REVIEW ARTICLE

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A practical guide to therapeutic drug monitoring in busulfan: recommendations from the Pharmacist Committee of the European Society for Blood and Marrow Transplantation (EBMT)

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Busulfan (Bu) is an important component of many conditioning regimens for allogeneic hematopoietic cell transplantation. The therapeutic window of Bu is well characterized, with strong associations between Bu exposure and the clinical outcome in adults (strongest evidence in myelo-ablative setting) and children (all settings). We provide an overview of the literature on Bu as well as a step-by-step guide to the implementation of Bu therapeutic drug monitoring (TDM). The guide covers the clinical, pharmacological, laboratory and administrative aspects of the procedure. Through this document, we aim to support centers in implementing TDM for Bu to further enhance the success rates of HCT and improve patient outcomes. The Pharmacist Committee of the European Society for Blood and Marrow Transplantation (EBMT) encourages all centers to perform TDM for Bu in the aforementioned indications.

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INTRODUCTION

Busulfan (Bu) is an alkyl sulfonate alkylating agent that has been used in a variety of hematological settings since the 1950s. Although its use for some conditions (e.g. chronic myeloid leukemia, polycythemia vera) has decreased over time with the emergence of more effective, targeted therapies, it is still widely used (in both intravenous [i.v.] and oral formulations) as part of high dose conditioning chemotherapy prior to hematopoietic cell transplantation (HCT) [1]. Following on from initial work in the 1980s combining oral Bu with i.v. cyclophosphamide (Cy) prior to allogeneic HCT in acute myeloid leukemia (AML) [2], results from a number of randomized trials led to the widespread adoption of Bu/Cy as an alternative to the well-established combination of Cy and total body irradiation (TBI) in the management of myeloid leukemias [3]. More recently, with the availability of an i.v. formulation of Bu and the development of reduced-intensity conditioning (RIC) protocols, a variety of newer Bu-based schedules have been introduced into clinical practice [4–7].

There are numerous publications describing a very high inter-individual variability between weight-based dosing and resulting exposure of Bu [8–11]. This applies not only to oral administration with assumed erratic absorption, but also unpredictable Bu clearance [10]. Furthermore, the clearance of Bu is suggested to decrease after the first doses [9].

The relationship between Bu exposure and patient outcomes has been studied extensively [12–16]. Personalized dosing with TDM has been shown to decrease severe toxicities, graft rejection rates and relapse rates [12–16] (Fig. 1). Consequently, Bu TDM is recommended, especially for children [13], for myeloablative conditioning (MAC) and for those conditioning regimens that have been developed with TDM [11]. However, according to a survey by the European Bone Marrow Transplant (EBMT) in 2017, few centers in Europe regularly use TDM for Bu: 17/102 for MAC and 9/88 for RIC conditioning regimens [17].

Bu is extensively hepatically metabolized, mainly via conjugation with glutathione. Some PK drug-drug interactions are known which may have a significant effect on the exposure of Bu, so that TDM is particularly important in these situations [8, 18].

Pharmacists and clinical pharmacologists are well trained and experienced in medication management as their focus is on

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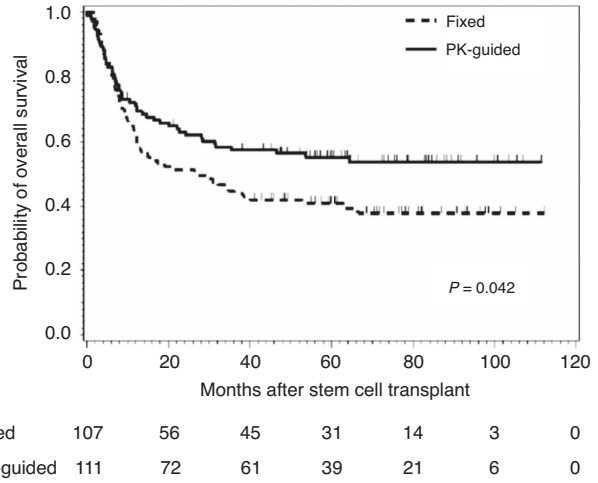

Fig. 1 Overall survival according to fixed or PK-guided Bu. Randomized controlled trial investigating i.v. Bu (in combination with fludarabine) at either fixed dose (dashed lines) or PK-guided adaptation (solid lines) in adult AML/MDS patients. Adapted from: Andersson et al. [12].

optimizing drug treatment in terms of indication, dosing, administration and the avoidance or management of interactions and adverse drug reactions [19]. In particular, they are often experts in performing TDM, pharmacometrics and model informed precision dosing (MIPD).

The pharmacist committee of the EBMT aims to improve pharmacological care for patients undergoing HCT or CAR-T cell therapies. Thus, this practical guideline is intended to provide recommendations on implementation and operational management of Bu TDM.

The current paper gives an overview of the pharmacokinetics and the therapeutic window of Bu in both pediatric and adult patients, as well as a step-by-step guide on how to perform TDM of Bu. We envisage this could lead to an increased number of centers implementing TDM for Bu as a standard of care.

CONCISE REVIEW OF LITERATURE ON BUSULFAN

Pharmacokinetic properties

Bu is a potent cytotoxic agent interfering with DNA by alkylation and cross-linking of the strands of DNA. To quantify exposure, the area under the concentration time curve (AUC) is the most commonly used measure. Clearance and volume of distribution serve as the central parameters for describing PK. The typical clearance and volume of distribution of Bu are approximately 0.2 L/kg total body weight (TBW) and 0.7 L/kg TBW, respectively, translating into an elimination half-life of 2–3 h [10, 20]. After i.v. administration, approximately 30% of Bu irreversibly binds to plasma proteins. The unbound portion distributes via passive diffusion both into cells [21], where it alkylates DNA after hydrolysis, and into the cerebrospinal fluid [22], which explains the increased risk of seizures during therapy [23, 24]. Metabolism is primarily hepatic and begins by conjugation of Bu with glutathione (GSH) either spontaneously or catalyzed by various isoenzymes of glutathione S-transferase (GST) [25, 26]. Conversion of the conjugate and further intermediate oxidative steps by flavin-containing monooxygenase 3 (FMO3) [27] and cytochrome P450 2C9 (CYP2C9) [28] ultimately produce the four inactive metabolites - tetrahydrothiophene (THT), THT-1-oxide, sulfolane, and 3-hydroxysulfolane -, of which 30% are excreted in the urine within 48 h of administration [21]. PK interactions occur when one drug affects the rate or extent of absorption, distribution, metabolism, or elimination of another drug, resulting in a decreased or increased exposure of the drug. The most common and relevant interactions of Bu relate to its metabolism [8]. Clinically relevant drug interactions are summarized in Table 1. The mechanisms underlying the interactions are, like the metabolism of Bu, complex and in some cases not yet fully understood.

Other than drug-drug-interactions, there are patient-specific factors that may have a varying degree of influence on the individual exposure to Bu. Numerous PK-models have identified that there is a relationship between total body weight, age, ethnicity or having a GSTA1 variant and the clearance or volume of distribution of Bu [29–31]. These patient-specific factors contribute to the observed inter-patient and between-dose variability of Bu-clearance by 20% (body weight, age and ethnicity) and 11% (GSTA1 variants) [20, 32, 33]. We further elaborate on the decreased clearance of Bu in the "How to calculate the AUC" section.

Therapeutic window in adult patients

We performed a mini review, carrying out an extensive search in PubMed and Google Scholar using the following search terms: "busulfan AND (AUC OR steady state plasma concentration) AND adult" as well as non-MeSH terms including myeloablative conditioning, reduced intensity conditioning. We present Bu exposure as the cumulative AUC in mg*h/L, in line with the exposure harmonization effort [34].

The European Medicines Agency (EMA) recommends a cumulative drug exposure of i.v. Bu of 59.0–98.4 mg*h/L, given four times daily during 4 days [35]. The US Food and Drug Administration (FDA) sets the recommended Bu cumulative AUC values at 72.2–88.6 mg*h/L also dosed once or four times daily over 4 days [36].

Unfortunately, there is no definitive consensus regarding Bu target exposure for all HCT conditioning regimens as no prospective randomized trials have addressed this topic [11]. A summary of the literature on the therapeutic window of Bu is given in Supplemental Table S1. In myeloablative conditioning, the cumulative AUC of busulfan is an important predictor for outcome for various underlying diseases and conditioning regimens. Underexposure to Bu is associated with relapse and impaired overall survival, with a minimal cumulative Bu exposure of 60 mg*h/L being needed (Fig. 2) [11, 13, 37–41]. Overexposure is mostly associated with toxicity including sinusoidal obstruction syndrome/veno-occlusive disease (SOS/VOD) and an increased incidence of non-relapse mortality. The maximum tolerated Bu exposure varies between studies, with some reporting maximum cumulative Bu AUC of 80 mg*h/L [37, 39, 40], while other studies show that exposures up to 100 mg*h/L are safe [11, 13, 42–45]. Some studies suggest that a higher Bu AUC of 100 mg*h/L can be tolerated when replacing Cy with Flu, thereby reducing the number of alkylating drugs. Some other studies have not found associations between Bu exposure and outcome [46].

Few studies are available investigating the optimal Bu AUC in RIC. This is likely due to the reduced number of dosing days, making TDM challenging, which reduces the opportunity for retrospective analyses of the optimal exposure to Bu in this setting.

In a non-malignant setting, two studies of a mainly pediatric cohort (but including some adult patients) treated for hemophagocytic lymphohistiocytosis (HLH) [47] and chronic granulomatous disorder (CGD) [48] targeted Bu at a cumulative AUC of 45–65 mg*h/L. Both showed acceptable results when compared to outcome following historical conditioning regimens.

In conclusion, the quantity and quality of most existing information precludes arriving at a consensus regarding the optimal Bu AUC. According to the discussed studies and clinical experience, a cumulative Bu AUC between 80 and 100 mg*h/L is commonly used for myeloablative therapy and an AUC between

approximately 65 and 85 mg*h/L does at least not seem to be associated with lower overall survival, relapse-free survival, therapy related mortality and GvHD. Very few qualitative studies are available for optimal Bu exposure in a RIC-setting. Cohort and randomized studies that provide more information on exposure in adults are necessary if a conclusive AUC interval for malignant as well as non-malignant diseases is to be established.

Therapeutic window in pediatric patients

We conducted a mini review, scanning literature in Pubmed for "busulfan[all fields] AND (children[all fields] OR pediatric[all fields]), including all papers investigating relationship between exposure of Bu (AUC or concentrations at steady state [Css]) and clinical outcome parameters in children. A total of 9 papers were included, which were all retrospective series (Supplemental Table S2).

There is strong evidence that optimal exposure to Bu is associated with superior outcomes in children, including survival, GvHD, SOS/VOD, graft failure and relapse. The largest published pooled series of 674 children and young adults showed that the optimal exposure to Bu in the myeloablative setting is 78–101 mg*h/L [13]. In this analysis there was no restriction on conditioning regimen or cell source, however most patients received single-alkylator conditioning. Patients with lower Bu exposures had higher chances on graft failure and relapse as compared to optimal exposure. On the other hand, over-exposure to Bu was associated with toxicity in terms of acute GvHD, SOS/VOD and higher incidence on transplant-related mortality (TRM). As compared to optimal exposure, patients with under- or over-exposure had a worse event free survival (Fig. 3). Other factors including cell source, underlying disease and the number of alkylators did not impact on the optimum Bu exposure, although the baseline risk for toxicity did increase with multiple alkylators. These results are mainly in line with those found in other, smaller, series [37, 49–55].

Some papers in the setting of immune deficiencies are outside the scope of the mini review as these do not correlate Bu exposure to outcome but deserve to be discussed. There is evidence that a lower target AUC of Bu may be beneficial in these indications, as full myeloablative conditioning may not be necessary. Pivotal papers in chronic granulomatous disease [48], hemophagocytic lymphohistiocytosis [47] and severe combined immune deficiency [56] suggest a lower target Bu exposure. This is in line with the Bu dosing recommendation by the EBMT inborn error working party, advising an AUC of 60–70 mg*h/L for RIC allografts (protocol C) [57]. In a pooled analysis in immune deficiencies, a Bu AUC of 70–90 mg*h/L is associated with improved EFS, with an AUC of 50–70 mg*h/L being advised for patients with a high co-morbidity index (personal communication J.J. Boelens).

Closing the gap on defining the therapeutic window

The therapeutic window for Bu is well-defined in some settings, while for other transplant settings the target AUCs for Bu are still a matter of debate. The strongest evidence towards a specific therapeutic window in our opinion would be a large study, preferably multicenter, where the AUC of Bu is correlated with clinical outcome, as was performed in the Bartelink paper [13]. Bu AUC would optimally be based on raw individual concentration data, and the AUC for the analyses should be calculated using Bayesian modeling. This gives a stronger evidence base as compared to separate studies comparing outcome after aiming for certain cumulative AUCs, as (1) the AUCs are calculated in the most accurate way in all patients, (2) the optimal AUC may be outside the target AUC in the studies, and (3) the number of patients is generally much higher in pooled analyses.

As such, the Bu therapeutic window is best defined for children receiving myeloablative conditioning [13], and in adults

Table 1. Clinically relevant drug-drug interactions with busulfan.

Interacting drug	Proposed mechanism	Effect	Recommended action	Reference
Blinatumomab	Unclear (probably cytokine-mediated CYP3A4-inhibition)	Increase of Bu plasma levels	If possible, avoid consecutive use of Bu after Blinatumomab or use TDM	[132]
Deferasirox	Unclear (probably inhibition of CYP2C8 and CYP1A2)	Increase of Bu plasma levels	Concurrent administration possible, however TDM necessary	[18]
Ketobemidone	Unclear (probably competition for CYP2C9 and CYP3A4)	Increase of Bu plasma levels	Use alternative analgesic	[8]
Paracetamol (acetaminophen)	Competition for glutathione	Increase of Bu plasma levels	Avoid paracetamol within 72 h prior to or concurrently with Bu	[8]
Metronidazole	CYP3A4 inhibition	Increase of Bu plasma levels	Monitor for increased Bu concentrations/toxicity when used concurrently	[133]
Itraconazole Posaconazole Voriconazole	Unclear (probably CYP3A4 inhibition)	Increase of Bu plasma levels	Use alternative antifungal depending on clinical situation	[133]
Phenytoin	CYP3A4/glutathione-S-transferase induction	Conflicting data	No clear advise, an alternative antiepileptic can be considered	[134–136]

Bu busulfan, *TDM* therapeutic drug monitoring.

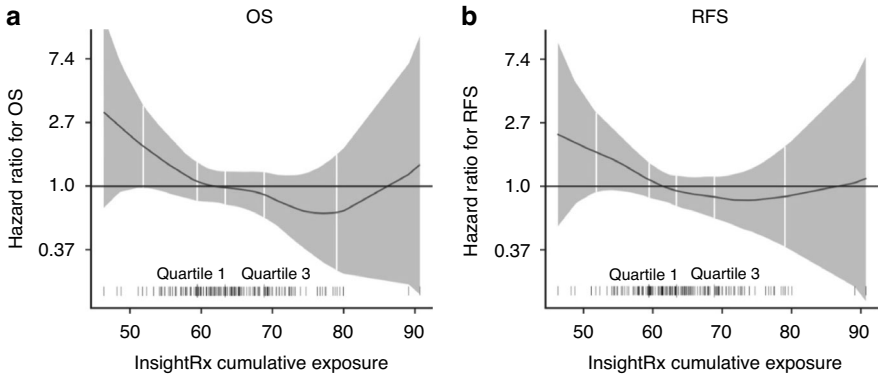

Fig. 2 Determination of optimal Bu exposure in adults receiving a CD34+ selected stem cell transplantation. Hazard ratio for overall survival (**a**) and relapse-free survival (**b**) according to cumulative Bu AUC, showing worse outcome with lower AUC < 59.5 mg*h/L. Adapted from: Tamari et al. [37].

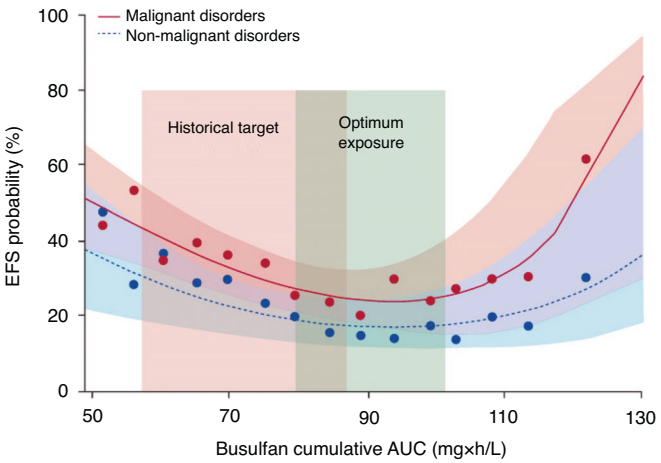

Fig. 3 Determination of the optimal Bu exposure in children. Polynomial Weibull models of the probability of event free survival according to Bu cumulative area under the curve, stratified by malignant (solid line) and non-malignant (dashed line) underlying disease shows that the AUC for optimal EFS lies between 78 and 101 mg*h/L, and does not depend on indication. The left rectangle show the historical target, as defined in previous studies. The right shaded rectangle show the target defined in the present study. Shaded areas around Weibull curves represent 95% CIs. Adapted from: Bartelink et al. [13].

undergoing CD34-selected transplants with intermediate intensity conditioning [37]. One analysis focusing on children with immune deficiencies was presented in a conference but has not been published (personal communication J.J. Boelens). For other indications, including children receiving a HCT with reduced intensity conditioning, triple alkylators or haplo-identical transplant, and for adults in most settings other than CD34-selected grafts, the evidence is less definitive. Of note, in reduced intensity conditioning in adults, the Bu AUC is less well correlated with outcome. Therefore, we would like to encourage the hematology community to perform large, multicenter analyses using raw Bu concentration data and outcomes to determine a more definitive therapeutic window for Bu in all settings.

Oral Bu

Since the i.v. preparation of Bu was developed more than twenty years ago, many centers switched from the oral to the i.v. formulation. The benefits of i.v. Bu include a more predictable PK and stable exposure [58–60]. The unpredictable bioavailability of oral Bu is likely partly due to the large number of tablets that must be taken, as the tablets were mostly available in 2 mg strengths only. A dose of 1 mg/kg QID (which is not unusual) would require a 70-kg adult patient to take 35 tablets every 6 h [61]. Moreover, food intake may lead to intra-patient variations in absorption over time. This variability makes TDM challenging, as today's measurements in a patient are a poor predictor for tomorrows PK [42, 60]. The unpredictable PK of oral Bu also translates into clinical outcomes. When compared to i.v. Bu, oral Bu is associated with a higher incidence of mucositis [62], SOS/VOD [15, 63, 64], acute GvHD [65], relapse [66], and worse survival [15, 66, 67]. Oral Bu is associated with worse outcome both in children [67] and adult patients [15, 62–64, 66].

In conclusion, we strongly advise against using oral Bu due to unpredictable PK and worse outcome. However, there are centers where the i.v. Bu formulation is unavailable. In these cases, the risks of oral Bu should be weighed against the possibility to use other alkylating agents such as treosulfan.

BUSULFAN DOSING AND TDM: A STEP-BY-STEP GUIDE

Choosing the initial dose

Adult patients. Initial Bu doses for adults can be prescribed according to the EMA's summary of product characteristics (SmPC) [11, 68]. As per the SmPC, the recommended i.v. Bu dose for patients with BMI < 30 kg/m^2 is 0.8 mg/kg actual body weight (ABW), given as a two-hour infusion four times a day (QID): a total daily dose of 3.2 mg/kg [41, 68, 69]. This dosing is suggested to target a cumulative AUC of 59–99 mg*h/L [70, 71]. Once-daily (QD) doses of 3.2 mg/kg/day, infused over three hours, can also be considered as they should yield equivalent cumulative AUC and outcomes [11, 72–75]. In overweight or obese individuals (BMI > 25 kg/m^2), Bu dose calculations should be based on the adjusted ideal body weight (AIBW25), which incorporates both the ideal body weight (IBW) and 25% of the excess weight over IBW (Supplemental Eqs. 1–3) [11, 68, 69, 71, 76]. A recent study indicated that AIBW25-based doses require significant dose adjustments for most included obese patients [77]. Until new data are available, TDM of Bu may be particularly advisable for obese patients [77, 78]. It is currently uncertain whether patients with hepatic impairment require an initial dose adjustment. Standard Bu doses (3.2 mg/kg/day in once daily or divided over four doses) and dose adjustments with TDM can be considered for patients with a significant decrease in hepatic function [78]. Standard doses are also to be considered for adult RIC regimens, with Bu treatment duration shortened to two or three days [11, 79–81].

Table 2. Selected available tools for pediatric initial Bu dose calculation.

Dosing recommendation	Dose calculation method	Targeted exposure	Variables required for the calculation
EMA SPC (Nguyen et al.) [68, 70, 71]	Actual body weight (ABW)-based nomogram [a]: <9 kg : 1.00 mg/kg/6 h 9 to <16 kg: 1.20 mg/kg/6 h 16 to 23 kg: 1.10 mg/kg/6 h >23 to 34 kg: 0.95 mg/kg/6 h >34 kg: 0.80 mg/kg/6 h	1125 µM*min 74 mg*h/L (over a 4-day treatment)	ABW
FDA drug information (Booth et al.) [69, 89]	ABW-based nomogram [a]: ≤12 kg: 1.10 mg/kg/6 h >12 kg: 0.80 mg/kg/6 h	1125 µM*min 74 mg*h/L (over a 4-day treatment)	ABW
Bartelink et al. High dose [13, 83]	ABW-based nomogram [b], and implemented in *InsightRx Nova software*	90 mg*h/L (over a 4-day treatment)	ABW
Bartelink et al. Reduced intensity [83]	ABW-based nomogram [b], and implemented in *InsightRx Nova software*	60 mg*h/L (over a 3-day treatment)	ABW
Poinsingnon et al. [91]	ABW-based nomogram [a]: ≤11 kg: 1.15 mg/kg 11 to 17 kg: 1.25 mg/kg 17 to 25 kg: 1.05 mg/kg 25 to ≤40 kg: 0.90 mg/kg >40 kg: 0.80 mg/kg	1200 µM*min 79 mg*h/L (over a 4-day treatment)	
McCune et al. [87]	Model-based prediction: Implemented in *NextDose*, and *PrecisePK* software	Flexible [c]	Normal fat mass (calculated from ABW, height, and gender), age (post-menstrual)
Shukla et al. [30]	Model-based prediction: Implemented in *InsightRx Nova* software	Flexible [c]	Fat-free mass (calculated from ABW, height, and gender), age (post-natal), fludarabine and clofarabine co-administration
Ben Hassine et al. [85]	Model-based prediction: Implementation in GUI software (*Tucuxi*) is ongoing.	Flexible [c]	Body weight, age (post-menstrual), *GSTA1* promoter polymorphisms, fludarabine co-administration

ABW actual body weight, AUC_{cum} cumulative AUC, *GSTA1* Glutathione-s-transferase A1, *GUI* graphical user interface, *SPC* summary of product characteristics.
[a]Nomogram's doses multiplied by 4 in case of choosing once-daily dosing.
[b]Nomogram's doses divided by 4 in case of choosing four times daily dosing.
[c]Target exposure and treatment duration can be defined by the prescriber.

Pediatric patients (< 18 years old). Bu dosing in children is more variable due to the non-linear change in Bu clearance over childhood and the ontogeny of GST, resulting in decreasing optimal doses on a mg/kg basis. Table 2 presents various initial dose calculation tools that could be considered for pediatric patients. These calculation methods are derived from population PK modeling and simulation studies. Some of these tools consist of nomograms designed to aim for a specific exposure target, while others are based on predictions using population PK models.

To our knowledge, the EMA ABW-based pediatric dosing nomogram [68, 71] is the most widely recommended and adopted Bu dose calculation tool [11]. This nomogram is designed to target a cumulative AUC of 74 mg*h/L [70, 71]. Initial doses from the EMA nomogram might not be appropriate when aiming for the optimized cumulative AUC target of 90 mg*h/L. In contrast, the dosing nomogram proposed by Bartelink et al. was designed to specifically aim at the latter target [13, 82, 83]. Nevertheless, the EMA nomogram might result in a significant proportion of sub- or supratherapeutic initial exposures [84]. Later, more dosing nomograms were introduced all aiming to reach that same AUC of 90 mg*h/L [30, 71, 85–91]. The dosing nomograms show variable performance in achieving optimal AUC (Fig. 4) [85]. There is some evidence that achieving optimal Bu exposure early in treatment (e.g. after the first dose with minimal adjustments) leads to improved outcomes [54]. While a dosing nomogram should be chosen to achieve optimal exposure, the use of TDM can correct for any under- or overdosing. This underscores the need for early TDM, preferably after the first dose, using state-of-the-art Bayesian dose adaptation tools.

An alternative to dosing algorithms, usually in the form of dosing tables, may be model-based initial dose prediction, integrated into TDM support software. These tools offer flexibility in selecting target exposures and estimating required individualized dosing regimens. The three model-based dose prediction tools in Table 2 have shown a commendable a priori predictive performance [30, 84, 85, 87]. Still, initial dose prediction with these models should not replace TDM, especially considering the random between-patient PK variability with respect to the narrow optimal therapeutic window [13].

Dosing frequency

Most alkylating agents are administered once daily, however oral Bu, due the availability of only 2 mg tablets, has been administered in the HCT setting every 6 h (QID) to improve patient compliance [92]. In the early 2000s, an i.v. formulation was marketed to overcome the disadvantages of the original oral compound's bioavailability and the initial clinical studies with i.v. Bu were performed with QID dosing program previously established with the oral formulation [75, 93]. Over time, several potential advantages of the QD administration have been highlighted: more tolerable and convenient for both caregivers and patients (reducing pharmacy, delivery and nursing resources, reduce waste and costs) [93], the possibility to perform transplantation procedures in the outpatient setting, and higher peak of Bu concentration and thus better penetration of poorly

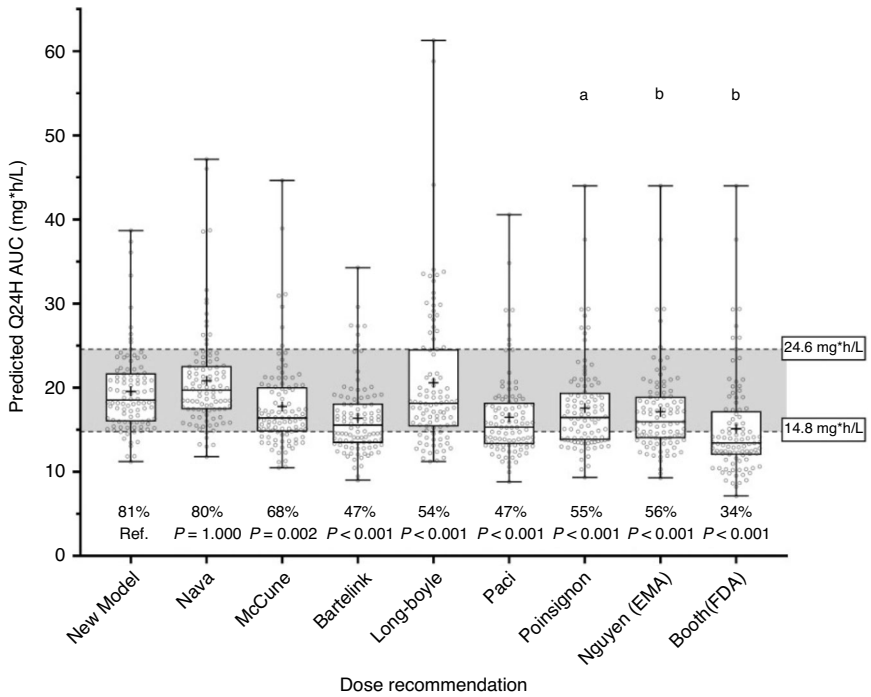

Fig. 4 Box plots of simulated AUCs with predicted q24h doses using the evaluated dosing guidelines. The boxplot's central line represents the median. The plus sign represents the mean value. The bottom and top edges of the boxplots indicate the 25th and 75th percentiles. The whiskers represent the full range of predicted AUCs. The shaded area represents the conventional therapeutic window of busulfan (60–100 mg*h/L). The percentage of patients within the target window and the *p* values from the pairwise comparison with the McNemar test for related samples are displayed below the boxes. Adapted from: Ben Hassine et al., Clinical Pharmacology and Therapeutics: Pharmacometrics and System Pharmacology [85] (https://doi.org/10.1002/psp4.12683).

vascularized sites. Also, regarding hepatic SOS/VOD, a QD approach may contribute to a reduction in hepatic injury, by allowing glutathione-S-reductase and glutathione-S-transferase recovery between doses [94]. The PK parameters seem to be similar between QID and QD dosing, in line with linear kinetics [75, 94, 95].

Multiple studies have shown that the clinical outcomes in adults did not differ after traditional QID versus QD Bu dosing in terms of toxicity including SOS/VOD and GvHD and relapse incidence [11, 72, 92–94, 96–98]. One conflicting study was presented at EBMT 2024 (personal communication Marc Ansari).

In terms of cost-efficacy, adult patients who received QD Bu dosing had similar or superior outcomes compared with those receiving QID dosing, with an estimated average annual cost reduction of $19,990 per patient (which included all costs in the first year after HCT) [73]. This suggests that QD dosing may be associated with a better cost-effectiveness profile.

In conclusion, several studies have demonstrated that the PK profiles and post-transplant complications do not depend on the schedule of i.v. Bu administration and suggest that once-daily, twice-daily and four times daily i.v. Bu regimens are equally safe and effective.

What are the optimal time-points to sample blood for Bu TDM?

Estimating a patient's Bu exposure accurately relies on obtaining PK samples within a suitable timeframe. Therefore, the dose frequency (QID, BID or QD), the half-life of Bu (usually between 2 and 3 h), as well as logistical challenges regarding sample storage [99] and quantification all need to be taken into account. The optimal number of samples and times between samples also depends on the method used for the AUC calculation. In general, non-compartmental analysis (NCA) needs a denser sampling scheme as compared to MIPD using Bayesian forecasting [11, 13, 30, 52, 100–102]. To strike a balance between the importance of achieving Bu's therapeutic window and practical considerations, we recommend a sampling schedule with four blood samples in case one or more cannot be used due to mishandling. A proposition (only to be used in combination with MIPD) for adequate sampling times dependent on the dose frequency can be found in Table 3.

Table 3. Sampling times after end of infusion (4 samples).

Sample	Time after end of infusion
1	+0–5 min
2	+1 h
3	+2 h
4	+3.5 h to +4 h

How to sample blood for Bu TDM?

Bu should be administered via an i.v. central line over either 2 h (if given QID) or over 3 h (if given QD). Care must be taken to ensure that the infusion systems are free of polycarbonate. It is recommended to use an administration set with a minimal residual priming volume (2 to 5 mL), although infusion systems with a significantly larger residual volume are often used in clinical practice.

In any case, the infusion line needs to be flushed after each infusion with normal saline (NS) at the same infusion rate as the Bu infusion to ensure that the patient receives the complete dose of Bu in the correct infusion time. The end time of the Bu infusion is therefore only reached when the volume of NS corresponding to the residual volume has been infused. If the infusion line is not filled with Bu before starting the Bu infusion, the time required for flushing the line must be added to the start time of the Bu

infusion in order to calculate the actual start time needed for AUC calculation.

The samples for the determination of the Bu concentration must not be taken via the Bu infusion line to avoid contamination, but it is possible to draw them using a different line of a multi-lumen central catheter. Busulfan is unstable at room temperature, therefore blood samples for Bu analysis should be kept in a refrigerator directly after collection of each individual sample. To avoid degradation, samples need to be centrifuged within 12 h of collection and plasma should be stored at −20 or −80 degrees celcius [103]. Alternatively, Bu can be stored for up to 24 h at 4 °C in whole blood samples, or for up to 2 years at −80 °C in plasma samples [99].

There are two small studies in the literature suggesting that Bu PK is subject to diurnal differences [104, 105]. Both studies were performed using oral Bu, meaning the observed diurnal variation could be due to clearance, absorption, or bioavailability (first pass effect). The latter two causes of variability are eliminated when using iv Bu, which is strongly recommended. In once daily dosing, potential diurnal differences will not pose problems as TDM is performed at the same time the next dose is given. In QID dosing, the diurnal differences lead to further variability in AUC, both in terms of TDM in current patients and reporting of AUC for research purposes. We advise to perform the TDM on the same daily dose of Bu in case of multiple TDM sampling episodes in QID dosing.

How to measure Bu concentrations?

Accurate measurement of Bu levels is crucial to ensure therapeutic efficacy while minimizing toxicity. Several analytical methods have been reported in the literature for the quantification of Bu in human plasma samples and in other biological fluids [106, 107].

The most commonly used techniques for the quantification of Bu are based on chromatographic methods. Gas Chromatography (GC) was initially developed in the 1990s [106], and involves the vaporization and separation of Bu on a chromatographic column. Detection is undertaken using a flame ionization detector (FID) [108], electron capture detector [108, 109] or mass spectrometry (MS) [110].

High-Performance Liquid Chromatography (HPLC) is another chromatographic technique widely used and detection is performed using ultraviolet (UV) or fluorescence detectors [107]. Liquid chromatography methods coupled with mass spectrometry (LC-MS) have been the most used technique for clinical care because of the sensitivity, specificity, and accuracy of their results [106]. This technique combines the separation power of liquid chromatography with the sensitivity and specificity of mass spectrometry [106, 111].

More recently, Tandem Mass Spectrometry (LC-MS/MS or GC-MS/MS) methodologies have been developed, which enhance specificity by measuring multiple mass-to-charge ratios, allowing rapid Bu plasma level monitoring without derivatization procedures and using relatively small amounts of plasma and other biological fluids like saliva [112–115]. Also, improved methods employing Ultra-Performance Liquid Chromatography (UPLC-MS/MS) [116], turbulent flow extraction technology [117] and Dried Blood Spots (DBS) sampling method [112, 118] have been developed. The DBS sampling method seems to be less invasive, and more cost-effective in terms of sample collection, storage, time for analysis and management. Also, the risk of infection by pathogens seems to be minimal. The potential clinical application of this method for routine TDM of Bu has been evaluated [112, 119].

Although infrequently used in clinical practice, several automated assays have also been studied, such as Enzyme-Linked Immunosorbent Assay (ELISA), to obtain quick and reliable results that would facilitate on-site Bu quantification [120]. Recently, novel antibodies were developed and applied to a nanoparticle immunoassay format with the advantages of high sensitivity to its target analyte, reagent stability and instrument flexibility [121].

The choice of method depends on factors such as equipment availability and the specific requirements of the clinical or research setting. It is important to follow established guidelines and quality control measures when using any analytical method for Bu measurement in a clinical context. Additionally, relevant regional or national standards and regulations should be considered. Given the sensitivity, specificity and accuracy of LC-MS/MS, most centers rely on this assay for the determination of Bu.

It is generally recognized that cross-validation of methods and long-term performance is crucial to monitoring and maintaining the quality of analytical methods. In 2019, an interlaboratory proficiency test program for the quantitation, PK modeling, and Bu dosing in plasma was developed by the Drug Analysis and Toxicology Division of the Dutch Foundation for Quality Assessment in Medical Laboratories (SKML). This program provides valuable insights into the performance of laboratories involved in the TDM of Bu worldwide and is a helpful tool to improve Bu quantitation methods [122].

Some centers may not have an assay available locally, and there may be obstacles such as logistical challenges in setting-up an in-house assay for Bu. Furthermore, many centers worldwide have set up collaborations for measuring Bu, where samples are shipped to another partner center to be analyzed. We invite centers unable to set-up an in-house assay who do not have current collaborations with other centers to contact the corresponding author, so that a list of nearby centers currently offering an assay for Bu can be shared.

How to predict the AUC?

In a recent survey by the Transplant Complications Working Party of the EBMT, a lack of consistency in PK-guided dose adjustment practices was identified [17]. This applies not only to the desired area under the curve (AUC) of Bu, but also to the calculation of the AUC [17]. The two most commonly used methods for AUC estimation are non-compartmental analysis (NCA) and MIPD using Bayesian forecasting. A schematic overview of the calculation of the AUC is shown in Fig. 5 [13]. The most important imprecisions in NCA are found in the underprediction of the peak concentration (shaded areas in both bottom plots of Fig. 5) and an underprediction of the tail-end of concentrations (shaded area of bottom left plot of Fig. 5). The MIPD-method accurately describes the true concentration-time curve both in peak concentration and tail-end concentrations and can handle variability in concentrations (top right plot of Fig. 5; introduced through assay variability, inaccuracy in registration of times etc.).

A comparison of these methods revealed that MIPD provides more accurate and precise AUC-estimations (with underestimations by NCA by up to 25%) using the same limited sampling scheme, and therefore provides a significant advantage in terms of achieving the target AUC [11, 13, 123]. In a direct comparison by Shukla et al., it was found that attaining the targeted cumulative AUC was achieved in 100% of all patients with MIPD, 88% using NCA and only in 66% of all cases if conventional dosing guidelines were used (Fig. 6) [30]. In addition, MIPD enables limited sampling strategies, which not only reduces stress for patients due to fewer blood draws but results in less costs due to fewer bioanalytical assays as well.

Another important aspect in calculating the AUC of Bu is the decrease in clearance. Multiple PK studies have shown that the clearance of Bu observed during the first day of dosing is higher than the clearance in subsequent dosing days in the same patient [9, 20, 30, 33, 83, 87, 88]. This observation is thought to be due to depletion of glutathione, thereby limiting the metabolic pathway of Bu [20]. On a population level, the clearance of Bu decreases ~10% from the first dosing day to subsequent dosing days, and as such this should be taken into account when calculating the

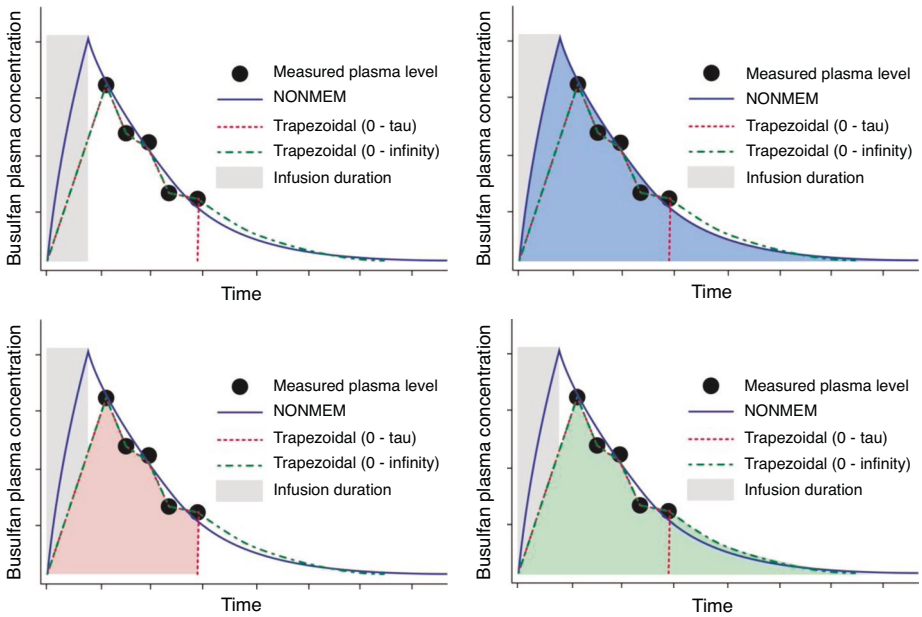

Fig. 5 Schematic representation of the different methods of calculating AUC. Plots showing individual concentration observations derived in individuals (black dots), the calculated AUC using model-informed precision dosing (shaded area in top right figure) and non-compartmental analysis to calculate the exposure up to the final observation (shaded area in bottom left figure) and AUC to infinity (shaded area in bottom right figure). NONMEM is a method of MIPD. Adapted from: Bartelink et al., Lancet Hematology 2016 [13] (https://doi.org/10.1016/s2352-3026(16)30114-4).

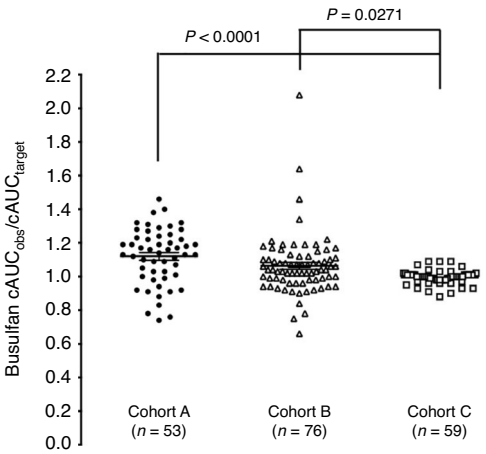

Fig. 6 Comparison of the ratio of Bu observed cumulative AUC compared to the pre-defined target cumulative AUC in 188 pediatric patients. Group A received Bu with TDM as per the shaded area in the bottom left of Fig. 5; cohorts B and C received Bu with TDM as per the shaded area in the top right of Fig. 5 (group B first generation model, group C second generation model). Adapted from Shukla et al., Frontiers in Pharmacology 2020 [30] (https://doi.org/10.3389/fphar.2020.00888) under CC BY 4.0 license (https://creativecommons.org/licenses/by/4.0/deed.en).

correct dose. Decreasing clearance is accounted for in most, if not all, MIPD software packages. Finally, MIPD allows for a somewhat easier conversion of harmonized Bu plasma exposure units (BPEU) in the comparison of TDM-based exposure data and thus AUC targets between transplant centers [13, 34]. There are many MIPD software packages available to perform TDM of Bu. A Bu dosing module is included in most commercial software packages, often for children and adults, while some packages are freely available. A comparison of the available MIPD packages has been made, with all packages performing reasonably well in terms of quality and validation [124]. Employing Bu TDM on at least two days might be reasonable to better attain the target AUC, yet there is no strong evidence supporting two day sampling [33]. We recognize that the calculation of the AUC and dose adjustments require specialized software and pharmacokinetic expertise, which may not be available in all centers. There may be centers who are willing to implement an in-house software package, or centers who want to set up collaborations with centers to perform the computational part of TDM. We invite those centers to contact the corresponding author for advice and/or assistance, or to send a list of nearby centers who currently have experience in AUC calculation and dose adjustments.

In conclusion, dose adaptation of Bu using MIPD is superior in comparison to NCA approaches. As such, we highly recommend the use of MIPD for the TDM of Bu to obtain accurate, reliable and translatable results.

Choosing the right AUC for the right indication

As described in the *Concise Review of Literature* above, there is no single correct Bu AUC for all HCT conditioning regimens. The effects of other conditioning regimen drugs, the intended bone marrow suppression, the underlying disease, factors such as risk of graft rejection or relapsed risk and baseline patient characteristics such as age or liver status should be considered when selecting the target BU exposure to optimize clinical outcomes. We suggest choosing the target Bu AUC based on these factors, and record this clearly in the patient's medical notes.

Calculate the next dose based on the AUC

After calculation of the $AUC_{0-\infty}$ after the first dose of Bu, dosing should be amended if the actual AUC falls out of the range of the desired target AUC. With the availability of software packages, the next dose of Bu will be suggested by the software. Most software packages will also show the AUC after each dosing day and the projected cumulative AUC.

However, to give an insight on how the next dose is calculated, the steps described in Supplemental Table S3 should be followed to manually calculate the next dose. This calculation is based on the linearity between dose and AUC.

In some cases, large adjustments in dosing (we suggest >25% change of dose) have to be made based on TDM. This could be due to outliers in terms of PK, but could also be due to errors in the TDM procedure (patient mix-up, wrong dose, assay issues etc). For safety, we advise centers to perform another TDM procedure the next day in case of dose adjustments >25% if logistics allow this.

Registration of the TDM-procedure and the subsequent dosing advice

When all doses of Bu have been administered and the TDM procedure has been completed, a final report should be written stating the target AUC, the starting dose, the measured concentrations, the actual AUC and the percentage dose change, if any, and the final overall estimated AUC. It is important that this information is included in the patient's medical record as it increases the acceptability of the recommendations, improves the quality of the process in terms of efficacy and safety, and meets the accreditation criteria required by JACIE [19].

Infrastructure and financial considerations

While all processes for TDM including the special considerations for Bu are described, the infrastructural and financial considerations have not been touched upon. The feasibility of setting up an in-house pipeline for Bu TDM depends on the volume of patients needing Bu TDM, and the use of the analytical equipment for other purposes such as TDM for other drugs: a higher volume of samples makes the costs per patient lower.

The most important aspects of performing in-house TDM for Bu include the acquisition of the equipment for the assay, most frequently an LC-MS [106, 111]. However, as described in the paragraph "How to measure Bu concentrations", the assay can also be performed using GC-MS [106, 110], HPLC [107] or ELISA-based assays [120]. The assay should be implemented locally, preferably including inter-lab assay proficiency testing such as provided by SKML [122]. We estimate that the costs for equipment and local assay implementation would cost approximately € 200.000, although would be vastly reduced if the analytical equipment is already available.

Another option for measuring Bu concentrations could be to outsource the assay to other hospitals or commercial parties. Costs per sample are reported to be $ 125–225 dollar in the United States [11]; in Europe prices are around € 50–100 per sample. Of note, this price may or may not include calculation of AUC and a dose advise. Moreover, most protocols advise to repeat TDM in case of major dose adjustments (> 25% dose change), which will result in double costs. Finally, the price for outsourcing does not include shipping costs.

For the determination of the projected AUC and the resulting dose advise, centers would need to acquire software for MIPD. Several packages are available, some commercial, and some free of charge [124]. It is advisable that the staff members that perform the AUC calculation and subsequent dosing advise are adequately trained to correctly interpret results and to recognize errors in data entry and/or dosing advise. The costs for software and training highly depend on the software package and the current amount of knowledge on TDM. As free software packages are available, and trainings for TDM and PK are available free of charge (for instance by the European Association of Hospital Pharmacists [EAHP]), this aspect of TDM should not form a significant barrier in terms of costs. Commercial software packages and/or commercial trainings may cost € 10,000–50,000 depending on the choice and desired functionalities of the software package, and size of the hospital.

Finally, to improve quality and reduce human errors, we advise to write standard operating procedures (SOPs) and protocols that capture all points addressed in this guide combined with all practical issues, applied to the center at hand. This may take the pharmacy team some time to produce, however no or relatively low costs are involved.

CONCLUSIONS AND PERSPECTIVES

In this overview, we highlight the importance of personalized Bu dosing in HSCT. Recognizing the high inter-individual variability in Bu PK, this paper underscores the necessity of TDM to ensure effective and safe therapy, in both adults and children. Part of the variability is attributed to factors like individual patient characteristics such as body weight, and thus can be accounted for in dosing. However, some of this variability remains unexplained and thus unpredictable. Emphasizing the value of MIPD, we advocate for its broader adoption as a more accurate approach to achieving desired Bu exposure. Interdisciplinary collaboration is essential for standardizing treatment protocols and expanding our collective understanding of Bu's optimal application in transplantation.

This position paper serves as a critical resource for pharmacists, clinicians and nurses offering practical guidance for TDM-guided dosing for Bu specifically, but which also mostly applies to TDM for other drugs. It also promotes a deeper understanding of the complexities of Bu therapy in pre-HCT conditioning and a shift towards more personalized medicine approaches in transplantation, ensuring that patients receive the most effective and safest treatment tailored to their individual needs.

In the field of HCT, the strongest evidence supporting the need for TDM is for Bu. The need for TDM is underlined by Bu's narrow therapeutic window with important consequences of under- and overexposure in combination with the large and unpredictable inter-patient variability. In current clinical care, Bu is the only drug used in HCT conditioning where TDM is routinely implemented in most centers. Other drugs used in conditioning that are suggested to have a critical therapeutic window (in terms of impacting survival) include fludarabine [125–127], melphalan [128] and anti-thymocyte globulin [129, 130]. All three share the large inter-patient variability. For fludarabine, a prospective randomized study in adults of TDM failed to show improvement in the primary outcome of viral reactivations (De Witte et al., abstract OS12-01 at presented at EBMT 2024); for anti-thymocyte globulin, model-based dosing did lead to improved outcomes [131]. Other drugs used in HCT outside of conditioning where TDM is frequently used include cyclosporin, tacrolimus, voriconazole, mycophenolate mofetil, aminoglycosides and vancomycin.

In conclusion, we strongly encourage centers to implement Bu TDM. This paper aims to give a step-by-step overview of how to perform TDM. Through this approach, we aim to enhance the success rates of HCT and improve patient outcomes, while harmonizing practices between transplant centers.

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AUTHOR CONTRIBUTIONS

VD conceptualized and designed the research, performed the investigation and wrote and reviewed the manuscript; KNH performed the investigation and wrote and reviewed the manuscript; AD performed the investigation and wrote and reviewed the manuscript; MEMM performed the investigation and wrote and reviewed the manuscript; KBH performed the investigation and wrote and reviewed the manuscript; VP performed the investigation and wrote and reviewed the manuscript; NK performed the investigation and wrote and reviewed the manuscript; TB performed the investigation and wrote and reviewed the manuscript; MH performed the investigation and wrote and reviewed the manuscript; ND performed the investigation and wrote and reviewed the manuscript; KK performed the investigation and wrote and reviewed the manuscript; MA performed the investigation, supervised the research and wrote and reviewed the manuscript; CL conceptualized and designed the research, performed the investigation, supervised the research and wrote and reviewed the manuscript; RA conceptualized and designed the research, performed the investigation, supervised the research and wrote and reviewed the manuscript.

COMPETING INTERESTS

MEMM declares honoraria as a speaker, consultancy or advisory role from Daiichy Sankio España, GSK, Gilead, Incyte, Seagen, Servier, all not related to the current topic. ND declares honoraria as a speaker from Novartis and Gilead, and travel grants from Novartis. KK declares honoraria as a speaker from Medac, Novartis, Pierre Fabre and travel grants from Pierre-Fabre, all not related to the current topic. RA declares an unrestricted research grant from Sanofi, not related to the current topic. VD, KNH, AD, KBH, VP, NK, TB, MH, MA and CL declare no conflicts of interest.

ADDITIONAL INFORMATION

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