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

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Abstract:
Transplant-associated thrombotic microangiopathy (TA-TMA) is a fatal post-transplant complication of hematopoietic stem cell transplantation (HSCT). 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 (GI) bleeding showed poor survival even when treated with more frequent dosing. Our objective was to develop separate models in bleeding and non-bleeding TA-TMA patients and propose precision dosing algorithms. Eculizumab PK/PD were analyzed in 19 bleeding and 38 non-bleeding patients (0.5-29.9 years). A complement activation biomarker (sC5b-9) and bodyweight were identified as significant determinants of eculizumab clearance regardless of bleeding. Eculizumab clearance after the first dose was higher in bleeding patients than in non-bleeding patients (83.8 vs. 61.3 mL/h/70kg, p=0.07). The high clearance was maintained over treatment doses in bleeding patients, whereas non-bleeding 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 aHUS had less than 15% probability of eculizumab target concentration attainment of >100 μg/mL in non-bleeding patients. This study 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 support for precision dosing to improve outcomes in children and young adults with TA-TMA.

Conflict of interest: COI declared - see note

COI notes: SJ holds US patent No: US 10,815,296 B2, lead PI for NIH funded multi-institutional study investigating TA-TMA (R01HD093773) and received travel support and honoraria for lectures from Omeros and Sobi. CD has received honoraria from Omeros. SMD has received research support from Alexion Pharmaceuticals. CED received honoraria from Omeros. SJ and KM have U.S. provisional patent application no. 62/172,987 entitled "Dosing Algorithm for Eculizumab". The remaining authors have no conflict of interest to declare.

Preprint server: No;

Author contributions and disclosures: KM, AAV and SJ structured the analysis, designed the optimal dosing algorithms, and wrote the manuscript; KM and AAV performed eculizumab PK/PD modeling and simulation; SJ and SMD monitored eculizumab therapy for all patients, collected and analyzed the data; CED provided vital contributions in study planning and multidisciplinary team integration in prospective TA-TMA screening and collected and analyzed the clinical data; ATC monitored eculizumab administration and summarized the pharmacology data; All authors read, provided feedback, and approved the final manuscript.

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Figure 1

Eculizumab concentration (µg/mL) vs. Time after each dose (hours) for 1st, 2nd, 3rd, 4th, and 5th doses.
Figure 2

(A) One-way ANOVA:
  Bleeding: NS
  Non-bleeding: p<0.01

(B) One-way ANOVA:
  Bleeding: p<0.01
  Non-bleeding: p=0.05

- Red circles: Bleeding (n=19)
- Gray squares: Non-bleeding (n=38)
Figure 3

- **Dose: 300 mg**
  - WT: 8 kg

- **Dose: 600 mg**
  - WT: 25 kg

- **Dose: 900 mg**
  - WT: 70 kg

**Eculizumab concentration (µg/mL)**

**Time after dose (hours)**

**sC5b-9 levels**
- 200 ng/mL
- 400 ng/mL
- 600 ng/mL
- 800 ng/mL
**Figure 4**

| Dose amount: | Current dosing recommended for aHUS | Proposed optimal dosing protocol for HSCT recipients with TA-TMA |
|--------------|------------------------------------|---------------------------------------------------------------|
| Dose interval: | Original dose | Original dose | Adjusted dose | Original dose | Adjusted dose | Adjusted dose |
| 70-<100kg    | Weekly interval | 3 days interval | 2100 mg / 3 days | 900 mg / day | 1200 mg / day |
| 40-<70 kg    | 900 mg / week | 900 mg / 3 days | 1500 mg / 3 days | 900 mg / day | 1200 mg / 2 days |
| 30-<40 kg    | 1200 mg / 3 days | 600 mg / day | 600 mg / 2 days | 900 mg / 2 days |
| 20-<30 kg    | 600 mg / week | 600 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 |
| <10kg        | 300 mg / week | 300 mg / 3 days | 300 mg / 3 days |

*Probability of target attainment (PTA %)*

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*Original* dose used for Protocol 1 and Protocol 3 is the current dosing amount (mg) by weight groups approved for aHUS but administered at intensified dosing intervals. Protocol 2 and Protocol 4 evaluated “Optimized” dose (mg) achieving 80% target attainment considering dosage strength of 300 mg per vial.
Figure 5

The figure illustrates the probability of target attainment over different dosing intervals for various Eculizumab doses in different weight categories. Each graph shows the probability of target attainment (%) against the dosing interval (days) for different weight ranges: <10 kg, 10-<20 kg, 20-<30 kg, 30-<40 kg, 40-<70 kg, and 70-<100 kg. The x-axis represents the dosing interval in days, and the y-axis represents the probability of target attainment. Different Eculizumab doses are indicated by distinct colors and line styles, allowing for comparison of efficacy across different dosages and weight categories.
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Short title for the running head: Eculizumab PK/PD-based precision dosing for TA-TMA

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Key Points:

- This is the largest study examining eculizumab pharmacokinetics/pharmacodynamics (PK/PD) in bleeding and nonbleeding patients with TA-TMA.
- PK/PD model-based eculizumab dosing is needed for bleeding TA-TMA patients, while fixed-dose regimens are effective in nonbleeding patients.
Abstract

Transplant-associated thrombotic microangiopathy (TA-TMA) is a fatal post-transplant complication of hematopoietic stem cell transplantation (HSCT). 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 (GI) bleeding showed poor survival even when treated with more frequent dosing. Our objective was to develop separate models in bleeding and non-bleeding TA-TMA patients and propose precision dosing algorithms. Eculizumab PK/PD were analyzed in 19 bleeding and 38 non-bleeding patients (0.5-29.9 years). A complement activation biomarker (sC5b-9) and bodyweight were identified as significant determinants of eculizumab clearance regardless of bleeding. Eculizumab clearance after the first dose was higher in bleeding patients than in non-bleeding patients (83.8 vs. 61.3 mL/h/70kg, p=0.07). The high clearance was maintained over treatment doses in bleeding patients, whereas non-bleeding 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 aHUS had less than 15% probability of eculizumab target concentration attainment of >100 µg/mL in non-bleeding patients. This study 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 support 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 activated terminal complement as measured by elevated blood sC5b-9 and proteinuria, have dismal outcomes with one-year post-transplant overall survival of 16.7%. 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. Eculizumab, the first available monoclonal antibody against complement C5, showed promising effectiveness for TA-TMA treatment. We demonstrated significantly improved overall survival of 66% compared to 16.7% one year after HSCT in patients with high-risk TA-TMA treated with eculizumab using pharmacokinetic/pharmacodynamically (PK/PD)-guided drug dosing as compared to those without targeted therapy.

In HSCT recipients with high-risk complement-mediated TA-TMA, blood soluble terminal complement complex activity (sC5b-9) serves as a surrogate pharmacodynamic biomarker for enhanced C5 production. We previously developed an eculizumab population PK model after the first dose of therapy which allows us to predict optimal initial dose as well as the optimal timing for the subsequent dose based on the individual pre-treatment sC5b-9 levels and body weight. However, this model only considers 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 gastro-intestinal bleeding on eculizumab PK/PD is another important factor to consider as part of dose individualization. We demonstrate that TA-TMA patients with bleeding have the fastest eculizumab clearance, required the highest number of eculizumab doses (20 vs. 9, p=0.0015), and had lower 1-year survival than those without bleeding (44% vs. 78%, p=0.01). Based on these observations, we identified HSCT subjects with TA-TMA, and 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 both bleeding patients and non-bleeding patients.

Model-informed precision dosing is an attractive and clinically feasible dosing strategy to improve treatment success by optimizing the drug target exposure, which has been recognized to be associated with significant treatment success. 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 non-bleeding and bleeding patients using the same patient cohort with recently reported clinical outcomes.

Methods

Study Subjects

A clinical cohort of sixty-four 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. 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
ml/kg/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 ≥1000 µg/mL and eculizumab
measurement results when patients were treated with therapeutic plasma exchange (TPE) were
excluded from the analysis. The measurement methodologies for eculizumab serum
concentrations and sC5b-9 have been previously described.6,7

**Eculizumab Treatment and Therapeutic Monitoring**

The details of eculizumab dosing, response monitoring methods, and clinical outcomes
assessment are described in a previous publication for the same patient cohort.6 Briefly, the first
eculizumab dose (mg) was based on bodyweight 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 time of eculizumab therapy start, and loading doses were continued
until blood sC5b-9 level normalized (normal <244 ng/mL). Maintenance and taper schedules were
only considered after sustaining therapeutic eculizumab concentrations and CH50 <10% for at
least two 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 two consecutive doses after starting the taper schedule.6

Eculizumab PK/PD Modeling

Pharmacokinetic (PK) models for non-bleeding and bleeding TA-TMA patients were developed
using nonlinear mixed-effect modeling (NONMEM version 7.4, ICON Development Solutions,
Ellicott City, MD, USA) interfaced with Perl-speaks-NONMEM (PsN 4.9.0) and Pirana 2.9.9.14-16
According to the criteria of 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 using age, sex, body weight, albumin, glomerular filtration rate (GFR), number
of eculizumab doses, and sC5b-9 levels. The effect of body size increase on eculizumab
clearance was evaluated using allometric scaling to a bodyweight of 70kg.7,18 Inter-occasion
variability was also considered. Missing sC5b-9 levels were substituted by an interpolated value
between the previous and subsequent values assuming a linear change. The selection of
covariates was based on a significant reduction of the objective function value by stepwise
forward inclusion (p<0.05), backward elimination (p<0.01), and a graphical 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 non-bleeding 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 non-bleeding patients. Considering the
currently recommended starting dose as approved for aHUS (300mg for <10kg, 600mg for 10-<40kg, and 900mg for 40 kg and above), representative patients were selected for PK simulations to cover the bodyweight range of the cohorts for young, median and older children/young adults as follows: 8-kg patients receiving 300mg, 25-kg patients receiving 600 mg, 70-kg patients receiving 900mg.

Optimal loading dose schedules for TA-TMA non-bleeding patients were explored by evaluating the probability of achieving target trough concentration attainment (PTA%) using a Monte Carlo Simulation (MCS) analysis. PTA% was defined as the proportion of patients achieving the pre-dose eculizumab concentration target of ≥100 µg/mL. A total of 12,000 age-bodyweight-matched subjects was randomly sampled from the CDC-NHANES database using bodyweight cohorts of <10kg, 10-<20kg, 20-<30kg, 30-<40kg, 40-<70, and 70-<100kg. Under the assumption of a similar data distribution as observed in the current study, realistic pre-dose sC5b-9 levels were generated using MCS 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 (300 mg for patients <10kg, 600 mg for patients with 10-<40 kg, and 900 mg for 40-<100 kg). In consideration of the dosage strength of 300 mg per vial, 5 different dosing scenarios were tested as follows: 1; the currently recommended dosing amount for each bodyweight cohort approved for aHUS but with shortening of the dosing interval to 3 days (protocol 1), 2; an increased dosing amount with a 3 day-dosing interval (protocol 2), 3; the currently recommended dosing amount for each bodyweight cohort approved for aHUS but with adjusted dosing interval depending on bodyweight cohort (protocol 3), 4; a combination of increased dosing amount and shortened dosing interval to avoid an extremely high dose (protocol 4), and 5; a mg/kg-based dosing (protocol 5).
Results

Study Subjects

Full eculizumab PK/PD data throughout multiple doses of therapy were available in 19 bleeding and 38 non-bleeding patients for the model development. Response to eculizumab therapy and clinical outcomes for this cohort of patients were recently published by Jodele et al in Blood. Relevant demographic data for the population PK model development are summarized in Table 1. Bleeding patients received significantly higher RBC and platelet transfusion than non-bleeding patients during eculizumab therapy time (79-84% of bleeding patients vs. 5% of non-bleeding patients). Pre-treatment sC5b-9 levels in this population were quite variable with a median of 217 ng/mL (107 - 1641 ng/mL). Bleeding patients had significantly lower albumin and AST values than non-bleeding patients. All other laboratory parameters, including age, bodyweight, pre-dose sC5b-9 levels, were not significantly different between bleeding and non-bleeding patients (Table 1).

Eculizumab PK/PD differences between non-bleeding and bleeding patients

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

Eculizumab PK/PD differences between non-bleeding and bleeding patients were also evaluated by using individual eculizumab clearance estimates and observed pre-dose sC5b-9 levels.
Median eculizumab clearance after the first dose tended to be higher in bleeding patients than in non-bleeding patients (83.8 vs. 61.3 mL/h/70kg, p=0.07). It is important to note that high clearance was maintained over time in bleeding patients, whereas non-bleeding patients showed a decrease in clearance over time. Pre-dose sC5b-9 levels were highest during the first week of therapy and decreased over time regardless of bleeding events (Figure 2B). Predose sC5b-9 levels tended to be higher in bleeding patients during the first week, followed by suppression to comparable levels as in non-bleeding patients during subsequent therapy.

**Eculizumab population PK modeling**

Separate population PK models were developed for non-bleeding and bleeding patients. A one-compartment model best described the data. Eculizumab clearance (CL) was described by a nonspecific linear clearance (CL\textsubscript{L}) component representing the neonatal Fc receptor-mediated clearance and a non-linear clearance (CL\textsubscript{NL}) component for target-mediated clearance.\textsuperscript{22-24} In bleeding patients, CL\textsubscript{L} and CL\textsubscript{NL} could not be estimated independently due to no observed eculizumab clearance changes over time. Considering very high total clearance observed in bleeding patients, the effect of CL\textsubscript{L} difference between bleeding and non-bleeding patients could be ignored. Therefore, CL\textsubscript{L} estimated in non-bleeding patients was used for the PK modeling for bleeding patients.

Our analysis showed that regardless of bleeding events, sC5b-9 level and bodyweight were significant covariates predictive of eculizumab clearance with comparable power exponent estimates. Non-bleeding patients showed a mean eculizumab clearance estimate after the first dose of 58.5 mL/h (CL\textsubscript{NL}; 31.3 mL/h, CL\textsubscript{L}; 27.2 mL/h) in a 70-kg patient and at the upper normal range of the sC5b-9 level of 244 ng/mL. 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.\textsuperscript{7} The eculizumab non-linear 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% over the doses of 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 the mean clearance estimate in non-bleeding patients. The eculizumab non-linear clearance remained constant over the multiple doses of therapy when bodyweight 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 non-bleeding patients. The amount of RBC correlated with the amount of platelet transfusion. 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, this effect was not significant and was not retained in the final model.

Eculizumab PK simulations

We expanded our previously reported eculizumab concentration-time simulations covering the 7 days after the first dose of therapy based on our newly developed PK model for patients with bleeding complications (Figure 3). The simulations predicted the timing of the next dose required to maintain eculizumab target concentrations at $\geq 100 \, \mu g/mL$ 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 non-bleeding patients receiving 600 mg eculizumab with a pre-dose sC5b-9 level of 400 ng/mL, the mean eculizumab concentration was predicted to decline below 100 $\mu g/mL$ at around 4 days after the first dose. The mean eculizumab concentration was maintained above the target for 5 days when a lower pre-treatment sC5b-9 level of 200 ng/mL would be present. The 8-kg
non-bleeding patients receiving 300 mg eculizumab were predicted to maintain eculizumab concentration above the target for a period ranging from 5 to 7 days depending on pre-dose sC5b-9 levels. This target attainment period was longer than what was observed in 25-kg patients receiving 600 mg eculizumab. In contrast, 70-kg non-bleeding 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 approximately 0.5-1 day earlier than what was 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 non-bleeding patients. Lower PTA% was predicted for patients with higher body weights (≥20kg) compared to those <20 kg. When intensifying the eculizumab dosing by using the same amount of drug (mg) per dose but given every 3 days (Protocol 1), a PTA% of at least 80% was achieved in subjects <20 kg. PTA% was below 50% in patients ≥20 kg. Therefore, Protocol 2 evaluated an optimal dose, especially for patients ≥ 20 kg with a fixed 3 day-dosing interval to reach at least 80% PTA by subdividing body-weight cohorts. With 3 day-dosing intervals, the optimal doses were 900 mg for patients weighing 20-<30 kg, 1200 mg for 30-<40kg, 1500 mg for 40-<70kg. 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 dosing interval to reach 80% PTA when the recommended aHUS dose amount (mg) was selected for each body-weight cohort. The predicted optimal interval was 3 days for patients weighing <20 kg, 2 days for 20-<30 kg, and 1 day for ≥30kg. 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 they were administered the dose on a daily basis. Protocol 4 evaluated the optimal dosing protocol by combining increased dosing amount (mg) and dose intensification. The predicted optimal dose protocol was 900 mg every 3
days for patients weighing 20-<30 kg, and 900 mg every 2 days for 30-<40 kg, 1200 mg every 2 days for 40-<70kg, and 1200 mg every 2 days for 40-<70kg and 1200 mg daily for ≥70 kg. Lastly, the optimal mg/kg-based dose to reach 80% PTA is proposed in Protocol 5 as an option. The optimal dose for patients weighing less than 10 kg was 40 mg/kg every 3 days, and 30 mg/kg every 2 days for patients of 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 (one vial) in each bodyweight cohort of protocol 4. The effect of pre-dose 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-<500, 500-<750 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 multi-organ injury and death. 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. This newly developed eculizumab loading and induction dosing algorithm confirms that the dosing regimen currently approved for patients with aHUS is not suitable for HSCT recipients with TA-TMA due to significantly low target attainment. These results strongly suggest that eculizumab dosing for HSCT patients with TA-TMA needs to be optimized.
Here we report population PK models by using an enriched eculizumab PK/PD dataset 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. The updated model can be used for the precision dosing of eculizumab not only for the first dose but also for subsequent doses. The enriched eculizumab PK/PD dataset allowed us to develop models considering the mechanistic operational concepts of the elimination pathways of the monoclonal antibodies: i.e., Brambell receptor-mediated elimination and target mediated-elimination pathways. Currently approved aHUS dosing guidelines use the same induction dose in patients with body weights ranging from 10 to 40kg. The simulations in HSCT patients showed that the target attainment for patients with ≥20kg was significantly lower compared to those with <20kg when they were treated with the same dosing protocol. These observations suggest that further subdivided bodyweight groups are beneficial in HSCT patients to avoid below target concentrations in higher bodyweight patients.

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 using fixed or variable dosing intervals or even more precise drug dosing using mg/kg dosing. In addition, the current models characterized eculizumab disposition in bleeding and non-bleeding 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 due to lower study subject numbers. There is a great benefit to optimize the eculizumab dose depending on the bleeding complications since our preliminary analysis showed that bleeding patients show different pharmacological and disease characteristics than what is observed in non-bleeding patients.

This newly developed model is also applicable for eculizumab dose optimization during the maintenance phase. Eculizumab predicted 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. This suggests that more frequent dosing may be required in TA-TMA patients than the currently recommended dosing intervals approved 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. As 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 drop 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 to non-bleeding patients (44% vs. 78%) and the need to further characterize eculizumab pharmacokinetics in the patients with bleeding events. In the current study, we characterized eculizumab PK/PD in bleeding patients and compared it to those in patients without bleeding. Since it’s 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 non-bleeding patients required transfusions after initiating eculizumab therapy indicating good control of hemolysis and platelet consumption, while 84% of bleeding patients were transfusion dependent. Consistent with higher transfusion requirements across treatment dosing intervals observed in bleeding patients, eculizumab clearance remained high over the doses of therapy, whereas clearance decreased over time in non-bleeding patients. Interestingly, there was no significant difference in time-dependent sC5b-9 reduction between bleeding and non-bleeding patients. This 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. 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 still 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 required to further improve clinical outcomes.

One of the important goals of this study was to elucidate the mechanism of high eculizumab
clearance in bleeding patients. In this study, pre-dose 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
due to 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
sustained excessive C5 generation from injured bowel tissue providing 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 sC5b-9 value normalized, potentially indicating ongoing C5 generation
that continues 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 monoclonal antibodies (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 non-bleeding patients. However, the low albumin may cause increased protein turnover,
resulting in facilitating degradation of IgG, including mAb, 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 were receiving total parenteral nutrition containing albumin, which could have masked the effect. This suggests that high drug clearance is likely multi-factorial in bleeding patients and such patients require personalized PK/PD-based dosing of eculizumab.

In summary, 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. While fixed-dose or “blanket” dosing regimens can be derived for eculizumab dosing in non-bleeding HSCT recipients, those with clinically significant bleeding require personalized dose adjustments using continues PK/PD dose modification in order to provide adequate eculizumab exposures based on disease activity. Bleeding patients also a need much longer loading and induction therapy course, likely due to sustained C5 generation from the injured bowel and some drug loss due to bleeding. We are in the process of preparing personalized dosing tools to be used in clinical practice for bleeding patients to further improve post-transplant outcomes.
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Authorship Contribution: KM, AAV and SJ structured the analysis, designed the optimal dosing algorithms, and wrote the manuscript; KM and AAV performed eculizumab PK/PD modeling and simulation; SJ and SMD monitored eculizumab therapy for all patients, collected and analyzed the data; CED provided vital contributions in study planning and multidisciplinary team integration in prospective TA-TMA screening and collected and analyzed the clinical data; ATC monitored eculizumab administration and summarized the pharmacology data; All authors read, provided feedback, and approved the final manuscript.

Conflict-of-interest disclosure: SJ holds US patent No: US 10,815,296 B2, lead PI for NIH funded multi-institutional study investigating TA-TMA (R01HD093773) and received travel support and honoraria for lectures from Omeros and Sobi. CD has received honoraria from Omeros. SMD has received research support from Alexion Pharmaceuticals. CED received honoraria from Omeros. SJ and KM have U.S. provisional patent application no. 62/172,987
entitled “Dosing Algorithm for Eculizumab”. The remaining authors have no conflict of interest to declare.
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### Table 1.

|                         | Non-bleeding | Bleeding | p-value |
|-------------------------|--------------|----------|---------|
| **Number of patients**  | 38           | 19       |         |
| **Gender**              |              |          |         |
| Male                    | 22           | 14       |         |
| Female                  | 16           | 5        |         |
| **Race**                |              |          |         |
| Caucasian               | 28           | 16       |         |
| Black/African American  | 4            | 3        |         |
| Asian                   | 3            | 0        |         |
| Mixed                   | 3            | 0        |         |
| **Age (years)**         | 4.9 (0.5-25.9) | 10.0 (0.6-29.9) | n.s.    |
| **Body weight (kg)**    | 21.3 (5.0-105) | 29.1 (5.5-91.6) | n.s.    |
| **GFR (ml/min/1.73m^2)**| 175 (21.6-501) | 136 (19.7-894) | n.s.    |
| **SCR (mg/dL)**         | 0.4 (0.2-2.2) | 0.34 (0.18-1.6) | n.s.    |
| **BIL (mg/dL)**         | 0.7 (1.0-12.5) | 1.2 (0.2-42.2) | n.s.    |
| **ALB (mg/dL)**         | 3.2 (2.0-4.2) | 2.7 (2.1-3.4) | <0.01   |
| **AST (units/L)**       | 62 (13-432)  | 38 (14-135) | <0.05   |
| **ALT (units/L)**       | 39 (14-643)  | 34 (11-248) | n.s.    |
| **Pre-dose sC5b9 (ng/mL)** | 217 (107-1035) | 339 (126-1641) | n.s.    |
| **RBC transfusion**     |              |          |         |
| Number of patients      | 5% (n=2/38)  | 84% (n=16/19) | <0.0001 |
| Total amount (ml/kg)\(^1\) | 0 (0-113) | 30 (0-151) | <0.0001 |
| Number of transfusion days\(^2\) | 0 (0-7) | 4 (0-34) | <0.01   |
| **Platelet transfusion**|              |          |         |
| Number of patients      | 5% (n=2/38)  | 79% (n=15/19) | <0.0001 |
| Total amount (ml/kg)\(^1\) | 0 (0-328) | 48 (0-220) | <0.0001 |
| Number of transfusion days\(^2\) | 0 (0-23) | 7 (0-65) | <0.0001 |
| **First loading dose**  |              |          |         |
| 300 mg                  | 6            | 4        |         |
| 600 mg                  | 23           | 6        |         |
| 900 mg                  | 9            | 9        |         |

**Patient demographics**

GFR, glomerular filtration rate adjusted by 1.73 m\(^2\) of body surface area; SCR, serum creatinine, BIL, serum bilirubin, ALB, albumin; AST, aspartate aminotransferase; ALT, alanine aminotransferase, RBC, red blood cell; PLTS, platelet;
1. Total amount, sum of RBC/PLTS transfusion (mg/kg/day) during the first 5 treatment doses;
2. Number of transfusion, sum the RBC/PLTS transfusion days during the first 5 treatment doses

Table 2 Population PK parameter estimates

|                        | Non-bleeding                  | Bleeding                       |
|------------------------|-------------------------------|--------------------------------|
| $\text{CL}_{\text{tot, pop}}$ (ml/h/70kg) | $\text{CL}_{\text{NL, pop}}, \text{CL}_{\text{L, pop}}$ | $\text{CL}_{\text{L, pop}}$ = $\text{CL}_{\text{L, pop}} \times (\text{WT/70})^{0.97}$, $\text{CL}_{\text{NL, pop}} \times (\text{sC5b-9/244})^{0.53} \times (\text{WT/70})^{0.97}$, $\text{Vd} = \text{Vd}_{\text{pop}} \times (\text{WT/70})^{0.63}$. |
| 1\textsuperscript{st} dose | 58.5 (31.3, 27.2) | 87.2 (60.0, 27.2 fixed) |
| 2\textsuperscript{nd} - 5\textsuperscript{th} dose | 44.7 (17.5, 27.2) | |
| $\geq$5\textsuperscript{th} dose | 41.6 (14.4, 27.2) | |
| $\text{Vd}_{\text{pop}}$ (L/70kg) |                                     | 4.4 |
| 1\textsuperscript{st} dose | 5.5 | |
| 2\textsuperscript{nd} - 5\textsuperscript{th} dose | 6.5 | |
| $\geq$5\textsuperscript{th} dose | 8.0 | |
| Exponent for pre-dose \(\text{sC5b-9}\) | 0.53 | 0.52 |
| Exponent for body weight | 0.97 for CL 0.63 for Vd | 1.03 for CL 0.74 for Vd |
| Inter-individual variability | 44.7% for CL 33.6% for Vd | 31.6% for CL 34.5% for Vd |
| Residual proportional variability | 0.11 | 0.084 |

Final model for non-bleeding patients is as follows:

$\text{CL} = \text{CL}_{\text{L}} + \text{CL}_{\text{NL}}, \text{CL}_{\text{L}, \text{pop}} = \text{CL}_{\text{L, pop}} \times (\text{WT/70})^{0.07}, \text{CL}_{\text{NL}, \text{pop}} \times (\text{sC5b-9/244})^{0.52} \times (\text{WT/70})^{0.97}$,

$\text{Vd} = \text{Vd}_{\text{pop}} \times (\text{WT/70})^{0.63}$.

Final model for bleeding patients is as follows:

$\text{CL} = \text{CL}_{\text{L}} + \text{CL}_{\text{NL}}, \text{CL}_{\text{L}, \text{pop}} \times (\text{WT/70})^{1.03}, \text{CL}_{\text{NL}, \text{pop}} \times (\text{sC5b-9/244})^{0.52} \times (\text{WT/70})^{1.03}$,

$\text{Vd} = \text{Vd}_{\text{pop}} \times (\text{WT/70})^{0.74}$.

where $\text{CL}_{\text{NL, pop}}$ is the eculizumab population mean nonlinear clearance for a 70 kg-patient, $\text{CL}_{\text{L, pop}}$, is the eculizumab population mean linear clearance for a 70 kg-patient, $\text{CL}_{\text{tot, pop}}$ is the
population mean total clearance defined as sum of $\text{CL}_{\text{NL, pop}}$ and $\text{CL}_{\text{L, pop}}$, $\text{Vd}_{\text{pop}}$, is the population mean volume of distribution for a 70 kg-patient, and $\text{WT}$ is actual body weight (kg).
Figure Legends

Figure 1. Eculizumab concentration-time profiles in TA-TMA patients with or without bleeding complications

The Y-axis shows eculizumab observed concentrations and the X-axis shows time after each dose. The black circles represent the data for non-bleeding 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.

Figure 2. Eculizumab clearance and sC5b-9 change over the doses of therapy

PK and PD changes over treatment doses were evaluated using individual eculizumab clearance estimates (A) and pre-dose sC5b-9 levels (B). The red and black circles represent the the data in bleeding patients and non-bleeding patients respectively. Eculizumab individual clearance values at each week were estimated by Bayesian estimation method with consideration of inter-occasion variability and adjusted by allometrically scaled bodyweight of 70kg. As the study included a wide age range (0.5-29.9 years), the effect of body size increase on eculizumab clearance was taken into account by adjusting it by allometric scaling to a bodyweight of 70kg to allow comparison across pediatric and young adult patients with different body sizes as previously described. Eculizumab clearance estimates and sC5b-9 level changes over time were statistically analyzed using one-way ANOVA (R 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 non-bleeding 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 pre-dose 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.

Figure 4. Simulation for optimal dosing algorithms for non-bleeding 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 bodyweight cohort. A total of 12,000 age- bodyweight-matched subjects was randomly sampled from the CDC-NHANES database. Six bodyweight cohorts were defined as follows: <10kg, 10-<20kg, 20-
<30kg, 30-<40kg, 40-<70, and 70-<100 kg. Realistic pre-dose sC5b-9 levels were generated using 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 using NONMEM.

**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 of target attainment % to reach eculizumab target trough concentration ≥100 μg/mL. The probability of target attainment was predicted for dosing protocols with different dose amount (mg) ranged from 300 to 2100 mg and are shown in lines with different colors. PTA% close to 100% could be achieved by increasing the dose by 300 mg (one vial) in each bodyweight cohort of protocol 4 shown in Figure 4.