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blood advances

Eculizumab precision-dosing algorithm for thrombotic microangiopathy in children and young adults undergoing HSCT

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Key Points

- This large study examined eculizumab PK/PD in bleeding and nonbleeding patients with TA-TMA.
- PK/PD model-based eculizumab dosing is needed for bleeding in TA-TMA, whereas fixed-dose regimens are effective in nonbleeding patients.

Transplant-associated thrombotic microangiopathy (TA-TMA) is a fatal posttransplant complication of hematopoietic stem cell transplantation. We recently reported that survival for TA-TMA has been improved by early intervention with eculizumab, a complement C5 inhibitor, guided by pharmacokinetic/pharmacodynamic (PK/PD) model-informed precision dosing. However, patients with gastrointestinal bleeding showed poor survival, even when treated with more frequent doses. Our objective was to develop separate models in bleeding and nonbleeding patients with TA-TMA and to propose precision dosing algorithms. Eculizumab PK/PD was analyzed in 19 bleeding and 38 nonbleeding patients (0.5-29.9 years of age). A complement activation biomarker (sC5b-9) and body weight were identified as significant determinants of eculizumab clearance regardless of bleeding. Eculizumab clearance after the first dose was higher in bleeding than in nonbleeding patients (83.8 vs 61.3 mL/h per 70 kg; $P = .07$). The high clearance was maintained over treatment doses in bleeding patients, whereas nonbleeding patients showed a time-dependent decrease in clearance. sC5b-9 levels were highest before the first dose and decreased over time, regardless of bleeding complications. A Monte Carlo Simulation analysis showed that the current dosing protocols recommended for atypical hemolytic uremic syndrome had <15% probability of attaining the target concentration of >100 μg/mL eculizumab in nonbleeding patients. We identified an intensified loading protocol to reach 80% target attainment. Our data clearly showed the need for individualized dosing for patients with significant bleeding and for ongoing dose adjustments to optimize outcomes. The developed models will be incorporated into a clinical decision guideline for precision dosing to improve outcomes in children and young adults with TA-TMA.

Introduction

Transplant-associated thrombotic microangiopathy (TA-TMA) is a life-threatening complication after hematopoietic stem cell transplantation (HSCT) in pediatric patients and young adults. In our first prospective observational study, we reported that patients with high-risk TA-TMA features, including

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The full-text version of this article contains a data supplement.

activated terminal complement, as measured by elevated blood-soluble terminal complement complex (sC5b-9) and proteinuria, have dismal outcomes, with 1-year posttransplant overall survival of 16.7%.[1] There are no uniformly agreed on treatment approaches for TA-TMA, but complement dysregulation has been shown to be an important pathogenic pathway with potential for clinical intervention.[2-4] Eculizumab, the first available monoclonal antibody (mAb) against complement C5, has shown promising effectiveness for TA-TMA treatment.[5] We demonstrated a significantly improved overall survival of 66% compared with 16.7% 1 year after HSCT in patients with high-risk TA-TMA treated with eculizumab using pharmacokinetic/pharmacodynamically (PK/PD)–guided drug dosing, as compared with the survival without targeted therapy.[6]

In HSCT recipients with high-risk, complement-mediated TA-TMA, sC5b-9 activity serves as a surrogate pharmacodynamic biomarker for enhanced C5 production. We developed an eculizumab population PK model after the first dose of therapy that enables us to predict the optimal initial dose, as well as the optimal timing of the subsequent dose based on individual pretreatment sC5b-9 levels and body weight.[7] However, this model considers only elevated sC5b-9 levels at the start of therapy and cannot reflect contextual changes in disease progression and improvement in the subsequent course of therapy.

The significant effect of gastrointestinal bleeding on eculizumab PK/PD is another important factor to consider as part of dose individualization. In our studies, bleeding patients with TA-TMA had the fastest eculizumab clearance, required the highest number of eculizumab doses (20 vs 9; $P = .0015$), and had a lower 1-year survival than those without bleeding (44% vs 78%, $P = .01$).[6,7] Based on these observations, we identified subjects with TA-TMA who had undergone HSCT and had clinically significant bleeding as an ultra–high-risk group in need of personalized drug dosing to improve survival. Therefore, there is an urgent need to develop a personalized dosing algorithm for bleeding and nonbleeding patients.

Model-informed precision dosing is an attractive and clinically feasible strategy for improving treatment success by optimizing drug target exposure, which has been recognized to be associated with significant treatment success.[8-11] The development of PK/PD model–informed eculizumab precision dosing throughout the therapy promises to improve not only treatment outcome but also cost effectiveness. The goal of this study was to extend our previously developed model by using the largest enriched PK/PD data set collected during multiple treatment doses in nonbleeding and bleeding patients in the same cohort who had recently reported clinical outcomes.[6]

Methods

Study subjects

A clinical cohort of 64 patients with high-risk TA-TMA treated with eculizumab was available for analysis. All study subjects were prospectively and uniformly monitored for TA-TMA and underwent real-time eculizumab PK/PD monitoring. Clinical outcomes of this cohort were recently published in *Blood* by Jodele et al.[6] Informed consent was obtained from all study subjects participating in the Bone Marrow Transplant Tissue Repository. The institutional review board at our center approved a retrospective analysis of the PK/PD data.

Patient demographics, transplant information, clinical data, and laboratory studies were captured from the electronic medical record and transplant data repository. Red blood cell (RBC) and platelet transfusions were reviewed for each day of eculizumab therapy and documented as milligrams per kilogram per day administered for each study subject. Patients with any clinical evidence of lower intestinal bleeding were marked as having clinically significant bleeding and assigned to the bleeding patient group for analysis.

Subjects with incomplete eculizumab concentrations and/or incomplete sC5b-9 data were excluded from the analysis. Eculizumab concentrations $\geq$1000 μg/mL and eculizumab measurement results when patients who were treated with therapeutic plasma exchange were excluded from the analysis. The measurement methodologies for eculizumab serum concentrations and sC5b-9 have been described.[6,7]

Eculizumab treatment and therapeutic monitoring

The details of eculizumab dosing, response monitoring methods, and clinical outcomes assessment have been described in a previous publication for the same patient cohort.[6] In brief, the first eculizumab dose (in milligrams) was based on body weight, as suggested in the eculizumab drug label and published data.[3,6,12] During the loading and induction phases of therapy, the dosing intervals were adjusted based on eculizumab concentrations and CH50 levels to maintain eculizumab trough concentration in blood at or above 100 μg/mL and trough CH50 levels below 10% of the normal value.[13] Eculizumab loading doses were given at least every 72 hours to subjects with elevated blood sC5b-9 levels at the beginning of eculizumab therapy, and loading doses were continued until t blood sC5b-9 level normalized (normal, $<$244 ng/mL). Maintenance and tapering schedules were considered only after therapeutic eculizumab concentrations were sustained and CH50 level was $<$10% for at least 2 consecutive dosing intervals after normalization of sC5b-9. Eculizumab therapy was discontinued after resolution of hematologic TA-TMA and stabilization/improvement in affected organ function when therapeutic eculizumab concentrations and normal sC5b-9 levels were documented for 2 consecutive doses after the taper schedule was started.[6]

Eculizumab PK/PD modeling

PK models for nonbleeding and bleeding patients with TA-TMA patients were developed with nonlinear mixed-effect modeling (NONMEM version 7.4; ICON Development Solutions, Ellicott City, MD) interfaced with Perl-speaks-NONMEM (PsN 4.9.0) and Pirana 2.9.9.[14-16] According to the criteria for good modeling practice, observations of |conditional weighted residual error | $>$6 were excluded from the modeling.[17] The effects of potential covariates on eculizumab PK were evaluated according to age, sex, body weight, albumin, glomerular filtration rate, number of eculizumab doses, and sC5b-9 levels. The effect of increase in body size on eculizumab clearance was evaluated by using allometric scaling to a body weight of 70 kg.[7,18] Interoccasion variability was also considered. Missing sC5b-9 levels were re[placed with an interpolated value between the previous and subsequent values, assuming a linear change. The selection of covariates was based on a significant reduction in the objective function value by stepwise forward inclusion ($P < .05$), backward elimination ($P < .01$), and a graphic evaluation of goodness-of-fit plots. The model evaluation was performed by bootstrap analyses and a prediction-corrected visual predictive check (supplemental Figure 1).[19]

Simulation of eculizumab concentration-time profiles for nonbleeding and bleeding patients

Eculizumab concentration-time profiles after the first dose were simulated to predict the optimal timing of subsequent doses in representative patients using Edsim++ ver. 1.9, based on the developed population PK models for bleeding and nonbleeding patients. Considering the currently recommended starting dose as approved for acute hemolytic uremic syndrome aHUS (300 mg for <10 kg, 600 mg for 10 to <40 kg, and 900 mg for ≤40 kg), representative patients were selected for PK simulations to cover the body weight range of the cohorts for young, median, and older children/young adults as follows: 8-kg patients receiving 300 mg, 25-kg patients receiving 600 mg, and 70-kg patients receiving 900 mg.[7]

Simulation of optimal loading-dose schedules

Optimal loading-dose schedules for nonbleeding patients with TA-TMA were explored by evaluating the probability of achieving target trough concentration attainment (PTA%) using a Monte Carlo simulation analysis.[20,21] PTA% was defined as the proportion of patients achieving the predose eculizumab concentration target of ≥100 μg/mL. A total of 12 000 age-body weight–matched subjects were randomly sampled from the CDC-NHANES database, by using body weight cohorts of <10 kg, 10 to <20 kg, 20 to <30 kg, 30 to <40 kg, 40 to <70, and 70 to <100 kg. Under the assumption of a data distribution similar to that observed in the current study, realistic predose sC5b-9 levels were generated by Monte Carlo simulation to be matched to the observed sC5b-9 distribution in this study (R 3.0.3). Five optimal dosing scenarios were selected based on the simulation results for the current recommended loading dose scenarios labeled for aHUS, which are 300 mg for patients <10 kg, 600 mg for patients with 10 to ≤40 kg, and 900 mg for 40 to <100 kg. In consideration of the dosage strength of 300 mg per vial, the 5 different dosing scenarios tested included protocol 1: the currently recommended dose amount for each body weight cohort approved for aHUS, but with shortening of the dose interval to 3 days; protocol 2: an increased dosing amount with a 3-day dose interval; 3: the currently recommended dose amount for each body weight cohort approved for aHUS, but with an adjusted dose interval depending on body weight cohort; protocol 4: a combination of increased dose amount and shortened dose interval, to avoid an extremely high dose; and protocol 5: a milligram per kilogram–based dose.

Results

Study subjects

Full eculizumab PK/PD data throughout multiple doses of therapy were available in 19 bleeding and 38 nonbleeding patients for the model development. Response to eculizumab therapy and clinical outcomes for this cohort were recently published in *Blood* by Jodele et al.[6] Relevant demographic data for the population PK model development are summarized in Table 1. Significantly more bleeding patients received RBC and platelet transfusions than nonbleeding patients during eculizumab therapy (79% to 84% of bleeding patients vs 5% of nonbleeding patients). Pretreatment sC5b-9 levels in this population were variable, with a median of 217 ng/mL (107-1641 ng/mL). Bleeding patients had significantly lower albumin and aspartate aminotransferase values than nonbleeding patients. All other laboratory parameters, including age, body weight, predose sC5b-9 levels, were not significantly different between bleeding and nonbleeding patients (Table 1).

Eculizumab PK/PD differences between nonbleeding and bleeding patients

We visualized eculizumab concentration-time profile changes during the first 5 doses of therapy in nonbleeding and bleeding patients (Figure 1). In bleeding patients, eculizumab concentrations fell below the target level more rapidly across treatment doses than in nonbleeding patients. Drug elimination gradually decreased across the doses of therapy in nonbleeding patients. By contrast, bleeding patients maintained high drug elimination across all 5 doses. Bleeding patients received more RBC and platelet transfusions across all first 5 doses of therapy compared with nonbleeding patients (supplemental Figure 2).

Eculizumab PK/PD differences between nonbleeding and bleeding patients were also evaluated by using individual eculizumab clearance estimates and observed predose sC5b-9 levels. Median eculizumab clearance after the first dose tended to be higher in bleeding patients than in nonbleeding patients (83.8 vs 61.3 mL/h per 70 kg; $P = .07$). It is important to note that high clearance was maintained over time in bleeding patients, whereas nonbleeding patients showed a decrease in clearance over time (Figure 2A). Predose sC5b-9 levels were highest during the first week of therapy and decreased over time, regardless of bleeding events (Figure 2B) and tended to be higher in bleeding patients during the first week, followed by suppression to comparable levels as in nonbleeding patients during subsequent therapy.

Eculizumab population PK modeling

Separate population PK models were developed for nonbleeding and bleeding patients. A 1-compartment model best described the data. Eculizumab clearance (CL) was described by a nonspecific linear clearance (CL_L) component representing the neonatal Fc receptor–mediated clearance and a nonlinear clearance (CL_{NL}) component for target-mediated clearance.[22-24] In bleeding patients, CL_L and CL_{NL} could not be estimated independently, because there were no observed eculizumab clearance changes over time. Considering the high total clearance observed in bleeding patients, the effect of the CL_L difference between bleeding and nonbleeding patients was ignored. Therefore, CL_L estimated in nonbleeding patients was used in the PK modeling for bleeding patients.

Our analysis showed that regardless of bleeding events, sC5b-9 level and body weight were significant covariates predictive of eculizumab clearance with comparable power exponent estimates. Nonbleeding patients showed a mean eculizumab clearance estimate after the first dose of 58.5 mL/h (CL_{NL}; 31.3 mL/h, CL_L; 27.2 mL/h) in a 70-kg patient and at the C5b-9 level of 244 ng/mL, the upper end of the normal range. These estimates are comparable to our previously published model, which derived a mean eculizumab clearance of 66.0 mL/h in a 70-kg patient with an sC5b-9 level of 244 ng/mL.[7] The eculizumab nonlinear time-dependent decrease in clearance was a function of the number of doses in addition to sC5b-9 changes. The mean volume of distribution after the first dose for a 70-kg patient was 5.5 L. The volume of distribution increased up to 63% during the protocol of the therapy.

Bleeding patients had a mean eculizumab clearance of 87.2 mL/h (CL_{NL}, 60.0 L/h; CL_L, 27.2 L/h) after the first dose in a 70-kg patient at an sC5b-9 level of 244 ng/mL, which was 50% higher than

Table 1. Patient demographics

	Nonbleeding	Bleeding	*P*
Number of patients	38	19	–
Sex			
Male	22	14	–
Female	16	5	–
Race			
White	28	16	–
Black/African American	4	3	–
Asian	3	0	–
Mixed	3	0	–
Age, y	4.9 (0.5-25.9)	10.0 (0.6-29.9)	NS
Bodyweight, kg	21.3 (5.0-105)	29.1 (5.5-91.6)	NS
GFR, mL/min per 1.73 m^2	175 (21.6-501)	136 (19.7-894)	NS
SCR, mg/dL	0.4 (0.2-2.2)	0.34 (0.18-1.6)	NS
BIL, mg/dL	0.7 (1.0-12.5)	1.2 (0.2-42.2)	NS
ALB, mg/dL	3.2 (2.0-4.2)	2.7 (2.1-3.4)	<.01
AST, U/L	62 (13-432)	38 (14-135)	<.05
ALT, U/L	39 (14-643)	34 (11-248)	NS
Predose sC5b-9, ng/mL	217 (107-1035)	339 (126-1641)	NS
RBC transfusion			
Patients, n/total in group	5 (2/38)	84 (16/19)	<.0001
Total amount, mL/kg*	0 (0-113)	30 (0-151)	<.0001
Transfusion days, n†	0 (0-7)	4 (0-34)	<.01
Platelet transfusions			
Patients, n/per total group	5 (2/38)	79 (15/19)	<.0001
Total amount, mL/kg*	0 (0-328)	48 (0-220)	<.0001
Number of transfusion days†	0 (0-23)	7 (0-65)	<.0001
First loading dose			
300 mg	6	4	–
600 mg	23	6	–
900 mg	9	9	–

ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BIL, serum bilirubin; GFR, glomerular filtration rate adjusted by 1.73 m^2 of body surface area; PLTS, platelet; RBC, red blood cell; SCR, serum creatinine.

*Total amount, sum of RBC/PLTS transfusions (mg/kg per day) during the first 5 treatment doses.

†Number of transfusions, sum of the RBC/PLTS transfusion days during the first 5 treatment doses.

the mean clearance estimate in nonbleeding patients. The eculizumab nonlinear clearance remained constant throughout the multiple doses of therapy when body weight and sC5b-9 levels did not change. Bleeding patients had a volume of distribution of 4.4 L (normalized to a 70-kg patient) across different dosing intervals, which was 20% lower than the volume of distribution after the first dose in nonbleeding patients. The number of RBCs correlated with the number of platelet transfusions. Patients who received RBC and/or platelet transfusion were likely to have higher eculizumab clearance (R^2 = 0.13 for RBC and R^2 = 0.20 for platelet transfusion, supplemental Figure 3). However, the effect was not significant and was not retained in the final model.

Eculizumab PK simulations

We expanded our previously reported eculizumab concentration-time simulations[7] covering the 7 days after the first dose of therapy based on our newly developed PK model for patients with bleeding complications (Figure 3; Table 2). The simulations predicted the timing of the next dose required to maintain eculizumab target concentrations at ≥100 μg/mL in representative cases. Figure 3 provides a summary of the simulated eculizumab concentration-time profiles for the dosing scenarios in 8-kg patients receiving 300 mg, 25-kg patients receiving 600 mg, and 70-kg patients receiving 900 mg.

In 25-kg nonbleeding patients receiving 600 mg eculizumab with a predose sC5b-9 level of 400 ng/mL, the mean eculizumab concentration was predicted to decline below 100 μg/mL at ~4 days after the first dose. The mean eculizumab concentration was maintained above the target for 5 days when a lower pretreatment sC5b-9 level of 200 ng/mL would be present. The 8-kg nonbleeding patients receiving 300 mg eculizumab were predicted to have an eculizumab concentration maintained above the target for a period ranging from 5 to 7 days depending on predose sC5b-9 levels. This target

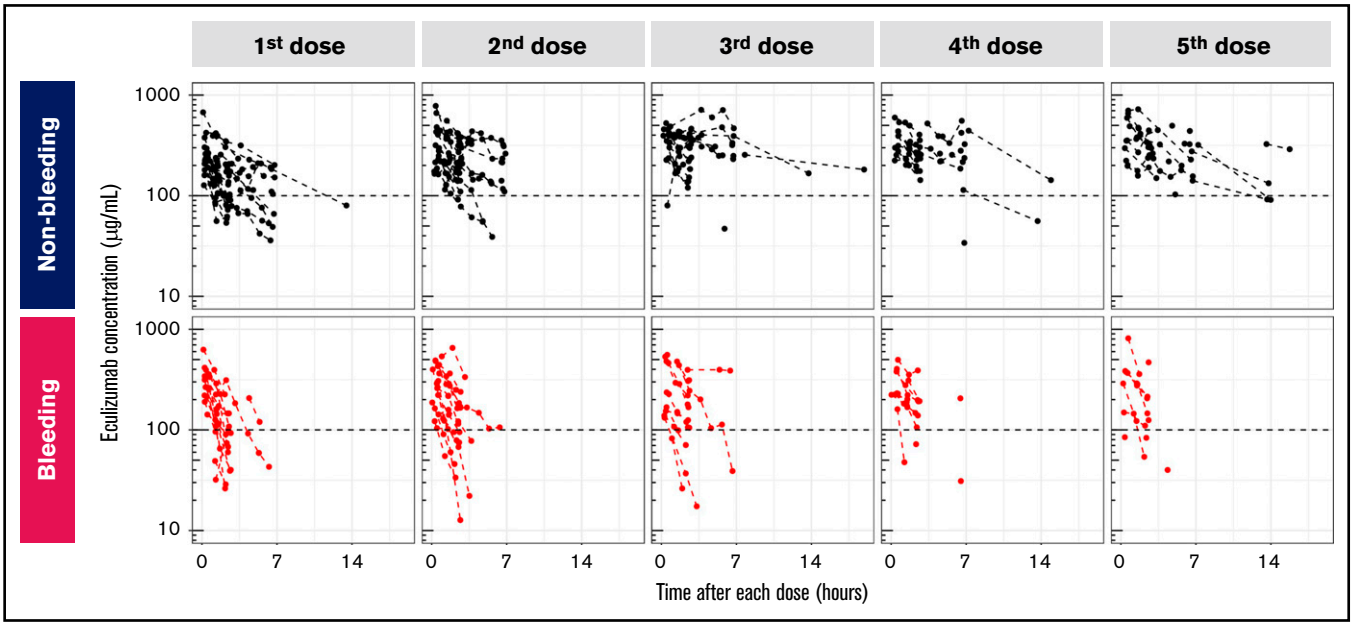


Figure 1. Eculizumab concentration-time profiles in patients with TA-TMA, with or without bleeding complications. The y-axis shows observed concentrations of eculizumab, and the x-axis shows time after each dose. The black circles represent the data for nonbleeding patients (n = 38) and the red circles represent the data for bleeding patients (n = 19). The data collected from the same patients are connected by dotted lines.

attainment period was longer than that observed in 25-kg patients receiving 600 mg eculizumab. In contrast, 70-kg nonbleeding patients receiving 900 mg were predicted to have their eculizumab concentration fall below target within 3 days after the first dose. In bleeding patients, eculizumab concentration was predicted to fall below the target ~0.5 to 1 day earlier than that predicted in non-bleeding patients.

Simulation of optimal dosing schedules

The currently recommended weekly induction dosing approved for aHUS resulted in a maximal probability of target attainment (PTA%) of 50% in nonbleeding patients. Lower PTA% was predicted for patients with higher body weights (≥20 kg) compared with those of lower weight (<20 kg). When intensifying the eculizumab dosing by using the same amount of drug (in milligrams) per dose but given

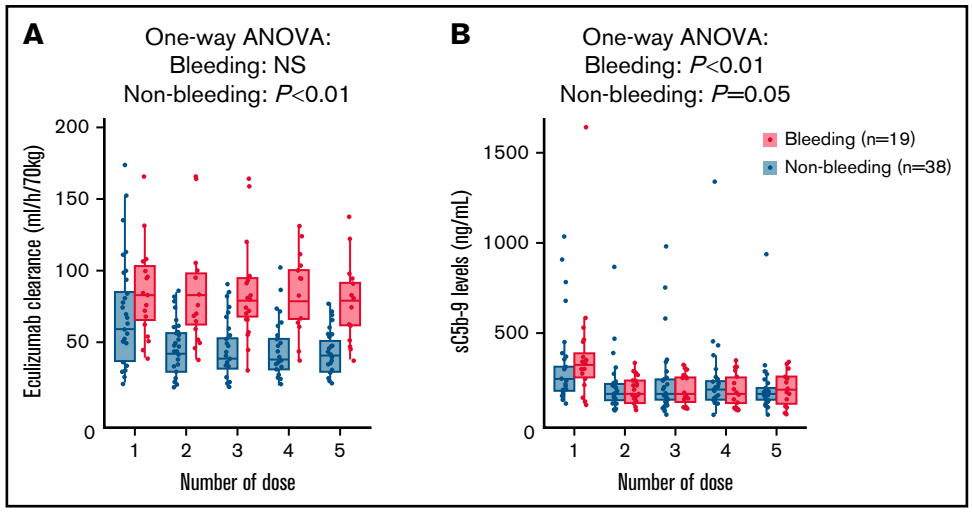


Figure 2. Eculizumab clearance and sC5b-9 change over the doses of therapy. *PK* and PD changes across treatment doses were evaluated using individual eculizumab clearance estimates (A) and predose sC5b-9 levels (B). Eculizumab individual clearance values at each week were estimated by the Bayesian estimation method with consideration of interoccasion variability and adjusted by an allometrically scaled body weight of 70 kg. Because the study included a wide age range (0.5-29.9 years), the effect of an increase in body size on eculizumab clearance was taken into account by adjusting it by allometric scaling to a body weight of 70 kg to allow for a comparison across pediatric and young adult patients with different body sizes as previously described.[7] Eculizumab clearance estimates and changes in sC5b-9 level over time were analyzed by 1-way analysis of variance (R, ver. 3.0.3).

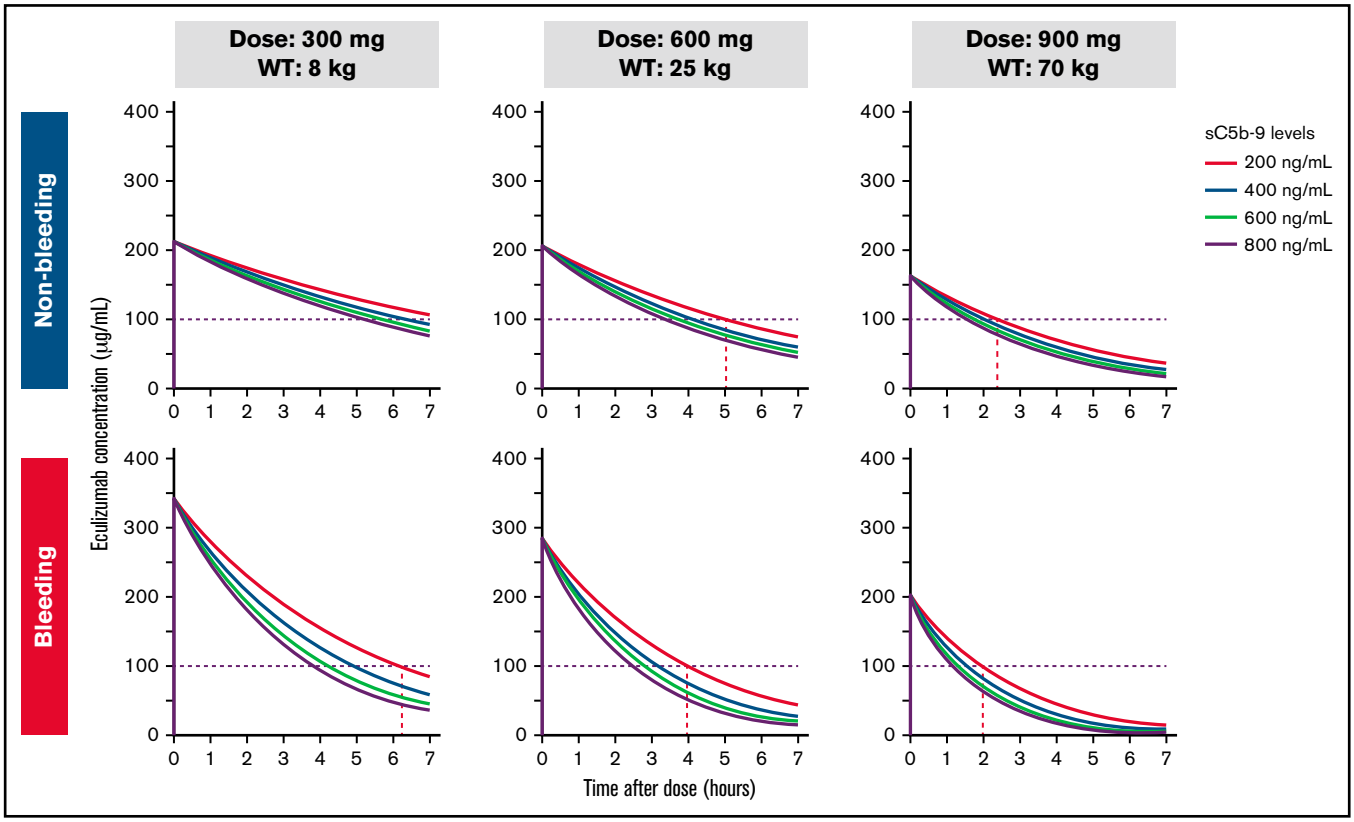


Figure 3. Simulation of eculizumab concentration-time profiles. Eculizumab concentration-time profiles over 7 days after the first dose were simulated based on our developed models for nonbleeding and bleeding patients shown in Table 2. The population PK parameter estimates were used for the simulations. Eculizumab concentration-time curves are shown in different colors and represent different predose sC5b-9 levels ranging from 200 to 800 ng/mL. The horizontal dotted pink line represents the suggested eculizumab target concentration of 100 µg/mL.

every 3 days (protocol 1), a PTA% of at least 80% was achieved in subjects <20 kg. PTA% was <50% in patients ≥20 kg. Therefore, protocol 2 evaluated an optimal dose, especially for patients ≥20 kg with a fixed 3-day dose interval to reach at least 80% PTA by subdividing body weight cohorts. With 3-day intervals, the optimal doses were 900 mg for patients weighing 20 to <30 kg, 1200 mg for those 30 to <40 kg, and 1500 mg for those 40 to <70 kg. In patients ≥70 kg, the optimal dose was predicted to be 2100 mg, which is significantly more than the currently recommended maximum induction dose of 900 mg. Protocol 3 evaluated the best dose interval to reach 80% PTA when the recommended aHUS dose amount (in milligrams) was selected for each body weight cohort. The predicted optimal interval was 3 days for patients weighing <20 kg, 2 days for 20 to <30 kg, and 1 day for ≥30 kg. It is important to note that the currently recommended 900-mg dose resulted in only up to 60% PTA in patients weighing 70 to 100 kg, even if the dose was administered daily. Protocol 4 evaluated the optimal dosing protocol by combining increased dose amount and dose intensification. The predicted optimal dose protocol was 900 mg every 3 days for patients weighing 20 to <30 kg, 900 mg every 2 days for those weighing 30 to <40 kg, 1200 mg every 2 days for those weighing 40 to <70 kg, and 1200 mg every 2 days for those weighing 40 to <70 kg and 1200 mg daily for those weighing ≥70 kg. Last, the optimal milligram-per-kilogram–based dose to reach 80% PTA is proposed in protocol 5 as an option. The optimal dose for patients weighing >10 kg was 40 mg/kg every 3 days and 30 mg/kg every 2 days for patients weighing 10 kg or more. A summary of the dosing protocols achieving various PTA% targets can be found in Figure 4. PTA% for the various dosing schedules with dosing amounts ranging from 300 to 2100 mg and dosing intervals ranging from 1 to 7 days are described. Figure 5 shows that a PTA% close to 100% could be achieved by increasing the dose by 300 mg (1 vial) in each body weight cohort of protocol 4. The effect of predose sC5b-9 levels on PTA% is summarized in supplemental Figure 4. The PTA% predictions for dosing protocol 4 were stratified by sC5b-9 cohorts of <250, 250 to <500, 500 to <750 ng/mL, and >750 ng/mL. PTA% declined along with increases in sC5b-9 levels. PTA% was predicted to be lower than 80% in patients with high sC5b-9 levels of ≥500 ng/mL.

Discussion

Sustained excessive complement activation is damaging to the endothelium and may result in multiorgan injury and death.[2,12,25-27] Our prior clinical observations clearly demonstrate that prompt complement blockade is needed to improve clinical outcomes in HSCT recipients with high-risk TA-TMA. In addition, the most precise drug dosing is required during the loading and induction phases of the therapy when complement and TA-TMA activity is the highest.[1,7] This newly developed eculizumab loading and induction dosing

Table 2. Population PK parameter estimates

	Nonbleeding	Bleeding
$CL_{tot,pop}$ (mL/h per 70 kg)($CL_{NL,pop}$, $CL_{L,pop}$)		
1st dose	58.5 (31.3-27.2)	87.2 (60.0, 27.2 fixed)
2nd to <5th dose	44.7 (17.5-27.2)	
≥5th dose	41.6 (14.4-27.2)	
Vd_{pop} (L/70 kg)		
1st dose	5.5	4.4
2nd to <5th dose	6.5	
≥5th dose	8.0	
Exponent for predose sC5b-9	0.53	0.52
Exponent for body weight	0.97 for CL0.63 for Vd	1.03 for CL0.74 for Vd
Interindividual variability	44.7% for CL33.6% for Vd	31.6% for CL34.5% for Vd
Residual proportional variability	0.11	0.084

The final model for nonbleeding patients is as follows:
$CL = CL_L + CL_{NL}$, $CL_L = CL_{L,pop} \times (WT/70)^{0.97}$, $CL_{NL} = CL_{NL,pop} \times (sC5b-9/244)^{0.53} \times (WT/70)^{0.97}$, $Vd = Vd_{pop} \times (WT/70)^{0.63}$.
Final model for bleeding patients is as follows:
$CL = CL_L + CL_{NL}$, $CL_L = CL_{L,pop} \times (WT/70)^{1.03}$, $CL_{NL} = CL_{NL,pop} \times (sC5b-9/244)$ $0.52x(WT/70)^{1.03}$, $Vd = Vd_{pop} \times (WT/70)^{0.74}$.
where CLNL,pop is the eculizumab population mean nonlinear clearance for a 70-kg patient; CLL_{pop}, is the eculizumab population mean linear clearance for a 70-kg patient; $CL_{tot,pop}$ is the population mean total clearance defined as sum of $CLNL_{pop}$ and CLL_{pop}; Vd_{pop}, is the population mean volume of distribution for a 70-kg patient; and WT is actual body weight (in kilograms).

algorithm confirms that the dosing regimen currently approved for patients with aHUS is not suitable for HSCT recipients with TA-TMA because of significantly low target attainment. These results strongly suggest that eculizumab dosing for patients with TA-TMA who undergo HSCT must be optimized.

Herein, we report population PK models by using an enriched eculizumab PK/PD data set collected throughout multiple eculizumab treatment doses in the largest TA-TMA cohort of HSCT recipients. The model updates have added several important features for future clinical applications to our previously published model.[7] The updated model can be used for the precision dosing of eculizumab, for the first dose and for subsequent doses. The enriched eculizumab PK/PD data set enabled us to develop models considering the mechanistic operational concepts of the elimination pathways of the mAbs: Brambell receptor-mediated elimination and target-mediated elimination pathways.[22,23] Currently approved aHUS dosing guidelines use the same induction dose in patients with body weights ranging from 10 to 40 kg. The simulations in HSCT patients showed that the target attainment for patients with ≥20 kg was significantly lower than that in those with <20 kg when they were treated with the same dosing protocol. These observations suggest that further subdivided body weight groups are beneficial in patients who undergo HSCT to avoid below-target concentrations in patients with higher body weight.

The larger PK/PD data set allowed us to propose several eculizumab dosing protocols for HSCT recipients with complement-mediated TA-TMA that can be adopted to clinical practice based on clinical needs by using fixed or variable dosing intervals or even more precise drug dosing using milligram per kilogram dosing. In addition, the current models characterized eculizumab disposition in bleeding and nonbleeding patients separately and clearly demonstrated that bleeding patients have much higher eculizumab drug clearance. Our previously published first-dose model did not address this question because of lower study subject numbers. There is a great benefit to optimize the eculizumab dose, depending on the bleeding complications because our preliminary analysis showed that bleeding patients show different pharmacological and disease characteristics than what is observed in nonbleeding patients.[7]

This newly developed model is also applicable for eculizumab dose optimization during the maintenance phase. Predicted eculizumab clearance during the maintenance phase was 27.2 mL/h when sC5b-9 levels are in the normal range and the target mediated clearance is negligible, which was still 23% higher than the reported clearance for aHUS, suggesting that more frequent dosing may be required in patients with TA-TMA than the currently recommended dosing intervals for aHUS suggest for the maintenance phase. The PK/PD model can also be used to support decisions on how to taper the eculizumab dose and when to discontinue therapy. Because the model includes a target-mediated clearance component, the winding down of the complement cascade as reflected by a sharp decrease in sC5b-9 concentration, will result in a decrease in eculizumab clearance. At that point the model will predict the resulting eculizumab concentration increase and allow for model-informed interpretation of the data to inform dose tapering and eventually discontinuation of the therapy.

We reported in our previous study that bleeding patients had significantly lower survival as compared with nonbleeding patients (44% vs 78%) and that there is a need to further characterize eculizumab pharmacokinetics in the patients with bleeding events.[6] In the current study, we characterized eculizumab PK/PD in bleeding patients and compared it to that in patients without bleeding. Because it is not possible to accurately determine blood loss in the stool, RBC and platelet transfusion requirements were used as surrogate markers for microangiopathic-hemolytic activity and blood loss. Impressively, only 5% of nonbleeding patients required transfusions after initiating eculizumab therapy, indicating good control of hemolysis and platelet consumption, whereas 84% of bleeding patients were transfusion dependent. Consistent with the higher transfusion requirements across treatment dosing intervals observed in bleeding patients, eculizumab clearance remained high across the doses of therapy, whereas clearance decreased over time in nonbleeding patients. Interestingly, there was no significant difference in time-dependent sC5b-9 reduction between bleeding and nonbleeding patients. This result can be explained by the more frequent dosing applied in the bleeding patients as the treatment study used real-time eculizumab concentrations and CH50 monitoring for dose adjustments.[6] These results suggest that a model-informed precision dosing strategy with consideration of bleeding has great potential to reduce sC5b-9 quickly and effectively by achieving eculizumab target concentrations. Adequate complement blockade in patients with intestinal bleeding may not be enough to improve survival, as these patients often have other transplant-related complications like graft-versus-host disease (GVHD) or infections. An early intervention preserving vascular endothelial health and prompt complement activation control along with effective GVHD prophylaxis or therapy and infection control is likely to be necessary to further improve clinical outcomes.

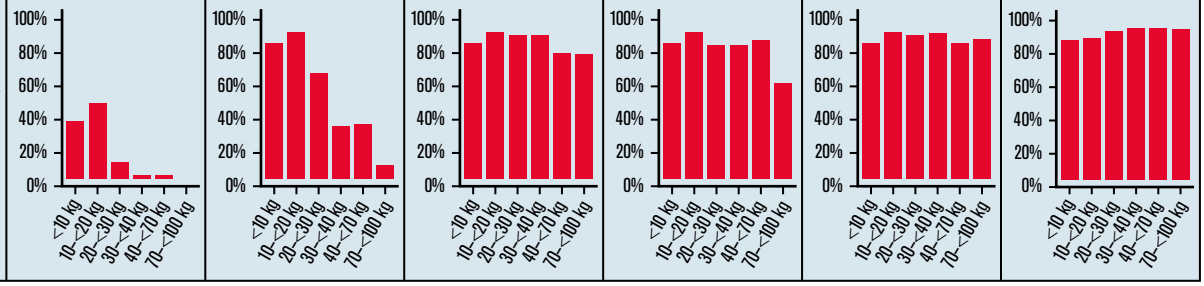
	Current dosing recommended for aHUS	Proposed optimal dosing protocol for HSCT recipients with TA-TMA				
		Protocol 1	Protocol 2	Protocol 3	Protocol 4	Protocol 5
Dose amount: Dose interval:	Original dose Weekly interval	Original dose 3 days interval	Adjusted dose 3 days interval	Original dose Adjusted interval	Adjusted dose Adjusted interval	mg/kg dose Adjusted interval
70–<100 kg	900 mg / week	900 mg / 3 days	2100 mg / 3 days	900 mg / day	1200 mg / day	30 mg/kg / 2 days
40–<70 kg			1500 mg / 3 days	900 mg / day	1200 mg / 2 days	
30–<40 kg	600 mg / week	600 mg / 3 days	1200 mg / 3 days	600 mg / day	900 mg / 2 days	
20–<30 kg			900 mg / 3 days	600 mg / 2 days	900 mg / 3 days	
10–<20 kg			600 mg / 3 days	600 mg / 3 days	600 mg / 3 days	
<10 kg	300 mg / week	300 mg / 3 days	300 mg / 3 days	300 mg / 3 days	300 mg / 3 days	40 mg/kg / 3 days
Probability of target attainment (PTA %)						

Figure 4. Simulation of optimal dosing algorithms for nonbleeding patients with TA-TMA. The y-axis shows the probability of target attainment determined as the proportion of patients who achieved eculizumab concentrations above the target, and the y-axis shows each body weight cohort. A total of 12 000 age-body weight–matched subjects was randomly sampled from the CDC-NHANES database. Six body weight cohorts were defined as follows: <10 kg, 10 to <20 kg, 20 to <30 kg, 30 to <40 kg, 40 to <70, and 70 to <100 kg. Realistic predose sC5b-9 levels were generated by simulation, to be matched to the observed sC5b-9 distribution. A Monte Carlo Simulation analysis was conducted to predict eculizumab trough concentrations for each dosing scenario according to NONMEM. The original dose used for protocols 1 and 3 is the current dose amount (in milligrams) by weight group approved for aHUS, but administered in intensified dose intervals. Protocols 2 and 4 evaluated the optimal dose (in milligrams) that achieved 80% target attainment considering a dosage strength of 300 mg per vial.

One of the important goals of this study was to elucidate the mechanism of high eculizumab clearance in bleeding patients. In this study, predose sC5b-9 level and patient weight remained significant covariates predictive of high drug clearance without any new covariates being identified. One possibility was that patients with severe blood loss had high eculizumab clearance caused by drug loss from the body. Our covariate analysis suggested that red blood cell transfusion as a surrogate marker for bleeding severity in bleeding patients could partly explain the high clearance, although it was not retained in the final model. Another potential mechanism is sustaining excessive C5 generation from injured bowel tissue providing a large number of targets for eculizumab to bind to.[28] Our PK/PD analysis showed that higher pretreatment sC5b-9 at the start of therapy reflected high drug clearance; however, eculizumab clearance in bleeding patients remained high, even after the sC5b-9 level normalized, potentially indicating ongoing C5 generation that continued to require eculizumab for blockade to maintain normal sC5b-9 level. The lower albumin levels in bleeding patients could be partly involved in the high clearance as reported for other mAbs, such as infliximab and anti-PD-L1 antibody.[29-32] In fact, our study showed that the baseline albumin levels in bleeding patients were significantly lower than in nonbleeding patients. However, the low albumin may cause increased protein turnover, facilitating degradation of IgG, including mAbs, and an increase in mAb neonatal Fc receptor–mediated clearance. In this study, there was no significant effect of albumin on eculizumab disposition, possibly because bleeding patients received total parenteral nutrition containing albumin, which could have masked the effect. This suggests that high drug clearance is likely multifactorial in bleeding patients and that such patients require personalized PK/PD-based dosing of eculizumab.

In summary, to our knowledge, this is the largest eculizumab PK/PD study to date in TA-TMA that shows that HSCT recipients require a dedicated drug dosing schedule suitable for this population. We identified several dosing strategies that can be incorporated into clinical care. Although fixed-dose or blanket dosing regimens can be derived for eculizumab dosing in nonbleeding HSCT recipients, those with clinically significant bleeding require personalized dose adjustments using continues PK/PD dose modification, to provide adequate eculizumab exposures based on disease activity. Bleeding patients also a need much longer loading and induction therapy course, most likely because of sustained C5 generation from the injured bowel and some drug loss caused by bleeding. We are in the process of preparing personalized dosing tools to be used in clinical practice for bleeding patients to further improve posttransplant outcomes.

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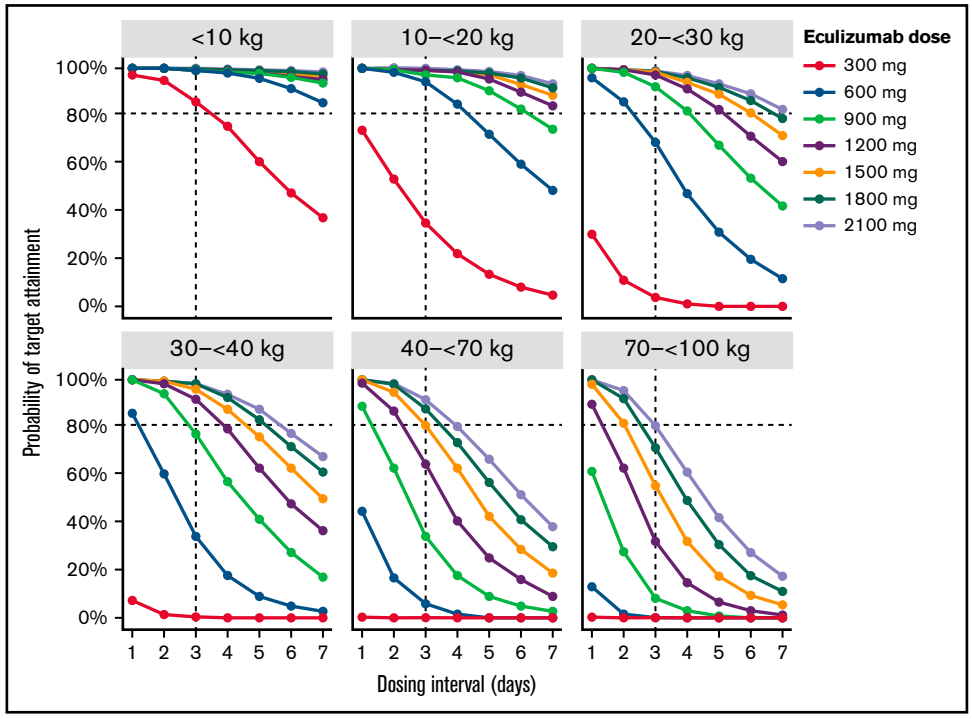

Figure 5. Probability of target attainment for various dosing schedules. The x-axis shows eculizumab dosing intervals ranged from 1 to 7 days. The y-axis shows the probability that the percent of target attainment would reach eculizumab the target trough concentration ≥100 μg/mL. The probability of target attainment was predicted for dosing protocols with different dose amount ranged from 300 to 2100 mg and are shown with different colored lines. PTA% close to 100% could be achieved by increasing the dose by 300 mg (1 vial) in each body weight cohort of protocol 4 (Figure 4).

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Authorship

Contribution: K.M., A.A.V., and S.J. structured the analysis, designed the optimal dosing algorithms, and wrote the manuscript; K.M. and A.A.V. performed eculizumab PK/PD modeling and simulation; S.J. and S.M.D. monitored eculizumab therapy for all patients and collected and analyzed the data; C.E.D. provided vital contributions in study planning and multidisciplinary team integration in prospective TA-TMA screening and collected and analyzed the clinical data; A.T.-C. monitored the administration of eculizumab and summarized the pharmacology data; and all authors read, provided critical feedback on, and approved the final manuscript.

Conflict-of-interest disclosure: S.J. holds US Patent 10 815 296 B2, principal investigator for the National Institutes of Health–funded multi-institutional study investigating TA-TMA and received travel support and honoraria from Omeros and Sobi for lectures. C.E.D. has received honoraria from Omeros. S.M.D. has received research support from Alexion Pharmaceuticals. C.E.D. received honoraria from Omeros. S.J. and K.M. have US provisional patent application 62/172 987 entitled "Dosing Algorithm for Eculizumab." The remaining authors declare no competing financial interests.

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References

1. Jodele S, Davies SM, Lane A, et al. Diagnostic and risk criteria for HSCT-associated thrombotic microangiopathy: a study in children and young adults. *Blood.* 2014;124(4):645-653.
2. Jodele S, Licht C, Goebel J, et al. Abnormalities in the alternative pathway of complement in children with hematopoietic stem cell transplant-associated thrombotic microangiopathy. *Blood.* 2013;122(12):2003-2007.
3. Jodele S, Laskin BL, Dandoy CE, et al. A new paradigm: Diagnosis and management of HSCT-associated thrombotic microangiopathy as multi-system endothelial injury. *Blood Rev.* 2015;29(3):191-204.

4. Jodele S. Complement in pathophysiology and treatment of transplant-associated thrombotic microangiopathies. *Semin Hematol.* 2018;55(3):159-166.
5. Zhang R, Zhou M, Qi J, et al. Efficacy and safety of eculizumab in the treatment of transplant-associated thrombotic microangiopathy: a systematic review and meta-analysis. *Front Immunol.* 2021;11:564647.
6. Jodele S, Dandoy CE, Lane A, et al. Complement blockade for TA-TMA: lessons learned from a large pediatric cohort treated with eculizumab. *Blood.* 2020;135(13):1049-1057.
7. Jodele S, Fukuda T, Mizuno K, et al. Variable eculizumab clearance requires pharmacodynamic monitoring to optimize therapy for thrombotic microangiopathy after hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant.* 2016;22(2):307-315.
8. Darwich AS, Ogungbenro K, Vinks AA, et al. Why has model-informed precision dosing not yet become common clinical reality? Lessons from the past and a roadmap for the future. *Clin Pharmacol Ther.* 2017;101(5):646-656.
9. Mizuno T, Emoto C, Fukuda T, Hammill AM, Adams DM, Vinks AA. Model-based precision dosing of sirolimus in pediatric patients with vascular anomalies. *Eur J Pharm Sci.* 2017;109S:S124-S131.
10. Vinks AA, Peck RW, Neely M, Mould DR. Development and implementation of electronic health record-integrated model-informed clinical decision support tools for the precision dosing of drugs. *Clin Pharmacol Ther.* 2020;107(1):129-135.
11. Barrett JS, Barrett RF, Vinks AA. Status toward the implementation of precision dosing in children. *J Clin Pharmacol.* 2021;61(suppl 1):S36-S51.
12. Legendre CM, Licht C, Muus P, et al. Terminal complement inhibitor eculizumab in atypical hemolytic-uremic syndrome. *N Engl J Med.* 2013;368(23): 2169-2181.
13. Jodele S, Fukuda T, Vinks A, et al. Eculizumab therapy in children with severe hematopoietic stem cell transplantation-associated thrombotic microangiopathy. *Biol Blood Marrow Transplant.* 2014;20(4):518-525.
14. Keizer RJ, Karlsson MO, Hooker A. Modeling and simulation workbench for NONMEM: tutorial on Pirana, PsN, and Xpose. *CPT Pharmacometrics Syst Pharmacol.* 2013;2(6):e50.
15. Lindbom L, Ribbing J, Jonsson EN. Perl-speaks-NONMEM (PsN)–a Perl module for NONMEM related programming. *Comput Methods Programs Biomed.* 2004;75(2):85-94.
16. Mould DR, Upton RN. Basic concepts in population modeling, simulation, and model-based drug development-part 2: introduction to pharmacokinetic modeling methods. *CPT Pharmacometrics Syst Pharmacol.* 2013;2(4):e38.
17. Byon W, Smith MK, Chan P, et al. Establishing best practices and guidance in population modeling: an experience with an internal population pharmacokinetic analysis guidance. *CPT Pharmacometrics Syst Pharmacol.* 2013;2(7):e51.
18. Anderson BJ, Holford NH. Understanding dosing: children are small adults, neonates are immature children. *Arch Dis Child.* 2013;98(9):737-744.
19. Marshall S, Madabushi R, Manolis E, et al. Model-informed drug discovery and development: current industry good practice and regulatory expectations and future perspectives. *CPT Pharmacometrics Syst Pharmacol.* 2019;8(2):87-96.
20. Dong M, Fukuda T, Selim S, et al. Clinical trial simulations and pharmacometric analysis in pediatrics: application to inhaled loxapine in children and adolescents. *Clin Pharmacokinet.* 2017;56(10):1207-1217.
21. Mouksassi MS, Marier JF, Cyran J, Vinks AA. Clinical trial simulations in pediatric patients using realistic covariates: application to teduglutide, a glucagon-like peptide-2 analog in neonates and infants with short-bowel syndrome. *Clin Pharmacol Ther.* 2009;86(6):667-671.
22. Mould DR, Green B. Pharmacokinetics and pharmacodynamics of monoclonal antibodies: concepts and lessons for drug development. *BioDrugs.* 2010;24(1):23-39.
23. Ryman JT, Meibohm B. Pharmacokinetics of monoclonal antibodies. *CPT Pharmacometrics Syst Pharmacol.* 2017;6(9):576-588.
24. Dirks NL, Meibohm B. Population pharmacokinetics of therapeutic monoclonal antibodies. *Clin Pharmacokinet.* 2010;49(10):633-659.
25. Waters AM, Licht C. aHUS caused by complement dysregulation: new therapies on the horizon. *Pediatr Nephrol.* 2011;26(1):41-57.
26. Noris M, Caprioli J, Bresin E, et al. Relative role of genetic complement abnormalities in sporadic and familial aHUS and their impact on clinical phenotype. *Clin J Am Soc Nephrol.* 2010;5(10):1844-1859.
27. Noris M, Remuzzi G. Atypical hemolytic-uremic syndrome. *N Engl J Med.* 2009;361(17):1676-1687.
28. Warren M, Jodele S, Dandoy C, et al. A complete histologic approach to gastrointestinal biopsy from hematopoietic stem cell transplant patients with evidence of transplant-associated gastrointestinal thrombotic microangiopathy. *Arch Pathol Lab Med.* 2017;141(11):1558-1566.
29. Fasanmade AA, Adedokun OJ, Blank M, Zhou H, Davis HM. Pharmacokinetic properties of infliximab in children and adults with Crohn's disease: a retrospective analysis of data from 2 phase III clinical trials. *Clin Ther.* 2011;33(7):946-964.
30. Fasanmade AA, Adedokun OJ, Olson A, Strauss R, Davis HM. Serum albumin concentration: a predictive factor of infliximab pharmacokinetics and clinical response in patients with ulcerative colitis. *Int J Clin Pharmacol Ther.* 2010;48(5):297-308.
31. Ogasawara K, Newhall K, Maxwell SE, et al. Population pharmacokinetics of an anti-PD-L1 antibody, durvalumab in patients with hematologic malignancies. *Clin Pharmacokinet.* 2020;59(2):217-227.
32. Xiong Y, Mizuno T, Colman R, et al. Real-world infliximab pharmacokinetic study informs an electronic health record-embedded dashboard to guide precision dosing in children with Crohn's disease. *Clin Pharmacol Ther.* 2021;109(6):1639-1647.