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Guideline

Personalizing Busulfan-Based Conditioning: Considerations from the American Society for Blood and Marrow Transplantation Practice Guidelines Committee

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A B S T R A C T

The Practice Guidelines Committee of the American Society of Blood or Marrow Transplantation (ASBMT) sought to develop an evidence-based review about personalizing busulfan-based conditioning. The Committee sought to grade the relevant published studies (June 1, 2008 through March 31, 2016) according to criteria set forth by the Steering Committee for Evidence Based Reviews from ASBMT. Unfortunately, the published literature was too heterogeneous and lacked adequately powered and sufficiently controlled studies for this to be feasible. Despite this observation, the continued interest in this topic led the Practice Guidelines Committee to develop a list of most frequently asked questions (FAQs) regarding personalized busulfan dosing. This "Considerations" document is a list of these FAQs and their responses, addressing topics of practical relevance to hematopoietic cell transplantation clinicians.

INTRODUCTION

The bifunctional alkylating agent busulfan (BU) has been used for approximately 40 years as a major component of chemotherapy-based conditioning before hematopoietic cell transplantation (HCT). High-dose BU is currently still used in many regimens for allogeneic HCT but is used to a much lesser extent when conditioning for autologous HCT. Historically, low-dose BU (2 mg to 6 mg orally daily) was used to treat myeloproliferative neoplasms, such as chronic myelogenous leukemia (CML), polycythemia vera [1,2], and essential thrombocytosis [3], because BU is cytotoxic to hematopoietic precursors and pluripotent stem cells. Now, high-dose BU-based conditioning is frequently used; however, because BU causes limited lymphotoxicity, it is unable to provide adequate immunosuppression as standalone conditioning. As

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Table 1
Frequently asked questions

Frequently Asked Questions (FAQs)	Summary of Answers
FAQ1. Why does personalized busulfan (BU) dosing need to be considered during hematopoietic cell transplantation (HCT)?	Personalized BU dosing is considered mainly because BU has a narrow therapeutic index and a specific BU exposure have been associated with important clinical outcomes in in HCT patients. Therefore, personalized BU dosing via therapeutic drug monitoring (TDM) needs to be considered to minimize sinusoidal obstruction syndrome, lower graft rejection rates, and lower relapse rates in certain situations.
FAQ2. Is personalized BU dosing always necessary?	No. BU TDM is currently considered to be unnecessary for reduced intensity conditioning (RIC) regimens where the balance of BU toxicity to BU efficacy is favorable. With RIC, data is needed to determine if lower BU doses or lower BU exposure compromise efficacy.
FAQ3. When should conditioning utilize BU TDM?	The first consideration to use BU TDM is when the specific BU exposure is associated with clinical outcome(s) in a homogenous patient population. BU TDM must be used in children receiving high-dose BU before allogeneic HCT to lower the risk of graft rejection. Another significant consideration for personalizing BU is when the regimen was developed with BU TDM.
FAQ4. Is oral or IV BU preferred?	Intravenous (IV) busulfan tends to be preferred on the basis of patient convenience and concerns about intrapatient pharmacokinetic variability because of unpredictable gastrointestinal absorption of oral BU and hepatic first-pass effects.
FAQ5. How should personalized BU dosing be achieved?	Personalized BU dosing should be achieved by using TDM after selecting and administering the initial dose of high-dose BU.
FAQ6. How is the initial BU dose best selected?	The initial IV BU dose should be based on the European Medicines Agency (EMA) nomogram for children with a target area under the curve (AUC) of 1125 μmolar × min. For adults with the same target AUC, the initial IV BU dose should be 0.8 mg/kg every 6 hours or 3.2 mg/kg every 24 hours. The initial IV BU dose may need adjustment for lower or higher target AUC. Oral BU dosing always begins at 1 mg/kg.
FAQ7. What is the optimal dosing frequency of BU?	The available IV BU data for adults do not suggest a significant difference in outcomes between Q6H and daily dosing, likely because BU clearance, volume of distribution and half-life appear to be similar regardless of dosing frequency. In children relevant studies are ongoing. Oral BU should be administered Q6H.
FAQ8. What is the best method for predicting BU clearance?	BU clearance is calculated based on the administered BU dose and an estimate of post-dose BU exposure using validated pharmacokinetic modeling tools (see Technical Appendix). Test dose strategies are not currently recommended.
FAQ9. How do other medications affect BU pharmacokinetics?	Ideally, there would be no changes to medications given concomitantly with BU in order to minimize any drug-drug interactions that alter BU pharmacokinetics. The following medications have affected IV BU clearance: fludarabine, deferasirox, metronidazole; or oral BU clearance: fludarabine, metronidazole, ketobemidone, and itraconazole. Phenytoin affects oral BU clearance but its effect upon IV BU clearance is unclear. By extrapolation, voriconazole or posaconazole would likely decrease BU clearance and should be avoided during conditioning.
FAQ10. Should the initial BU dose be personalized based on genetic polymorphisms?	Pharmacogenomics-based dosing of BU, either IV or oral, is not recommended.

a result, BU-induced myelotoxicity has been complemented by adding lymphotoxic agents (eg, cyclophosphamide [CY] or fludarabine [FLU]) and sometimes with agents that have additional activity against the tumor (eg, thiotepa, melphalan, or clofarabine). Seminal studies led by George Santos and Peter Tutschka [4-6] demonstrated that high-dose BU plus CY was effective conditioning [6-8] for allogeneic HCT. However, sinusoidal obstruction syndrome (SOS) was quickly understood to be a dose-limiting toxicity [9]. This observation provided an early hint of the narrow therapeutic index of BU and subsequent data led to the development of personalized BU dosing with the goal of improving patient outcomes.

Personalized dosing of BU using the patient-specific BU clearance, hereafter referred to as *BU therapeutic drug monitoring (TDM)*, is conducted by personalizing the BU dose to a target exposure based on TDM. Target exposure is reflected in the measurement *area under the plasma concentration-time curve* (AUC) or *concentration at steady state* (CSS). The CSS is simply the AUC divided by dose frequency. The practice guidelines committee of the American Society of Blood or Marrow Transplantation (ASBMT) sought to develop an evidence-based review of this complex topic but found that the published literature was too heterogeneous and lacked the necessary controlled studies for this to be feasible. This conclusion was reached after a comprehensive review of articles about the association of BU exposure with clinical outcomes, termed *pharmacodynamic associations* hereafter. Data published between June 1, 2008 and March 31, 2016 were reviewed, with earlier data included when deemed necessary. We searched the PubMed database using the terms *busulfan* and *pharmacokinetic* as well as topics relevant to each particular discussion section. Only finalized peer-reviewed publications were included for review. Initially, we sought to grade studies according to criteria set forth by the steering committee for evidence-based reviews from ASBMT [10]. However, those criteria could not be used because of the heterogeneity of the patient population, conditioning regimen, BU dosing, and BU pharmacokinetic data from studies of typically fewer than 100 patients. As a result, the purpose of this manuscript is to present and answer frequently asked questions (FAQs see Table 1) regarding personalized BU dosing; the answers try to take into consideration what is most practically relevant for offering guidance to HCT clinicians.

FAQ1: WHY DOES PERSONALIZED BU DOSING NEED TO BE CONSIDERED DURING HCT?

Personalized BU dosing is considered mainly because BU has a narrow therapeutic index and a specific BU exposure has been associated with important clinical outcomes in HCT patients. Therefore, personalized BU dosing via TDM needs to be considered to minimize SOS, lower graft rejection rates, and lower relapse rates in certain situations.

In the original BU/CY conditioning regimen, oral BU dosing was based on body weight (mg/kg). Shortly after that, Grochow et al. reported that higher BU exposure was

associated with more frequent hepatotoxicity in adults conditioned with BU/CY [11]. Over the next decade, case series of 50 or fewer patients confirmed this association [12,13] and Slattery et al [13]. were the first to report in children that low BU exposure during BU/CY conditioning was associated with more frequent graft rejection [13,14]. The results of subsequent BU/CY case series found that, compared with historically controlled weight-based dosing, the use of BU TDM was associated with a reduction in hepatotoxicity rates from 75% to 18% [15] and improved engraftment rates from 74% to 96% [16]. Higher BU exposure was associated with lower relapse rates [17] and targeting higher exposure through BU TDM lowered relapse rates [18] among patients conditioned with BU/CY with previously untreated CML, before the era of tyrosine kinase inhibitors. However, understanding the association of BU exposure with post-transplantation relapse in children with acute myeloid leukemia (AML) has been difficult because of small sample sizes and heterogeneity of the AML population [19,20]. The difficulty with understanding the pharmacodynamic association presumably contributes to the variable target exposure chosen by clinicians for children with AML [21]. An association of BU exposure with graft-versus-host disease (GVHD) was not found in BU/CY conditioned patients [22-25]. Thus, in the BU/CY regimen, BU TDM increases engraftment rates in children [16], lowers hepatotoxicity rates in adults [15], and lowers relapse rates in patients with previously untreated CML [18]. However, outside of these clinical situations, the benefit of BU TDM in BU/CY conditioned patients is less clear.

Furthermore, as explained in FAQ3, similar associations between BU exposure and outcomes were not found in patients receiving slightly different conditioning regimens [26,27]. Thus, there remains some controversy regarding the advantage of BU TDM for all conditioning regimens. It is unlikely that any randomized controlled trials will be conducted to understand the benefit of BU TDM. In the absence of such data, clinicians are left to consider whether the narrow therapeutic index of BU applies to their patient based on their conditioning regimen and whether pharmacodynamic associations might be relevant.

FAQ2: IS BU TDM ALWAYS NECESSARY?

No. BU TDM is currently considered unnecessary for reduced-intensity conditioning (RIC) regimens where the balance of BU toxicity to BU efficacy is favorable. With RIC, data are needed to determine if lower BU doses or lower BU exposure compromise efficacy.

BU TDM is only necessary for conditioning regimens that have a pharmacodynamic association of BU exposure with outcomes or, ideally, data showing that BU TDM improves outcomes, as explained in FAQ1. Notably, some studies have reported the unexpected association that low BU exposure is associated with worse nonrelapse mortality (NRM) in the BU/FLU/Thymoglobulin (Genzyme Polyclonals, Lyon, France) regimen [28]. In actuality, the usefulness of BU TDM in RIC (BU < 9 mg/kg oral or intravenous [i.v.] equivalent) has not been systematically evaluated. There are also substantial logistical barriers to BU TDM with these regimens because BU is only administered for 1 or 2 days, necessitating on-site BU TDM. Therefore, it has not been feasible to identify a total BU dose that is unsafe when dosed based on body weight or without TDM. A reduced-intensity FLU/BU/Thymoglobulin regimen has been successfully developed with BU TDM for infants with nonmalignant diseases (see FAQ7). However, outside of this patient population, weight-based BU dosing without TDM has been predominantly used in RIC [29-32]. In the interest of trying to determine whether BU dosing in the FLU/BU regimen had any effect on disease control, 1 group evaluated 6 different BU dose cohorts, ranging from 3.2 mg/kg to 12.8 mg/kg, and found that the 11.2 mg/kg dose cohort, compared with all other predominantly lower dose cohorts, had improved overall survival (OS) and relapse-free survival [50]. However, another group compared 3.2 mg/kg daily to 6.4 mg/kg daily and found no difference in OS, disease-free survival, GVHD, or NRM [29]. Only controlled trials will be able to answer adequately the question of whether there is the potential clinical benefit of BU TDM in RIC.

FAQ3: WHEN SHOULD CONDITIONING UTILIZE BU TDM?

The first consideration to use BU TDM is when the specific BU exposure is associated with clinical outcome(s) in a homogenous patient population. BU TDM must be used in children receiving high-dose BU before allogeneic HCT to lower the risk of graft rejection. Another significant consideration for personalizing BU is when the regimen was developed with BU TDM.

The initial data showing that BU exposure was associated with clinical outcomes and was generated by BU TDM after orally administered BU (see FAQ1). When Andersson et al [33]. led the development of i.v. BU in the 1990s, it was hoped that improved interpatient variability in BU pharmacokinetics would obviate the need for BU TDM. Evidently, this was not the case, as reflected by the product labeling for i.v. Busulfex (Patheon Manufacturing Services LLC, Greenville, NC) that clearly states: "Therapeutic drug monitoring and dose adjustment following the first dose of BUSULFEX is recommended" for pediatric HCT patients with CML conditioned with BU/CY [34]. The target i.v. BU exposure (AUC) is 1125 μmolar × minute with an acceptable range of 900 to 1350 ± 5% μmolar × minute after every 6 hour (Q6H) dosing (See FAQ5 and Technical Appendix for further details) [34]. Not surprisingly, the frequency of BU TDM increased shortly after the February 1999 Food and Drug Administration (FDA) approval of i.v. BU (Supplemental Figure 1 of McCune et al [21].). Currently, BU TDM is considered only for high-dose conditioning (BU > 9 mg/kg oral or i.v. equivalent [35]) with pharmacodynamic associations or when the regimen was developed with BU TDM (eg, BU/melphalan [36], vorinostat/gemcitabine/BU/melphalan [37], or CY/BU [38]). Unfortunately, there cannot be 1 BU target exposure for all HCT conditioning regimens because each regimen was developed based on the maximum tolerated BU dose (or systemic exposure) within unique multicomponent regimens. This issue is compounded by the underlying disease type and risk for graft rejection (eg, minimal pretransplantation therapy), which influences the optimal BU exposure. Ideally, the BU target exposure would be available for each high-dose regimen, but the published literature is often too heterogeneous with small case series. The section below describes confounding factors and general considerations for choosing the target exposure, making recommendations for those regimens used most often.

Confounders

When choosing the target BU exposure to optimize clinical outcomes, one also needs to consider the impact of other conditioning regimen components and the baseline patient characteristics. The heterogeneity in the conditioning regimens and the baseline patient characteristics has confounded most retrospective outcomes analysis evaluating the association of BU exposure to clinical outcomes. Interpretation of GVHD analyses is particularly difficult because of age and

GVHD practice variation across different centers [39]. The extent of HLA mismatch can also confound the risk for both graft rejection and GVHD. Although rates of SOS after BU/CY can be minimized through personalized BU dosing [14,15,40], there are alternative approaches to mitigating SOS. These approaches include replacing CY with FLU [30] or administering the CY before BU, as in the CY/BU regimen [38]. Delaying CY administration until after BU might be beneficial based on data from a cohort of patients who received BU without phenytoin followed by CY at varying time intervals [41]. Specifically, a higher incidence of SOS was observed when the first CY dose was administered 7 to 15 hours versus 24 to 48 hours after the last BU dose [41]. In other studies, BU exposure alone was not associated with SOS, but higher BU exposure was associated with SOS in patients receiving concomitant melphalan [42] or in patients with neuroblastoma who were conditioned for autologous HCT with BU, melphalan, and thiotepa [43]. Besides SOS, GVHD is a significant contributor to NRM in allogeneic HCT recipients. In general, BU exposure is not associated with GVHD, although this association is confounded by the additional conditioning regimen components. Specifically, GVHD rates after BU/CY have not been influenced by BU exposure [22-25], although 2 pediatric studies reported a higher incidence of GVHD when BU/CY was paired with melphalan [25,44].

General Considerations

In allogeneic HCT preceded by BU/CY conditioning, in general, the target BU CSS (see **FAQ5** for further explanation) is 600 ng/mL to 900 ng/mL when the underlying reason for HCT is hematologic malignancy other than previously untreated CML. More narrow target exposures (eg, 800 ng/mL to 900 ng/mL [38,45] or 900 ± 100 ng/mL [46,47]) have been used in the BU/CY or BU/FLU regimen, respectively. A large study by the Center for International Blood and Marrow Transplant Research (CIBMTR) showed that BU/CY conditioning is generally associated with superior outcomes compared with CY/total body irradiation (TBI) for first remission AML. Although a CIBMTR survey later found that 50% to 60% of reporting centers provide BU pharmacokinetic data, the AML study was unable to determine how BU TDM might have contributed to the reported outcomes [48]. Cautious interpretation is needed as these results are based on retrospective analysis of registration data.

It should be recognized that when BU is combined with agents other than or in addition to CY (eg, TBI, melphalan, or thiotepa), relationships between BU exposure and clinical outcomes are altered [14]. This has been observed in BU/CY/TBI and in children receiving BU plus various alkylating agents (ie, thiotepa alone, melphalan alone, CY/melphalan, CY/thiotepa). BU TDM should be conducted in children receiving BU-based conditioning for an allograft because personalizing doses reduces graft rejection (see FAQ1). In general, BU TDM should be conducted in adults receiving BU/CY, FLU/BU/Thymoglobulin ± TBI, and any novel regimens developed using BU TDM (eg, vorinostat/gemcitabine/BU/melphalan [37]). However, the use of BU TDM has varied with FLU/BU [49,50], clofarabine/BU [32,51,52], and BU/CY/etoposide (BuCyE) regimens mostly based on the magnitude of the BU dose [53,54].

Of keen interest to the development of novel high-dose conditioning regimens is the replacement of CY with FLU, a purine nucleoside inhibitor that is potentially less toxic yet with similar immunosuppressive efficacy as CY [30]. Data from a recent phase III trial in older patients with AML indicated that FLU/BU is associated with lower transplantation-related mortality than BU/CY but retains antileukemic activity, suggesting FLU/BU should be the standard of care for such patients [55]. A large meta-analysis of 15 clinical trials including 1830 patients reported FLU/BU was associated with a lower risk for day 100 NRM, no differences in all-cause mortality at 100 days, and lower SOS and microbiologically documented infections compared to BU/CY [30]. Notably, engraftment kinetics and risks of grade 3 to 4 mucositis, GVHD, relapse, and NRM at the end of the study were all similar between FLU/BU and BU/CY. These findings led to the conclusion that both regimens have similar efficacy profiles, whereas toxicity is lower with FLU/BU regimen [30]. The replacement of CY with BU has allowed for higher BU target exposures without SOS. Keeping BU CSS <900 ng/mL appears necessary for BU/CY conditioning [14], but a BU CSS of 800 ng/mL to 1000 ng/mL is well tolerated in the FLU/BU regimen [46,47,57]. Although centers developed the FLU/BU regimen with BU TDM, most others started with weight-based BU dosing, which provided sufficient variability in the BU exposure to allow for discovery of associations between BU exposure and clinical outcomes [56-59]. The need for BU TDM in the FLU/BU regimens is variable because of regimen permutations in the total BU dose, FLU dose, use of Thymoglobulin, and/or type of post-grafting immunosuppression. When combined with a cumulative FLU dose of 120 mg/m^2, i.v. BU (dosed every 12 hours or daily) can be dosed based on body weight without TDM. Within this regimen, the cumulative BU doses range from 3.2 [29] to 11.2 [50] mg/kg in adults. With similar FLU/BU regimens, BU exposure is associated with outcomes. For adolescents and adults who received FLU/BU, a BU AUC >9000 μmolar × minute/day led to SOS in all patients, whereas only 2 cases of SOS occurred among 69 patients with target AUC ≤ 7500 μmolar × minute/day [58]. A study of FLU/BU/alemtuzumab demonstrated increased risk of fatal SOS when maximum AUC exceeded 6800 μmolar × minute/day [60]. In the FLU/BU/Thymoglobulin ± TBI regimen, a BU CSS over 1026 ng/mL was associated with lower NRM, progression-free survival, and OS [28,61]. Interestingly, Russell et al [28], found in a study with a heterogeneous cohort of 158 patients who received FLU/BU/Thymoglobulin ± TBI, patients with BU exposure in the lowest quartile as well as the highest quartile had increased risk of NRM. Specifically, among those patients with a BU CSS <1026 ng/mL, the association of BU exposure with outcomes was evaluated over the 4 BU exposure quartiles. Those with a BU exposure of 759 ng/mL to 854 ng/mL has the lowest risk of NRM, the lowest risk of acute GVHD, a better disease-free survival, and better OS [28]. Engraftment did not differ between the BU exposure groups [28]. The data have been contradictory regarding the relationship between BU exposure and rates of GVHD; some studies showed higher rates of GVHD with low AUC [28], yet others showed higher rates of GVHD with higher AUC [42].

FAQ4: IS ORAL OR INTRAVENOUS BU PREFERRED?

I.V. BU tends to be preferred on the basis of patient convenience and concerns about intrapatient pharmacokinetic variability because of unpredictable gastrointestinal absorption of oral BU and hepatic first-pass effects.

The disadvantages of oral BU are the need for multiple tablets per dose (1 mg/kg dose with only 2 mg tablets available), delayed absorption that can confound BU TDM, the potential for emesis and the need for a standard practice around whether to replace oral BU doses after emesis, and

greater intrapatient (between dose) pharmacokinetic variability. There is a debate regarding whether oral BU has greater interpatient (between patient) variability in BU clearance.

The effects of oral versus i.v. BU on efficacy, toxicity, and pharmacokinetics on the outcomes of allogeneic HCT have been analyzed retrospectively [48,53,63-68]. These studies are often confounded by heterogeneous patient populations, the use of BU TDM for only 1 of the administration routes, differences in the other conditioning regimen components, and BU exposure data not being available. Only those studies with the largest samples sizes, specifically the CIBMTR studies either in allogeneic [48,63] or autologous HCT, are described [65]. Differences between patients who had received i.v. versus oral BU were compared in the CIBMTR study in patients with AML in first remission who underwent allogeneic transplantation after BU/CY conditioning. Compared with patients receiving oral BU/CY, multivariate analysis found that patients who received i.v. BU had lower rates of relapse after 1 year after HCT. As noted in FAQ3, whether patients in this study received BU TDM is unknown, but a survey of centers done shortly after the study suggested 50% to 60% of the centers who reported to CIBMTR used BU TDM [48]. This analysis contrasts with results that have not noted any increased risk in relapse rates when comparing similar regimens that differed only by the route of BU administration [67-70]. The effect of the administration route on SOS has also not been consistent: some reports found a significantly higher rate of SOS after oral BU when compared with after i.v. BU [63,64,66] and others found no major differences [67-70]. Additionally, there have been no reported differences in OS after allogeneic HCT between groups who received oral BU versus i.v. BU regimens [48,68,70]. This lack of difference in OS [48,68] supports the continued use of the less expensive oral BU by some HCT centers [38,68]. In a retrospective Japan registry analysis of 460 children, just over one-half of the study cohort had acute lymphoblastic leukemia and the remainder had AML, 262 had received oral BU, and 198 had received i.v. BU in combination with 1 or 2 of CY, melphalan, or etoposide. The data showed no significant impact of route of BU administration on rates of SOS, NRM, relapse, or OS for both acute lymphoblastic leukemia and AML [71]. This led to an accompanying editorial questioning if the adoption of i.v. BU occurred too quickly [72].

Differences between oral and i.v. BU were compared in patients with non-Hodgkin lymphoma who were conditioned with BuCyE for autologous HCT. Compared with oral BU, i.v. BU was associated with lower relapse rates and superior relapse-free survival and OS. Notably, BU TDM has not been consistently used in BuCyE conditioning for autologous HCT. Most publications show that weight-based BU dosing without BU TDM was used [54], although BU TDM can be used [70,73]. In patients with non-Hodgkin's lymphoma receiving BuCyE, improved OS with i.v. BU has been observed [65,70]; again, contradictory data exist [53].

One final point is that because engraftment is expected in the context of autologous HCT, the lower limit of the target BU exposure should not be based on data obtained from allogeneic HCT recipients showing low BU exposure is associated with poor engraftment (see FAQ1).

FAQ5: HOW SHOULD PERSONALIZED BU DOSING BE ACHIEVED?

Personalized BU dosing should be achieved by using TDM after selecting and administering the initial dose of high-dose BU.

Personalized BU dosing should be achieved using TDM, which is also referred to as *targeted BU*, *target concentration intervention*, or *pharmacokinetic-guided dosing*. The procedure for BU TDM follows general pharmacokinetic principles and has been previously published [74]. Close attention to detail, a validated analytical method to quantitate BU plasma concentrations, and expertise in pharmacokinetic modeling are necessary (see Technical Appendix).

For BU TDM, an initial dose of BU is chosen (see FAQ6) and administered. Next, sequential pharmacokinetic samples are drawn before the subsequent BU dose. Obtaining pharmacokinetic samples over an acceptable time period is critical for accurate estimation of a patient's BU exposure. An acceptable time period for BU pharmacokinetic sampling must balance the half-life of the drug (typically 2 to 3 hours), the dosing frequency (see FAQ7), and the practical logistical issue of obtaining the TDM results in a timely fashion to personalize the BU dose. For BU personalized dosing, an acceptable time period for BU pharmacokinetic sampling can be as short as 4 hours, which occurs with a 2-hour BU infusion and Q6H dosing, or as long as 8 hours, which occurs with a 3-hour BU infusion and every 24-hour (Q24H or daily) dosing. However, the acceptable time period for BU pharmacokinetic sampling can be shortened if population pharmacokinetic (popPK) modeling is used instead of the traditional noncompartmental analysis [75]. The BU clearance is calculated from the administered BU dose and the resulting BU exposure (AUC).

$$\mathrm{CL} = \frac{\text{Administered dose 1}}{\mathrm{AUC}_{0-\infty}} \quad (1)$$

$$\text{Personalized dose} = \mathrm{CL} \times \text{target AUC} \quad (2)$$

$$\mathrm{Css} = \frac{\mathrm{AUC}_{0-\infty}}{\text{dosing frequency}} \quad (3)$$

Firm knowledge of the BU dose and accurate estimation of the BU exposure are essential for predicting BU clearance. After the initial BU dose, the estimated BU exposure is the area under the plasma concentration-time curve to time infinity. The actual AUC value is a complex derived value that uses the pharmacokinetic sample data as detailed in the Technical Appendix. Using Equation 1, the patient's BU clearance is estimated and then used to estimate subsequent BU doses to achieve the desired patient target exposure, as described in FAQ3. With the subsequent Equations 2 and 3, the personalized dose and CSS are calculated, respectively.

In the United States, AUC is reported as micromoles/liter × minute (ie, μmolar × minute or μM × minute) and CSS is reported as ng/mL. A CSS of 900 ng/mL = AUC of 1315 μmolar × minute with Q6H dosing, or an AUC of 5260 μmolar × minute for daily dosing (Table 2). A more detailed table of the equivalents is included in the Technical Appendix. Harmonization of the method for reporting BU exposure and clearance is needed to minimize confusion with interpreting studies from different institutions.

FAQ6: HOW IS THE INITIAL BU DOSE BEST SELECTED?

The initial i.v. BU dose should be based on the European Medicines Agency (EMA) nomogram [76] for children with a target AUC of 1125 μmolar × minute. For adults with the same target AUC, the initial i.v. BU dose should be 0.8 mg/kg Q6H or 3.2 mg/kg Q24H. The initial i.v. BU dose may need adjustment for lower or higher target AUC. Oral BU dosing always begins at 1 mg/kg.

Table 2
BU AUC to CSS Equivalency Table

AUC	AUC	CSS *	AUC†	AUC
μMolar × min Q6H dosing	μMolar × min daily dosing	ng/ mL	mg/L × h Q6H dosing	mg/L × h daily dosing
877	3508	600	3.60	14.4
900	3800	650	3.90	15.6
1125	4500	770	4.62	18.5
1316	5262	900	5.40	21.6
1500	6000	1026	6.16	24.6

All BU plasma exposures are presented in this manuscript using the units within the original manuscript and, if needed, converted to BU concentration at steady state (CSS). The technical appendix and equations 1 to 3 in FAQ5 explain how to convert between the various BU exposure units.

* CSS = AUC divided by the dosing frequency.

† When the AUC is expressed in micromolar (micromoles/L) units, then the BU molecular weight (246.3 g/mol) must be used to calculate the AUC in mg/L units.

Children

When given orally, BU is given at 1 mg/kg every 6 hours for 4 days (a total of 16 mg/kg). Nowadays, children rarely receive oral BU, presumably because of the ease of i.v. administration over oral administration (see FAQ4), although recent data have suggested caution regarding the replacement of oral BU with i.v. BU in children [71,72].

An i.v. BU dose of 0.8 mg/kg results in similar BU exposure to 1 mg/kg of oral BU; a 2-hour i.v. infusion duration was chosen to mimic the time to the maximum plasma concentration after oral administration. Because the initial dose occasionally does not achieve the desired target BU exposure, dose adjustment is required during the conditioning regimen. In fact, over the course of a 4-day BU regimen, the daily BU exposure may fluctuate greatly. It is unclear whether these fluctuations over the entire course of the conditioning regimen will significantly impact outcomes, although achieving target exposure late in the 4-day course has been associated with worse hepatotoxicity in the context of the BU/CY regimen [12].

To identify the optimal pediatric dose, both the EMA and the United States' FDA created separate recommendations for weight-based dosing of i.v. BU with Q6H dosing frequency to achieve a target AUC of 1125 μmolar × minute, which equates to a BU CSS of 770 ng/mL. The FDA labeling advises that the initial dose is based on ideal or actual body weight (whichever is lower) and that 1.1 mg/kg be used for ≤12 kg and 0.8 mg/kg for >12 kg with an acceptable BU AUC being 900 to 1350 ± 5% μmolar × minute [34,77] (CSS = 650 ng/mL to 924 ng/mL). The EMA algorithm is more complicated because it has 5 dose cohorts (Table 3) that are based on actual body weight to achieve a BU AUC of 900 μmolar × minute to 1500 μmolar × minute (CSS = 650 ng/mL to 1026 ng/mL). These 2 different dosing recommendations were based on popPK modeling (see FAQ8 for description). Alternative dosing recommendations exist for children; many are also based on popPK models. A greater proportion of patients achieve the target exposure when using EMA dosing (70%) than when using the FDA dosing (57%), based on simulations using a popPK model built using data from 1610 HCT recipients (92% of whom were children) [78]. Thus, the EMA dosing is recommended (Table 3).

Table 3
European Medicines Agency's I.V. Busulfan Dose To Achieve a Plasma Busulfan AUC of 1125 (900-1500) Micromolar × Minute after Q6H Dosing (CSS = 770 [650 -1026] ng/mL)

Patient's Actual Body Weight	EMA Dosing with Q6H	Corresponding Dosing with Q24H
<9 kg	1 mg/kg/dose	4 mg/kg/dose
9 to <16 kg	1.2 mg/kg/dose	4.8 mg/kg/dose
16 to <23 kg	1.1 mg/kg/dose	4.4 mg/kg/dose
23 to 34 kg	0.95 mg/kg/dose	3.8 mg/kg/dose
>34 kg	0.8 mg/kg/dose	3.2 mg/kg/dose

Please note only Q6H dosing was evaluated by Nguyen [76] and only Q6H dosing is approved by the EMA and the FDA.

When the initial dose is based on EMA nomogram [76], the initial dose has achieved BU target exposures in 59% to 81% [81] of children [76]. In the United States, there has been substantive variability in the initial BU dose prescribed for children, with only a minority having received the FDA-approved dose. Unfortunately, in current clinical practice, the initial i.v. BU dosing has achieved desired target exposure in only 24.3% of children and improved approaches to selecting the preferred initial i.v. BU dose are desirable (eg, test dose and pharmacogenetics). These observations show that carefully selected initial BU dosing does not obviate a need for BU TDM (see FAQ5), especially if a narrow target exposure is desired.

Adults

Both the FDA and EMA recommend an initial i.v. Q6H BU dose of 0.8 mg/kg for adults; specifically, patients >12 kg per the FDA or >34 kg per the EMA. With once daily i.v. BU, the initial adult dose has been 3.2 mg/kg, 4 mg/kg [38,82], or 130 mg/m^2 [62,83]. BU target exposure influences the initial dose selection because a 4 mg/kg initial dose achieved target CSS of 800 ng/mL to 1000 ng/mL in a higher percentage of patients than the traditional 3.2 mg/kg dose. For obese adults, current ASBMT guidelines recommend dosing i.v. BU based on adjusted ideal body weight, as calculated using Equation 4 [84]. This equation should be used in adults receiving mg/kg dosing or mg/m^2 dosing with body surface area estimated using actual body weight [84].

$$\text{Adjusted ideal body weight} = \text{ideal body weight} + 0.25\,(\text{actual body weight} - \text{ideal body weight}) \quad (4)$$

For oral BU, the initial dose of 1 mg/kg Q6H continues to be appropriate for adults. In obese patients, oral BU should be dosed based adjusted ideal body weight (Equation 4). Hemodialysis has been shown to enhance BU clearance after oral BU administration [14], a similar effect would be expected after i.v. BU administration, as well. The costs of BU TDM, compared with the medication costs, are described in Table 4.

Liver Disease

For patients with liver disease, it is unclear what is the preferred initial dose and whether this matters if dose adjustment will be made using BU TDM. In general, high-dose BU-based conditioning is relatively contraindicated in patients with severe liver dysfunction. However, for patients with known liver fibrosis, hepatitis, or significant iron overload who are cleared for HCT, some regimens suggest lower initial doses and dose adjustment using BU TDM to avoid liver toxicity. An example would be the i.v. BU initial dose algorithm suggesting reduced initial BU doses for children > 8 years with hepatomegaly or serum ferritin > 5000 μg/L undergoing HCT conditioning with BU/CY/FLU/thiotepa for beta-thalassemia major [85].

FAQ7: WHAT IS THE OPTIMAL DOSING FREQUENCY OF BU?

The available i.v. BU data for adults do not suggest a significant difference in outcomes between Q6H and daily dosing, likely

Table 4
Costs of BU-based Conditioning and Therapeutic Drug Monitoring

Drug	Cost per mg	Typical Dose in High-dose HCT	Cumulative Dose (mg)	Cumulative ASP* [79]
Oral BU	$11.73 [79]	1 mg/kg p.o. Q6H × 16 doses	1280	$15,008
IV BU	$35.21 [79]	0.8 mg/kg i.v. Q6H × 16 doses†	1024	$36,055
IV CY	$0.42 [79]	60 mg/kg i.v Q24H × 2 doses	9600	$4,219
IV fludarabine	$1.29 [79]	40 mg/m^2 i.v Q24H × 4 doses	320	$4,124
	Cost per sample	Typical number of samples per AUC	Number of AUCs	
BU TDM	$25.22 [80]	6	1	$151‡

Center for Medicare & Medicare Services average sale price (ASP) for BU-based conditioning and fee schedule for BU TDM in a hypothetical patient who weighs 80 kg and is 6 feet tall, with a body surface area of 2.02 m^2.
ASP indicates average sales price; P.O., per oral.
* Reimbursement amount was based on the available dosage formulations, rounded to the nearest pill or vial size that is commercially available in the United States.
† FDA-approved dose.
‡ The charge per sample ranges from $125 to $225 in the United States and therefore, the charge per AUC, ranges from $750 to $1350.

because BU clearance, volume of distribution, and half-life appear to be similar regardless of dosing frequency. In children, relevant studies are ongoing. Oral BU should be administered Q6H.

BU dosing frequency has ranged from the traditional Q6H to Q24H [62,82] or as a continuous infusion [86]. For the first 20 years, BU was only available as 2-mg tablet that was administered Q6H. At least in adults, this dosing frequency allowed for a manageable number of pills per dose (eg, 80-mg dose would be 40 2-mg tablets). In infants and small children, a nasogastric tube is necessary to administer oral BU. Alternative dosing frequencies were obviously desired and, after the subsequent development of i.v. BU with Q6H dosing, the focus subsequently turned towards daily i.v. BU dosing. Daily administration is also more convenient and less resource-intensive than Q6H dosing.

In adults, a comparison of different dose frequencies has mostly been addressed only retrospectively in case series or as a subset analysis of phase I/II trials [50]. Not unexpectedly, the maximum plasma concentration is proportionally higher in adults receiving once-daily i.v. BU, but BU clearance, the volume of distribution, and half-life appear to be similar regardless of dosing frequency. In general, clinical outcomes in adults have not differed after traditional Q6H versus Q24H BU dosing. In particular, the rates of SOS [42,87,88], OS [42,88], and relapse [42] have not differed significantly between Q6H and Q24H dosing, but definitive conclusions were impossible given the heterogeneity in the patient population, concomitant medications, and inconsistent use of BU TDM. In children, it has clearly been demonstrated that Q24H i.v. BU administration is safe [89-91]. Most recently, in a prospective cohort study of children and adults with myeloid malignancies, the CIBMTR reported similar outcomes in HCT conditioning regimens using i.v. BU Q6H (n = 586) or Q24H (n = 427) in combination with CY or FLU [92].

FAQ8: WHAT IS THE BEST METHOD FOR ESTIMATING BU CLEARANCE?

BU clearance is calculated based on the administered BU dose and an estimate of post-dose BU exposure using validated pharmacokinetic modeling tools (see Technical Appendix). Test dose strategies are not currently recommended.

As explained in FAQ5, accurate estimation of a patient's individual BU clearance is essential to determine the personalized dose that is necessary to achieve target BU exposure for that patient (see Technical Appendix for details). BU TDM follows general pharmacokinetic principles, by conducting pharmacokinetic analysis of 1 patient's concentration-time data at a time. The techniques for BU TDM have remained essentially unchanged for the past 20 years.

PopPK models have great potential to improve the estimation of the preferred initial BU dose and an individual's BU clearance. PopPK modeling can characterize patient factors (covariates) such as weight and age that can be used to predict the initial (ie, before TDM results are available) dose. Between-subject variability and between-occasion variability (ie, between dose) of a drug's pharmacokinetics can be defined; these are useful for Bayesian dose adjustment [78]. After 1999, FDA guidance states an expectation that initial dose recommendations be based on popPK models [93]. Both FDA and EMA dosing strategies for initial i.v. BU (see FAQ6) were based on 2 different popPK models that led to the different dosing strategies in children. There have been many popPK models characterizing i.v. BU pharmacokinetics in children (reviewed in Table S1 of McCune et al.) [78]. PopPK models of i.v. BU have indicated that age and body size—either normal fat mass, actual body weight, body weight (not specified further), or body surface area—are associated with i.v. BU clearance in children.

Beyond initial dose estimation, popPK models can also be used to estimate an individual patient's BU clearance, but the latter currently still requires pharmacokinetic samples and concentration-time data from the patient. PopPK-based approaches have already been applied to the TDM of oral BU and i.v. CY in HCT recipients. PopPK models also facilitate the development of limited blood sampling schedules. For example, the use of an individual patient's concentration-time data with a popPK model—termed *maximum a posteriori dose personalization*—could allow, in the case of Q24H i.v. BU, for BU clearance to be accurately estimated using a pharmacokinetic sampling duration of less than 8 hours, which might make TDM feasible in the outpatient clinic [75]. However, the rate-limiting step for adopting this strategy is the creation of dashboards or clinical decision-support tools for clinicians to use popPK models for BU dose adjustment.

Another method to estimate an individual patient's BU clearance is to utilize a pre-HCT test dose. Most test-dose strategies evaluate BU clearance after a single small dose of BU, ranging from 0.25 mg/kg to 0.8 mg/kg. Although the use of a test dose has been able to minimize subsequent dose adjustments during the actual conditioning, the test-dose strategy does not predict clearance well enough to replace BU TDM.

FAQ9: HOW DO OTHER MEDICATIONS AFFECT BU PHARMACOKINETICS?

Ideally, there would be no changes to medications given concomitantly with BU to minimize any drug-drug interactions that alter BU pharmacokinetics. The following medications have affected i.v. BU clearance: FLU, deferasirox, metronidazole.

Medications that affect oral BU clearance include phenytoin, FLU, metronidazole, ketobemidone, and itraconazole. Phenytoin affects oral BU clearance, but its effect upon i.v. BU clearance is unclear. By extrapolation, voriconazole and posaconazole would likely affect BU clearance.

BU is hepatically metabolized through glutathione (GSH) conjugation by glutathione S-transferase (GST) enzymes; this process depletes hepatocyte GSH stores. Conjugation with GSH forms an unstable S-glutathione sulfonium conjugate (GS + THT). Recent data indicate that GS + THT undergoes β-elimination to form γ-glutamyldehydroalanylglycine, which may contribute to the narrow therapeutic index of BU through various mechanisms [94,95]. Plasma tetrahydrothiophenium ion (THT^+) [96], THT 1-oxide, sulfolane, and 3-OH-sulfolane [97,98] have also been reported in HCT recipients. GSTA1-1 is the most active human form of GST for BU conjugation; GSTM1-1 and GSTP1-1 also mediate i.v. BU conjugation, but their estimated in vivo contributions to i.v. BU conjugation are ~5% and 0.2%, respectively, after accounting for their lower activity for BU conjugation and lower hepatic expression relative to GSTA1 [99,100]. Various cytochrome P450 (CYP) enzymes may be involved in the metabolism of THT to sulfolane.

When evaluating a potential drug interaction with BU, the clinician should ideally complete BU administration before starting the potentially interacting drug. For example, many centers defer azole antifungal medications until after graft infusion when conditioning has been completed to avoid harmful drug interactions [101]. If BU must be administered with a potentially interacting drug, the interacting drug should not begin or stop during BU administration to minimize intrapatient (ie, between dose) changes in BU clearance. It logically follows that BU dose changes for a potential drug interaction are not advised without BU TDM because of the narrow therapeutic index of BU. Notably, drug interactions that occur with oral BU cannot also be assumed to occur with i.v. BU because i.v. BU predominantly undergoes hepatic metabolism, while oral BU can also have drug interactions at the level of the gastrointestinal tract.

There are several medications that have a documented or theoretical risk of BU interaction, the most notable of which are the antiepileptic drugs to prevent BU-induced seizures (Table 5). After recognition of its neurotoxicity, seizure prophylaxis concomitant with BU began shortly after that [110]. Characteristics of the ideal seizure prophylaxis include the following: (1) can load to therapeutic dose within 8 hours; (2) no overlapping toxicity with conditioning regimen; (3) does not interfere with donor cell engraftment; (4) toxicity cannot obscure a diagnosis of skin GVHD (ie, no to minimal dermatologic toxicity); (5) safe for outpatient administration; and (6) no to minimal pharmacokinetic interactions with BU [111]. Various antiepileptic drugs have been used as seizure prophylaxis for BU-induced seizures. Phenytoin has been the preferred medication to treat BU-induced seizures, but many HCT centers have replaced phenytoin with newer antiepileptic drugs (typically levetiracetam) [111,112]. Phenytoin is well known as a potent inducer of hepatic drug-metabolizing enzymes such as CYP2B6, 2C, and 3A and UDP glucuronosyltransferases [111]. Hassan et al. reported that patients receiving phenytoin had a higher clearance of oral BU as compared to those who received diazepam [106]. CYP2C9 may also play a role in the oxidation reactions of THT [113]. The effect of phenytoin on i.v. BU clearance is unclear; the package insert states that phenytoin increases i.v. BU clearance by 15% or more possibly due to induction of GST [34]. However, phenytoin administration has had either a slight effect [107] or no effect [62,108,109] on i.v. BU clearance. The clinical relevance of phenytoin's potential drug interaction is part of an ongoing CIBMTR study evaluating the association of seizure prophylaxis with clinical outcomes [114].

Table 5
Drugs that Affect Busulfan Clearance

Interacts with I.V. BU*	Interacts with Oral BU	Hypothetical or Presumed Interaction
Deferasirox	Fludarabine [102]	Acetaminophen
Fludarabine	Ketobemidone [103]	Posaconazole
Metronidazole	Itraconazole [104]	Voriconazole
	Metronidazole [105]	
	Phenytoin [106]	

* The effect of phenytoin upon i.v. BU clearance is unclear; the package insert states that phenytoin increases i.v. BU clearance by at least 15% [34]. However, phenytoin administration has had either a slight effect [107] to no effect [62,108,109] on i.v. BU clearance.

Beyond potential interactions with antiepileptics, i.v. BU clearance decreased by an average of 9.7% during concomitant FLU administration in some studies [58,82]; however, this has not been observed by others [83,115]. Other medications with reported drug-drug interactions with i.v. BU include metronidazole [116] and deferasirox [117]. Medications that have reported drug-drug interactions with oral BU include FLU (~30% increase in BU AUC) [102], itraconazole [104], ketobemidone [103], and metronidazole [105]. The underlying causes of these BU-drug interactions are known, making it difficult to extrapolate these interactions to other medications. Notably, interactions with BU and the newer azoles, including voriconazole, posaconazole, and isavuconazole, have not been reported. The presumed mechanism of itraconazole interacting with oral BU is CYP3A. All azoles inhibit CYP3A4 but with various potencies, with their potencies decreasing as follows: itraconazole, voriconazole, posaconazole (potent inhibitors), fluconazole and isavuconazole (moderate inhibitors). To err on the side of caution, one should assume that voriconazole and posaconazole also interact with BU [118].

Use of acetaminophen in combination with or within 72 hours before BU administration may cause a decrease in BU clearance by reducing GSH concentrations in the blood and tissues. Although the clinical significance of this interaction is not yet known, acetaminophen use should be avoided or minimized less than 72 hours before and avoided during BU administration and for 24 hours afterward [34].

FAQ10: SHOULD THE INITIAL BU DOSE BE PERSONALIZED BASED ON GENETIC POLYMORPHISMS?

Pharmacogenomics-based dosing of BU, either i.v. or oral, is not recommended.

There has been substantial interest in whether constitutional genetic polymorphisms are associated with BU-associated clinical outcomes in HCT. So far, the main focus of pharmacogenomics studies has been on the different GSTs. To date, none of the genes associated with GSTs have demonstrated a consistent effect on the efficacy, toxicity, or pharmacokinetics of BU. However, as with the pharmacodynamic studies, many of the observations came from single-institution case series; few studies had an a priori power calculation. Currently, personalizing BU doses based on genetic polymorphisms is not recommended for routine clinical practice. Meta-analyses of the existing BU pharmacogenomics data (Table S1) remains of interest, with the hope that the larger sample size could discover a genotype associated with outcomes of interest.

CONCLUSIONS

Although there have been multiple publications outlining some of the issues with BU and appropriate dosing, more work needs to be done. To optimize the use of BU in allogeneic HCT, there are several steps we could take. First, the collection of data by CIBMTR, Children's Oncology Group, and other organizations relevant to BU dosing and BU TDM would be of benefit for retrospective studies that seek to evaluate the association of BU exposure with post-transplantation outcomes. A collection of such data could mitigate the need for prospective multicenter studies that aim to evaluate different dosing strategies. Second, new conditioning regimens should be developed to identify the maximum tolerated systemic exposure, in which cohorts are defined by their target BU exposure and the BU exposure is sequentially increased to identify the exposure associated with maximum efficacy and least toxicity while simultaneously assessing the impact of other concomitant chemotherapy. This study design can provide greater clarity regarding the maximal exposure associated with the optimal outcomes. Third, advances in the methods of BU TDM are needed, including the use of popK and identifying novel predictors of BU clearance such as metabolomics. At present, harmonization of BU exposure units and how BU pharmacokinetic data are interpreted should be explored. In any case, all advances must be made with the intent of improving the efficacy of BU-based HCT conditioning.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2016.07.013.

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