

Busulphan in Blood and Marrow Transplantation: Dose, Route, Frequency and Role of Therapeutic Drug Monitoring

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Abstract: Busulphan (Bu) is an alkylating agent that, when combined with agents such as cyclophosphamide (Cy), ablates the bone marrow prior to blood or marrow transplantation. There is wide inter- and intra- patient variability in Bu pharmacokinetics. Early pharmacodynamic studies suggested a significant relationship between high Bu exposure and the occurrence of veno-occlusive disease of the liver, but were not performed in uniform patient populations and tended to use a Cy dose of 200mg/kg. Pharmacodynamic studies in uniform patient populations, with lower doses of Cy, contradict these results. Despite 20 years of clinical use, pharmacodynamic studies are still required to define the relationship between Bu exposure and optimal transplant outcome, and these may vary according to age, disease, transplant type and conditioning regimen. Given the availability now of an intravenous formulation, it is timely to review how and why we are giving Bu.

Administration of single daily doses of Bu has been shown to be safe and effective with oral Bu and has been used with i.v. preparations. This simple administration provides accuracy in measuring exposure, which is ideal for pharmacokinetic studies, and provides the possibility of defining a target exposure level associated with a good outcome. Only when this target is defined will therapeutic drug monitoring be useful to minimise toxicity, maximise efficacy and improve transplant outcome.

Key Words: Busulfan, pharmacokinetics, bone marrow transplantation, therapeutic drug monitoring, drug monitoring, veno-occlusive disease, hepatic.

INTRODUCTION

Busulphan (Bu) is an alkylating agent commonly used in high dose-chemotherapy regimens that require blood or marrow stem cell support. Up until 1996, when an intravenous (i.v.) preparation of Bu was first reported [1], Bu was only available as a tablet. The oral formulation was characterised by wide inter- and intra- patient variability in Bu pharmacokinetics. The study of oral Bu pharmacokinetics has been complicated by Bu dosing every 6 h (q.i.d.), with consequent difficulties in determining pharmacokinetic parameters in patients with late absorption of the drug [2]. The i.v. formulation exhibits less dose - to - dose variability, leading to more reliable and consistent pharmacokinetic estimations [3,4].

There is evidence to suggest that total exposure to Bu is an important determinant of graft rejection, regimen-related toxicity (RRT), relapse of disease and survival [3,5-7]. The therapeutic index of Bu is narrow and depends on disease, conditioning regimen and transplant type. Further pharmacodynamic studies in uniform disease populations are required to better define the relationship between Bu exposure and toxicity and efficacy and identify target exposure levels that are associated with good outcome. These studies will be facilitated by the consistent and accurate exposure estimates that can be obtained with i.v. Bu. It should also be noted that,

as i.v. Bu appears to have less toxicity than oral Bu [8,9] it may not be possible to extrapolate the results of pharmacodynamic studies conducted with oral Bu to i.v. Bu.

This review looks at:

- (1) the role of Bu in blood and marrow transplantation (BMT) for a variety of diseases;
- (2) use of oral and i.v. Bu when combined with cyclophosphamide as well as in alternative regimens;
- (3) the pharmacokinetics of oral and i.v. Bu;
- (4) the pharmacodynamic relationships that have been established between Bu exposure and RRT, relapse of disease, graft rejection and survival in uniform disease populations.
- (5) the role of therapeutic drug monitoring (TDM) in Bu therapy.

We hope this will assist us in identifying current gaps in our knowledge in the use of Bu.

BU IN BLOOD AND MARROW TRANSPLANTATION (BMT)

History and Uses of BMT

Blood and marrow transplantation (BMT) is a medical procedure in which stem cells that can reconstitute the haematopoietic system (Haematopoietic Stem Cells, HSC) are given to a patient. For the first 30 years of such procedures, these cells were almost invariably obtained from the bone marrow - hence Bone Marrow Transplantation - but in the

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last decade these HSC may be derived from the peripheral blood, umbilical cord blood or bone marrow, i.e. from blood or marrow. There are two main reasons why patients undergo BMT. The commonest indication is for malignancy. Chemo radiotherapy may be used to control the cancer, particularly haematological malignancies such as leukaemia, but toxicity to the bone marrow is dose limiting. Where there is evidence that the drug still has a dose response effect in killing the malignancy at doses that cause marrow toxicity, and where other organ toxicity does not limit the dose, then the dose can be escalated, as long as HSC are used to rescue the patient from the high dose therapy. Typically, a dose of irradiation that would myeloablate the marrow is combined with a chemotherapeutic agent (for additional immunosuppression) and this can control or eliminate leukaemia that is refractory to all other therapy. The organ toxicity that most often limits the dose of the chemotherapeutic agents is gastro-intestinal toxicity.

The second main group of conditions treated by BMT are for a variety of diseases which are caused by the failure of some haematopoietic-derived cell. In fact, these represent some of the earliest transplants performed in humans. The first examples were congenital disorders where the patient's immune system was absent or defective. HSC could be given to restore function. One example is that of severe combined immunodeficiency (SCID), where patients often have malfunctioning B cells (that normally produce immunoglobulin) because they require the co-operation of T cells, which are missing. An infusion of HSCs will replace the missing T cells. However if there were B cell progenitors occupying space in the marrow, these will persist and may prevent engraftment with productive donor B cells. It may be necessary to eliminate these defective B cells, making space in the marrow for the donor B cells to engraft as well as T cells, to obtain a full functioning immune system. The treatment given to facilitate engraftment is called BMT conditioning. For these patients with immune deficiencies, who already had a defective marrow, the doses of drugs used to obtain this space did not have to be very high – and can be what would now be described as non-myeloablative.

A third concept which needs to be introduced is that of non-myeloablative transplants. High dose therapy given to eliminate malignancy is very toxic, particularly in adults where the death rate in the first 100 days after the BMT (usually referred to as transplant-related mortality, TRM) is 20-40%. It was realised that, for some malignancies, rather than the high dose therapy itself producing the elimination of malignancy, the introduction of a new immune system could account for part or most of the anti-cancer effect. This allowed the possibility of using less toxic therapy, usually more immunosuppressive and less myelosuppressive, to facilitate engraftment of the new immune system, rather than myeloablative therapy. Such treatment has lower TRM, allowing it to be extended to older patients and those less likely to survive the intensity of conventional myeloablative conditioning.

Role of Bu in BMT

In the early days of clinical transplantation, much time was spent working out a conditioning regimen that treated

the patient's leukaemia and allowed allogeneic engraftment. Total Body Irradiation (TBI) combined with cyclophosphamide (Cy) emerged as a standard. However, many centres did not have access to TBI and a chemotherapy alternative was therefore sought. Alkylating agents are widely used as part of the preparative regimen prior to BMT. Due to dose-limiting marrow toxicity and, consequently, a requirement for bone marrow stem cell rescue, they are often not used as part of standard chemotherapy regimens. Thus, for patients with malignancies, any residual tumour would not have been previously exposed to the drug, and so would not have developed resistance. The marrow toxicity can be ignored when marrow stem cells are going to be infused as part of the BMT procedure. Their steep dose-response curve and lack of cross-resistance also makes them attractive. They are not cell cycle specific, and thus are most commonly given once a day.

Busulphan or busulfan (Bu) is 1,4 – di (methanesulfonyl)butane, a bifunctional alkylating agent. As such, the alkyl groups bind to and cross-link DNA. Bu was synthesised and found to be toxic to the rat bone marrow [10]. It is profoundly myelotoxic and has broad activity against a range of malignancies. In a series of rodent experiments, Santos and workers showed that Bu and its myeloablative or space making properties, when combined with the immunosuppressive properties of Cy, would allow engraftment of foreign cells. The use of Bu or Cy on their own was insufficient: animals with an intact haematopoietic system needed both drugs and both properties to allow engraftment [11,12].

Clinical Trials Using Busulphan and Cyclophosphamide (BuCy)

Clinical trials using BuCy commenced in 1978 and the seminal paper reporting the initial experience with BuCy was that of Santos *et al.* [13]. In this study 51 patients with AML and matched sibling donors were conditioned with 16 doses of 1 mg/kg Bu and 4 doses of 50 mg/kg Cy. Twelve patients were alive and in remission at the time of the report and 10 are long term survivors [14]. Acute graft-versus-host disease (GvHD) and CMV interstitial pneumonitis were the major causes of death. Only small numbers of patients developed haemorrhagic cystitis (n = 5) or hepatic veno-occlusive disease (VOD) (n = 3) post transplant – two typical complications of transplantation, frequently associated with the use of BuCy. VOD, a clinical syndrome of fluid retention, painful hepatomegaly, jaundice, ascites and / or unexplained weight gain, is of particular concern because it can often lead to multi-organ failure and death [15]. Following this report, BuCy was adopted widely, if only because of its avoidance of TBI, a therapy which was intrinsically felt to be toxic.

Despite 20 years of clinical use of Bu in conditioning regimens, with at least in part the feeling that as a non-radiotherapy based conditioning it must be better than using TBI, it took until the 1990s for 4 randomised studies to be completed that directly compared the efficacy and toxicity of Bu with TBI, with both combined with Cy. The 4 randomised studies have been reported in depth [16-19] and there have been several summary analyses of the findings [20-22]. In summary, BuCy is associated with more VOD, haemorrhagic cystitis and alopecia, but fewer cataracts long term. In

terms of disease control, BuCy and Cy-TBI seem comparable for CML, but Cy-TBI has equal or better control of AML, especially for advanced disease. As the studies looked at adults, ALL was not included, but other studies have shown the superiority of TBI-Cy over BuCy in this disease [23,24].

Evolution of Bu Dose in the BuCy Regimen

Over the same time period, the use of Bu for non-malignant diseases had also evolved. In patients with a variety of immune deficiencies and relatively "weak" marrow function, 4 doses of 2 mg/kg of Bu were used and this was adequate to achieve engraftment in diseases such as Wiskott - Aldrich syndrome. However, when this dose was used for patients with storage disorders, and therefore more intact haematopoietic and immune systems, there was a high likelihood of graft rejection [25]. For example, 4 of the first 5 children with such disorders transplanted at Westminster Children's Hospital, under the direction of John Hobbs and Ken Hugh-Jones in the early 1980s, rejected their graft. On the basis that 2 mg/kg for a 70 kg, 1.73m² adult was 80mg/m²/day, the Bu dose was changed to a total of 320mg/m² given over 4 days (and for Cy this was 50 mg/kg/day for a 70kg, 1.73m² adult = 2g/m²/day = total of 8g/m²). A whole series of patients were transplanted at this dose – all using Bu as a single daily dose [26,27]. The rate of engraftment was better with higher doses of Bu. Higher doses of Cy also appeared to be important for engraftment. Problems arose when this high dose of Cy was used in underweight patients with thalassaemia, as smaller patients were getting a significantly higher dose when expressed on a per kg basis. When 16 x 1 mg/kg Bu doses were used for malignancy, it was found that the standard 4mg/kg/day dose provided inadequate exposure in children, and thus a surface area dose was proposed, where 16mg/kg for an adult was equivalent to 600mg/m² for a child with malignancy [28]. A surface area – based Bu dose provides younger children with equivalent systemic exposure or area-under-the-concentration-versus-time curve (AUC) as older children and adults [29,30].

The majority of the work reported up to this date had been with the most widely used q.i.d. regimen of administering Bu at 1mg/kg 6 hourly for 4 days (16mg/kg total dose) followed by 2 to 4 days of Cy (total dose 120 – 200mg/kg). It is important that one remembers that, for many years, the only available formulation of oral Bu in the USA was 2mg tablets and so, purely because of the number of tablets required, the dose (which like most alkylating agents would normally be given once a day) was divided into 4 doses per day. However, at Westminster Children's Hospital, a single daily dose (SDD) had always been used. When we started to use BuCy for AML at our own institution, we also adopted a SDD and rapidly moved to a surface area based dose of 150mg/m²/day for 4 days (total dose 600mg/m²). In young children, especially where a nasogastric tube was necessary, the SDD is convenient and well tolerated. We have previously demonstrated that our SDD regimen produced equivalent total systemic exposure as the q.i.d. regimen [29] and that this was very effective at treating AML [31]. Of course, one of the main reasons we started determining Bu pharmacokinetics was to dose modify, but we rarely found an adequate reason to do so [30].

Aspects Concerning Bu Administration

In the past Bu has been administered as whole tablets (to older children and adults), [2,29,30] as crushed tablets in a water or glucose suspension delivered through a nasogastric tube [30 32] and in capsules containing Bu and lactose [33]. However, it should be noted that suspensions in glucose have limited stability (unpublished data) and should be administered immediately after preparation. The practice of preparing large batches of Bu suspension to be administered over the 4 days of treatment is therefore of concern because the patient may not be delivered the expected dose. Decreasing exposure to Bu with increasing dose number is highly likely under these circumstances.

After the 25 mg tablets were withdrawn from the Australian market in 2002, it was necessary for us to prepare suspensions even for older children as too many tablets were required for the SDD. The task of preparing Bu suspensions for each and every dose is quite arduous for the pharmacist. This problem was, however, resolved when intravenous Bu (i.v. Busulfex) became commercially available. This formulation, prepared in dimethylacetamide and polyethylene glycol (1:2, v/v), is stable for 180 days at room temperature and 7.5 years at 4°C [1]. When diluted with water to a final Bu concentration suitable for parenteral administration (3 mg/mL), i.v Bu is stable for more than 54 h at room temperature [1].

In following sections of this paper, the literature on oral and i.v. Bu are reviewed, to provide an insight into Bu pharmacokinetics, pharmacodynamics and the role of therapeutic drug monitoring in Bu therapy.

Methods of Pharmacokinetic Analysis

Pharmacokinetics is the study of the processes that determine the time course of concentrations of a drug within body fluids, including drug absorption, distribution and elimination. Both model-dependent and model-independent methods have been used to determine the pharmacokinetic parameters of Bu [2,28,34-36]. Both methods produce the same AUC results when appropriate concentration-versus-time data is collected [36,37]. However, Schuler *et al.* recommend the use of non-compartmental methods for analysing oral Bu given every 6 h, and point out that both methods are inaccurate in patients with late absorption [2].

Model independent methods rely on an accurate estimation of the area-under-the concentration versus time curve (AUC), which can then be used to determine clearance, volume of distribution, the elimination rate constant and the average concentration during a dosing interval at steady state (C_{ss}, or AUC/ dosing interval). C_{ss} is often quoted in papers describing Bu pharmacokinetics and is often expressed as an average of the results from multiple doses given over a 4 day period. Accurate AUC measurements are obtained by measuring plasma drug concentrations at regular intervals after the dose and for a sufficiently long period of time to fully characterise the terminal elimination phase. Linear regression analysis may then be used to derive a limited sampling model for the drug.

Model - dependent approaches involve fitting a pharmacokinetic model to the concentration versus time data. The

best model is selected based on a range of criteria including the observed fit, the sum of squares and the distribution of the weighted residuals. Information from the population can also be used in combination with limited blood sampling and Bayesian forecasting to derive individual patient's pharmacokinetic parameters.

PHARMACOKINETIC CHARACTERISTICS OF ORAL BU

Bu is generally well absorbed after oral administration. However there is the occasional patient with very poor bioavailability. In young children bioavailability estimates range from 22 – 120% ($n = 8$) and in older children and adults they range from 47 – 103% ($n = 8$) [38]. Peak concentrations vary widely between individuals, but are higher for the surface area – based dose compared with the weight – based dose [30]. In 27 children who received 150 mg/m² SDD Bu, peak concentrations were median 23.3 μM (interquartile range: 18.3 – 30.8 μM) and in 7 children who received 4 mg/kg SDD Bu, peak concentrations were median 12.4 μM (interquartile range: 10.8 – 16.8) [30]. Approximately 4 fold lower peak concentrations are obtained with the 4 fold lower doses used in q.i.d. regimens [28,39]. Bu (1 mg/kg) given q.i.d to 20 adults resulted in peak concentrations ranging from 2.5 to 11.5 μM , with a mean of 6.1 μM [2].

We have found that, in children, the time to reach peak Bu concentrations ranges from 0.5 h to 5 h (median 2 h) and that the lag time (time to absorption start) is shorter when tablets are crushed [29,30]. Similar observations have been made for adults [40,41]. A long time to reach peak concentration means that Bu C_{ss} and AUC cannot be determined due to inadequate characterisation of the elimination phase with a 6 h dosing interval. This difficulty has been highlighted previously [2], occurring 26% and 16% of the time in two series [41,42]. This should not be a concern for i.v. Bu.

There is a log-linear decline in Bu concentrations following the peak concentration and Bu disposition is well described by a one compartment pharmacokinetic model [29]. There is wide interpatient variability in apparent clearance of Bu and in one large study that included 279 adults, the coefficient of variation in apparent clearance was approximately 21% [43]. In that study 173 adults of normal body weight (body mass index range: 18 – 26.9 kg/m²) had apparent Bu clearance of (mean \pm s.d.) 2.9 \pm 0.62 mL/min/kg, while 89 obese adults (body mass index range: 27-35 kg/m²), had significantly lower apparent clearance of 2.56 \pm 0.55 mL/min/kg. This group also noted that patients with Non-Hodgkin's lymphoma ($n = 10$) had significantly lower apparent clearance than those with chronic myelogenous leukaemia (CML, $n = 73$). In children mean apparent clearance of oral doses range from 3.4 to 4.9 mL/min/kg in different studies [28,29,32]. Clearance of Bu tends to be higher in younger children (< 3 or 4 years) than in older children and adults [6,28,30]. This is probably a consequence of age-dependant changes in Bu metabolism [43,44].

Wide inter- and intra- patient variability in exposure is observed for a given Bu oral dose in both adults and children [2,45]. For the SDD oral Bu dose of 4 x 150 mg/m², AUCs

ranged from 4005 to 11278 $\mu\text{M}\cdot\text{min}$ in 27 children (median 7517 $\mu\text{M}\cdot\text{min}$), and this was found to be significantly higher than the AUCs obtained from 7 children receiving the weight-based SDD Bu dose of 4 x 4 mg/kg (median: 4675, range: 2465 – 5513) [30]. Approximately 4 fold lower AUCs are obtained when children receive the 4 fold lower doses used in oral q.i.d. regimens [28,39]. In 20 adults given 1 mg/kg Bu doses q.i.d for 4 days, mean AUC_(0-6h) achieved for dose numbers 1,2,5,13 or 14 ranged from 1050 to 1522 $\mu\text{M}\cdot\text{min}$ and varied with dose number and whether or not the dose was given with food or metoclopramide [2]. Typical median C_{ss} values for q.i.d Bu ranged from 473 to 917 ng/mL in different studies [5,6,46].

Mean apparent volume of distribution after administration of oral doses to adults or children range from 0.7 to 1.4 L/kg in different studies [28,29,32]. Bu is approximately 30 – 50 % covalently bound to plasma proteins and approximately 47% to red blood cells [47,48]. Bu distributes freely into the cerebrospinal fluid (CSF) [39] and this probably accounts for why some patients experience seizures. We have found that clonazepam is more effective in preventing seizures than carbamazepine [30]. Additionally, it does not interfere with Bu pharmacokinetics, unlike phenytoin [49].

METABOLISM OF BU AND CY AND THE DEVELOPMENT OF VOD

The pathophysiology of VOD involves many different factors including cytotoxic, immunologic, inflammatory and haemostatic events. There is extensive damage to centrilobular structures (zone 3 of the liver acinus), which can include hepatic venular occlusion, eccentric luminal narrow and/or phlebosclerosis, sinusoidal fibrosis and hepatic necrosis [50]. The cytochrome P450 metabolising enzymes and the glutathione S-transferase (GST) enzymes are predominantly located in zone 3 of the liver acinus. Both of these enzyme systems are involved in the metabolism of Bu and Cy. Agents such as Bu and Cy produce hepatic injury by damaging the sinusoidal endothelial cells and this can lead to the development of VOD. As Bu is highly lipophilic and minimally protein bound, it reaches high levels in the liver.

Bu is eliminated after conjugation with glutathione (GSH) in the liver, a reaction catalysed by the GST enzymes [51,52]. The main GSTs involved in the metabolism of Bu are GST α , μ and π , coded for by the genes GSTA1-1; GSTM1-1 and GSTP1-1. The conjugation reaction produces at least 12 metabolites, most of which are not cytotoxic, but one of them is a sulfonium ion of GSH, which decomposes to a lipophilic compound, tetrahydrothiophene, which is hepatotoxic [40,53]. This compound undergoes further oxidation by the cytochrome P450 enzymes [54]. Bu is conjugated with GSH mainly by GST α [51]. GSTA1 has at least 7 polymorphisms, but none are associated with altered hepatic expression or function. However, GSTM1 is highly polymorphic, including homozygous deletions in some populations. Differences in GST isoform expression within the population and within different age groups may therefore contribute to intersubject variability in Bu pharmacokinetics, and this may be relevant in patients with Thalassaemia (see below) as well as in other diseases.

Cy is metabolized by cytochrome P450 to the cytotoxic metabolite, 4-hydroxy cyclophosphamide, which is highly reactive and can damage hepatocytes. Detoxification of 4-hydroxy cyclophosphamide and other Cy metabolites (e.g. acrolein) involves conjugation with GSH and a dose – dependant depletion of hepatic GSH by Cy and its metabolite (acrolein) has been observed [55]. Bu influences Cy clearance [56] possibly by reducing GST and GSH levels or by inhibiting cytochrome P450 activity. This may lead to a build up of the hepatotoxic metabolites of Cy, which may contribute to liver damage and the development of VOD.

The sensitivity of the liver to damage by Bu or Cy may also depend on whether there is pre-existing GSH depletion or not [57]. Depletion of GSH may lead to an accumulation of the hepatotoxic metabolites of Bu and Cy, leading to the hepatic damage underlying the development of VOD in BuCy-containing regimens. Supplementation with GSH precursors (e.g. N-acetyl-L-cysteine) is one method that has been used to prevent or treat VOD [58].

Thus, important considerations for the development of VOD would include aspects related to both Bu and Cy administration (e.g. dose, pharmacokinetics and timing with respect to each other). Tutschka *et al.* reported that reducing the Cy dose from a total of 200 mg/kg to 120 mg/kg reduced the incidence of interstitial pneumonitis and VOD in AML patients without compromising the anti-leukaemic effect [59]. Hassan *et al.* (2000) reported significantly higher clearance of Cy, significantly greater exposure to a cytotoxic Cy metabolite and less VOD when Cy was administered at least 24 h after the last Bu dose, compared with administration 7 – 15 h after the last dose [56]. More intensive conditioning by the addition of a third alkylating agent (e.g. thiotepa or melphalan) is also associated with a higher incidence of VOD [33,60,61], perhaps by further depleting GSH levels.

PHARMACODYNAMICS OF ORAL BU

To design effective dosage regimens it is necessary to define the relationships between the administered dose of a drug, the resulting concentrations in body fluids and the intensity of toxic or therapeutic pharmacological effects (pharmacodynamics). In the following sections of this paper the literature is reviewed to provide an insight into the pharmacodynamics of oral Bu when combined with Cy and / or other agents. Table 1 provides a summary of the results of the key studies on oral Bu.

REGIMENS USING BU COMBINED WITH CY

Relationship Between Bu Pharmacokinetics and Toxicity

A relationship between high Bu AUC and the development of VOD was first reported by Grochow *et al.* [34]. Bu pharmacokinetic analysis was performed on 30 patients given 16 doses of 1 mg/kg Bu and 4 doses of 50 mg/kg Cy. They had a range of diagnoses and donor types. The AUC estimates ranged from 606 to 5144 $\mu\text{mol/L}\cdot\text{min}$ and the mean \pm s.d. was 2012 \pm 1223 $\mu\text{mol/L}\cdot\text{min}$. Of the 6 patients who developed VOD, all had an AUC value greater than the mean and, in 5 of the 6, this was > 1 s.d. above mean. In a subsequent study by the same group using the same dose of BuCy but a different AUC cutoff, it was found that VOD occurred

in 6 of 8 patients with BU AUCs above 1500 $\mu\text{M}\cdot\text{min}$ but in only 1 (of 27) patients with AUCs below 1500 $\mu\text{M}\cdot\text{min}$ [35]. Concerns about the clinical toxicity of Bu (16 x 1 mg/kg) Cy (4 x 50 mg/kg) had meant that clinicians had already responded by modifying the conditioning for many patients to Cy (2 x 60 mg/kg) and, importantly, no similar pharmacokinetic study showing a relationship between Bu pharmacokinetics and VOD has been published with two days of Cy instead of 4.

Another paper which is widely quoted as supporting the relationship between VOD and high Bu levels is that of Dix *et al.* [41]. They studied 68 patients aged 14–65 years with 5 different diagnoses with pre-transplant conditioning of Bu in combination with 3 different drugs and a mixture of autologous and allogeneic transplants. A variety of administration times were tried, in order to allow time for the pharmacokinetics to be analysed after a first dose and then to subsequently modify the dose. It was not possible to evaluate the pharmacokinetic parameters in 26% of patients due to slow absorption. An initial AUC of $> 1500 \mu\text{M}\cdot\text{min}$ was seen in 18/51 (35%) and 6 of these 18 got VOD.

There have also been a number of reports that have failed to demonstrate a relationship between Bu pharmacokinetics and VOD [2,5]. In the study of Schuler *et al.* Bu pharmacokinetic parameters were determined on multiple occasions over the 4 days in 20 adults who mainly had matched sibling donors [2]. Bu was given as 16 x 1 mg/kg doses. Cy doses were 2 x 60 mg/kg doses for 18 patients and 4 x 50 mg/kg doses for 2 patients. A total of 3 patients developed VOD, but there was no relation to any Bu pharmacokinetic parameter. The analyses provided 89 curves from the 20 patients. In 16 of these the values gave impossible or implausible estimates – mainly because of the late peaks sometimes seen with oral dosing. Regression from the AUC of the first dose to later doses was not acceptable. They highlighted the limitation of 6 hourly dosing and dose to dose variation. They also discuss how far it is reasonable to try and calculate the AUC based on extreme model fitting, and pointed out that this must have been used in the studies of Grochow *et al.* [34,35]. They concluded that there was no role for Bu monitoring outside of clinical studies designed to further elucidate the role of Bu.

The Relationship Between Bu Pharmacokinetics and Transplant Outcome

Two papers, mainly paediatric, with the pharmacokinetic analysis performed at the Fred Hutchinson Cancer Research Centre in Seattle, attempted to relate Bu C_{ss} to clinical outcome measures, such as graft rejection and relapse [46,62]. Low C_{ss} tended to be associated with an increased incidence of graft rejection and relapse. One problem, however, which limits the applicability of these results, is that Bu concentrations were measured in the 0–6h interval between doses after a number of doses and C_{ss} was expressed as a mean of all values obtained for each patient. This means that a retrospective analysis such as this cannot be used to determine a target C_{ss} for first dose pharmacokinetics in patients studied prospectively. Another problem is that both studies included patients with a very broad range of diagnoses (including both malignant and non-malignant diseases), stem cell sources,

Table 1. Summary of Key Studies Involving Oral Bu in Uniform Patient Populations

Indication	Regimen	Patients	Bu dose	Comedication	Bu exposure	RRT	Engraftment	Outcome	Comments	Ref.
CML	BuCy120	45 adults, all allo	1 mg/kg q.i.d. for 4 days	Antiemetics (ondansetron, diphenhydramine, lorazepam), Phenytoin	Mean C _{ss} : 917 ng/mL, range 642 – 1749 ng/mL	Mucositis (95% of patients) VOD (35% of patients)	All patients achieved functional engraftment.	34 patients alive at 4 years post transplant. Deaths from RRT (1), sepsis (6), GVHD (1), relapse (3)	Relapse was associated with C _{ss} < 917 ng/mL.	Slattery <i>et al.</i> 1997 [5]
AML	BuCy200	45 children 7 adults, Auto(25) Allo(27)	1 mg/kg q.i.d. for 4 days	Medication for seizure prophylaxis not given routinely	Median C _{ss} : 578 ng/mL, Median AUC: 846 μM.min	No information	No information	29 patients alive at median 5 years post transplant. Deaths from relapse (16) and non relapse (11) causes.	C _{ss} below 578 ng/mL associated with improved disease free survival, and lower non-relapse mortality. Relapse and GvHD not associated with Bu exposure	Baker <i>et al.</i> 2000 [6]
AML (59) ALL (4)	BuCy120	63 children Auto(40) Allo(23)	SDD Bu 4mg/kg(25) 150 mg/m ² (38)	Clonazepam(46) Phenytoin (2), Carbamazepine (2)	Median AUC 4675 μM.min for 4 mg/kg dose (n = 7), 7517 μM.min for 150 mg/m ² dose (n = 27).	3 children had RRT grade 3 or 4, 3 had VOD. Common side effects were mucositis (92%), hepatic toxicity (51%), GI toxicity (40%) and renal toxicity (13%).	1 patient failed allogeneic engraftment. This patient had a low AUC of 4005 μM.min.	42 patients alive and well at 6-18 years post transplant. Deaths were from relapse (16), GvHD (3), sepsis (1) and RRT (1).	No relationship found between Bu exposure and RRT or outcome. Safety and efficacy of 150 mg/m ² Bu dose similar to that of 4 mg/kg dose. 150 mg/m ² dose safe in children < 3 years.	Shaw <i>et al.</i> 2004 [30]
Inherited metabolic storage disease, including 20 with Hurlers syndrome	BuCy120	39 children, all allo	8 doses of 40 mg/m ² Bu	phenytoin	Median 4 th dose AUC: 1292 μM.min, min:458 μM.min, max: 2602 μM.min	3 patients developed VOD (all died).	Early engraftment occurred in 32 (of 38) evaluable patients. Late full engraftment occurred in 19 (of 27) evaluable patients.	1 year survival was 67%, 4 year survival was 56%.	No association found between Bu exposure and early engraftment.	Jacobson <i>et al.</i> 2001 [105]
Thalassaemia	Bu14Cy200 (n = 36) Bu14Cy120 (n = 17) Bu16Cy120 (n = 11)	64 children and young adults, all allo	Total dose 14 mg/kg or 16 mg/kg given q.i.d over 4 days	clonazepam(61) phenytoin (7), phenobarbital(1)	First dose AUC median: 650 μM.min 10 th dose AUC median 740 μM.min	3 patients had VOD, 7 patients had interstitial pneumonitis, 5 had early onset seizures	5 patients failed engraftment	Mortality rate was 15%.	No relationship found between Bu exposure and RRT or outcome. Graft rejection associated with Cy dose and GVHD prophylaxis. VOD associated with ferritin levels and degree of siderosis on liver biopsy.	Pawlowska <i>et al.</i> 1997 [67]

(Table 1. Contd....)

Indication	Regimen	Patients	Bu dose	Comedication	Bu exposure	RRT	Engraftment	Outcome	Comments	Ref.
Thalassaemia	BuCy200	94 children, all allo	Dose randomised, Total dose 600 mg/m ² (n = 47) and 16 mg/kg (n = 47), given q.i.d over 4 days.	No information	600 mg/m ² Bu dose group: mean AUC 1236 µM.min, 16 mg/kg dose group: mean AUC 760 µM.min.	600 mg/m ² Bu dose group: 45 patients had mucositis, 31 had GI haemorrhage, 8 had VOD 16 mg/kg dose group: 41 patients had mucositis, 20 had GI haemorrhage, 12 had VOD	600 mg/m ² Bu dose group: 2 failed engraftment, 16 mg/kg dose group: 4 failed engraftment	600 mg/m ² Bu dose group: overall survival 68% (32 patients), disease free survival 68%. 16 mg/kg dose group: overall survival 72%, disease free survival 64%	Incidence of GI haemorrhage significantly higher for 600 mg/m ² Bu dose compared with the 16 mg/kg dose. Graft rejection was associated with low C _{min} , C _{ss} and AUC values. VOD associated with low AUC values.	Chandy <i>et al.</i> 2005 [7]

CML = Chronic myelogenous leukaemia, AML = acute myeloid leukaemia, ALL = acute lymphoblastic leukaemia, Cy120 = cyclophosphamide dose 120mg/kg, Cy 200 = cyclophosphamide dose 200 mg/kg, SDD= single daily dose, auto= autologous transplant, allo = allogeneic transplant, RRT = regimen-related toxicity. VOD = venoocclusive disease of the liver, GI = Gastrointestinal, GVHD = Graft versus host disease.

transplant types (including autologous, related and unrelated), Bu dose (ranging from 11 to 30mg/kg) and Cy dose (ranging from 120-335mg/m²). These studies were important in developing the field but highlighted that, in order to better understand the relationship between Bu pharmacokinetics and outcome, one has to look at more uniform patient populations. Adults and children with the same disease should be treated as separate populations as they may respond to treatment differently.

Bu in CML

Slattery *et al.* studied the influence of Bu C_{ss} on transplant outcome in a cohort of 42 adults with CML conditioned with 16 doses of 1mg/kg Bu and 2 doses of 60 mg/kg Cy [5]. Bu levels were measured after any 2 days of doses 5, 9 or 13 and Bu C_{ss} was the mean of all values obtained. Patients with Bu C_{ss} less than median (of 917ng/ml) had a higher relapse rate. There was no relation between Bu C_{ss} and non-relapse mortality or severe regimen-related toxicity (RRT) – the latter may reflect the fact that a total Cy dose of 120mg/kg was given rather than the higher 200mg/kg dose used in the original papers. There was also no relation between C_{ss} and graft rejection in this cohort receiving grafts from HLA-identical siblings and where the minimum C_{ss} was 642 ng/mL. This is one of the few papers that makes clear recommendations based on a uniform patient population, and states that one should target a high Bu level, greater than the median (917 ng/mL). In this way the chance of disease relapse will be minimised, without an increased risk of severe RRT. Yet this recommendation is at odds with the majority of Bu concentration targeting being carried out in the USA. In a subsequent series of 131 HLA matched family donors and the same conditioning, Bu pharmacokinetics were analysed after the first dose and, if necessary, dose adjustments were then made after doses 1, 5 and 9, targeting a C_{ss} of > 900ng/ml. This series of patients had an excellent outcome (the 3 year non-relapse mortality was 14%) and there was no relationship between Bu level (again expressed

as the median of all the Bu C_{ss} measured) and outcome and no comment on any toxicity of the regimen [63].

Bu in Acute Myeloid Leukaemia (AML)

Baker *et al.* looked at a population of mainly paediatric AML CRI patients, receiving both allogeneic and autologous transplants [6]. Most patients received 16 doses of 1 mg/kg Bu and 4 doses of 50 mg/kg Cy, as per the original Grochow papers. Despite performing the pharmacokinetics in a large uniform cohort, the data could only suggest a trend to better disease free survival with a Bu C_{ss} below the median (< 578 ng/mL). There was no significant correlation between C_{ss} and relapse, and the incidence of non-relapse mortality was greater in patients with C_{ss} above the median, but mainly from GVHD rather than VOD. There was no comment on whether VOD was related to Bu C_{ss}.

We have examined the effects of Bu dose and pharmacokinetics on toxicity and outcome in 63 children with acute leukaemia given BuCy prior allogeneic or autologous transplantation [30]. Bu was administered 4 times as a SDD either based on weight (4 x 4 mg/kg) or on surface area (4 x 150 mg/m²), while all children received 2 doses of 60 mg/kg Cy. Only one child did not achieve full allogeneic engraftment and she had the lowest exposure to Bu (AUC = 4005 µM.min) in the group of children who had the 4 x 150 mg/m² dose. The median AUC for the 150 mg/m² dose group was 61% higher than for the 4 mg/kg dose group (7517 versus 4675 µM.min), while median peak Bu concentrations were 88% higher (23.3 versus 12.4 µM). While the 150 mg/m²/day dose represented a considerable escalation of dose and exposure we found that it was not associated with an increased risk of severe RRT, the incidence of which was low in our cohort. We also did not find any significant relationships between Bu exposure and GVHD or relapse. Our results with paediatric patients with acute leukaemia suggest that there is generally no need for any dose adjustments when the surface area –based dose is used, with only the odd patient with very low exposure needing a dose increase to

target an AUC closer to that of the median (7517 $\mu\text{M}\cdot\text{min}$) to ensure engraftment. It should be noted that our median AUC for the 150 mg/m^2 dose, was substantially higher than 6000 $\mu\text{M}\cdot\text{min}$, the SDD equivalent for the 1500 $\mu\text{M}\cdot\text{min}$ upper limit target for the q.i.d. regimen that was used by Grochow *et al.* [35]. A total of 67% of our children receiving the 150 mg/m^2 dose achieved AUCs above the 6000 $\mu\text{M}\cdot\text{min}$ upper limit, but still they did not experience significantly more toxicity. The lower Cy dose (2 x 60 mg/kg) probably contributed to the low rate of RRT.

Bu in Thalassaemia

Presumably due in part to the effect of iron overload on the liver, an active marrow and the lack of prior therapy, patients with thalassaemia have been found to suffer a high rate of non-engraftment and VOD post BMT [64,65]. This led an Italian group to modify the conditioning regimen based on patient risk factors including the degree of liver dysfunction and the degree of fibrosis and iron in the liver. Using a combination of a reduced Bu dose (often 14 mg/kg total dose) and Cy (120 -200 mg/kg total dose) it was found that there was reduced toxicity, but a higher rate of non-engraftment [66]. This suggests that, in thalassaemia, Bu pharmacokinetic analysis may be very important to see if targeting Bu levels would maintain a low toxicity, by avoiding low levels that could be associated with non-engraftment and also high levels, which, according to the work of Grochow *et al.*, would be associated with VOD (as high levels lead to more depletion of hepatic GSH, predisposing cells to damage by Cy).

Pawlowska studied 64 thalassaemia patients who underwent BMT with matched sibling donors using either Bu (14 mg/kg) Cy (200 mg/kg) or Bu (14-16 mg/kg) Cy (120 mg/kg) with methotrexate and Anti-Thymocyte Globulin (ATG), with Bu administered t.d.s. rather than q.i.d. The lower Cy dose and additional anti-rejection immunosuppression in the second regimen was used in those patients who were at higher risk of TRM, based on the Lucarelli model [67]. They found no relationship between Bu exposure and toxicity or rejection. Rejection was not seen in the 6 cases with a C_{ss} of < 200 ng/mL , reported by Slattery to be associated with rejection. Rejection was associated with the lower dose of Cy, but this dose was used for patients with Class 3 disease, who were more at risk of transplant-related mortality. VOD occurred in only 3 patients.

Poonkuzhali *et al.*, performing the only randomised study of Bu dose to date, in 23 thalassaemics, compared the Bu dose of (16 x 1 mg/kg) with ATG (A) with Bu (16 x 37.5 mg/m^2) (B), all followed by Cy (4 x 50 mg/kg) [65]. The surface area-based dose provided significantly higher AUCs than the weight-based dose, but there was no increase in toxicity. There was also no correlation between Bu C_{ss}, C_{max} or AUC and hepatic toxicity. In fact, the first dose AUC was higher in those that did not develop VOD, many of which had much higher exposure that would have been expected to cause VOD based on the threshold levels of Grochow. They postulated that polymorphisms of the GST genes may be associated with the risk of developing VOD in thalassaemia. This was confirmed in a subsequent study of 114 thalassaemia

patients, in which it was found that the GSTM1 null genotype and age were significant predictors of VOD using multivariate analysis [68]. Patients with the GSTM1 null genotype cleared Bu faster and had lower Bu exposure than those who were GSTM1-positive. This is because patients who are GSTM1 null have higher expression of GSTA1 [69]. Clearance of Bu was higher and first dose C_{ss} lower in those patients who developed VOD. They suggested that more rapid clearance of Bu from the blood leads to formation of a toxic metabolite of Bu which then contributes to development of VOD, either directly or indirectly through depletion of the GSH pool (although there was no correlation between the pre-transplant hepatic GSH levels and VOD). This may be further enhanced by the subsequent delivery of Cy, particularly if insufficient time has elapsed for the regeneration of GSH [56,70], or reduced by use of agents such as N-acetyl-L-cysteine [58]. This is in keeping with the theory of DeLeve and Wang, that in a liver that is not GSH depleted, it is the GSH-metabolite of Bu that can cause the liver damage – hence more rapid clearance of Bu from the blood and delivery into the liver is associated with more conjugation to the toxic metabolite [57]. Irrespective of which dose was used, 16 mg/kg or 600 mg/m^2 , low Bu exposure was more likely to be associated with rejection; data which supports the rationale of targeting a minimum exposure. Thus, in diseases such as Thalassaemia, it may be rational to screen patients for their GST genotype pre-transplant. It may also be necessary to routinely provide supplements such as N-acetyl-L-cysteine to ensure that the GSH pool is not depleted. Since GST polymorphism has been shown to be an important determinant of Bu pharmacokinetics and toxicity in Thalassaemia, there may be other diseases where the pharmacogenomics of Bu is relevant to its metabolism, toxicity and outcome.

REGIMENS USING BU COMBINED WITH AGENTS OTHER THAN CY

Most of the preceding work has been based on BuCy, with the occasional patient having a third drug added in some of the series. We have pointed out that one should not extrapolate from studies using BuCy (200 mg/kg) to BuCy (120 mg/kg), and this is even more true if data derived from BuCy is used to suggest modifications in dose when Bu is combined with other agents. This is well illustrated in a series of 45 patients \leq 20years given Bu combined with melphalan (MLP) or thiotepa (TT) [71]. The overall rate of VOD was 36%, making it potentially easier to determine a relationship between Bu exposure and this toxicity. VOD occurred more with BuMLP than with BuTT. However a number of problems were highlighted in the study, including (1) Bu pharmacokinetic parameters could not be determined in 15% of patients because of slow absorption, (2) with BuMLP there was a trend to lower AUC in those who developed VOD, as seen in the thalassaemia patients previously mentioned, (3) patients treated with BuTT who got VOD had higher Bu AUCs, (4) 6 of the 13 with no VOD had AUC above the toxicity threshold of Slattery [72], (5) mean Bu troughs were lower in those with VOD than those without and (6) there was a poor correlation between the AUC determined after dose 13 and the limited sampling estimate after the first dose. It was their conclusion that the therapeutic

tic window defined for BuCy (and we would argue if this has in fact been defined adequately) cannot be used to prospectively adjust the dose with BuMLP or BuTT. In addition, since VOD was not associated with high Bu exposure, one cannot develop a model for TDM with these combinations. As in the thalassaemia work, [68,73] they postulated that the prior therapy the BuMLP patients had received, and the fact that both Bu and MLP deplete GSH means that the more rapid clearance of Bu into the liver predisposes it to further depletion by the MLP, as in the model proposed by Deleve and Wang [57].

Thus, we are left with the situation where the one combination that has been tested in adults (Bu 16 x 1mg/kg and Cy 4 x 50 mg/kg) is very rarely used apart from in paediatrics, and we do not know how many patients of this age group were included in the series of Grochow showing the relationship between levels and VOD [34,35]. When looking at uniform patient populations, the data is inconclusive for toxicity and cannot be extrapolated from BuCy to other combinations. Despite this, there is a high expectation, particularly in the USA, that TDM will and should be done for Bu-containing regimens. With the advent of i.v. Bu, it is timely to ensure we do not propagate the mistakes of the past.

INTRAVENOUS BU

A Bu formulation suitable for parenteral administration was first reported in 1996 [1]. Clinical trials in humans then began and the results of some of these studies are summarised in Table 2. The i.v. Bu preparation has been shown to be safe in humans and usually associated with less toxicity compared with oral Bu [8,74-77]. Less VOD has been reported with i.v. doses (usually 0.8mg/kg IV) that are felt to be equivalent doses to oral Bu (1 mg/kg) given q.i.d. [9,78]. Reductions in VOD and 100 day mortality have also been seen with i.v. BuCy conditioning in a uniform adult patient cohort with CML (n = 47) when compared with data from the Centre for International Blood and Marrow Transplant Research (CIBMTR) [8]. Similar observations have also been made in patients with a broader range of diagnoses. For example, Sobocinski *et al.*, in a matched pair analysis, compared the outcome of patients given i.v. Bu Cy 2 x 60mg/kg conditioning as part of 4 clinical trials conducted by the manufacturer with CIBMTR data on patients receiving oral Bu [79]. Overall incidence of VOD or mortality in the first 28 days was 4.6% (4/83) with i.v. Bu and 20.3% (38/149) with oral Bu (p<0.001); 100-day mortality for autologous transplant recipients was 0% for i.v. and 9.3% for oral Bu; amongst allogeneic BMTs it was 8.7% with i.v. Bu and 22.5% with oral Bu patients (p=0.015). In logistic regression analysis, only the mode of Bu administration was a significant factor for the risk of VOD, with i.v. Bu associated with a greatly reduced risk (p=0.004) compared with oral Bu. It was felt that although TDM was not used for the i.v. Bu, it would have been routine to use this for the patients receiving oral Bu, at least for those in the USA.

INTRAVENOUS BU GIVEN AS A SINGLE DAILY DOSE

Intravenous Bu has also been used in a SDD schedule. A SDD of 3.2 mg/kg/day (n = 20) was compared to 0.8mg/kg q.i.d. (n =11) and an oral q.i.d. comparison group (n = 25);

all followed by Cy (2 x 60 mg/kg) in adults with haematological malignancies. The dosing formula used was highly predictive of AUC and it was suggested that dose adjustments, and so monitoring, may be unnecessary [80]. When using SDD i.v. Bu combined with Cy (2x 60 mg/kg), there is a note of caution with one series of patients (16 – 60 years) who did show a high rate of VOD [81]. However, this series of patients were high risk: 9 had relapsed or refractory disease at the time of transplant, and all patients had a very short time interval between the last Bu dose and subsequent Cy – all less than 24h and some less than 6 hours. As discussed above, we know that depletion of hepatic GSH by Bu can impact on the subsequent clearance and toxicity of the second agent, including Cy [56]. Fernandez *et al.* reported on using i.v. Bu either twice a day or as a SDD. Despite achieving similar AUCs to the series of Williams *et al.* [81] only 1 patient developed clinical VOD which resolved [82]. The results of other studies also support the safety profile of i.v. Bu given as a SDD. For example, Benn *et al.* reported that 20 adults (mixed diagnoses) conditioned with SDD of i.v. Bu had a good outcome with 18 (of 19 evaluable) surviving 100 days post transplant and two with mild clinical VOD [83]. Shaughnessy *et al.* compared 20 adults given SDD i.v. Bu with 16 given Bu q.i.d. and obtained similar results [70]. Pharmacokinetically – guided dose adjustments were not made in any of these studies.

The evaluation of i.v. Bu has also coincided with the introduction of fludarabine (Flu) and non-myeloablative conditioning regimens. Bu Flu is now a well accepted combination of therapy for myeloablative conditioning and SDD i.v. Bu as part of Bu Flu is safe and effective, without pharmacokinetic-based dose modifications [4,84].

PHARMACOKINETICS OF I.V. BU

Following the administration of an intravenous dose, there is a monophasic log-linear decline in busulphan concentrations with time [85], which is consistent with a one compartment model. Mean clearance of i.v. Bu is typically 3.3 mL/min/kg in adults [80] and 4-5 mL/min/kg in children [76]. Similar to oral Bu, clearance of i.v. Bu tends to be greater in younger children (< 3 or 4 years) compared with older children and adults [76]. Mean estimates for volume of distribution range from 0.62 L/kg to 0.84 L/kg [76,85,86].

Bu given intravenously was associated with AUCs of 1130 ± 353 µM.min (mean ± s.d.) in 35 adults given 0.8 mg/kg Bu q.i.d. [74], while in 45 adults who had SDD IV Bu (4 x 130 mg/m²), the median AUC was 4871 µM.min (range: 2931 – 8271 µM.min) [84]. It has been our observation that the 130 mg/m² i.v. Bu dose tends to provide slighter lower, but still adequate, AUCs than the oral 150 mg/m² dose for most children (median 5602 versus 7517µM.min), but we have had 1 child with SCID and 1 with Omenn Syndrome who have had extremely high AUCs (of 12253 and 13422 µM.min, respectively) [87]. Therefore, there is still a high degree of variability in exposure to i.v. Bu in some disease populations, although there is less dose to dose variability compared with oral Bu [4,42]. Bu given i.v. is unaffected by food, unlike oral Bu [2].

Table 2. Summary of Key Studies Involving i.v. Bu

Indication	Regimen	Patients	Bu dose	Comedication	Bu exposure	RRT	Engraftment	Outcome	Comments	Ref.
CML, Acute leukaemia, MDS, MM	BuCy120 Retrospective comparison of oral versus i.v. Bu	88 adults (50 oral Bu 38 i.v. Bu)	Oral Bu: 1 mg/kg or i.v. Bu: 0.8 mg/kg given q.i.d. for 4 days	Phenytoin, antiemetics	No information	VOD: 6 patients (20%) for oral Bu, (all died), 3 patients for i.v. Bu (2 died)	No information	100-day mortality: 10 (33%) for oral Bu and 6 (13% for i.v. Bu)	i.v. Bu associated with less VOD and overall 100-day mortality than oral Bu.	Kashyap <i>et al.</i> 2002 [9]
AML, MDS	BuCy120	35 adults, all allo	0.8 mg/kg given q.i.d for 4 days	Phenytoin	Dose 1 Mean AUC: 1130 $\mu\text{M}\cdot\text{min}$ Dose 9 Mean AUC: 1169 $\mu\text{M}\cdot\text{min}$	7 patients died in first 100 days post BMT from VOD(2), CMV pneumonia (1), interstitial pneumonitis (1) GVHD (1) and leukemic progression (2). No serious CNS toxicity.	All patients engrafted.	33 of 35 patients in CR at 1 month post BMT. 3 (of 8) CR1 patients remain in CR at 21-24 months. In high risk patients (18 AML, 9MDS), overall survival is 64% and disease free survival 31% at 2 years post BMT.	Toxicity profile of i.v. Bu similar to that observed with oral. Low incidence of VOD observed and no serious CNS toxicity.	Andersson <i>et al.</i> 2000 [92]
CML	i.v. Bu Cy120 (47) oral BuCy120 (35%) CyTBI (45%)	1812 adults, all allo	i.v. Bu: 0.8 mg/kg given q.i.d for 4 days.	No information	No information	No patients receiving i.v. Bu died prior to 100 days.	No information	100 – day mortality significantly lower for i.v. BuCy compared with alternative regimens (0% versus 20%)		Thall <i>et al.</i> 2004 [8]
AML (11) ALL (7) MDS (1) JMML (1)	TTPBuCy120 (n=17) i.v. BuCy120 (n = 3)	20 children, all allo	0.8 mg/kg for 2 doses, then dose modification	lorazepam	Target AUC: 1000 - 1300 $\mu\text{M}\cdot\text{min}$. 13 (of 18 patients achieved the target AUC.	10 patients (50%) had RRT grades 2 or 3. Mucositis, esophagitis and diarrhea were most common side effects. 7 patients had hyperbilirubinemia, none had VOD	1 patient failed engraftment.	All patients survived beyond day 30. 13 patients survived after median 651 days. Deaths from GVHD(1), recurrent leukaemia (6).		Tran <i>et al.</i> 2004 [42]
CML	BuCy120	36 adults, all allo	0.8 mg/kg q.i.d for 4 days (25 patients), 1mg/kg for 2 doses, followed by targeted doses (11 patients).	phenytoin	Median AUC 1265 $\mu\text{M}\cdot\text{min}$ Target AUC for last 11 patients: 1250 $\mu\text{M}\cdot\text{min} \pm 20\%$	No grade 4 RRT. No CNS or lung toxicity. Main toxicities were mucositis (47%), diarrhea (17%). No VOD, but 10 patients (28%) had hyperbilirubinemia.	All patients showed full engraftment at median 12 days post BMT. Acute GVHD in 12 patients (33%).	11 patients died from acute GVHD (8) or recurrent disease (3)	Probability of GI toxicity, hyperbilirubinemia, mucositis and GVHD increased with increasing AUC. Risk of death lower for patients with Bu AUC between 950 – 1520 $\mu\text{M}\cdot\text{min}$.	Andersson <i>et al.</i> 2002 [3]
CML (16) AML (39) MDS (1) HD (1) NHL (4) CLL(8) HES (1)	Bu Flu250	70 adults, all allo	SDD Bu 3.2 mg/kg for 4 days.	phenytoin	First dose mean AUC: 4867 $\mu\text{M}\cdot\text{min}$ (n = 12). Fourth dose mean AUC: 4980 $\mu\text{M}\cdot\text{min}$ (n = 12)	1 patient had a seizure. Mucositis occurred in 49 patients (70%), haemorrhagic cystitis in 9 patients (13%). No VOD. There were 3 early deaths (before day 73).	2 patients failed engraftment	After 2 years, projected relapse was 21% for low risk patients, 66% for high risk AML, 18% for other high risk patients. Projected 2 year overall survival was 88% for low risk patients, 37% for high risk AML and 71% for other high risk groups.	SDD i.v. Bu was reasonably well-tolerated and provides predictable blood concentrations.	Russell <i>et al.</i> 2002 [4]

(Table 2. Contd....)

Indication	Regimen	Patients	Bu dose	Comedication	Bu exposure	RRT	Engraftment	Outcome	Comments	Ref.
AML (74) MDS (22)	BuFlu160	96 adults, all allo (20% in CR1, 56% with active disease)	SDD Bu 130 mg/m ² for 4 days	phenytoin	Median AUC: 4871 µM.min. Range: 2931 – 8271 µM.min. (n = 45)	1 death from RRT. 2 patients had VOD. Grade 3-4 elevation of transaminases and bilirubin occurred in 18% and 9% of cases respectively. Grade 3 mucositis, diarrhea and abdominal pain occurred in 13% of patients, and haemorrhagic cystitis in 3%	1 patient failed engraftment 62 (of 89) evaluable patients (70%) were complete chimeras by day +30.	CR rate was 85% (n = 47 responders) of 54 patients with active disease. 33 patients (34%) relapsed by 1.5 – 12 months. 62 patients alive at median follow up of 12 months. Deaths from GVHD (2), disease relapse (31) and RRT (1).	BuFlu regimen well tolerated with only 1 death due to RRT.	De Lima <i>et al.</i> 2004 [84]

MDS = myelodysplastic syndrome, MM = multiple myeloma, JMML = juvenile myelomonocytic leukaemia., HD = Hodgkins disease, NHL = Non-Hodgkins lymphoma, CLL = chronic lymphocytic lymphoma, HES = Hypereosinophilic syndrome, CML = Chronic myelogenous leukaemia, AML = acute myeloid leukaemia, ALL = acute lymphoblastic leukaemia, Flu250 = fludarabine 250 mg/m². Flu160 = fludarabine 160 mg/m², Cy120 = cyclophosphamide dose 120mg/kg, SDD= single daily dose, auto= autologous transplant, allo = allogeneic transplant, RRT = regimen-related toxicity. VOD = venoocclusive disease of the liver, GI = Gastrointestinal, GVHD = Graft versus host disease, CR = Complete response, CNS = Central nervous system.

There are a number of factors which may contribute to the observed interpatient variability in exposure to i.v. Bu including weight, [88] co-medication and variability in Bu metabolism. While phenytoin and metoclopramide have been shown to influence Bu clearance [2,49], the impact of other medications has not been studied adequately.

PHARMACODYNAMICS OF I.V. BU

There have been few pharmacodynamic studies conducted with i.v. Bu in uniform patient populations. As previously mentioned, VOD and 100-day mortality appears to have a lower incidence for i.v. Bu compared with oral Bu, so results of pharmacodynamic studies performed with oral Bu cannot be extrapolated to i.v. Bu. With a low frequency of VOD in patients with haematological malignancies given i.v. Bu (Table 2), it would be difficult to demonstrate a significant relationship with i.v. Bu exposure. Studies in patients with other diagnoses that tend to have higher incidence of VOD (e.g. thalassaemia) have not yet been reported. However, in adult CML patients, the probability of other regimen-related toxicities, including gastro-intestinal toxicity, hyperbilirubinemia, mucositis and GVHD has been found to increase with increasing AUC [3]. In that study the risk of death was lower for patients with Bu AUC values between 950 – 1520 µM.min, with risk of death rising sharply when AUC values were above or below these levels.

For disease populations in which pharmacodynamic studies with oral Bu have demonstrated a significant relationship between Bu exposure and graft rejection (e.g. Thalassaemia [7]) or relapse (e.g. CML [5]), it is likely that the target exposure levels that were established would also be relevant for i.v. Bu. However, many of these studies quote C_{ss} levels, which are relevant only for Bu given q.i.d. AUC measurements would be more relevant for SDD i.v. Bu. Thus further studies are required with i.v. Bu in uniform disease populations to establish the target AUC levels that are associated with a high rate of engraftment and a low rate of toxicity, relapse and mortality.

The elegance of a SDD i.v. dose is that you measure the AUC easily, as with SDD oral. There is minimal error. In marked contrast to the difficulties inherent in accurately determining meaningful Bu exposure on the oral q.i.d. regimen with all the problems such as delays in absorption and marked interpatient and inpatient variability, we finally have a more consistent exposure and can start to look at if there is any relationship between Bu exposure with the i.v. formulation and outcome. Only then, if such a relation is found, is there any need to consider targeting a particular Bu AUC.

THERAPEUTIC DRUG MONITORING (TDM) FOR BU – TARGETING WHAT?

TDM can be useful for improving outcome in cancer chemotherapy if:

- (1) there is a demonstrated relationship between drug exposure and outcome in drugs with relatively narrow therapeutic indexes,
- (2) a target exposure level has been identified that provides the optimal outcome,
- (3) there is substantial interpatient variability in drug exposure that may be reduced by TDM,
- (4) blood concentrations and drug exposure can be measured in a precise, reproducible and timely manner.

Busulphan fits some of these criteria in that it is a toxic drug with a relatively narrow therapeutic index showing considerable intersubject variability in the pharmacokinetic parameters. However, there are a number of challenges for TDM of Bu. Firstly, it is often not possible to obtain reliable estimates of Bu exposure within the 6 hour dosing interval with oral Bu given q.i.d [2]. Dose to dose variability in exposure is also high for oral Bu [2], so that even after dose adjustments are made, the target exposure levels are not reached in a significant proportion of patients. This has occurred in a number of studies investigating TDM of Bu [42,89-91]. However, it should be noted that less dose to

dose variability in AUC has been observed for i.v. Bu,[92] suggesting that more successful targeting of Bu exposure may be possible.

An additional problem is that the instrumentation and expertise necessary for measuring Bu concentrations are not readily available in every institution (gas chromatography with electron capture detection, gas chromatography with mass – spectrometric detection, high performance liquid chromatography). These assays, which often include a derivatisation step, are difficult and errors in the assay have occurred [93]. Another demanding aspect of TDM is that, as Bu is given over 4 days only, Bu concentrations need to be measured on the same day as the first dose samples are collected, to maximise the benefit of the targeting procedure. There are therefore logistical difficulties if institutions need to send samples away to be measured. Excessive vomiting after oral doses can also complicate TDM, particularly if Bu is not the drug administered first, as has been observed by Tran *et al.* [90].

One of the most important concerns about TDM for Bu is that target exposure levels that provide optimal outcomes have not been properly identified for uniform disease populations receiving uniform conditioning. In situations where TDM and dose adjustments are made in populations where an acceptable target exposure range has not been established, outcomes are often not improved and may even be worse in the targeted patients compared with those who did not receive dose adjustments. This is illustrated by the results of some of the TDM studies discussed below for oral and i.v. Bu.

TDM of Oral Bu

Grochow *et al.* observed that targeting Bu AUCs within 1 s.d. of the median reduced the incidence of VOD in patients receiving Bu 16 x 1 mg/kg and Cy 4 x 50 mg/kg; however there were no details of the diagnoses, the type of transplant, or other outcome data for any of these patients [35]. Dix *et al.* also attempted TDM, in that if they found an initial AUC > 1500 $\mu\text{M}\cdot\text{min}$, they targeted 1200 $\mu\text{M}\cdot\text{min}$ for subsequent doses [41]. An initial AUC of > 1500 $\mu\text{M}\cdot\text{min}$ was seen in 18 (of 51) patients (35%). Of these, 10 patients received reduced doses targeting AUCs of 1200 $\mu\text{M}\cdot\text{min}$ from the 10th to 15th dose onwards. VOD occurred in 4 (of 10) patients who had the dose reduction compared with 2 (of 8) patients who did not receive a reduced dose. These results suggest that Bu AUC is only one risk factor for VOD and that by merely reducing Bu exposure, you may not reduce VOD.

In a prospective study in 32 children with both malignant and non-malignant diseases undergoing allogeneic transplantation with matched sibling donor grafts, Bolinger *et al.* adjusted the Bu dose to provide a C_{ss} within a 600 – 900 ng/mL target range, with the Cy dose being mainly 4 x 50mg/kg [94]. When compared with a historical cohort that had not had C_{ss} levels targeted, they found a significantly improved rate of engraftment (94% compared with 74%). However, it should be pointed out that there was an overall increased incidence of grade 3 or 4 RRT in the target group despite the fact that many (10 of 14 who required dose modifications) had dose reductions. It is difficult to comment on

the Bu dose as, despite these reductions, the total dose of Bu given ranged from 10.9 to 28.9 mg/kg with only 3 patients receiving significantly less than 16 mg/kg. These results do not support a role for targeting to avoid toxicity, because, here again, we are plagued by the fact that the population consisted of children with a wide range of diagnoses with different risks of transplant-related mortality, RRT and non-engraftment (for example, there were fewer patients with Thalassemia in the targeted series), and possibly requiring different Bu doses. This highlights the need to look at the effect of Bu targeting in uniform patient populations.

In response to their own attempted TDM study, Krivoy *et al.* recommended a uniformly higher Bu dose for all children [89].

Bleyzac *et al.* compared the outcome of 29 children who had targeted Bu doses with 29 prior children who received 16 x 1mg/kg Bu, as they felt that randomisation was unethical once the assay became available. TDM led to a Bu dose reduction in 69% of patients, which was a rather high percentage compared with other studies. The rate of VOD rate was also reduced, from 24% to 3.4%, but again 24% seems high compared to most paediatric series. They suggested that the 3 month engraftment rate was also better, despite the reduced Bu dose. However the number of children receiving a low cell dose (<3.5 x 10⁸/kg) was significantly higher in the historical control group and the mean Bu AUC was no different for those with full engraftment and mixed chimerism, suggesting that other factors than Bu exposure, such as cell dose, may be more important. This study highlights the difficulty of such non-controlled comparisons [95].

If one turns to a series of individual diagnoses, then the Seattle series for CML has already been summarised above, with the clear recommendation to target a high level of Bu, as targeting a C_{ss} > 900 ng/mL was associated with an excellent outcome [5,63]. Similar data has been reported for adults with myelodysplasia, where a good outcome, including a good rate of engraftment, was observed in spite of the fact that the Bu dose was reduced in 78% of patients, with there being no data to support that the good outcome was due to Bu TDM [96]. Targeted Bu has also been reported with Bu Fludarabine (Flu) conditioning [97] but many studies have already shown that Bu Flu is well tolerated without targeting [4,84]. Similarly, as we move away from myeloablative doses of Bu to its use at a reduced dose in combination therapy for non-myeloablative conditioning, the evidence of the need to target a certain AUC is lacking [4].

In summary, TDM of oral Bu is based on incomplete data with Bu 16 x 1mg/kg and Cy 4 x 50 mg/kg. This has been used to justify TDM for lower doses of Cy and also with other combinations. When looking at individual diseases, the data often contradicts the general recommendation to target a certain Bu exposure.

TDM OF i.v. BU

Like other regimens, the ease of i.v. administration and use in a single daily dose schedule means that it is entirely feasible to target the Bu AUC, or C_{ss} (although C_{ss} is not really relevant for SDD i.v. Bu [98]). It has already been suggested that there may be a target AUC for i.v. Bu in adult

CML patients (950 – 1520 μ M.min for i.v Bu given q.i.d.) but this was based on a good outcome with low TRM and no pharmacokinetic -based dose adjustments [3]. Tran *et al.* had a narrower target range, of 1000 – 1300 μ M.min, when they performed TDM of i.v. Bu in children with haematological malignancies receiving i.v. Bu, Thiotepa and Cy (2 x 60 mg/kg) [42]. A total of 72% of patients achieved AUCs within the target range. The regimen was well tolerated and no case of VOD was encountered. Madden *et al.*, in a report on 60 patients receiving SDD i.v. Bu, also observed a low level of inpatient variability (< 20%) and there was no accumulation over the 4 days [99].

While the studies described above have demonstrated reliable and consistent exposure between i.v. Bu doses, suggesting that target exposure levels can be easily achieved using TDM and dose adjustments, there was one study which had difficulty in achieving target Bu exposure levels in children < 2 years [100]. In that study, Bu exposure derived from a test dose (0.8 mg/kg) was used to determine the dose required to target an AUC of 3200 – 4800 μ M.min (for two doses of SDD Bu) and a total of 7 (of 30) children failed to achieve AUCs within the target range. While the median AUC achieved was 3798 μ M.min, the range was wide: (1511 – 7254 μ M.min). The authors noted that 6 of the 7 children were < 2 years old. In total there were 9 children < 2 years and all but 1 had non-malignant disease (5 with severe combined immunodeficiency). Children with immunodeficiencies often have multiple infections and are probably on many drugs, including antimicrobial medication. It is possible that one (or more) of the medications may interfere with Bu pharmacokinetics.

Therefore TDM of i.v. Bu is feasible in most disease populations. However, AUC-targeting may still be difficult in some disease populations (e.g. children with non-malignant diseases). Additionally, as noted by Tran *et al.* [42], there is no uniformly accepted target AUC range or dosing method (weight-based versus surface area-based), which are important factors to consider when performing TDM.

ROLE OF TDM FOR THE FUTURE

In disease populations where interpatient variability in Bu exposure is low and where the Bu-containing regimen is well tolerated and effective (e.g. children with acute leukaemia [30]), TDM and Bu dose adjustments may not be necessary. However, in high risk disease populations with low rates of engraftment, high rates of regimen-related toxicity and wide interpatient variability in Bu exposure (e.g. children with genetic disease [87]), TDM with Bu dose adjustments would be very useful once target exposure levels that are associated with good outcome have been identified.

There already appears to be a target AUC range, of 950 – 1520 μ M.min, for adult CML patients given i.v. Bu q.i.d. [3]. As previously mentioned, the risk of mortality rose sharply when AUC values were above or below this range. This population would therefore be a good candidate population for TDM and subsequent dose adjustments. Dose adjustments should only be performed routinely in such patient populations with an established target range. In those disease populations where a target range has not been established

pharmacokinetic studies should be observational, conducted as part of clinical trials designed to establish a target range. In such studies, dose adjustments should only be made in those patients with very high or very low exposure (e.g. > 2 standard deviations from the median). In the absence of an established target AUC associated with good outcome, the median of the population may be targeted.

TDM of Bu can best be done by giving a test dose of i.v. Bu and then the full dose. The models used may allow a smaller test dose to be used, or, if there is sufficient variation between clearance of a smaller test dose used and a full dose, as suggested in one report, [101] then it may be necessary to give a full dose with a 24 to 48h gap to allow pharmacokinetic studies to be done and then adjust the dose for days 2 to 4. It may be useful to perform a randomised study of Cy combined with i.v. Bu on a SDD versus q.i.d., and analyse the pharmacokinetics but not dose-adjust based on the results. Only if such a study shows a relationship between Bu AUC and outcome is there any rationale for proposing a TDM strategy.

TDM AND LIMITED SAMPLING MODELS

TDM, if performed, would be facilitated by the use of limited sampling models. Some limited sampling models for oral and i.v. Bu are provided in Table 3. Many of these formulas employ 2 or 3 samples, even for oral Bu given q.i.d. Balasubramanian *et al.* evaluated a number of the published limited sampling models for oral Bu in children with thalassaemia major [102]. They found that the three sample models of Chattergoon *et al.* [103] and Schuler *et al.* [2] had the highest correlation to AUC values determined using non-compartmental methods ($r^2 = 0.98$ and 0.94 , respectively). However, Hassan *et al.* [36] reported that the formula of Schuler *et al.* [2] underestimated AUCs by approximately 25% (range 2-50%), while the formula of Vassal *et al.* [28] had a high degree of variation and a tendency to overestimate high AUCs. Similar observations were made by Chattergoon *et al.* [103]. It is our opinion that 2 or 3 sample limited sampling models are of limited value for oral Bu, as there would be a high proportion of patients (e.g. those with delayed absorption) where the limited sampling AUC would be totally inaccurate. Bullock *et al.*, using D-optimality, also observed that sampling strategies with fewer than 4 samples had poor precision [104]. Use of i.v. Bu makes limited sampling entirely possible. Given the log-linear decline in concentrations following the end of the infusion, in theory, it should be possible to determine an AUC from two concentration points. However, Vaughan *et al.* recommend the use of at least 4 concentrations to provide assurance of reliability in clinical decision making [37]. In their study, AUCs determined from samples taken at 1, 2, 3 and 4 h post infusion were in very good agreement with, and had greater precision than, AUCs determined using 11 concentration time points. It is also our recommendation that a minimum of 4 concentration – time points be used to determine the AUC of i.v. Bu, especially when difficult assay methods are used.

CONCLUSIONS

In this review, we have summarised the current knowledge of the clinical use, pharmacokinetics and pharmacody-

Table 3. Limited Sampling Models for Estimation of AUC for Oral and i.v. Busulphan

Reference	Oral / i.v. Bu	No. patients	Dose and schedule	Limited sampling formula	Pearson correlation ¹
Vassal <i>et al.</i> 1992 [28]	Oral	27 children	37.5 mg/m ² q.i.d for 4 days	Two sample: $AUC(0-\infty) = 122 + 0.97 C_{0.5h} + 13.94 C_{6h}$	$r^2 = 0.93$
Schuler <i>et al.</i> 1994 [2]	Oral	19 adults 1 child	1 mg/kg q.i.d for 4 days	Two sample: $*AUC_{(0-6h)} = 782 + 1.42 C_{1h} + 3.74 C_{4h}$ Three sample: $*AUC_{(0-6h)} = 289 + 1.16 C_{1h} + 1.06 C_{2h} + 3.16 C_{4h}$	$r^2 = 0.94$ $r^2 = 0.97$
Hassan <i>et al.</i> 1996 [36]	Oral	20 children	2 - 6 mg/kg/day, q.i.d, SDD or twice daily	Three sample: $AUC_{(0-\infty)} = 1.78C_{1h} + 1.44C_{3h} + 7.35C_{6h}$	$r^2 = 0.99$
Chattergoon <i>et al.</i> 1997 [103]	Oral	9 children	Test dose: 1.1-1.8 mg/kg.	Two sample: $AUC_{(0-\infty)} = 30 C_{1h} + 300C_{1h}/(\ln C_{1h} - \ln C_{6h})$ Three sample: $AUC_{(0-\infty)} = 45C_{1h} + 15C_{1.5h} + 270C_{1.5h}/(\ln C_{1.5h} - \ln C_{6h})$ Four sample: $AUC_{(0-\infty)} = 45C_{1h} + 30C_{1.5h} + 15C_{2h}/(\ln C_{2h} - \ln C_{6h})$	$r^2 = 0.95$ $r^2 = 0.98$ $r^2 = 0.98$
Vaughan <i>et al.</i> 2002 [37]	i.v	59 adults	27.5 mg/m ² infused over 2 h and given q.i.d	*AUCs determined using four samples taken at 1,2, 3 and 4 h post infusion were similar to, and had greater precision, than AUCs determined using 11 samples (with some taken during the infusion)	
Cremers <i>et al.</i> 2002 [86]	i.v.	6 children	0.8 mg/kg over 2 h q.i.d for 4 days	A two sample limited sampling model was evaluated with sampling at 2.5 and 6 h post dose. Monte Carlo simulations were performed using pharmacokinetic parameters estimated using a population approach.	$r^2 = 0.94$
Bullock <i>et al.</i> 2006 [104]	Oral	12 adults	1 mg/kg q.i.d. for 4 days	D-optimality used to evaluate limited sampling strategies, ranging from 2 to 5 samples per patient. Precise estimates only obtained with 4 (0.5,2,4,6 h) and 5 sample (0.5,1,2,4,6 h) strategies. Precision of C_{ss} estimates < 7% for 4 and 5 sample strategies.	

$C_{x,h}$ is the Bu concentration at x hours after the dose ¹Correlation coefficient between AUC estimated using the limited sampling model and AUC determined using full sampling (7 - 12 samples). * These models were validated using a different data set to that used for model development.

namics of Bu in blood and marrow transplantation. Our review has highlighted the fact that despite 20 years of clinical use, pharmacodynamic studies are still required in uniform disease populations to better define the relationship between Bu exposure and transplant outcome, to determine the levels required to achieve engraftment but avoid toxicity, and these may differ according to disease, transplant type and conditioning regimen. For patients with malignancy, the whole rationale of BMT is to cure their disease. Therefore, subsequent relapse rate and leukaemia free survival, as well as overall survival, are important end points. Many papers quote short term toxicity and do not look at long term outcome. It is also important to establish the minimum effective AUC to achieve engraftment, and this may be different for patients with different diagnoses. Such pharmacodynamic studies will be facilitated by the consistent and accurate exposure estimates that can be obtained with i.v. Bu, using 4 sample limited sampling.

It is our recommendation that TDM with dose modifications should be performed only in those disease populations that (1) have an established target Bu exposure range that is associated with good outcome, (2) exhibit wide interpatient variability in Bu pharmacokinetics and (3) have a high rate

of RRT, relapse or engraftment failure that can be improved by Bu TDM. For disease populations without an established optimum target Bu exposure range, pharmacokinetic studies should be observational, without dose modifications, to be conducted as part of trials intended to establish such a range. TDM and Bu dose adjustments may not be necessary in disease populations that have good outcome with low RRT and low interpatient variability in Bu exposure.

We would also like to recommend the use of single daily dosing schedule for Bu. SDD Bu has been shown to be safe and effective for both the oral and i.v. preparations. With its ease of administration and increased accuracy in measuring AUC, it is an ideal administration method when performing pharmacokinetic studies or TDM. It is important to remember that the widespread use of the q.i.d. dosing regimen simply occurred to cope with the large number of 2 mg tablets which needed to be ingested by an adult. Once this limitation is removed, the logic of using a q.i.d. regimen is not only gone but makes the pharmacokinetic analysis unnecessarily complicated. With the availability of an i.v. preparation, it is timely to review how and why we are giving Bu and what we can do to minimise toxicity and maximise effect.

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