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Test Dose Pharmacokinetics in Pediatric Patients Receiving Once-Daily IV Busulfan Conditioning for hematopoietic stem cell transplant: A Reliable Approach?

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Abstract

Intravenous (IV) busulfan test dose pharmacokinetics (PK) has been shown to accurately predict once-daily dose requirements and improve outcomes in adult transplant patients, with limited data to support this approach in children. Test doses of busulfan ~0.8 mg/kg were infused over 2–3 hours, followed by serial sampling to 4–6 hours post-infusion in pediatric hematopoietic stem cell transplant recipients (n=5). Once-daily busulfan doses were calculated based on a myelosuppressive AUC target of ~3700 – 4000 uM*min, and assumed dose-proportionality to the test dose. PK analysis was then repeated at full daily doses within 6–8 days of test dose administration. Plasma PK samples collected under test and full dose conditions were analyzed using validated commercial assays and noncompartmental methods. In 4 out of 5 patients, PK estimates after once-daily IV busulfan administration differed in comparison to test dose estimates (AUC range: –38.2% to +49.7%, CL range: –34.3% to +61.8%). Patients 1, 2, and 3 required increases in remaining daily busulfan doses to achieve AUC targets, and no adjustment was required in patient 4. Patient 5's AUC was 49.7% higher than expected, and he subsequently developed fatal sinusoidal obstruction syndrome. In our experience with pediatric patients, test dose PK failed to reliably predict daily dosing requirements with large discrepancies from predicted AUC targets. This report highlights the necessity for therapeutic drug monitoring of IV

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Conflict of Interest

The authors declare no relevant conflicts of interest.

busulfan and inadvisability of relying solely on test-dose busulfan PK in pediatric patients. Furthermore, clinicians should consider strategies to expedite dose adjustments in real-time.

Keywords

busulfan; therapeutic drug monitoring; test dose; pharmacokinetics; pediatrics

Introduction

Busulfan is an alkylating agent used in combination with cyclophosphamide, fludarabine, and/or other tumor-specific agents in hematopoietic stem cell transplant (HSCT) conditioning regimens. Both oral and IV formulations of busulfan are available, though the IV formulation is preferred as there is less variability in drug exposure subsequent to alterations in absorption.¹ Patient-specific busulfan dosing can be tailored according to the results of therapeutic drug monitoring (TDM), particularly in patients receiving high-dose busulfan or regimens that were developed on the basis of TDM, though this practice varies between institutions. Busulfan is typically dosed to reach a total area-under-the-curve (AUC) target of ~3600–5400 uM*min over a 24-hour period.^{2, 3} Strategies to optimize attaining and maintaining busulfan exposures within a defined therapeutic window are of high importance as subtherapeutic levels may result in engraftment failure, and supratherapeutic exposure may result in severe and sometimes fatal toxicities, such as sinusoidal obstruction syndrome.^{4, 5}

Several strategies have been proposed for dosing IV busulfan, including altering the frequency of administration, or incorporating patient-specific factors, such as age or weight, into the starting dose. Another method has been to perform a test-dose prior to the full conditioning regimen, which involves the administration of IV busulfan at a dose of ~0.8 mg/kg to assess the patient's pharmacokinetics (PK), with full once-daily doses adjusted to target exposures assuming dose-linearity. This approach has been shown to accurately predict once-daily dosing requirements and improve outcomes in adult HSCT patients.^{6–9} However, data on the reliability of this approach in children are limited. Available studies in pediatric populations reached target levels in 60–84% of cases where both test and full doses were examined.^{5, 10} This test dose strategy has been applied to pediatric patients at the NIH Clinical Center receiving IV busulfan without routine follow-up PK assessment to confirm target attainment. Thus, we performed TDM following administration of test and full daily IV busulfan doses to examine the accuracy of this approach in a subset of pediatric patients receiving busulfan as part of their standard conditioning regimen.

Methods

These studies were reviewed and approved by the National Institute of Allergy and Infectious Disease (NIAID) or National Cancer Institute (NCI) Institutional Review Boards as applicable. Clinical research was conducted according to guidelines for human experimentation as specified by the US Department of Health and Human Services. Data were obtained from pediatric patients at the NIH Clinical Center undergoing HSCT for X-linked chronic granulomatous disease (CGD) (NCT01306019), dedicator of cytokinesis 8

immunodeficiency syndrome (DOCK8) (NCT01176006), or acute lymphoblastic leukemia (ALL) (NCT01287104). Parents of participants provided written informed consent, and assent was obtained from patients as appropriate under each respective protocol.

Busulfan Administration, Sampling Strategy & Analytical Methods

Busulfan test doses were infused over 2 hours, followed by serial PK sampling through at least 4 hours post-infusion. PK samples for patients 1, 2, and 4 were collected at time 0 (immediately post-infusion), 15, 30, 60, 120, 180, 240, and 360 minutes post-infusion. PK samples for patients 3 and 5 were collected at time 0 (immediately post-infusion), 60, 120, and 240 minutes post-infusion. Plasma samples were analyzed in commercial CLIA-certified laboratories using validated liquid chromatography-tandem mass spectrometry (Mayo Clinic, Rochester, MN) or gas chromatography methods (Quest Diagnostics, Baltimore, MD).

Full once-daily busulfan doses were administered every 24 hours and were initiated within 4–8 days of test dose assessments. Busulfan doses during the conditioning regimen were calculated assuming linearity to test dose estimates, with individualized $AUC_{0-\infty}$ targets of ~3700–4000 $\mu\text{M}\cdot\text{min}$ over a 24-hour period. Once-daily busulfan doses were infused over 2 hours in patients 1, 2, and 4, and over 3 hours in patients 3 and 5. Serial sampling was again completed through at least 4 hours post-infusion using the same time points collected during test dose conditions following the first dose in subject 1–4, and the last dose in subject 5. Subsequent dosing modifications were made as permitted if measured full-dose AUC estimates deviated from the target AUC by more than 10%.

PK Analysis

PK results of test and full daily busulfan doses were analyzed by the NIH Clinical Center Pharmacy Department as part of TDM performed at the discretion of investigators for patients under these protocols. PK parameters were calculated using noncompartmental methods (Phoenix WinNonlin, Pharsight v6.4, Certara, St. Louis, MO). AUC from time 0 through the last sampling time point ($AUC_{0-\text{last}}$) was calculated using linear up-log down trapezoidal rule, and extrapolation of AUC through infinity ($AUC_{0-\infty}$) was estimated as the sum of $AUC_{0-\text{last}}$ and the concentration at the last time point (C_{last}) divided by the elimination rate constant (k_e). k_e was determined based on log-linear regression of at least 3 sampling time points during the elimination phase, and half-life ($t_{1/2}$) was calculated as $0.693/k_e$. Busulfan clearance (CL) estimates were calculated using the equation

$CL = \text{Dose} / AUC_{0-\infty}$, and the volume of distribution (V_d) was calculated using the equation $V_d = CL / k_e$.

Plots comparing predicted full-dose estimates against actual measures were created using GraphPad Prism v7.03. Predicted concentrations were generated using the concentrations at each time point measured under test dose conditions, which were then scaled proportionally by the factor used to reach individual AUC targets.

Drug-Drug Interactions

Concomitant medications administered prior to and during test and daily dose conditions were reviewed to identify potential drug-drug interactions that may have contributed to discrepancies in PK results. All concomitant medications were administered as standard of care under the respective protocols.

Results

A total of five patients were evaluated (Table 1). Busulfan exposures after once-daily dosing differed by more than 10% in comparison to test dose predictions in 4 of 5 patients (AUC range: -38.2% to +49.7%, CL range: -34.3% to +61.8%) (Table 2 and Figure 1). Patients 1, 2, and 3 required increases in remaining daily busulfan doses to achieve AUC targets, and no adjustment was required in patient 4. Engraftment, as defined by an absolute neutrophil count >500/L for 3 consecutive days was achieved in all patients, all of whom attained 99% donor chimerism. Patient 5's AUC was 49.7% higher than expected, though no adjustments could be made as TDM was conducted following the final IV busulfan dose. He subsequently developed sinusoidal obstruction syndrome, was initially managed with defibrotide, but ultimately suffered a fatal pulmonary hemorrhage.

Evaluation of concomitant medications with the potential to interact between test and full dose conditions revealed that patients 1, 2, and 4 were initiated on IV dexamethasone daily ~48 hours prior to daily busulfan. In patient 3, acetaminophen was administered 24 and 48 hours prior to and on the same day as the busulfan test dose, and daily IV dexamethasone was initiated on the first day of full-dose busulfan. Patients 1, 2, and 4 received cyclophosphamide and fludarabine during their conditioning regimens, and patients 3 and 5 received fludarabine alone. No other differences in drug-drug interactions between dosing conditions were identified.

Discussion

Busulfan test dose PK failed to predict optimal daily dosing requirements in 4 out of 5 pediatric patients examined. Clearance estimates in these patients were within the range of what has been previously reported in pediatric patients (mean, ~3.6 mL/min/kg; range, 2.4–7.3 mL/min/kg).¹¹ However, changes in clearance within patients varied widely between test and full dose conditions. This is in contrast to adult populations in whom the test dose strategy has been applied and shown to improve attainment of busulfan target exposures.^{6–9} Mean (range) clearance in adults is ~2.5 mL/min/kg (~1.5–4.3 mL/min/kg), with low intrasubject variabilities reported.² The exact underlying reasons for the discrepancies seen in these pediatric patients in comparison to adult populations is unclear, but may be attributable to a number of factors.

High intra-subject variability in these patients is one likely explanation as this issue has been reported with the IV formulation of busulfan in pediatric patients¹, particularly those with immunodeficiencies.¹¹ Intra-subject variabilities up to 44% in busulfan clearance have been reported in this population, the exact underlying mechanisms of which have not been identified.^{11, 12} A number of covariates and drug-drug interactions were examined in these

studies, which did not reveal any clear associations between the examined factors and the variabilities in drug exposures that were seen. The magnitude of change in clearance estimates between test and full dose conditions in our patients ranged from 10.6% to 61.8%, which is higher than previously reported. Though high intra-subject variability is one explanation for the discrepancies between test- and full-dose conditions, a number of other factors may have influenced the changes in busulfan PK that occurred.

Drug-drug interactions, particularly those that differ between test and full dose conditions, can be a significant source of deviations in PK estimates. These may have played a partial role in the deviations observed in these patients. Dexamethasone, a corticosteroid sometimes used for antiemetic purposes, is an inducer of cytochrome P450 (CYP450) enzymes.¹³ Though busulfan is primarily metabolized by glutathione-S-transferases (GST), CYP450 inducers have been shown to decrease busulfan exposure through unidentified mechanisms. However, it is unlikely that the addition of this medication alone would account for the ~60% increase in busulfan clearance observed in patients 1 and 2. Phenytoin, one of multiple options for seizure prophylaxis in this patient population, is another medication that has been implicated in reducing busulfan exposures via induction of drug-metabolizing enzymes.¹⁴ All patients detailed in this report received levetiracetam, which has not been implicated in altering busulfan exposures.¹⁵ Acetaminophen is another medication that is theorized to interact with busulfan through competition with glutathione, resulting in decreased busulfan clearance,¹ but this is in contrast to the increased clearance observed in patient 3 who received acetaminophen. Azole antifungals can also decrease busulfan clearance through enzyme inhibition.¹⁶ However, all patients received posaconazole consistently at the same dose throughout the test and conditioning regimen periods.

There is mixed evidence for whether other agents used as part of HSCT conditioning can affect busulfan levels. Patients detailed in this report received fludarabine with or without cyclophosphamide. Some studies suggested that fludarabine may slightly decrease busulfan clearance,^{17, 18} though others did not find an effect.^{19, 20} Cyclophosphamide is primarily metabolized by the liver, and glutathione is required for detoxification of toxic metabolites, similarly to busulfan. Combined use of cyclophosphamide with busulfan can result in hepatic injury and competition for clearance of toxic metabolites via glutathione depletion.²¹ Patients 1, 2, and 4 in this report received cyclophosphamide separately from busulfan (48 and 24 hours prior), which may have decreased glutathione stores. However, increased busulfan clearance was measured in these patients, which is the opposite of what would have been expected.

Aside from drug-drug interactions, there are other sources for alterations to the capacity of patients to metabolize and clear busulfan between doses. Busulfan is primarily metabolized by GST, and thus differences in baseline GST expression^{22, 23} and glutathione levels²⁴ can influence busulfan clearance. How increases or decreases in the expression of GST or glutathione stores may change within pediatric patients following multiple doses of busulfan concomitantly with other agents has not been extensively studied. Busulfan levels, particularly in pediatric children under 5 years of age, have been reported to vary up to three-fold between day and night, though these studies were performed with the oral formulation of busulfan that is known to have highly variable absorption.^{25, 26} This

phenomenon was not seen adults.²⁵ There is also some evidence that busulfan may induce its own metabolism or the synthesis of glutathione.^{25, 27} However, it is unlikely that a single 0.8 mg/kg dose 4–8 days prior to initiation of the full conditioning regimen would have caused significant induction in these patients. Additionally, patient 5 had higher levels than expected. As busulfan is primarily metabolized by the liver, changes in organ function may alter the PK of this agent. However, no changes in hepatic or renal function were detected in these patients between the PK assessments performed during test and full once-daily doses, with the exception of patient 3 who had a single elevation in her serum creatinine 2 days prior to the first full dose of busulfan, which normalized by the next day (prior to receiving busulfan).

Multiple publications have shown that busulfan demonstrates linear PK, and dose adjustments at many institutions are made under the assumption of linear PK with this agent. The U.S. Food & Drug Administration (FDA) package labeling utilizes this approach as well.² However, more recent population PK modeling has revealed that busulfan clearance may demonstrate non-linearity in pediatric populations.^{28, 29} This effect may be particularly pronounced at concentrations between 250 to 2000 ng/mL, where clearance can be decreased by up to 20%.¹⁰ Peak concentrations in our patients ranged between 2512 to 4013 ng/mL (or 10.2 to 16.6 μ M). However, in comparison to test dose results, decreased busulfan clearance was only observed in patient 5. The mechanism behind this patient's markedly higher exposures in comparison to test dose predictions is unclear. No changes in renal or hepatic function were detected. However, this was the second transplant in this patient, which is associated with an increased risk of sinusoidal obstruction syndrome.³⁰ In addition, the PK assessment was performed with the final once-daily busulfan dose, at which point a reduction in glutathione stores and reduced ability to effectively detoxify and clear busulfan may have resulted in increased drug exposure.

Several dosing strategies for pediatric patients have been published, each of which incorporate various patient-specific characteristics such as age, weight (actual and ideal), and body surface area.³¹ Many of these strategies also employ the use of shorter dosing intervals (e.g., every 6 or 12 hours), which provides the ability to adjust busulfan doses earlier in the conditioning regimen once PK results from the first dose become available.^{2, 31–34} However, a recent review attempted simulations based on several published models, and revealed that an AUC target of 900–1500 μ M*min was still only reached in 51–74% of pediatric patients.³¹ The FDA package insert provides a suggested dosing nomogram for IV busulfan dosing based on actual body weight from a population PK model developed by Booth et al.,³⁴ and is predicted to reach AUC targets of 900–1350 μ M*min in ~60% of patients.¹ The European Medicines Agency (EMA) employs a more complicated every-6-hour dosing algorithm based on actual body weight with five separate weight bands.³³ This approach appears moderately improved as it is projected to reach AUC targets in ~70% of patients³⁵, and thus was recently recommended by the American Society for Blood and Marrow Transplantation as the preferred method for initially dosing IV busulfan.¹ However, as illustrated in Table 3, use of the FDA or EMA dosing algorithms may still not have provided optimal busulfan exposures in our patients, and without real-time TDM, dose adjustments would not have been made.

There are limitations to the analyses detailed in this report. These assessments were performed in a small number of patients with different underlying conditions. Notably, 4 of the 5 patients had genetic immune deficiencies, which has been associated with high intra-subject variability in busulfan clearance estimates. Interestingly, these patients all deviated towards an increased clearance following the test dose. Sampling strategies also varied between patients, both with regard to the number of samples collected (range 4–8), and time points through which samples were collected following the end of infusion. A review by McCune et al examined TDM practices for IV busulfan in pediatric HSCT patients, and found that institutions vary in the number of samples collected (range 3–8).²⁹ The most common number of sampling points collected was 7, though the authors noted that this was higher than expected given that busulfan typically follows a 1-compartment model. Notably, none of the 51 institutions included in the analysis utilized the FDA package insert recommendation of collecting a minimum of 3 time points at 2, 4, and 6 hours post-dose. Despite these variations in approaches, limited sampling strategies through 4 hours post-dose have previously found that these methods were able to accurately predict busulfan PK estimates.^{36, 37} Furthermore, it does not appear that AUC extrapolation resulted in any significant bias, as there was no relationship between the percent extrapolation and AUC discrepancies between test and full dose in our participants. Notably, patient 1 had a very low percent extrapolation of less than 10% at both test and full doses, and had the second largest percent deviation in AUC estimates (38%) and the largest change in clearance (+60%) between test and full doses. However, noncompartmental and compartmental analyses can result in different AUC estimates and subsequent conflicting dosing recommendations.³⁸ Differences between approaches were not assessed in this small subset of patients.

The test-dose strategy was not predictive of full-dose busulfan PK in this small number of patients, and without real-time TDM following the first dose, the need for real-time dose adjustments would not have been identified. Several dosing algorithms exist for IV busulfan dosing during HSCT. However, real-time dose adjustments may still be necessary to achieve optimal busulfan exposures and patient outcomes. Utilization of strategies that allow for rapid assessment of busulfan PK following the first dose and the ability to adjust subsequent doses in real-time during the administration of full conditioning regimens should be used. Alteration of factors that may affect busulfan PK between doses should be minimized and avoided.

Conclusions

Reliance solely on test dose estimates to determine full IV busulfan dosing requirements for pediatric patients undergoing HSCT should be avoided. Clinicians should use real-time TDM of full-dose IV busulfan to efficiently review and adjust subsequent doses and optimize patient outcomes, regardless of the initial dosing strategy used.

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Figure 1.
Actual and Predicted Busulfan Concentration-vs-Time Curves with Once-Daily Dosing
Based on Test Dose Estimates

Table 1

Patient Characteristics

Subject	1	2	3	4	5
Age (yr)	13	12	10	16	15
Weight (kg)	38.2	36.2	19.9	59.8	34.7
Sex	Male	Male	Female	Male	Male
Diagnosis	X-CGD	X-CGD	DOCK8 deficiency	X-CGD	ALL
HSCT Conditioning Regimen	CY 14.5 mg/kg days -6 to -5 FLU 30 mg/m ² days -6 to -2 BU (per test-dose) days -4 to -2	CY 14.5 mg/kg days -6 to -5 FLU 30 mg/m ² days -6 to -2 BU (per test-dose) days -4 to -2	FLU 40 mg/m ² days -6 to -3 (per test-dose) days -6 to -3	CY 14.5 mg/kg days -6 to -5 FLU 30 mg/m ² days -6 to -2 BU (per test-dose) days -4 to -2	FLU 40 mg/m ² days -5 to -2 BU (per test-dose) days -5 to -2
Number of BU Doses Administered	3	3	4	3	4

Abbreviations: ALL = acute lymphoblastic leukemia, BU = busulfan, CY = cyclophosphamide, DOCK8 = dedicator of cytokinesis 8, FLU = fludarabine, X-CGD = X-linked chronic granulomatous disease

Table 2**Results & Comparisons of Test and Full Dose IV Busulfan PK**

Subject	1	2	3	4	5
<i>Test-Dose PK Results</i>					
Administered Dose (mg (mg/kg))	31.8 (0.83)	29 (0.8)	16.6 (0.83)	37.1 ^a (0.8)	27.8 (0.8)
AUC _{0-∞} (uM*min)	1047	1135	745.8	1483	822
AUC _{0-∞} % extrapolation	10%	26%	17%	26%	23%
CL/Weight (mL/min/kg)	3.23	2.87	4.54	2.18	3.38
Vd/Weight (mL/kg)	503.2	942.8	748.5	519.6	776.6
t _{1/2} (min)	108	228	114	212	136
<i>Once Daily Dose PK Results</i>					
Time Post-Test Dose (days)	7	8	7	6	7
AUC _{0-∞} Target	3983	4000	3735	4000	4000
Administered Dose (mg (mg/kg))	121 (3.17)	102 (2.82)	82.8 (4.29)	100.1 (2.15)	133 (3.86)
AUC _{0-∞} (uM*min)	2461	2521	3116	3619	5988
AUC _{0-∞} % extrapolation	8%	17%	29%	22%	31%
CL/Weight (mL/min/kg)	5.23	4.54	5.43	2.41	2.60
Vd/Weight (mL/kg)	752.7	1092.1	746.9	486.6	701.6
t _{1/2} (min)	101	170	93	186	186
<i>Test Dose Predictions vs. Once Daily Dose PK Results</i>					
Percent Change vs. Target AUC _{0-∞} ^b	-38.2%	-37.0%	-16.6%	-9.5%	+49.7%
Percent Change vs. Test-Dose CL	+61.8%	+58.4%	+19.5%	+10.6%	-34.3%
Corrected Dose (mg (mg/kg)) ^c	197 (5.16)	161.8 (4.47)	96 (4.82)	n/a	n/a

Abbreviations: AUC_{0-∞} = area-under-the-concentration-time curve from time 0 to infinity; CL = clearance; Vd = volume of distribution; t_{1/2} = half-life

^aBased on ideal body weight

^bActual AUC measures in comparison to full dose AUC targets based on test dose PK predictions

^cCorrected dose (mg and mg/kg) refers to the corrected full daily dose calculated for the original AUC target, based on the PK results of the full daily dose. n/a = not applicable, no adjustments made.

Table 3

Comparisons to FDA and EMA busulfan dosing nomograms

Subject	1	2	3	4	5
<i>Recommended Dosing Based on Nomograms^a</i>					
FDA Dose (mg/kg) ^{a,c}	0.8 (q 6h) 3.2 (q 24h)	0.8 (q 6h) 3.2 (q 24h)	0.8 (q 6h) 3.2 (q 24h)	0.8 (q 6h) 3.2 (q 24h)	0.8 (q 6h) 3.2 (q 24h)
EMA Dose (mg/kg) ^{b,c}	0.8 (q 6h) 3.2 (q 24h)	0.8 (q 6h) 3.2 (q 24h)	1.1 (q 6h) 4.4 (q 24h)	0.8 (q 6h) 3.2 (q 24h)	0.8 (q 6h) 3.2 (q 24h)
<i>Comparisons to Test-Dose PK Results (e.g., every-6-hour dosing)</i>					
Actual Dose (mg/kg)	0.83	0.8	0.83	0.8 ^a	0.8
Measured AUC _{0-∞} (μM*min)	1047	1135	746	1483	822
Adjust dose per FDA? ^d	No	No	Yes – ↑	Yes – ↓	Yes – ↑
Adjust dose per EMA? ^e	No	No	Yes – ↑	No	Yes – ↑
<i>Comparisons to Once-Daily Dose PK Results (e.g., every-24-hour dosing)</i>					
Actual Dose (mg/kg)	3.17	2.82	4.29	2.15	3.86
Measured AUC _{0-∞} (μM*min)	2461	2521	3116	3619	5988
Adjust dose per FDA? ^d	Yes – ↑	Yes – ↑	Yes – ↑	No	Yes – ↓
Adjust dose per EMA? ^e	Yes – ↑	Yes – ↑	Yes – ↑	No	No
<i>Comparisons to Corrected Once-Daily Dose PK Results</i>					
Actual Dose (mg/kg)	5.16	4.47	4.82	n/a	n/a

^aThe US Food & Drug Administration dosing nomogram for IV busulfan suggests dosing based on actual body weight in pediatric patients, with doses of 1.1 mg/kg at weights 12 kg, and 0.8 mg/kg >12 kg for every-6-hour busulfan regimens.

^bThe European Medicines Agency employs a more complicated every-6-hour dosing algorithm based on actual body weight of 1 mg/kg for patients <9 kg, 1.2 mg/kg for 9 to <16 kg, 1.1 mg/kg for 16 to <23 kg, 0.95 mg/kg from 23–34 kg, and 0.8 mg/kg for those weighing >34 kg based on data obtained from pediatric patients with malignant and non-malignant disease.

^cCorresponding doses for every-24-hour doses included for comparison – every-24-hour dosing regimens have not been evaluated or approved by the FDA or EMA.

^dBased on an AUC target (range) of 1125 (900–1350) μM*min for every-6-hour regimens, and 4500 (3600–5400) μM*min for every-24-hour regimens as indicated in the package insert

^eBased on an AUC target (range) of 1125 (900–1500) μM*min for every-6-hour regimens, and 4500 (3600–6000) μM*min for every-24-hour regimens as indicated by the dosing nomogram