Therapeutic drug monitoring of intravenous busulfan in Thai children undergoing hematopoietic stem cell transplantation: A pilot study

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ABSTRACT

Busulfan (Bu) is commonly used in myeloablative conditioning regimens for children undergoing hematopoietic stem cell transplantation. The standard target area under the concentration-time curve (AUC) of Bu is approximately 900–1500 μM·min. In previous studies using five fixed doses (0.8–1.2 mg/kg) for Bu without dose adjustment, 75% patients achieved the target AUC. The aim of this pilot study was to determine the percentage of target AUC for intravenous (IV) Bu in Thai children. IV Bu was administered every 6 h over 16 doses. Blood samples were collected for pharmacokinetic (PK) analysis after the first, ninth, and thirteenth doses of Bu. Seven patients (2–14 years; median 6 years) were diagnosed with thalassemia (n = 4), acute myeloid leukemia (n = 2), and pure red cell aplasia. Three, two, and two patients received Bu at 1.1, 1.2, and 0.8 mg/kg, respectively. The AUC of Bu varied from 292–1714 μM·min (median = 804). Nine (42.86%), eleven (52.38%), and one (4.76%) AUC values were within, below, and above the target, respectively. The median (range) Bu clearance was 5.93 (1.91–14.65) ml/min/kg. In this study, 42.86% AUC value achieved the target, which was lower than that in previous studies. Therapeutic drug monitoring (TDM) of Bu should be considered in Thai children receiving five fixed doses of IV Bu, and dose adjustment should be performed as necessary. Further PK studies for Bu with a larger sample size are warranted for confirming the necessity of TDM in every step dose of Bu.

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Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is the treatment of choice for various malignant disorders (e.g., acute myeloid leukemia; AML) and nonmalignant disorders (e.g., severe thalassemia). Busulfan (Bu) is an alkylating agent used as a standard compound in myeloablative conditioning regimens for children undergoing HSCT.\textsuperscript{1,2} The intravenous (IV) formulation is now widely used for HSCT because it exhibits low inter- and intra-individual pharmacokinetic (PK) variations and lower toxicity and mortality at day +100 compared to the oral formulation.\textsuperscript{3,4} Bu has a narrow therapeutic index. The standard target area under the concentration-time curve (target AUC\textsubscript{0-\infty}) of Bu is approximately 900–1500 μM min, when administered on a conventional dosing schedule (16 doses over 4 days).\textsuperscript{1} AUC values below 900 μM min have been correlated with graft rejection and disease relapse.\textsuperscript{5-7} A higher exposure to Bu (AUC above 1500 μM min) has been associated with an increased risk of toxicity, such as mucositis, sinusoidal obstruction syndrome of the liver, graft versus host disease (GVHD), neurologic toxicity, and transplant-related mortality.\textsuperscript{7-10} Personalized IV Bu dosing via therapeutic drug monitoring (TDM) needs to be considered to achieve the target AUC and improve HSCT outcomes.\textsuperscript{11-13} Five fixed doses of Bu are now recommended in the European Medicines Agency (EMA) labeling.\textsuperscript{14} The American Society for Blood and Marrow Transplantation (ASBMT, ASTCT) guidelines\textsuperscript{15} recommend that the initial IV Bu dose should be based on the five fixed doses recommended by EMA. However, PK-guided dosing should be used in patients in whom the target AUC cannot be achieved.\textsuperscript{11} Previous population PK studies using five fixed doses without dose adjustment achieved the target AUC in approximately 75% patients (74–78%).\textsuperscript{14-17} One study in Thai children with thalassemia supported the safety and efficacy of using five fixed doses IV Bu without performing TDM.\textsuperscript{18} As per our knowledge, there is no report on the target AUC and PK parameters of Bu in Thailand, which has limited resources and few hospitals for performing TDM to adjust the dose of Bu dynamically. Therefore, the objective of this pilot study was to analyze the percentage of the target AUC and report the PK parameters and early clinical outcomes of five fixed doses IV Bu in Thai children undergoing HSCT.

Materials and methods

Patients and objectives

This pilot study was a prospective, single center, open-label study conducted at the Department of Pediatrics, Faculty of Medicine Siriraj hospital, Mahidol University, Thailand from April 2017 – April 2019. Bu was administered for 2 h via IV infusion into patients every 6 h for 16 doses over 4 days. Five fixed doses of Bu schedules were calculated on the basis of actual body weight (ABW) following the preceding EMA labeling: 1.0, 1.2, 1.1, 0.95, and 0.8 mg/kg for patients with ABW <9 kg, 9–<16 kg, 16–23 kg, >23–34 kg, and >34 kg, respectively.\textsuperscript{14} Blood samples were collected after the first, ninth, and thirteenth doses at 14 separate points for analyzing the Bu level using gas chromatography-mass spectrometry (GC-MS). The primary objective was to analyze the percentage of the target AUC for IV Bu; the secondary objectives were to report the PK parameters of IV Bu while the drug level was in steady state from the initial dose and early clinical outcomes in the first month after HSCT.
Eligibility criteria

Pediatric patients (1–18 years of age) undergoing allogeneic HSCT were enrolled in this study. Histocompatibility was determined via DNA typing with intermediate- or high-resolution in class I (A, B, C) and II (DR, DQ) for 10 loci using standard procedures. The eligibility criteria included patients who presented normal cardiac and pulmonary function, serum creatinine <2 times the upper limit of normal (ULN) values, serum total bilirubin <1.5 times the ULN, and AST and ALT <3 times the ULN. Patients who had previously undergone HSCT were excluded. Written informed consent was obtained before enrollment in this study. The protocol and informed consent forms were approved by the Institutional Review Board, Mahidol University (COE. No. Si 112/2017), and they were in full compliance with international Guidelines for Human Research Protection. This study was registered in TCTR under TCTR20190528003.

Conditioning regimens and supportive care

IV Bu (BUSULFEX®, Otsuka Pharmaceuticals) was administered over five fixed doses based on ABW every 6 h; each dose was infused for 2 h using a central venous catheter, for a total of 16 doses over 4 days. The conditioning regimen for nonmalignant diseases consisted of IV Bu, followed by IV cyclophosphamide (Cy) 50 mg/kg/day for 4 days. Rabbit anti-thymocyte globulin (ATG) 2.5 mg/kg/day for 3 days was added for unrelated HSCT. For AML patients, the conditioning regimen consisted of IV Bu, IV Cy 60 mg/kg/day for 2 days, and IV melphalan (Mel) 140 mg/m²/day for 1 day. Dose adjustment for IV Bu was not performed in any patient because the objective of this pilot study was to study the percentage of target AUC and PK parameters of IV Bu while the drug level was in steady state from the initial dose.

For seizure prophylaxis, phenytoin was administered per oral (PO) at a loading dose of 10 mg/kg and subsequently at 5 mg/kg/day divided into 2 doses, starting 12 h before the initiation of Bu until 48 h after completion of Bu. Filgrastim was administered intravenously 10 μg/kg/dose once daily, starting 24 h after the stem cell infusion (day +1) until neutrophil engraftment. GVHD prophylaxis included a short course of methotrexate (MTX) at 2 mg/kg/course divided into 4 doses, which were administered once daily on day +1, +3, +6, and +11 after stem cell infusion, and IV cyclosporine A (CSA) 3 mg/kg/day, which was divided into 2 doses every 12 h, starting 24 h before stem cell infusion. CSA level was monitored regularly to ensure maintenance at 150–250 μg/L. MTX on day +11 was omitted if the patients developed severe oral mucositis or severe infection. Ursodeoxycholic acid was administered for VOD prophylaxis at 15 mg/kg/day divided into 2 doses PO every 12 h on day +1 until day +30 post HSCT. Oral antibacterial (gentamicin or ciprofloxacin) and antifungal (fluconazole or posaconazole) prophylaxis was administered in accordance with the institutional guidelines. Trimethoprim-sulfamethoxazole 5 mg/kg/day divided into 2 doses for 3 days/week was initiated after stable engraftment and continued until the immunosuppressive drugs were terminated for 3 months.

PK sampling and analysis

PK blood samples (1 mL/sample) were collected for the analysis of Bu level after 1, 9, and 13 doses for 14 separate points using sodium heparin tubes. For the first dose, six blood
samples were collected pre-infusion, 1.5, 2.0, 2.5, 4.0, and 6.0 h after the start of the infusion. For doses 9 and 13, four blood samples were collected pre-infusion, 2.0, 4.0, and 6.0 h after infusion. After centrifugation, the plasma samples were stored at −20°C and were analyzed within 72 h. Bu plasma concentrations were determined using a GC-MS assay method,\textsuperscript{19} which was externally validated by the Siriraj Poison Control Center, Siriraj hospital in terms of selectivity, accuracy, precision, linearity ($r^2 > 0.9994$), and stability.\textsuperscript{20} The calibration curves were linear over 10 (limit of quantification)–5000 ng/mL. The within- and between-run coefficients of variation were always within 15% of the nominal value.\textsuperscript{20}

The Bu concentration–time data were fitted using a one-compartment PK model with first-order elimination and zero-order input as the infusion rate.\textsuperscript{14–17} The molecular weight of Bu is 246.3 g/mol. The PK parameters [AUC, clearance (Cl), volume of distribution (Vd), and half-life ($t_{1/2}$)] of Bu were analyzed by a non-compartmental analysis model using the Phoenix WinNonlin\textsuperscript{®} software, version 8 (USA). The AUC was calculated according to the linear trapezoidal rule. The AUC from time 0 to infinity (AUC$_{0-\infty}$) was calculated after the first Bu dose. The AUC from 0 to the dosing interval (AUC$_{0-d}$) was calculated from doses 9 and 13. Bu Cl was calculated by dividing the dose by the AUC (Cl = dose/AUC). In total, 21 (seven patients × AUC (1, 9, and 13 doses)) AUC measurements were available.

**Evaluation of clinical outcomes**

The engraftment was determined through the complete blood count by calculating the absolute neutrophil count (ANC) for neutrophil engraftment and DNA chimerism to evaluate the percentage of donor’s DNA. Neutrophil engraftment was defined as the first of 3 consecutive days with an ANC $\geq$ 0.5 x 10$^9$/L. Engraftment failure was defined as the failure to achieve an ANC of 0.5 x 10$^9$/L by day +28 post HSCT. Chimerism analysis was performed periodically using 16 loci autosomal short tandem repeat methodology. Full or complete chimerism (CC) generally refers to complete (95–100%) replacement of the host DNA by the donor DNA. Mixed chimerism (MC) is the presence of both donor (<95%) and recipient cells. The chimerism analysis was performed using bone marrow (BM) or peripheral blood samples on day +30 post HSCT or earlier upon the time of neutrophil engraftment.

Complications were assessed by Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v4.0) according to the National Cancer Institute (NCI). Grading based on the CTCAE scale, such as febrile neutropenia, seizure, diarrhea, oral mucositis, vomiting, hyperbilirubinemia, and elevated transaminases (AST or ALT), was recorded in each organ system.\textsuperscript{21} VOD was evaluated as per the modified Seattle (1992) and Baltimore (1987) diagnostic criteria.\textsuperscript{22} Acute GVHD (aGVHD) was graded according to the established criteria.\textsuperscript{23}

**Results**

**Patients and transplant characteristics**

Seven patients (four boys, three girls; median age 6 years, range 2-14 years) were enrolled in this study. Thalassemia was the most common underlying disease (n = 4, 57.14%), followed
Table 1. The PK parameters for Bu.

<table>
<thead>
<tr>
<th>PK parameters (units)</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>Range</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSS (ng/mL)</td>
<td>563</td>
<td>378</td>
<td>443</td>
<td>89–1700</td>
<td>488</td>
<td>381</td>
<td>366</td>
<td>53–1700</td>
</tr>
<tr>
<td>AUC (μM min)</td>
<td>841</td>
<td>361</td>
<td>777</td>
<td>341–1271</td>
<td>879</td>
<td>403</td>
<td>804</td>
<td>292–1714</td>
</tr>
<tr>
<td>CI (mL/min/kg)</td>
<td>6.37</td>
<td>3.91</td>
<td>5.93</td>
<td>2.55–13.44</td>
<td>6.36</td>
<td>3.92</td>
<td>5.93</td>
<td>2.55–13.44</td>
</tr>
<tr>
<td>τ½ (h)</td>
<td>2.20</td>
<td>1.22</td>
<td>2.18</td>
<td>0.31–4.43</td>
<td>2.35</td>
<td>1.39</td>
<td>1.85</td>
<td>0.31–6.40</td>
</tr>
<tr>
<td>Vd (L/kg)</td>
<td>1.14</td>
<td>0.92</td>
<td>0.64</td>
<td>0.25–2.54</td>
<td>1.19</td>
<td>0.90</td>
<td>0.91</td>
<td>0.25–3.69</td>
</tr>
</tbody>
</table>


*Total of 7 AUC (7 patients × AUC of first dose).
**Total of 21 AUC (7 patients × AUC of all doses).

by AML (n = 2, 29%) and pure red cell aplasia (PRCA). All patients with thalassemia exhibited iron overload pre-HSCT (serum ferritin: median 2050 ng/mL; range 1466–3050 ng/mL). Six patients underwent HLA-matched-related HSCT (85.71%); one was 9/10 HLA-matched (one antigen mismatched at the DRB1 locus) and five 10/10 HLA-matched with their sibling donors. One patient underwent 10/10 HLA-matched unrelated HSCT. Four (57.14%) received BM stem cells. Both AML patients and one thalassemia patient received PBS. The median number of CD34+ cells was 7.33 × 10⁹/kg. Five patients received Bu/Cy ± ATG conditioning regimen; two received Bu/Cy/Mel. All patients received GVHD prophylaxis with CSA and a short course of MTX. The median ABW was 18.60 (10.70–62.70) kg. Three, two, and two patients received Bu at 1.1 mg/kg, 1.2 mg/kg, and 0.8 mg/kg, respectively. No patient received Bu at 1.0 or 0.95 mg/kg.

**Target AUC and PK parameters**

The AUC values for Bu varied from 292–1714 μM min (median = 804). Nine (42.86%), 11 (52.38%), and one AUC values were within, below, and above the target, respectively (Table 1 and 2). Among 7 patients receiving Bu at 0.8, 1.1, and 1.2 mg/kg, 83%, 33%, and 17% AUC values, respectively, achieved the target (Table 3).

The median (range) values for the steady-state plasma concentration (CSS), CI, τ½, and Vd were 366 (53–1700) ng/mL, 5.93 (1.91–14.65) mL/min/kg, 1.85 (0.31–6.30) h, and 0.91 (0.25–3.69) L/kg, respectively. The median (range) values for CSS, AUC, and CI for the first dose of Bu were 443 (89–1700) ng/mL, 777 (341–1271) μM min, and 5.93 (2.55–13.44) mL/min/kg, respectively.

Two of seven (28.57%; numbers 4 and 7) patients achieved the target AUC of all 3 doses. In one patient (number 5), 2 doses of Bu level reached the target AUC; however, one AUC measurement (dose 13), was above the target. In four patients (numbers 1, 2, 3, and 6), the AUC for Bu was lower than the target; however, one AUC measurement (dose 9, patient number 3), reached the target. The Bu PK parameters are summarized in Table 1. The AUC for each patient is shown in Figure 1.
Table 2. The PK parameters of Bu in thalassemia and non-thalassemia patients (n = 21).

<table>
<thead>
<tr>
<th>PK Parameters (μM·min)</th>
<th>Thalassemia*</th>
<th>Non-thalassemia*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 12 (%)</td>
<td>n = 9 (%)</td>
<td></td>
</tr>
<tr>
<td>Target AUC (900–1500)</td>
<td>3 (25.00)</td>
<td>6 (66.67)</td>
</tr>
<tr>
<td>Lower AUC (&lt;900)</td>
<td>9 (75.00)</td>
<td>2 (22.22)</td>
</tr>
<tr>
<td>Higher AUC (&gt;1500)</td>
<td>Median (range)</td>
<td>Median (range)</td>
</tr>
<tr>
<td>AUC (μM·min)</td>
<td>576 (292–1301)</td>
<td>1204 (758–1714)</td>
</tr>
<tr>
<td>CI (ml/min/kg)</td>
<td>7.98 (3.42–14.65)</td>
<td>2.69 (1.91–6.50)</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>1.67 (0.31–5.07)</td>
<td>2.26 (1.32–6.30)</td>
</tr>
<tr>
<td>Vd (L/kg)</td>
<td>1.17 (0.25–3.69)</td>
<td>0.59 (0.43–1.76)</td>
</tr>
</tbody>
</table>

AUC: area under the curve, Bu: busulfan, CI: clearance, Css: steady-state plasma concentration, n: number of AUC, PK: pharmacokinetic, t1/2: half-life, Vd: volume of distribution.

*Total of 12 AUC values [4 patients × AUC (for doses 1, 9, and 13)].

*Total of 9 AUC values [3 patients × AUC (for doses 1, 9, and 13)].

Table 3. The AUC and PK parameters for Bu in our study compared with that from previous studies.15,16

<table>
<thead>
<tr>
<th>Bu doses (ABW)</th>
<th>Pael et al.*</th>
<th>Michel et al.16</th>
<th>Our study</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8–1.2 mg/kg</td>
<td>NA</td>
<td>Target AUC (%)</td>
<td>43b</td>
</tr>
<tr>
<td>1.0 mg/kg</td>
<td>59</td>
<td>78c</td>
<td>NA</td>
</tr>
<tr>
<td>1.2 mg/kg</td>
<td>73</td>
<td>79</td>
<td>17</td>
</tr>
<tr>
<td>1.1 mg/kg</td>
<td>79</td>
<td>93</td>
<td>33</td>
</tr>
<tr>
<td>0.95 mg/kg</td>
<td>90</td>
<td>77</td>
<td>NA</td>
</tr>
<tr>
<td>0.8 mg/kg</td>
<td>90</td>
<td>92</td>
<td>33</td>
</tr>
<tr>
<td>Mean AUC (%)</td>
<td>1256 (23); 808–2410*</td>
<td>879 (35); 292–1714*</td>
<td>636 (35); 1,91–14,65*</td>
</tr>
<tr>
<td>CI (ml/min/kg)</td>
<td>3.43 (24); 1.69–5.07*</td>
<td>6.36 (35); 1,91–14.65*</td>
<td></td>
</tr>
</tbody>
</table>


*Five fixed doses (0.8–1.2 mg/kg) of Bu in accordance with EMA labeling guidelines.

**Five fixed doses (0.8–1.2 mg/kg) of Bu in accordance with EMA labeling guidelines; none of the patients were administered Bu at 1.0 and 0.95 mg/kg.

Both AML patients received Bu at 0.8 mg/kg. All samples achieved the target AUC, except one, in which the AUC was greater than the target (number 5, dose 13). Thalassemia patients received Bu at 1.1–1.2 mg/kg. Thalassemia and non-thalassemia patients exhibited median (range) AUC values of 576 (292–1301) μM·min and 1204 (758–1714) μM·min, respectively; the median values (range) of CI were 7.98 (3.42–14.65) and 2.69 (1.91–6.50) mL/min/kg, respectively (Table 2).

Clinical outcomes

All patients exhibited neutrophil engraftment at a median of 20 days (11–22 days). Six patients (85.71%) exhibited CC during the first month of HSCT. Two of three patients who reached the target AUC exhibited CC (66.67%). One patient (number 7) whose Bu level reached the target AUC group exhibited early MC since the first month of HSCT. This patient was severe thalassemia and presented alloantibodies (platelet antibodies) since pre-HSCT. Immunosuppressive drugs were discontinued for him; chimera studies improved to stable MC with a percentage of donor >90%. No patient in this low-AUC group presented alloantibodies pre-HSCT. Early complications (AEs ≥ grade 3 of NCI-CTCAE v4.0) consisted of febrile neutropenia (n = 7), mucositis (n = 4), vomiting
(n = 3), and elevated transaminases (n = 2). Patient number 5, with one AUC value above the target, suffered all the above early complications; however, the grades were not different from that of the other patients. No patient presented seizure, VOD, or aGVHD.

**Discussion**

According to our knowledge, this pilot study is the first report on PK parameters of IV Bu in Thai children. The following dose of Bu was not adjusted by the level of the 1\textsuperscript{st} dose because this study aimed to calculate the percentage of AUC values and PK parameters of steady-state five fixed doses IV Bu. In Thailand, there are few hospitals where the Bu levels can be monitored. The situation may be the same in other developing countries; therefore, we aimed to determine which of the five fixed doses can be applied without TDM monitoring. In this study, only 42.16% AUC values and 28.57% patients reached the target, compared to 75% (74–78%) patients in previous population PK studies in Americans,\textsuperscript{14} Caucasians,\textsuperscript{15,16} and Asians.\textsuperscript{17} The group receiving 0.8 mg/kg showed a higher percentage of achieving the target AUC (83%) than other groups. This result was similar to that from previous studies, as described in Table 3.\textsuperscript{3,16} However, in our study, the target AUC was achieved in only 33% and 17% AUC values receiving Bu at 1.1 and 1.2 mg/kg, respectively; this percentage was considerably lower than that from previous reports (Table 3).\textsuperscript{3,16}

In our study, inter-individual variability percent coefficients of variation (%CV) were 35% for both the estimated AUC and Cl (Table 3), which was higher than that from the study in pediatrics by Michel et al.\textsuperscript{16} They reported that the %CV for AUC and Cl were 23% and 24%, respectively. Another study\textsuperscript{14} using five fixed doses of Bu reported 19% CV for Cl. This high %CV for AUC and Cl in Thai children administered five fixed doses of Bu in our study postulated the necessity to perform TDM. Several studies have reported the factors that might influence Bu Cl, including age,\textsuperscript{11,12} underlying diseases,\textsuperscript{11,12} metabolomics (e.g., serum ferritin),\textsuperscript{24} genetic polymorphism of GST (glutathione-sulfate-transferase) enzymes,\textsuperscript{25} and drug-drug interactions (e.g., phenytoin).\textsuperscript{11,12,26}

In our study, all patients received phenytoin for seizure prophylaxis. This medication is well known as a strong inducer of hepatic metabolizing enzymes such as CYP2B6, 2C,
3A, and UDP-glucuronosyltransferases. In contrast, Bu is mainly metabolized by conjugating GST enzymes, primarily GSTA1, with a minor form of GSTM1 and GSTP1. Therefore, the Bu level should not be effected by phenytoin. However, Hassan et al. reported that patients receiving phenytoin exhibited a higher Cl for oral Bu compared to those who received diazepam; thus, the effect of phenytoin on Bu Cl according to various studies are controversial.

In terms of the underlying disease, patients with thalassemia exhibited a higher risk of unpredictable Bu metabolism because of liver dysfunction, possibly due to disease and iron overload, and genetic variations of the GST enzymes. Chiesa et al. reported that, in 53 Middle East Asian children with thalassemia, the median (range) of AUC for Bu after the first dose was 1083 (669–2698) μM·min. Thirty-one patients (58%) achieved the target AUC. Nine patients (17%) exhibited AUC greater than the target AUC; 13 patients (25%) exhibited AUC values lower than the target AUC and required dose escalation. Some hypotheses have explained that the Bu level is lower in thalassemia because the patients present GSTA levels 5–10 times higher than normal controls and age-matched leukemic patients. Iron overload in patients with thalassemia might induce GST via activation of the Nrf2/keap1 pathway, which also affected the Bu level. Our patients with thalassemia exhibited iron overload pre-HSCT. This might explain the low level of AUC after the first dose of Bu in most (75%) of them (Figure 1). Both patients with AML presented body weights above 34 kg and received Bu at 0.8 mg/kg. Almost all samples (83%) achieved the target AUC. One possible explanation is that children with higher body weights exhibited a physiological maturation of GST enzymes similar to adults; therefore, the same dose of Bu administered in adults (0.8 mg/kg) can be administered in patients with body weights above 34 kg.

Bu is primarily metabolized by GSTA1. The protein expressed by the homozygous genotype GSTA1*A/A (wild type) metabolizes Bu more rapidly than that expressed by heterozygous GSTA1*A/B and homozygous GSTA1*B/B. However, the effect of GST polymorphism upon Bu Cl is unclear. Asian populations with malignancy exhibited high frequencies of GSTA1*A/A. Homozygous GSTA1*A/A was detected in 71–80% Japanese, Korean, and Chinese patients. Patients with homozygous GSTA1*A/A showed a significantly lower level of AUC (or higher Cl) than the heterozygous GSTA1*A/B group in the Asian population. In a study on GSTA1 polymorphisms in adults with breast cancer in Thailand, the distribution of GSTA1*A/A was approximately 87.5%, which is similar to the results from other studies in the Asian population. However, GSTA1 polymorphism in Thai children was not reported. A recent study in patients with malignancies from China did not demonstrate any definite association between GST polymorphism and clinical outcome. The authors concluded that the GSTA1 genotype was not clinically relevant to Bu PK and did not recommend utilization of the GSTA1 genotype to determine the initial Bu dose in Chinese adult patients. Concordant with the ASBMT guidelines, the authors did not recommend personalized therapy with Bu based on genetic polymorphisms in routine clinical practice.

In our study, all patients exhibited successful neutrophil engraftment; however, only 28.57% patients achieved the target AUC of all 3 doses. One patient (number 7) achieved the target AUC but exhibited early MC since the first month of HSCT. This result might be explained by the presence of alloantibodies since pre-HSCT in this
patient. Early complications observed in this study were febrile neutropenia, mucositis, vomiting, and elevated transaminases, which are common side effects of Bu-conditioning regimens, similar to the results from other studies.\textsuperscript{12} However, these side effects were well tolerated and reversible. One patient with high AUC exhibited complications that were not different from other patients. In addition, VOD was not observed in our study possibly because 95% of the AUC values were $<1500 \mu M \text{min}$. Our pilot study, demonstrated the importance of TDM for five fixed doses of Bu in Thai children as 52.38% AUC values was lower than the target. The AUC for the first dose of Bu could predict the trend of the AUC of doses 9 and 13. Three patients (number 4, 5, 7) who had the first dose AUC of Bu in the target had 83.3% AUC values of doses 9 and 13 in the target whereas 4 patients (number 1–3, 6) who had the first dose AUC lower than the target had only 12.5% AUC values of doses 9 and 13 in the target (Figure 1). Therefore, personalized Bu dosing should be considered by using TDM after the first dose of Bu, as recommended in the ASBMT guidelines.\textsuperscript{11} PK-guided dosing adjustment should be performed in a patient who does not achieve the target AUC. The dose adjustment can be calculated by an equation recommended by ASBMT as follows: adjusted dose (mg) = actual dose (mg) $\times$ target AUC (μM min)/actual AUC (μM min). The target AUC for this equation was 1125 μM min.\textsuperscript{11}

In conclusion, 42.86% Thai pediatric patients receiving five fixed doses of IV Bu in this pilot study achieved the target AUC values; this percentage was lower than that from previous studies. Therefore, TDM of Bu should be considered in Thai children receiving five fixed doses of IV Bu, and dose adjustment should be performed as necessary. The limitations of our pilot study were the small number and heterogeneous group of patients, not covered every step doses of Bu in the five fixed doses for the AUC analysis, and absence GST polymorphism data for analyzing their association with Bu metabolism. However, this study might emphasize the necessity of TDM for five fixed doses of IV Bu. Further studies on Bu PK utilizing a larger sample size and the GST polymorphisms in Thai children are warranted for confirming the necessity of TDM at every step dose of IV Bu.

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Declaration of interest

The authors report no conflict of interest.

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