

# Busulfan Interlaboratory Proficiency Testing Program Revealed Worldwide Errors in Drug Quantitation and Dose Recommendations

Dina M. Kweekeel, PhD PharmD,\*† Jeannine S. McCune, PhD PharmD,‡ Arjen M. Punt, BS,§¶  
Matthijs van Luin, PhD PharmD,†|| and Eric J.F. Franssen, PhD PharmD†\*\*

**Background:** The clinical outcomes of busulfan-based conditioning regimens for hematopoietic cell transplantation (HCT) have been improved by personalizing the doses to target narrow busulfan plasma exposure. An interlaboratory proficiency test program for the quantitation, pharmacokinetic modeling, and busulfan dosing in plasma was developed. Previous proficiency rounds (ie, the first 2) found that 67%–85% and 71%–88% of the dose recommendations were inaccurate, respectively.

**Methods:** A proficiency test scheme was developed by the Dutch Foundation for Quality Assessment in Medical Laboratories (SKML) and consisted of 2 rounds per year, with each round containing 2 busulfan samples. In this study, 5 subsequent pro-

iciency tests were evaluated. In each round, the participating laboratories reported their results for 2 proficiency samples (ie, low and high busulfan concentrations) and a theoretical case assessing their pharmacokinetic modeling and dose recommendations. Descriptive statistics were performed, with  $\pm 15\%$  for busulfan concentrations and  $\pm 10\%$  for busulfan plasma exposure. The dose recommendations were deemed accurate.

**Results:** Since January 2020, 41 laboratories have participated in at least 1 round of this proficiency test. Over the 5 rounds, an average of 78% of the busulfan concentrations were accurate. Area under the concentration–time curve calculations were accurate in 75%–80% of the cases, whereas only 60%–69% of the dose recommendations were accurate. Compared with the first 2 proficiency test rounds (PMID 33675302, October, 2021), the busulfan quantitation results were similar, but the dose recommendations worsened. Some laboratories repeatedly submit results that deviated by more than 15% from the reference values.

**Conclusions:** The proficiency test showed persistent inaccuracies in busulfan quantitation, pharmacokinetic modeling, and dose recommendations. Additional educational efforts have yet to be implemented; regulatory efforts seem to be needed. The use of specialized busulfan pharmacokinetic laboratories or a sufficient performance in busulfan proficiency tests should be required for HCT centers that prescribe busulfan.

**Key Words:** busulfan, proficiency testing, therapeutic drug monitoring, pharmacokinetics, quality control

(*Ther Drug Monit* 2023;00:1–6)

## INTRODUCTION

The chemotherapy drug busulfan is used in conditioning regimens that are administered before allogeneic hematopoietic cell transplantation (HCT). Usually, busulfan doses range from 2 to 4 mg/kg/d and are administered for 1–4 days.<sup>1</sup> Because busulfan pharmacokinetics (PK) vary among patients, it is common practice to conduct busulfan therapeutic drug monitoring (TDM). In general, this process involves quantitating busulfan plasma concentrations after administration of the first dose and calculating the area under the concentration–time curve (AUC). The individual patient's busulfan clearance is then calculated ( $CL = \frac{\text{dose}}{\text{AUC}}$ ) and subsequently used to estimate the dose personalized to the target AUC using this patient's clearance. This allows the dose to be adjusted before the next busulfan administration to reach the target AUC. This is

Received for publication February 14, 2023; accepted March 31, 2023.

From the \*Department of Clinical Pharmacy and Toxicology, Leiden University Medical Center, Leiden, The Netherlands; †Drug Analysis and Toxicology Division (KKG) of the Dutch Foundation for Quality Assessment in Medical Laboratories (SKML), Utrecht, The Netherlands; ‡Department of Hematologic Malignancies Translational Sciences and Department of Hematologic Malignancies & HCT, Beckman Research Institute of City of Hope, Duarte, California; §Department of Clinical Pharmacy, Division of Laboratory Medicine and Pharmacy, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands; ¶Central Diagnostic Laboratory, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands; ||Department of Clinical Pharmacology, University Medical Center Utrecht, Utrecht, The Netherlands; and \*\*Department of Clinical Pharmacy OLVG, Amsterdam, The Netherlands.

The authors declare no conflict of interest.

D.M. Kweekeel is the primary author and, since May 2020, coordinator of the Busulfan PTS at the Drug analysis and Toxicology Division (KKG) of the Dutch Foundation for Quality Assessment in Medical Laboratories (SKML), Utrecht, The Netherlands. J.S. McCune is co-founder of the busulfan proficiency test and coauthor. A. Punt was involved in the production of busulfan samples. M.V. Luin is coauthor and board member of KKG/SKML, Utrecht, The Netherlands. E.J. Franssen is chair of the KKG/SKML, Utrecht, The Netherlands.

Compliance with Ethical Standard: Yes (if applicable).

Correspondence: Dina M. Kweekeel, PharmD, PhD, Leiden University Medical Center, Department of Clinical Pharmacy & Toxicology, Albinusdreef 2, Leiden 2333 ZA, The Netherlands (e-mail: d.m.kweekeel@lumc.nl).

Copyright © 2023 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the International Association of Therapeutic Drug Monitoring and Clinical Toxicology. This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

important because low AUC values have been linked to worsened patient outcomes,<sup>2–5</sup> graft rejection,<sup>6–8</sup> or relapse.<sup>9</sup> Conversely, high AUC values are associated with increased nonrelapse mortality<sup>10</sup> and liver toxicity.<sup>7</sup>

Busulfan plasma concentrations must be measured rapidly because the time between doses (ie, 6–24 hours) and the duration of treatment are short ( $\leq 4$  days). These logistical constraints allow very little time to quantitate busulfan concentrations, conduct pharmacokinetic modeling of these concentrations, and recommend an accurate dose to achieve the target AUC. In addition to these practical challenges, the quantitation of busulfan is challenging. Busulfan is an unstable compound in plasma and requires robust quality control of analytical materials and procedures within the laboratory. Before 2019, no worldwide proficiency testing program was available, making it difficult to verify results in other laboratories. In 2021, we described the results of the first 2 rounds of an international proficiency testing program for busulfan.<sup>11</sup> It was found that approximately 15% of busulfan concentrations were inaccurately quantitated, and over 10% of dosing recommendations were inaccurate. Here, we describe the findings of 5 subsequent rounds of testing and provide insights into the current status of busulfan quantitation, PK modeling, and dose recommendations.

## MATERIALS AND METHODS

As previously described,<sup>11</sup> a busulfan proficiency testing program (PTS) was developed by the Drug Analysis and Toxicology Division of the Dutch Foundation for Quality Assessment in Medical Laboratories (SKML, www.skml.nl). Since our previous publication in 2021, 5 additional PTS rounds (ie, rounds 3–7) with 2 samples per round were conducted between January 2020 and September 2022. The results of the subsequent rounds are presented here.

The sample preparation and distribution procedures were validated as previously described.<sup>11</sup> In brief, because busulfan is an unstable analyte, a nonaqueous formula was developed that could be shipped on ice packs. The

participating laboratories were masked to the busulfan concentrations in the PTS samples. Immediately before quantitation, the participating laboratories diluted the sample containing a precise amount of busulfan (dissolved in 0.09 mL of N,N-dimethylacetamide) with 1 mL of blank calf serum. The authors electronically submitted their results to the SKML database. Results were considered accurate if they fell within 85%–115% of the reference value (ie, the theoretical value of the added busulfan in N,N-dimethylacetamide). Busulfan concentrations in the 10 PTS samples (2 samples per round) ranged from 0.7 to 3.5 mg/L (Table 1).

In addition to busulfan quantitation, we provided theoretical or actual clinical (anonymized) cases to assess the capabilities of the laboratories for PK modeling and dose recommendations. The clinical cases described the patients characteristics (weight, age, and sex), target AUC window, and known plasma busulfan concentrations. Therefore, the clinical cases could be assessed accurately, even if the actual quantitation of the samples was incorrect (ie, they were separate assignments). The participating laboratories were asked to complete 2 rounds (rounds 5 and 7). Answers within a 10% range of the reference value (ie, 90%–110%) were deemed accurate. The reference values for the PK modeling results (ie, the AUC calculation) and dose recommendations were the average of the answers provided by 3 or 4 busulfan PK experts. These experts were masked to each other's answers and were asked to answer clinical case questions. The PTS coordinator compared the individual expert answers and used the average as the reference value.

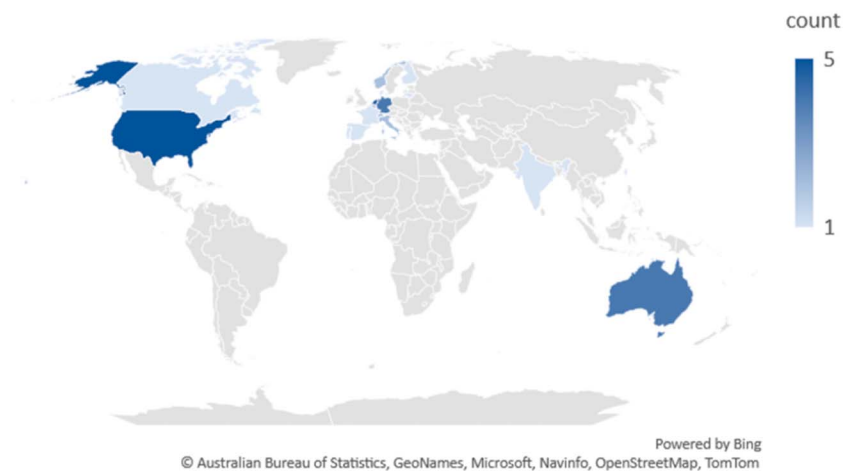
After each round, a report summarizing the results was sent to each participating laboratory. For reference, the report included anonymized data from all participating laboratories, as well as the individual results of that particular participating laboratory compared with the reference values. Thus, the reports provide valuable insights into the performance of each laboratory's busulfan quantitation, PK modeling, and dose recommendations. Data were then submitted through the digital QBase portal and analyzed using Microsoft Excel (Redmond, WA).

**TABLE 1.** Busulfan Quantification Results

Month–Year of PTS	October 2020		December 2020		April 2021		November 2021		April 2022	
	2020.1A	2020.1B	2020.2A	2020.2B	2021.1A	2021.1B	2021.2A	2021.2B	2022.1A	2022.1B
Reference value (mg/L)	3.220	3.303	0.826	2.147	0.991	3.468	0.710	1.998	1.503	3.501
No. of participating laboratories	28	28	30	30	30	30	29	29	38	38
Percentage of laboratories using LC-MS	89%	89%	83%	83%	90%	90%	93%	93%	89%	89%
Percentage (n) laboratories with accurate concentrations*	79% (22)	71% (20)	70% (21)	77% (23)	70% (21)	77% (23)	86% (25)	76% (22)	87% (33)	87% (33)
Median (range), as a % of the reference value	104% (47–128)	104% (39–125)	106% (62–179)	103% (70–158)	105% (73–227)	104% (64–137)	93% (75–117)	94% (70–116)	104% (36–143)	101% (48–116)

Each PTS had a low (shaded gray) and high busulfan concentration. The participating laboratories were blinded to the concentrations when the PTS samples were quantitated.  
\*Accuracy, defined as 85%–115% of the reference value.

### Participating Laboratories' locations



**FIGURE 1.** Locations of laboratories participating in the busulfan PTS conducted in April 2022.

### RESULTS

A total of 41 laboratories participated in 1 or more PTS rounds since January 2020. The participating laboratories were distributed worldwide (Fig. 1), with most located in Europe (22), Australia (4), and the United States (5). Nearly all participating laboratories used LC–MS/MS to quantify busulfan.

#### Drug Quantitation

The participating laboratories received 157 shipments; only 1 participant reported that they received the samples at ambient temperature (round 2020.1). For these samples, the results did not differ significantly from those reported by other laboratories (interquartile range test) and were therefore included in the current analysis. In addition, data were adjusted in case of obvious typographical errors, such as a 1000-fold mistake (1 laboratory in 2020.1, 3 laboratories in 2021.1, 2 laboratories in 2021.2, and 3 laboratories in 2022.1) or when laboratories switched the PTS samples (1 laboratory in 2020.2 and 1 laboratory in 2021.2).

Overall, busulfan concentrations were accurately measured in 70%–87% of the PTS samples (Table 1). In the subgroup of 15 laboratories that had participated in all rounds since January 2020 and submitted a >15% deviation in at least 1 of those rounds, there was a trend toward smaller and fewer deviations in each subsequent round (Fig. 2).

#### Pharmacokinetic Modeling and Dose Recommendations

Only 16 (53%) and 20 (53%) participating laboratories provided PK modeling (ie, AUC) and dose recommendations, respectively (Table 2). In 3 responses, the participating laboratories reported a 4-day AUC, whereas only the AUC on day 1 was requested. Those responses were corrected by dividing them by 4 (2 laboratories in 2021.1 and 1 laboratory in 2022.1). In round 2022.1, 1 laboratory reported both trapezoidal and Bayesian results; only its Bayesian estimation was used for data analysis.

Overall, the AUC was accurate in 75%–80% of the 2 cases, whereas only 60%–69% of the dose recommendations were accurate. Even in the most recent (2022.1) case, there were considerable differences between the AUC results (Fig. 3A). This resulted in large differences in the dose recommendations (Fig. 3B). This PTS did not evaluate how [eg, post hoc Bayesian estimation, compartmental modeling, or noncompartmental (trapezoidal) analysis] the AUC and clearance were calculated by the participating laboratories. However, some laboratories that reported low AUC values for day 1 commented that they used noncompartmental analysis (ie, a trapezoidal PK analysis). These data suggest that some laboratories use PK models that may result in divergent AUC values, but unfortunately, there are not enough data to arrive at a definite conclusion on this point.

It is expected that laboratories that calculate low AUCs would recommend high doses for days 2–4 and vice versa; however, this does not seem to be the case. Strikingly, some laboratories that calculated low AUCs provided accurate dose recommendations, whereas it was expected that they would advise a higher dose. On the other hand, a higher dose was also recommended by laboratories with an accurate AUC for day 1. In addition, laboratories with normal AUC calculations recommended very low doses, which shows that, apart from the PK model used, there were significant differences in the interpretation of the AUC, individual patient clearance, and dose recommendations.

### DISCUSSION

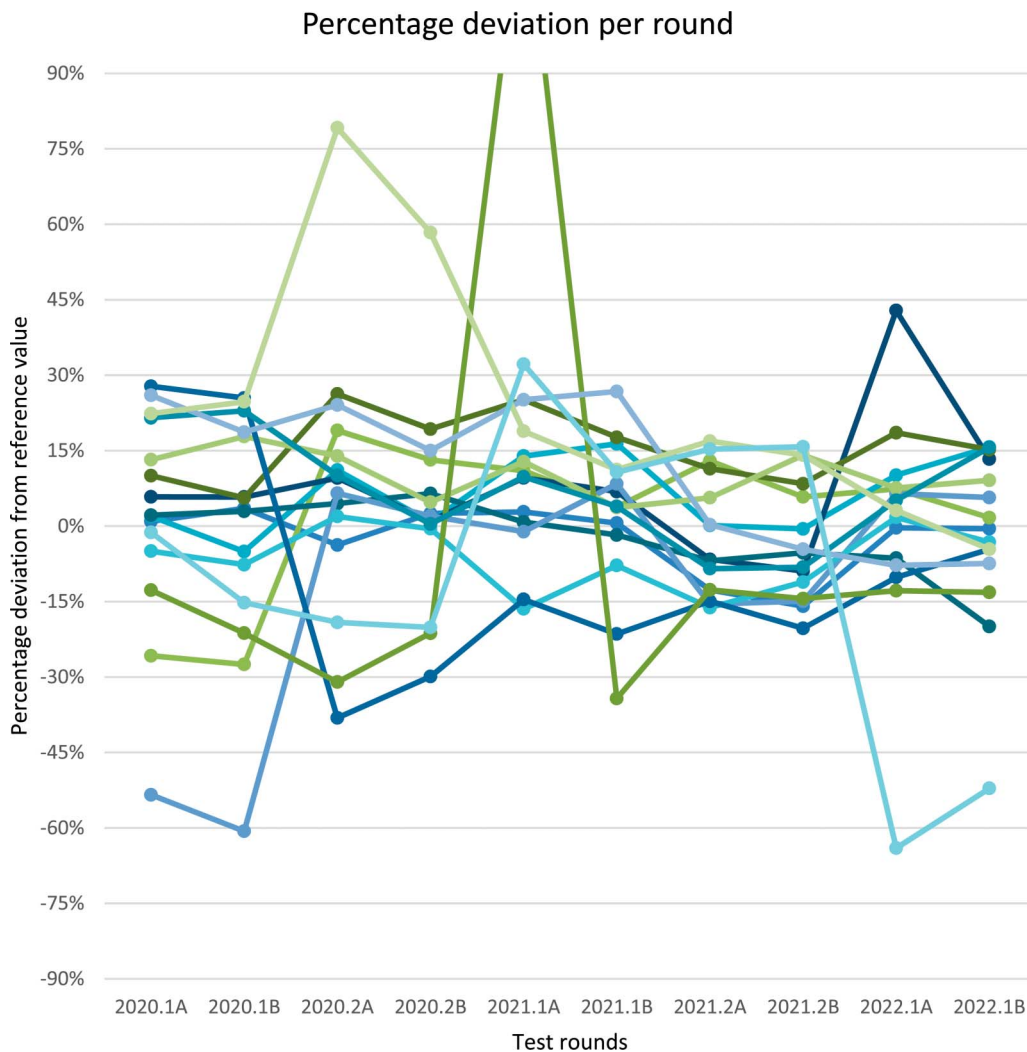
This study provides valuable insights into the performance of laboratories involved in the TDM of busulfan in patients with HCT worldwide. If the results represent the day-to-day quantitation of clinical samples, approximately 1 in 5 quantitations (22%) of these laboratories deviate by more than 15% from the reference value. In addition, there are considerable differences in dose recommendations even when laboratories used the same busulfan concentration data set. This article shows that approximately 1 in 3 dose recommendations (36%) differ by more than 10% from the expert panel.

Because busulfan is a highly potent and toxic agent, overdosing and underdosing must be avoided, and these observations are extremely relevant.

Compared with our previous results,<sup>11</sup> the accuracy of busulfan quantitation was comparable; the first 2 PTS rounds had 67%–85% accurate results, compared with 70%–87% in the subsequent 5 PTS rounds. For laboratories that participated in all rounds (ie, 10 samples), a trend was visible toward fewer and smaller deviations with time. This indicates that participation in a proficiency testing schedule is a helpful tool to improve busulfan quantitation methods, as was found in studies of other drugs as well.<sup>12,13</sup> In addition, for ISO15189-accredited medical laboratories, participation in interlaboratory comparisons is a prerequisite for accreditation.<sup>14</sup> Many regional accreditation agencies (eg, the College of American Pathologists in the United States) also require this type of interlaboratory comparison. Our PTS service has 2 unique capabilities. First, the global PTS is economically feasible because it avoids the shipping of proficient

samples on dry ice. Our PTS samples can be sent using an ice pack, which saves international fees for shipping packages with dry ice. The busulfan PTS sample contained a small amount of N,N-dimethylacetamide to prevent the decomposition of busulfan. This chemical disturbs the immunoagglutination reaction in certain automated analyzers, resulting in a result that is 3–10 times higher than LC-MS and reference values, as published previously.<sup>15</sup> In the current study, none of the participating laboratories used an immunoanalytic method. In addition to LC-MS/MS, a few laboratories have used HPLC-UV or GC-MS to quantify busulfan. The second unique capability is our infrastructure for assessing the PK modeling and dose recommendations.

Although additional local regulations exist worldwide, our global PTS could facilitate more rigorous multicenter international studies to evaluate the association between busulfan AUC and clinical outcomes. Because the published literature was too heterogeneous and lacked adequate support and sufficiently



**FIGURE 2.** Accuracy of busulfan quantification over time. The y axis shows the % deviation from the reference value, and the x axis shows the PTS round. Each line represents 1 participating laboratory; only laboratories that submitted results for all samples and had >15% deviation in at least 1 concentration are shown.

Downloaded from http://journals.lww.com/drug-monitoring by BhdMf5ePHkav1zEoun1tQIN4a+kULNEZgshH04XM on 06/08/2023

**TABLE 2.** Accuracy of Responses to the Pharmacokinetic Modeling and Dose Recommendation Questions Provided by Participating Laboratories

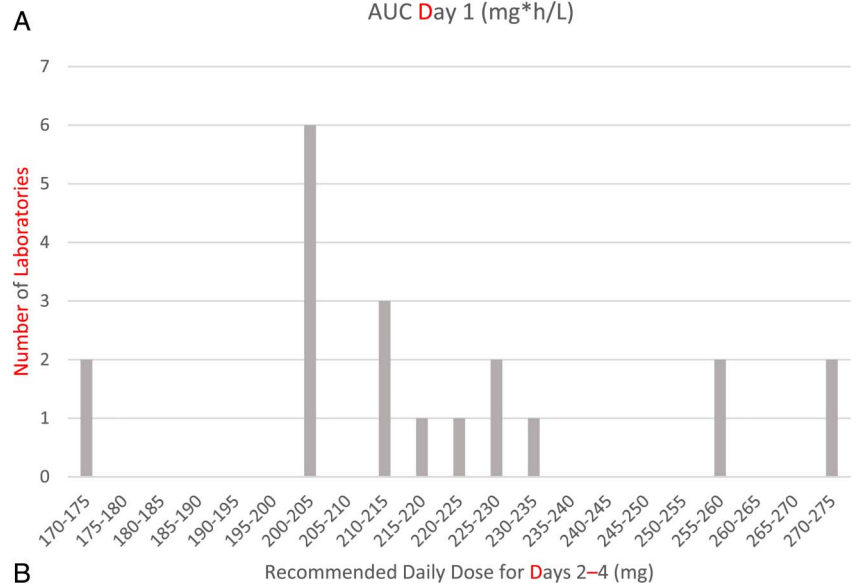
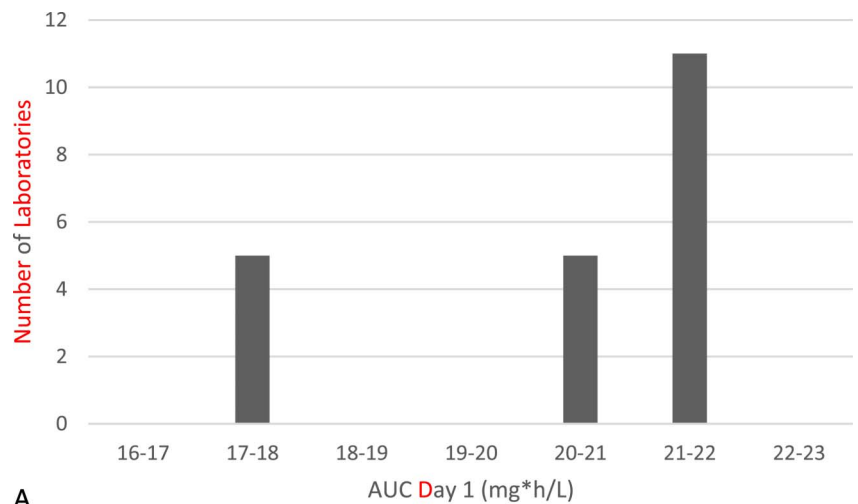
PTS Round, as Month-Year	April 2021	April 2022
Number of participating laboratories responding to AUC questions	16	20
Percentage of accurate AUC calculations (n)*	75% (12)	80% (16)
Median (range) of AUC calculation, as a % of the reference value	100% (89–119)†	100% (95–112)
Percentage of accurate dose recommendations (n)*	69% (11)	60% (12)
Median (range) of dose recommendations, as a % of the reference value	101% (88–115)	100% (81–131)

\*Accuracy within 10% of the reference value.

†Note: excluding 1 outlier of 328% for the AUC calculation.

controlled studies, the American Society for Cellular Therapy and Transplantation Clinical Practice Guideline Committee could not establish target busulfan AUCs.<sup>3</sup> Combining busulfan AUC data in the Center for International Blood and Marrow

Transplant Research database is likely to be even more fruitful. Collecting such data is important, especially because a survey suggested that 50%–60% of HCT centers use busulfan TDM.<sup>16</sup> Such studies will permit the development of evidence-based



**FIGURE 3.** (A) Busulfan AUC values (x axis) by the number of participating laboratories (y axis) for PTS round 2022.1. One laboratory submitted 2 results; both of which are shown in this figure. The number of accurate responses, as determined by the expert panel, was 19.4–23.7 mg × h/L. (B) Recommended daily busulfan dose for days 2–4 (x axis) based on the number of participating laboratories (y axis) for PTS 2022.1. The accurate responses, as determined by the expert panel, were 200–225 mg.

Downloaded from http://journals.lww.com/drug-monitoring by BhdMf5aPHkav1zEoun1tQIN4a+kULNEZgphsH0d4XM on 06/08/2023

guidelines for the target busulfan AUC for various HCT conditioning regimens in specific disease settings.

It is generally recognized that the PTS is a crucial means of monitoring and maintaining the quality of analytical methods, such as busulfan quantification.<sup>17</sup> The number of participating laboratories has increased from 27 in 2019 to 38 in 2022. Although this increase is encouraging, we suspect that not all laboratories that quantitate busulfan participate in the PTS. In addition, over the past 4 years since the first busulfan PTS, some laboratories have discontinued participating in this PTS (reasons unknown).

The percentage of accurate dose recommendations was lower than that in the previous study (60%–69% vs. 71%–88%).<sup>11</sup> Participating laboratories using the noncompartmental analysis for the clinical case in round 2022.1 calculated a significantly lower AUC than those using a population PK model. The use of population PK-guided dosing for busulfan has been suggested for over 15 years and we suggest that it should be immediately adopted.<sup>18,19</sup> Because an actual clinical case with real-life busulfan concentrations was used for this round, these observations are extremely relevant. Although unexpected and disappointing, these results indicate an urgent need for more education on this topic and the use of contemporary (ie, population PK-guided) dosing tools. HCT centers that prescribe busulfan are required to participate in and accurately perform busulfan PTS. Centers without accurate performance should use specialized busulfan PK laboratories that accurately perform busulfan PTS. With the lack of improvement between our first publication and this study, accrediting boards for HCT programs (eg, Foundation for the Accreditation of Cellular Therapies or FACT) may require HCT centers to accurately perform the busulfan PTS over a consistent period before the center can prescribe busulfan and its TDM.

## CONCLUSIONS

Inaccuracies persist in busulfan quantitation, PK modeling, and dose recommendations. Busulfan is a potent and toxic drug used in vulnerable patients. Therefore, it is extremely important that laboratories produce reliable results for therapeutic drug monitoring and that these results are interpreted accurately. The new busulfan PTS may have helped some laboratories improve their TDM. Overall, many inconsistent results were obtained, requiring internal investigation by the laboratories involved. Importantly, there are important differences between the PK analysis methods used, and more education on this topic is necessary. Besides the differences in PK models, finding an accurate busulfan dose with PK modeling depends not only on the expertise of the person conducting the analysis but also on accurate measurements. Therefore, contemporary population PK models, additional education, and regulatory efforts are recommended to improve the accuracy of PK modeling to estimate the AUC and clearance, along with busulfan dose recommendations.

## ACKNOWLEDGMENTS

In memory of Erik van Maarseveen, who started the proficiency test in 2019. The authors acknowledge the

laboratories that participated in the proficiency rounds. The authors thank all the Dutch Foundation for Quality Assessment in Medical Laboratories (SKML) personnel for their cooperation with busulfan PTS, especially Linda Wisselo and Anneke Harteveld-Pauw. Please email office@skml.nl to inquire about their participation in future busulfan proficiency rounds.

## REFERENCES

- Deeg HJ, Maris MB, Scott BL, et al. Optimization of allogeneic transplant conditioning: not the time for dogma. *Leukemia*. 2006;20:1701–1705.
- Bartelink IH, Lalmohamed A, van Reij EM, et al. Association of busulfan exposure with survival and toxicity after haematopoietic cell transplantation in children and young adults: a multicentre, retrospective cohort analysis. *Lancet Haematol*. 2016;3:e526–e36.
- Palmer J, McCune JS, Perales MA, et al. Personalizing busulfan-based conditioning: considerations from the American society for Blood and marrow transplantation practice guidelines committee. *Biol Blood Marrow Transpl*. 2016;22:1915–1925.
- McCune JS, Gibbs JP, Slattery JT. Plasma concentration monitoring of busulfan: does it improve clinical outcome? *Clin Pharmacokinet*. 2000;39:155–165.
- McCune JS, Holmberg LA. Busulfan in hematopoietic stem cell transplant setting. *Expert Opin Drug Metab Toxicol*. 2009;5:957–969.
- Bolinger AM, Zangwill AB, Slattery JT, et al. An evaluation of engraftment, toxicity and busulfan concentration in children receiving bone marrow transplantation for leukemia or genetic disease. *Bone Marrow Transpl*. 2000;25:925–930.
- Slattery JT, Sanders JE, Buckner CD, et al. Graft-rejection and toxicity following bone marrow transplantation in relation to busulfan pharmacokinetics. *Bone Marrow Transpl*. 1995;16:31–42.
- McCune JS, Gooley T, Gibbs JP, et al. Busulfan concentration and graft rejection in pediatric patients undergoing hematopoietic stem cell transplantation. *Bone Marrow Transpl*. 2002;30:167–173.
- Slattery JT, Clift RA, Buckner CD, et al. Marrow transplantation for chronic myeloid leukemia: the influence of plasma busulfan levels on the outcome of transplantation. *Blood*. 1997;89:3055–3060.
- Geddes M, Kangaroo SB, Naveed F, et al. High busulfan exposure is associated with worse outcomes in a daily i.v. busulfan and fludarabine allogeneic transplant regimen. *Biol Blood Marrow Transpl*. 2008;14:220–228.
- McCune J, Punt A, Yeh R, et al. Quality control of busulfan plasma quantitation, modeling, and dosing: an interlaboratory proficiency testing program. *Ther Drug Monit*. 2021;43:657–663.
- Wallenburg E, Brüggemann RJ, Asouit K, et al. First international quality control programme for laboratories measuring antimicrobial drugs to support dose individualization in critically ill patients. *J Antimicrob Chemother*. 2021;76:430–433.
- van Luin M, Wit FW, Smit C, et al. Adherence to HIV therapeutic drug monitoring guidelines in The Netherlands. *Ther Drug Monit*. 2011;33:32–39.
- European Committee for Standardization. *Medical Laboratories—Requirements for Quality and Competence*. Brussels: Comité Européen de Normalisation; 2012. ISO 15189:2012.
- Verougstraete N, Stove V, Verstraete A, et al. Automation in busulfan therapeutic drug monitoring: evaluation of an immunoassay on two routine chemistry analyzers. *Ther Drug Monit*. 2022;44:335–339.
- Copelan EA, Hamilton BK, Avalos B, et al. Better leukemia-free and overall survival in AML in first remission following cyclophosphamide in combination with busulfan compared to TBI. *Blood*. 2013;122:3863–3870.
- Choong E, Uppugunduri C, Marino D, et al. Therapeutic drug monitoring of busulfan for the management of pediatric patients: cross-validation of methods and long-term performance. *Ther Drug Monit*. 2018;40:84–92.
- Bertholle-Bonnet V, Bleyzac N, Galambrun C, et al. Influence of underlying disease on busulfan disposition in pediatric bone marrow transplant recipients: a nonparametric population pharmacokinetic study. *Ther Drug Monit*. 2007;29:177–184.
- Salinger D, Vicini P, Blough D, et al. Development of a population pharmacokinetics-based sampling schedule to target daily intravenous busulfan for outpatient clinic administration. *J Clin Pharmacol*. 2010;50:1292–1300.