemotherapies. Our model also indicates that gender and race could explain portions of IIV for SLOPE and MTT, respectively.

With respect to our model's ability to accurately predict DOSN after ASCT with HDM, the model performed well overall as was demonstrated in the diagnostic plots for all patients (**Figure 2,c,d**), VPCs (**Figure 3**), and summaries of model performance (**Table 4**). However, despite the overall generally good performance and the ability of the model to fit observed data, regardless of when G-CSF starts, we do point out that the model will not be useful in predicting DOSN in patients receiving G-CSF starting on day +7 (**Figure S5,f**). Based on data from this trial, we had previously concluded that G-CSF administration needs to start on day +1 after ASCT due to the prolonged DOSN observed when G-CSF was started later.[9] Therefore, we would not anticipate using the model for G-CSF started later than day +1. Nonetheless, this highlights that the model may not be applied generally across different G-CSF dosing regimens and needs additional modification that will require additional data, such as prospective gathering of G-CSF levels in the HDM, ASCT setting to better understand how ANC responds to HDM, both endogenous and exogenous G-CSF, and corticosteroids in patients with MM undergoing ASCT.

In conclusion, a population PK/PD model for HDM in patients with MM undergoing ASCT followed by G-CSF was developed. The newly developed PK/PD model combined previously published neutropenia models by incorporating a marginated pool compartment and a separate gamma factor on the G-CSF feedback loop. This model is expected to enable prediction of ANC profiles and DOSN in patients with MM undergoing HDM in ASCT with G-CSF starting day +1 after transplantation. Further, prospective studies and data will be needed to refine the model for more generalized use with different G-CSF regimens.

Supporting Information. Supplementary information accompanies this paper on the *CPT: Pharmacometrics & Systems Pharmacology* website (www.psp-journal.com).

Table S1. Individual Covariates with significant influence on PD parameters ($p<0.05$)

Figure S1. Diagnostic plots of predicted vs. observed ANC and duration of severe neutropenia.

Figure S2. Observed vs. population predictions (PRED) for duration of severe neutropenia for all patients.

Figure S3. Dosing simulation.

Figure S4. VPC plots of the model presented by Quartino and colleagues (Pharm Res (2014) 31:3390–3403) applied to our dataset.

Figure S5. Observed vs. population predictions for duration of severe neutropenia (DOSN).

Funding. This research was supported by Multiple Myeloma Opportunities for Research and Education (MMORE), a Pelotonia IDEA award (46050-502048), the Ohio State University Comprehensive Cancer Center Core Grant (P30 CA016058), and an Eli-Lilly fellowship. The content is solely the responsibility of the authors and does not represent the official views of the National Cancer Institute or the National Institutes of Health.

Conflict of Interest. The authors declared no competing interests for this work.

Author Contributions. Y.C., D.J.I., and M.A.P. wrote the manuscript. C.C.H., M.P., and M.A.P. designed the research. Y.C., J.L., D.W., and A.E.R. performed the research. Y.C., D.J.I., J.L., M.B., A.D., D.R.M., S.F., E.D.H., and M.A.P. analyzed the data.

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