CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

20-954/S-004

Administrative

SECTION 13
PATENT INFORMATION

13.0 PATENT INFORMATION

December 20, 2001

The following information is provided in accordance with the Drug Price Competition and Patent Term Restoration Act of 1984 and 21 CFR 314.53.

- Trade Name: Busulfex®
- NDA #: 20-954
- Active Ingredient(s): busulfan
- Strength(s): 6 mg/mL
- Dosage Form: intravenous

13.1 Individual Patents

13.1.1 U.S. PATENT NUMBER: 5,430,057

Expiration Date: September 30, 2013

Type of Patent-Indicate all that apply:

1. Drug Substance (Active Ingredient): No
2. Drug Product (Composition/Formulation): Yes
3. Method of Use: Yes

The method(s) of use for which approval is being sought that are covered by the patent:

- A method of treating a malignant condition through intravascular administration of busulfan.

- A method for treating leukemia or a lymphoma in a patient undergoing a bone marrow transplant through intravenous administration of busulfan.

Name of Patent Owner: Board of Regents, The University of Texas System, Austin; University of Houston-University Park, Houston.

13.1.2 U.S. Patent Number: 5,559,148

Expiration Date: May 24, 2015

Type of Patent-Indicate all that apply:

1. Drug Substance (Active Ingredient): No
2. Drug Product (Composition/Formulation): Yes
3. Method of Use: Yes

The method(s) of use for which approval is being sought that are covered by the patent:

- A method of treating a malignant disease through parenteral administration of busulfan.

- A method for treating a patient undergoing a bone marrow transplant through intravascular administration of busulfan.

Name of Patent Owner: Board of Regents, The University of Texas System, Austin; University of Houston-University Park, Houston.

13.2 Declaration Statements

The undersigned declares that the above stated United States Patent Number 5,430,057 covers the composition, formulation and/or method of use of Busulfex® (busulfan) Injection. This product is the subject of this supplemental application for which approval is being sought.

The undersigned declares that the above stated United States Patent Number 5,559,148 covers the composition, formulation and/or method of use of Busulfex® (busulfan) Injection. This product is the subject of this supplemental application for which approval is being sought.

Carol S. Curme

Date: 12/4/01

Carol S. Curme, J.D., RAC
Senior Manager of Regulatory Affairs
(952) 513-6974

EXCLUSIVITY SUMMARY for NDA # 20-954 SUPPL # 004

Trade Name Busulfex Generic Name busulfan

Applicant Name Orphan Medical, Inc. HFD- 150

Approval Date January 13, 2003

PART I: IS AN EXCLUSIVITY DETERMINATION NEEDED?

1. An exclusivity determination will be made for all original applications, but only for certain supplements. Complete Parts II and III of this Exclusivity Summary only if you answer "YES" to one or more of the following questions about the submission.

   a) Is it an original NDA? YES/___/ NO /__X__/

   b) Is it an effectiveness supplement? YES /__X__/ NO /___/

      If yes, what type(SE1, SE2, etc.)? **SE2**

   c) Did it require the review of clinical data other than to support a safety claim or change in labeling related to safety? (If it required review only of bioavailability or bioequivalence data, answer "NO.")

      YES /__X__/ NO /___/

      If your answer is "no" because you believe the study is a bioavailability study and, therefore, not eligible for exclusivity, EXPLAIN why it is a bioavailability study, including your reasons for disagreeing with any arguments made by the applicant that the study was not simply a bioavailability study.

      If it is a supplement requiring the review of clinical data but it is not an effectiveness supplement, describe the change or claim that is supported by the clinical data:

      Addition of pediatric dosing guidelines to the special populations section.

d) Did the applicant request exclusivity?

YES /__X__/ NO /___/

If the answer to (d) is "yes," how many years of exclusivity did the applicant request?

Sponsor requested only 6-month Pediatric Exclusivity

e) Has pediatric exclusivity been granted for this Active Moiety?

YES /__X__/ NO /___/

IF YOU HAVE ANSWERED "NO" TO ALL OF THE ABOVE QUESTIONS, GO DIRECTLY TO THE SIGNATURE BLOCKS ON Page 9.

2. Has a product with the same active ingredient(s), dosage form, strength, route of administration, and dosing schedule previously been approved by FDA for the same use? (Rx to OTC) Switches should be answered No - Please indicate as such).

YES /___/ NO /__X__/

If yes, NDA # _____ Drug Name

IF THE ANSWER TO QUESTION 2 IS "YES," GO DIRECTLY TO THE SIGNATURE BLOCKS ON Page 9.

3. Is this drug product or indication a DESI upgrade?

YES /___/ NO /__X__/

IF THE ANSWER TO QUESTION 3 IS "YES," GO DIRECTLY TO THE SIGNATURE BLOCKS ON Page 9 (even if a study was required for the upgrade).

PART II: FIVE-YEAR EXCLUSIVITY FOR NEW CHEMICAL ENTITIES
(Answer either #1 or #2, as appropriate)

1. Single active ingredient product.

Has FDA previously approved under section 505 of the Act any drug product containing the same active moiety as the drug under consideration? Answer "yes" if the active moiety (including other esterified forms, salts, complexes, chelates or clathrates) has been previously approved, but this particular form of the active moiety, e.g., this particular ester or salt (including salts with hydrogen or coordination bonding) or other non-covalent derivative (such as a complex, chelate, or clathrate) has not been approved. Answer "no" if the compound requires metabolic conversion (other than deesterification of an esterified form of the drug) to produce an already approved active moiety.

YES /_X_/ NO /___/

If "yes," identify the approved drug product(s) containing the active moiety, and, if known, the NDA #(s).

NDA # N009386 MYLERAN

NDA #

NDA #

2. Combination product.

If the product contains more than one active moiety (as defined in Part II, #1), has FDA previously approved an application under section 505 containing any one of the active moieties in the drug product? If, for example, the combination contains one never-before-approved active moiety and one previously approved active moiety, answer "yes." (An active moiety that is marketed under an OTC monograph, but that was never approved under an NDA, is considered not previously approved.)

YES /___/ NO /_X_/

If "yes," identify the approved drug product(s) containing the active moiety, and, if known, the NDA #(s).

NDA #

NDA #

NDA #

IF THE ANSWER TO QUESTION 1 OR 2 UNDER PART II IS "NO," GO DIRECTLY TO THE SIGNATURE BLOCKS ON Page 9. IF "YES," GO TO PART III.

PART III: <u>THREE-YEAR EXCLUSIVITY FOR NDA'S AND SUPPLEMENTS</u>

To qualify for three years of exclusivity, an application or supplement must contain "reports of new clinical investigations (other than bioavailability studies) essential to the approval of the application and conducted or sponsored by the applicant." This section should be completed only if the answer to PART II, Question 1 or 2, was "yes."

1. Does the application contain reports of clinical investigations? (The Agency interprets "clinical investigations" to mean investigations conducted on humans other than bioavailability studies.) If the application contains clinical investigations only by virtue of a right of reference to clinical investigations in another application, answer "yes," then skip to question 3(a). If the answer to 3(a) is "yes" for any investigation referred to in another application, do not complete remainder of summary for that investigation.

YES /_X_/ NO /___/

IF "NO," GO DIRECTLY TO THE SIGNATURE BLOCKS ON Page 9.

2. A clinical investigation is "essential to the approval" if the Agency could not have approved the application or supplement without relying on that investigation. Thus, the investigation is not essential to the approval if 1) no clinical investigation is necessary to support the supplement or application in light of previously approved applications (i.e., information other than clinical trials, such as bioavailability data, would be sufficient to provide a basis

for approval as an ANDA or 505(b)(2) application because of what is already known about a previously approved product), or 2) there are published reports of studies (other than those conducted or sponsored by the applicant) or other publicly available data that independently would have been sufficient to support approval of the application, without reference to the clinical investigation submitted in the application.

For the purposes of this section, studies comparing two products with the same ingredient(s) are considered to be bioavailability studies.

(a) In light of previously approved applications, is a clinical investigation (either conducted by the applicant or available from some other source, including the published literature) necessary to support approval of the application or supplement?

YES /__X__/ NO /___/

If "no," state the basis for your conclusion that a clinical trial is not necessary for approval **AND GO DIRECTLY TO SIGNATURE BLOCK ON Page 9:**

(b) Did the applicant submit a list of published studies relevant to the safety and effectiveness of this drug product and a statement that the publicly available data would not independently support approval of the application?

YES /___/ NO /__X__/

(1) If the answer to 2(b) is "yes," do you personally know of any reason to disagree with the applicant's conclusion? If not applicable, answer NO.

YES /___/ NO /__X__/

If yes, explain:

(2) If the answer to 2(b) is "no," are you aware of published studies not conducted or sponsored by the applicant or other publicly available data that could independently demonstrate the safety and effectiveness of this drug product?

YES /___/ NO /__X__/

If yes, explain:

(c) If the answers to (b)(1) and (b)(2) were both "no," identify the clinical investigations submitted in the application that are essential to the approval:

Investigation #1, Study # **OMC-BUS-5**

Investigation #2, Study #

Investigation #3, Study #

3. In addition to being essential, investigations must be "new" to support exclusivity. The agency interprets "new clinical investigation" to mean an investigation that 1) has not been relied on by the agency to demonstrate the effectiveness of a previously approved drug for any indication and 2) does not duplicate the results of another investigation that was relied on by the agency to demonstrate the effectiveness of a previously approved drug product, i.e., does not redemonstrate something the agency considers to have been demonstrated in an already approved application.

(a) For each investigation identified as "essential to the approval," has the investigation been relied on by the agency to demonstrate the effectiveness of a previously approved drug product? (If the investigation was relied on only to support the safety of a previously approved drug, answer "no.")

Investigation #1	YES /___/	NO /__X__/
Investigation #2	YES /___/	NO /___/
Investigation #3	YES /___/	NO /___/

If you have answered "yes" for one or more investigations, identify each such investigation and the NDA in which each was relied upon:

NDA # _____ Study #
NDA # _____ Study #
NDA # _____ Study #

(b) For each investigation identified as "essential to the approval," does the investigation duplicate the results of another investigation that was relied on by the agency to support the effectiveness of a previously approved drug product?

Investigation #1 YES /___/ NO /_X_/

Investigation #2 YES /___/ NO /___/

Investigation #3 YES /___/ NO /___/

If you have answered "yes" for one or more investigations, identify the NDA in which a similar investigation was relied on:

NDA # _____ Study #

NDA # _____ Study #

NDA # _____ Study #

(c) If the answers to 3(a) and 3(b) are no, identify each "new" investigation in the application or supplement that is essential to the approval (i.e., the investigations listed in #2(c), less any that are not "new"):

Investigation #__, Study # **OMC-BUS-5**

Investigation #__, Study #

Investigation #__, Study #

4. To be eligible for exclusivity, a new investigation that is essential to approval must also have been conducted or sponsored by the applicant. An investigation was "conducted or sponsored by" the applicant if, before or during the conduct of the investigation, 1) the applicant was the sponsor of the IND named in the form FDA 1571 filed with the Agency, or 2) the applicant (or its predecessor in interest) provided substantial support for the study. Ordinarily, substantial support will mean providing 50 percent or more of the cost of the study.

(a) For each investigation identified in response to question 3(c): if the investigation was carried out under an IND, was the applicant identified on the FDA 1571 as the sponsor?

Investigation #1	
IND # **46232** YES /**X**/	NO /___/ Explain:
Investigation #2	
IND # _____ YES /___/	NO /___/ Explain:

(b) For each investigation not carried out under an IND or for which the applicant was not identified as the sponsor, did the applicant certify that it or the applicant's predecessor in interest provided substantial support for the study?

Investigation #1	
YES /___/ Explain _____	NO /___/ Explain _____
_____	_____
_____	_____
Investigation #2	
YES /___/ Explain _____	NO /___/ Explain _____
_____	_____
_____	_____

(c) Notwithstanding an answer of "yes" to (a) or (b), are there other reasons to believe that the applicant should not be credited with having "conducted or sponsored" the study? (Purchased studies may not be used as the basis for exclusivity. However, if all rights to the drug are purchased (not just studies on the drug), the applicant may be considered to have sponsored or conducted the studies sponsored or conducted by its predecessor in interest.)

YES /___/ NO /_X_/

If yes, explain: _____

_____

_____

_____
Signature of Preparer Date
Title: Regulatory Health Project Manager

Signature of Office or Division Director Date

CC:
Archival NDA
HFD-150/Division File
HFD-150/Sean Bradley
HFD-093/Mary Ann Holovac
HFD-104/PEDS/T.Crescenzi

Form OGD-011347
Revised 8/7/95; edited 8/8/95; revised 8/25/98, edited 3/6/00

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Richard Pazdur
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PEDIATRIC EXCLUSIVITY DETERMINATION CHECKLIST

PART I - TO BE COMPLETED BY THE REVIEWING DIVISION.

Date of Written Request from FDA **3/27/00** . Application Written Request was made to: NDA#**20-954**
Timeframe Noted in Written Request for Submission of Studies **12/31/01**.
NDA# **20-954** Supplement #**004** Choose one: SE1 **SE2** SE3 SE4 SE5 SE6 SE7 SE8 SLR
Sponsor **Orphan Medical, Inc.**
Generic Name **busulfan** Trade Name **Busulfex**
Strength **6 mg/mL (10 mL ampoules containing 60 mg busulfan)** Dosage Form/Route **Injectable / Intravenous**
Date of Submission of Reports of Studies **12/28/01**.
Pediatric Exclusivity Determination Due Date (60 or 90 days from date of submission of studies) **3/28/02**.

Was a formal Written Request made for the pediatric studies submitted?	Y _✓_	N _ _
Were the studies submitted after the Written Request?	Y _✓_	N _ _
Were the reports submitted as a supplement, amendment to an NDA, or NDA?	Y _✓_	N _ _
Was the timeframe noted in the Written Request for submission of studies met?	Y _✓_	N _ _
If there was a written agreement, were the studies conducted according to the written agreement? OR If there was no written agreement, were the studies conducted in accord with good scientific principles?	Y _✓_	N _ _
Did the studies fairly respond to the Written Request?	Y _✓_	N _ _

SIGNED **Ramzi Dagher, M.D.** DATE **February 6, 2002**
(Reviewing Medical Officer)

Do not enter in DES - FORWARD TO PEDIATRIC EXCLUSIVITY BOARD, HFD-960

PART II - TO BE COMPLETED BY THE PEDIATRIC EXCLUSIVITY BOARD

Pediatric Exclusivity ✓ **Granted** ___ **Denied**

Existing Patent or Exclusivity Protection:

NDA/Product #	Eligible Patents/Exclusivity	Current Expiration Date
20-954	NDF	4-Feb-2002
	ODE	4-Feb-2006
	5430057	30-Sep-2013
	5559148	24-May-2015

SIGNED /S/ DATE 3/12/02

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Grace Carmouze
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PROJECT MANAGER REVIEW OF LABELING

NDA: 20-954/S004

Drug: Busulfex® (busulfan) Injection

Applicant: Orphan Medical Incorporated

Submission Dates: December 21, 2001; July 22, November 6, and December 13, 2002.

Receipt Dates: December 28, 2002; July 24, November 8, and December 16, 2002

BACKGROUND:

On December 21, 2001, Orphan Medical submitted a request for Pediatric Exclusivity (supplement 004) that included changes to the package insert labeling incorporating pediatric information on dosing, pharmacokinetics and safety.

This supplement received an "Approvable" decision during the first review cycle pending a re-analysis of the pharmacokinetic data that was submitted with the supplement. Orphan submitted their amended supplement on July 22, 2002, which included updated pharmacokinetic data requested by the Agency.

DOCUMENTS REVIEWED:

1. Final Approved Labeling (FA) dated February 11, 1999
2. SLR-004 dated December 21, 2001
3. AZ to SLR-004 dated July 22, 2002
4. BL to SLR-004 dated November 6, 2002
5. BL to SLR-004 dated December 13, 2002 (which sponsor concurred with the FDA's proposed labeling (sent via e-mail on November 21, 2002).
6. FDA approved labeling included in the recently approved supplement 001

The only changes found were those noted below.

REVIEW:

The following **bolded-text** labeling changes are the finalized changes agreed upon by both the FDA and Orphan Medical Inc.

1. The following paragraph was added to the **CLINICAL PHARMACOLOGY** section, **Pharmacokinetics** subsection:

.....and a low coefficient of variation for this parameter.

In a pharmacokinetic study of Busulfex in 24 pediatric patients, the population pharmacokinetic (PPK) estimates of Busulfex for clearance (CL) and volume of distribution (V) were determined. For actual body weight, PPK estimates of CL and V were 4.04 L/hr/20 kg (3.37 ml/min/kg; inter-patient variability 23%); and 12.8 L/20 kg (0.64 L/kg; inter-patient variability 11%).

2. The **PRECAUTIONS** section, **Special Populations** subsection has been updated

from:

Pediatric: The safety and efficacy of BUSULFEX in children have not been established. Busulfan clearance has been demonstrated to be higher in children than in adults. This has necessitated the development of alternative dosing regimens for oral busulfan in this population. Studies are underway to define the pharmacokinetics of BUSULFEX in children. Currently the recommended dose of BUSULFEX in children has not been defined.

to:

Pediatric: The effectiveness of BUSULFEX in the treatment of CML has not been specifically studied in pediatric patients. An open-label, uncontrolled study evaluated the pharmacokinetics of BUSULFEX in 24 pediatric patients receiving BUSULFEX as part of a conditioning regimen administered prior to hematopoietic progenitor cell transplantation for a variety of malignant hematologic (N=15) or non-malignant diseases (N=9). Patients ranged in age from 5 months to 16 years (median 3 years). BUSULFEX dosing was targeted to achieve an area under the plasma concentration curve (AUC) of 900-1350 μM•min with an initial dose of 0.8 mg/kg or 1.0 mg/kg (based on ABW) if the patient was > 4 or ≤ 4 years, respectively. The dose was adjusted based on plasma concentration after completion of dose 1.

Patients received BUSULFEX doses every six hours as a two-hour infusion over four days for a total of 16 doses, followed by cyclophosphamide 50 mg/kg once daily

for four days. After one rest day, hematopoietic progenitor cells were infused. All patients received phenytoin as seizure prophylaxis. The target AUC (900-1350 ±5% μM•min) for BUSULFEX was achieved at dose 1 in 71% (17/24) of patients. Steady state pharmacokinetic testing was performed at dose 9 and 13. BUSULFEX levels were within the target range for 21 of 23 evaluable patients.

All 24 patients experienced neutropenia (absolute neutrophil count < 0.5 x 10^9/L) and thrombocytopenia (platelet transfusions or platelet count < 20,000/mm^3). Seventy-nine percent (19/24) of patients experienced lymphopenia (absolute lymphocyte count < 0.1 x 10^9). In 23 patients, the ANC recovered to > 0.5 x 10^9/L (median time to recovery = BMT day +13; range = BMT day +9 to +22). One patient who died on day +28 had not recovered to an ANC > 0.5 x 10^9/L.

Four (17%) patients died during the study. Two patients died within 28 days of transplant; one with pneumonia and capillary leak syndrome, and the other with pneumonia and veno-occlusive disease. Two patients died prior to day 100; one due to progressive disease and one due to multi-organ failure.

Adverse events were reported in all 24 patients during the study period (BMT day -10 through BMT day +28) or post-study surveillance period (day +29 through +100). These included vomiting (100%), nausea (83%;), stomatitis (79%;), hepatic veno-occlusive disease (HVOD) (21%), graft-versus host disease (GVHD) (25%;), and pneumonia (21%).

Based on the results of this 24-patient clinical trial, a suggested dosing regimen of BUSULFEX in pediatric patients is shown in the following dosing nomogram:

BUSULFEX Dosing Nomogram

Patient's Actual Body Weight (ABW)	BUSULFEX Dosage

≤ 12 kgs	1.1 (mg/kg)
> 12 kgs	0.8 (mg/kg)

Simulations based on a pediatric population pharmacokinetic model indicate that approximately 60% of pediatric patients will achieve a target BUSULFEX exposure (AUC) between 900 to 1350 µM•min with the first dose of BUSULFEX using this dosing nomogram. Therapeutic drug monitoring and dose adjustment following the first dose of BUSULFEX is recommended.

Dose Adjustment Based on Therapeutic Drug Monitoring
Instructions for measuring the AUC of busulfan at dose 1 (see Blood Sample Collection for AUC Determination), and the formula for adjustment of subsequent doses to achieve the desired target AUC (1125 µM•min), are provided below.

Adjusted dose (mg) = Actual Dose (mg) x Target AUC(µM•min)/Actual AUC(µM•min)

For example, if a patient received a dose of 11 mg busulfan and if the corresponding AUC measured was 800 µM•min, for a target AUC of 1125 µM•min, the target mg dose would be:

Mg dose = 11 mg x 1125 µM•min / 800 µM•min = 15.5 mg

Busulfex dose adjustment may be made using this formula and instructions below.

Blood Sample Collection for AUC Determination:

Calculate the AUC (µM•min) based on blood samples collected at the following time points:

For dose 1: 2 hr (end of infusion), 4 hr and 6 hr (immediately prior to the next scheduled Busulfex administration). Actual sampling times should be recorded.

For doses other than dose 1: Pre-infusion (baseline), 2 hr (end of infusion), 4 hr and 6 hr (immediately prior to the next scheduled Busulfex administration).

AUC calculations based on fewer than the three specified samples may result in inaccurate AUC determinations.

For each scheduled blood sample, collect one to three mL of blood into heparinized (Na or Li heparin) Vacutainer® tubes. The blood samples should be placed on wet ice immediately after collection and should be centrifuged (at 4°C) within one hour. The plasma, harvested into appropriate cryovial storage tubes, is to be frozen immediately at -20°C. All plasma samples are to be sent in a frozen state (i.e., on dry ice) to the assay laboratory for the determination of plasma busulfan concentrations.

Calculation of AUC:

Busulfex AUC calculations may be made using the following instructions and appropriate standard pharmacokinetic formula:

Dose 1 $AUC_{infinity}$ Calculation: $AUC_{infinity} = AUC_{0\text{-}6hr} + AUC_{extrapolated}$,

where $AUC_{0\text{-}6hr}$ is to be estimated using the linear trapezoidal rule and $AUC_{extrapolated}$ can be computed by taking the ratio of the busulfan concentration at Hour 6 and the terminal elimination rate constant, λ_z. The λ_z must be calculated from the terminal elimination phase of the busulfan concentration vs. time curve. A "0"pre-dose busulfan concentration should be assumed, and used in the calculation of AUC.

If AUC is assessed subsequent to Dose 1, steady-state AUC_{ss} (AUC_{0-6hr}) is to be estimated from the trough, 2 hr, 4 hr and 6 hr concentrations using the linear trapezoidal rule.

Instructions for Drug Administration and Blood Sample Collection for Therapeutic Drug Monitoring:

An administration set with minimal residual hold up (priming) volume (1-3 mL) should be used for drug infusion to ensure accurate delivery of the entire prescribed dose and to ensure accurate collection of blood samples for therapeutic drug monitoring and dose adjustment.

Prime the administration set tubing with drug solution to allow accurate documentation of the start time of Busulfex infusion. Collect the blood sample from a peripheral IV line to avoid contamination with infusing drug. If the blood sample is taken directly from the existing central venous catheter (CVC), DO NOT COLLECT THE BLOOD SAMPLE WHILE THE DRUG IS INFUSING to ensure that the end of infusion sample is not contaminated with any residual drug. At the end of infusion (2 hr), disconnect the administration tubing and flush the CVC line with 5 cc of normal saline prior to the collection of the end of infusion sample from the CVC port. Collect the blood samples from a different port than that used for the Busulfex infusion. When recording the Busulfex infusion stop time, do not include the time required to flush the indwelling catheter line. Discard the administration tubing at the end of the two-hour infusion.

See Preparation for Intravenous Administration section for detailed instructions on drug preparation.

3. The **DOSAGE AND ADMINISTRATION** section, **Adult (BuCY2)** subsection has been reformatted (no new information added) to now read:

When BUSULFEX is administered as a component of the BuCy conditioning regimen prior to bone marrow or peripheral blood progenitor cell replacement, the recommended doses are as follows:

Adults (BuCy2): The usual adult dose is 0.8 mg/kg of ideal body weight or actual body weight, whichever is lower, administered every six hours for four days (a total of 16 doses). For obese, or severely obese patients, BUSULFEX should be administered based on adjusted ideal body weight. Ideal body weight (IBW) should be calculated as follows (height in cm, and weight in kg): IBW (kg; men)= 50 + 0.91x(height in cm -152); IBW (kg; women)= 45 + 0.91x(height in cm -152). Adjusted ideal body weight (AIBW) should be calculated as follows: AIBW = IBW +0.25x(actual weight - IBW). Cyclophosphamide is given on each of two days as a one-hour infusion at a dose of 60 mg/kg beginning on BMT day –3, no sooner than six hours following the 16th dose of BUSULFEX.

BUSULFEX clearance is best predicted when the BUSULFEX dose is administered based on adjusted ideal body weight. Dosing BUSULFEX based on actual body weight, ideal body weight or other factors can produce significant differences in BUSULFEX (busulfan) Injection clearance among lean, normal and obese patients.

BUSULFEX should be administered intravenously via a central venous catheter as a two-hour infusion every six hours for four consecutive days for a total of 16 doses. All patients should be premedicated with phenytoin as busulfan is known to cross the blood brain barrier and induce seizures. Phenytoin reduces busulfan plasma AUC by 15%. Use of other anticonvulsants may result in higher busulfan plasma AUCs, and an increased risk of VOD or seizures. In cases where other anticonvulsants must be used, plasma busulfan exposure should be monitored (See DRUG INTERACTIONS). Antiemetics should be administered prior to the first dose of BUSULFEX and continued on a fixed schedule through administration of BUSULFEX. Where available, pharmacokinetic monitoring may be considered to further optimize therapeutic targeting.

4. In the **DOSAGE AND ADMINISTRATION** section, the following **Pediatrics** subsection was added:

   Pediatrics: The effectiveness of BUSULFEX in the treatment of CML has not been specifically studied in pediatric patients. For additional information see Special Populations-Pediatric section.

5. In the **DOSAGE AND AMINISTRATION** section, **Preparation and Administration Precautions** subsection, the following sentence was added:

   An administration set with minimal residual hold-up volume (2-5 cc) should be used for product administration.

   As with other cytotoxic compounds, caution should be exercised in.....

CONCLUSION-RECOMMENDED REGULATORY ACTION:

As a result of the communications between Orphan Medical Inc. and the Agency regarding the changes the package insert; in addition to Orphan Medical's December 13, 2002 letter in which they concurred with the Agency's proposed labeling, this supplement should be approved and FPL requested.

Sean Bradley, R.Ph./09JAN03
Project Manager

Concurrence:

Dotti Pease/13JAN03
Chief, Project Management Staff

Brian Booth, Ph.D./10JAN03
Biopharm Reviewer

Atiqur Rahman, Ph.D./10JAN03
Biopharm Team Leader

Ramzi Dagher, M.D./10JAN03
Medical Reviewer

Ann Farrel, M.D./10JAN03
Medical Team Leader

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Sean Bradley
1/14/03 02:06:40 PM
CSO

ORPHAN MEDICAL

December 20, 2001

Food and Drug Administration

RE: NDA 20-954, Busulfex® (busulfan) Injection

GENERIC DRUG ENFORCEMENT ACT OF 1992 CERTIFICATION

This information is submitted in accordance with Section 306(k)(1) of the Act [21 U.S.C. 335a (k)(1)].

I certify that Orphan Medical, Inc. did not and will not use in any capacity the services of any person debarred under subsections (a) or (b) [section 306(a) or (b)], in connection with this supplemental New Drug Application for Busulfex® (busulfan) Injection.

Carol S. Curme 12/4/01

Carol S. Curme, J.D., RAC
Senior Manager of Regulatory Affairs
(952) 513-6974

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952-513-6900 • Fax: 952-541-9209 • www.orphan.com

MEMO OF TELECON

IND: NDA 20-954/S004 **DATE:** Busulfex (busulfan) Injection

DATE: December 17, 2002 **TIME:** 11:10 AM, EST

SPONSOR: Orphan Medical Incorporated/952-513-6900 (OMI-Conf. Rm A)

PARTICIPANTS:

FDA

Anne Farrell, M.D.	Medical Team Leader
Ramzi Dagher, M.D.	Medical Reviewer
Atik Rahman, Ph.D.	Biopharm Team Leader
Brian Booth, Ph.D.	Biopharm Reviewer
Sean Bradley, R.Ph.	Project Manager

ORPHAN MEDICAL

Dayton Reardan, Ph.D.	Vice President of Regulatory Affairs
David Fuller, M.D.	Vice President of Medical Affairs
Shari Lennon	Director of Busulfex Development
Carol Curme, J.D., R.A.C.	Senior Manager of Regulatory Affairs

BACKGROUND:

During a visit to Munster, Germany, Dr. Hirschfeld (DODP) was informed about a 19-patient pediatric study with Busulfex. After hearing about this trial, he informed the Division of the existence of the trial and in lieu of the pending pediatric supplement for Busulfex currently being reviewed by the Division, felt it would be in the Division's best interest to retrieve and review the results of this study. An internal meeting was held and the decision was made to contact the sponsor to request further information related to the 19 patients.

DISCUSSION SUMMARY:

Shari Lennon of Orphan Medical informed the Agency that they did not sponsor the activities and have no access to the data. Lennon continued by stating that Orphan Medical was only peripherally aware of this information. The 19 patients in the German cohort were not part of any clinical trial. These patients received Busulfex in Europe via a name-patient basis. These patients were then individually treated with Busulfex in addition to other therapies in a variety of regimens and infusion schedules.

Orphan Medical informed us that [redacted]

The teleconference concluded at 11:43 AM, EST.

/S/

Sean Bradley, R.Ph., Project Manager

/S/

Ramzi Dagher, M.D., Medical Reviewer

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Ramzi Dagher
12/31/02 01:54:47 PM

ORPHAN MEDICAL

December 13, 2002

Richard Pazdur, M.D.
Food and Drug Administration
Center for Drug Evaluation and Research
Division of Oncology Drug Products [HFD-150]
Woodmont Office Complex II, Room 2055
1451 Rockville Pike
Rockville, MD 20852
(301) 594-2473

Subject: **Busulfex® (busulfan) Injection; NDA #20-954/S-004**
Response to FDA's proposed Labeling dated November 21, 2002
User Fee #3,396, Orphan Designation #94-830

Dear Dr. Pazdur:

Orphan Medical is submitting this response to the FDA's proposed labeling dated November 21, 2002 (sent via e-mail on November 22, 2002) in regards to the efficacy supplement, NDA 20-954/S-004. Orphan Medical concurs with the FDA's version of the proposed labeling as presented in the file "21NOV02FDAV6clean", which also included the agreed upon labeling changes from NDA 20-954/S-001.

On behalf of Orphan Medical, I wish to extend our thanks to the Division for the flexibility and thoughtful consideration shown throughout the labeling negotiation process.

If you have any questions or concerns, please contact me directly.

Sincerely,

Carol S. Curme, J.D., R.A.C.
Senior Manager of Regulatory Affairs
Phone: (952) 513-6974

cc: Dayton Reardan, Ph.D., Vice-President Regulatory Affairs
Sean Bradley, R. Ph., FDA Project Manager

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ORPHAN MEDICAL

November 6, 2002

Richard Pazdur, M.D.
Division of Oncology Drug Products [HFD-150]
Center for Drug Evaluation and Research
Food and Drug Administration
Woodmont II
1451 Rockville Pike
Rockville, MD 20852
Ph: (301) 827-1537

Subject: **Busulfex® (busulfan) Injection; NDA #20-954/S-004 (Pediatric)**
Response to the FDA's proposed labeling dated October 15, 2002
User Fee #3,396, Orphan Designation #94-830

Dear Dr. Pazdur:

Orphan Medical provides this response to the FDA's proposed labeling, sent by e-mail on October 18, 2002, in regards to the efficacy supplement NDA 20-954/S-004. Reference is also made to the original efficacy supplement (pediatric information) dated December 21, 2001, FDA correspondences dated May 24, 2002 and June 28, 2002, and Orphan Medical correspondences June 17, 2002 and July 22, 2002.

Orphan Medical accepts the FDA's proposed labeling as presented in the file "101502 FDAclean," except for Orphan Medical's proposed revisions described below.

1. Under **Dose Adjustment Based on Therapeutic Drug Monitoring,** the FDA's proposed sentence

[ ]

desired target AUC of 1125 μM•min:"

was revised as follows:

*"Instructions for measuring the AUC of busulfan at dose 1 (see **Blood Sample Collection for AUC Determination**), and the formula for adjustment of subsequent doses to achieve the desired target AUC,*

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Rationale for revision:

- The term "[redacted]" would be confusing to physicians. The revised sentence directs the physician's attention to the specific instructions for measuring the AUC of busulfan at dose 1.

- Reference to the "desired target AUC of 1125 μM•min" was removed to allow physicians to determine target concentrations based on institutional practices. The target value (1125 μM•min) is included in the calculation as an example.

2. Changes were made to the subsection ***Instructions for Drug Administration and Blood Sample Collection for Therapeutic Drug Monitoring*** to describe collection of the end of infusion sample from the CVC port and to remove verbiage from the last sentence.

3. The term "Dose 1" was revised to "dose 1" to be consistent with the rest of the package insert.

We appreciate the FDA's adoption of most of Orphan Medical's proposed revisions to labeling as presented in our response (dated July 22, 2002) to the FDA's approvable letter dated June 28, 2002. If you have any questions about the package insert presented in this submission, please contact me directly.

Sincerely,

Carol S. Curme, R.A.C.
Senior Manager of Regulatory Affairs
Phone: (952) 513-6974

cc: Dayton Reardan, Ph.D., Vice-President Regulatory Affairs
Sean Bradley, R. Ph., FDA Project Manager

Date :	10/02/02
Subject :	Addendum to Medical Review
Supplement :	20954/S-004
Medical Officer :	Ramzi Dagher, M.D.
Medical Team Leader :	Donna Griebel, M.D.

The sponsor originally submitted this S-004 supplement on 12/10/01. The submission consisted of data from a pharmacokinetic study of busulfex injection in 24 children with a variety of malignant and non-malignant disorders receiving busulfex injection and cyclophosphamide as a preparative regimen for stem cell transplantation. The submission was also in response to a pediatric written request, which was issued to the sponsor on 3/27/00. Based on the contents of this submission, a 6-month extension of exclusivity was granted to the sponsor on 3/12/02.

During the review process, agreement was reached with the sponsor on pediatric dosing recommendations to be added to the Special Populations section of the label. However, the sponsor had not provided suggested guidelines for therapeutic drug monitoring (TDM) in the original S-004 supplement. Although the sponsor did submit suggested guidelines for TDM, FDA reviewers concluded that these were not supported by the pharmacokinetic data. Therefore, an approvable letter was issued to the sponsor on 6/28/02, with a request to submit data supporting TDM guidelines. The applicant's response to FDA's approvable letter, forwarded 7/22/02, includes justification for selecting the three sampling times at 2,4, and 6 hours to monitor busulfan exposure and evidence supporting the use of these sampling times to accurately determine a target AUC for dose-adjustment.

With respect to the justification for using the three suggested sampling times and FDA's view of the re-analysis submitted by the sponsor, the clinical pharmacology and biopharmaceutics review team has concluded that the information submitted supports the instructions for TDM proposed by the sponsor.

This amendment also includes proposed changes to the FDA's last revision of the package insert forwarded to the sponsor on 6/25/02. Included in the proposed changes, in the pediatric component of the Special Populations section, is a modification to the statement regarding lymphopenia. As lymphopenia was defined as an absolute lymphocyte count $< 0.1 \times 10^9$ / L and there were five patients who had total white count nadirs of $0.1\text{-}0.2 \times 10^9$ / L without having a differential count recorded, the medical reviewer agrees with the sponsor's proposed statement that ' Seventy-nine percent (19/24) of patients experienced lymphopenia (absolute lymphocyte count $< 0.1 \times 10^9$ / L).

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Ramzi Dagher
11/7/02 08:43:29 AM
MEDICAL OFFICER

Donna Griebel
11/29/02 08:49:34 AM
MEDICAL OFFICER

ORPHAN MEDICAL

July 22, 2002

Richard Pazdur, M.D.
Division of Oncology Drug Products [HFD-150]
Center for Drug Evaluation and Research
Food and Drug Administration
Woodmont II
1451 Rockville Pike
Rockville, MD 20852
Ph: (301) 827-1537

Subject: **Busulfex® (busulfan) Injection; NDA #20-954/S-004 (Pediatric)**
Amendment in Response to FDA's "Approvable" Letter,
dated June 28, 2002
User Fee #3,396, Orphan Designation #94-830

Dear Dr. Pazdur:

In response to the FDA's "approvable" letter dated June 28, 2002, Orphan Medical submits this amendment to its supplemental new drug application (s-NDA) (S-004). This amendment includes a report of pharmacokinetic data requested by the FDA in the facsimile dated June 26 and the "approvable" letter dated June 28, 2002.

This amendment should qualify as a Class 1 resubmission as it contains a re-analysis of pharmacokinetic data that was submitted in the s-NDA. The amendment, submitted electronically, is comprised of two main sections:

- Labeling: This section includes Orphan Medical's proposed changes to the FDA's last revision of the Busulfex® package insert (June 25, 2002). Instructions for taking blood samples and calculating the AUC have been added. The values for clearance and volume of distribution of busulfan were corrected per the FDA's facsimile dated June 26.

- Pharmacokinetics: This section includes Orphan Medical's response to requests stated in the FDA's correspondences dated June 28, 2002 and June 26, 2002. Support for Orphan Medical's response is found in the Pharmacokinetics Report titled "Therapeutic Drug Monitoring of Busulfex® (busulfan) Injection in Pediatric Patients Utilizing a Limited Sampling Strategy," including:
  - Justification for selecting the three sampling times at 2, 4, 6 hr to monitor busulfan systemic exposure after Busulfex dosing; and
  - Evidence to support that the use of these sampling times can accurately determine a target AUC to calculate dose-adjustment.

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We appreciate the FDA's consideration of our request to provide instructions for dosage adjustment that reflects current clinical practice in the field of bone marrow transplantation.

If you have any questions or issues with the information presented, please contact me directly.

Sincerely,

Carol S. Curme, J.D., R.A.C.
Senior Manager of Regulatory Affairs
Phone: (952) 513-6974

cc: Dayton Reardan, Ph.D., Vice-President Regulatory Affairs
Sean Bradley, R. Ph., FDA Project Manager

Fax

DIVISION OF ONCOLOGY DRUG PRODUCTS

Center for Drug Evaluation and Research, HFD-150
Woodmont Office Complex - Two
1451 Rockville Pike, Rockville, MD 20852

To:	Carol Curme	**From:**	Sean Bradley
Fax:	952-541-9209	**Fax:**	301-827-4590
Phone:	952-513-6974	**Phone:**	301-594-5750
Pages, including cover sheet:	4	**Date:**	June 28, 2002
Re:	NDA 20-954/S-004		

☐ **Urgent** ☐ **For Review** ☐ **Please Comment** ☐ **Please Reply** ☐ **Please Recycle**

Carol –

Please see the attached letter regarding your supplemental new drug application for Busulfex® (busulfan) Injection.

If you have any questions regarding this transmission, please contact me at 301-594-5750.

Sean Bradley, R.Ph.

Regulatory Project Manager

67 pages redacted from this section of the approval package consisted of draft labeling

Fax

DIVISION OF ONCOLOGY DRUG PRODUCTS

Center for Drug Evaluation and Research, HFD-150
Woodmont Office Complex - Two
1451 Rockville Pike, Rockville, MD 20852

To:	Carol Curme	**From:**	Sean Bradley
Fax:	952-541-9209	**Fax:**	301-827-4590
Phone:	952-513-6974	**Phone:**	301-594-5750
Pages, including cover sheet:	2	**Date:**	June 26, 2002
Re:	NDA 20-954/S-004 FDA Draft Labeling		

☐ **Urgent** ☐ **For Review** ☐ **Please Comment** ☐ **Please Reply** ☐ **Please Recycle**

Carol –

Regarding Therapeutic Drug Monitoring:

As discussed during our teleconference this afternoon, you will need to provide the following information regarding your approach for therapeutic drug monitoring:

1. Justify the choice of the 2, 4 and 6 hr times for sampling Busulfex concentrations.

2. Demonstrate that the use of these three samples can be used to accurately determine AUC. It would be useful if you provided a comparison of the Busulfex AUC derived from the complete data for each patient in the OMC-BUS-5, with the AUC derived using the three samples at the proposed time points for each patient.

3. Provide Busulfex labeling instructions that explain how to take the samples and how to calculate the AUC.

Regarding CL and Vd Data:

In the CL and Vd values in the PK section of the FDA proposed labeling, we failed to use the appropriate normalization. The correct values are:

CL= 4.04 L/hr/20 kg (3.37/ml/min/kg)
Vd = 12.8 L/20 kg (0.64 L/kg)

Our apologies for this error. Please add the correct values to the labeling.

If you have any questions regarding this transmission, please contact me at 301-594-5750.

Sean Bradley, R.Ph.

Regulatory Project Manager

Memo of Teleconference

MEETING DATE: June 26, 2002 **TIME:** 1:45 PM, EST

LOCATION: Woodmont Office Complex-2, Conference Room A

NDA #20-954/S-004

DRUG: Busulfex® (busulfan)

SPONSOR/APPLICANT: Orphan Medical

PARTICIPANTS:

FDA

Donna Griebel, M.D.	Medical Team Leader
Ramzi Dagher, M.D.	Medical Reviewer
Atiq Rahman, Ph.D.	Biopharm Team Leader
Brian Booth, Ph.D.	Biopharm Reviewer
Jogarao V Gobburu, Ph.D.	Team Leader, Pharmacometrics
Sean Bradley, R.Ph.	Project Manager

ORPHAN MEDICAL

David Fuller, M.D.	Vice-President of Medical Affairs
Shari Lennon	Director of Busulfex Development
Carol Curme	Senior Manager of Regulatory Affairs
	Consultant

MEETING OBJECTIVE: Orphan Medical to discuss labeling issues regarding the dose adjustment and pharmacokinetic values for clearance and volume of distribution included in the FDA's suggested labeling for Busulfex® (busulfan).

DISCUSSION SUMMARY

REGARDING DOSE-ADJUSTMENT STRATEGY

FDA's recommendation:

For Pediatric patients ≤ 12 kgs:

For Pediatric patients > 12 kgs:

Rationale: ***"Therapeutic Drug Monitoring: Although dose adjustment based on several samples/AUC is scientifically preferable, it is believed that this recommendation would not be practicable/feasible in a clinical setting. Therefore, dose adjustment based on the original FDA suggestion of C2hr has been re-inserted, but in the mathematical form used by Orphan Medical (instead of the dose adjustment nomogram)"***

ORPHAN MEDICAL:

OMI agrees with FDA's suggestion to include a formula to assist in dose adjustment however we suggest that a dose-adjustment formula based on AUC is more appropriate to reflect current clinical & pharmacokinetic practice in the USA. In addition use of AUC improves accuracy and precision and thereby ensures increased safety in interpretation and clinical application of these time-critical clinical data. These points are discussed below.

Current Clinical / Pharmacokinetic Practice:

The internationally accepted standard for dose-adjustment of busulfan based regimens is AUC or estimation of C_{ss} values derived from AUC. (C_{ss} = concentration at steady state = AUC divided by dosing interval).

In the US several established institutions (Attachment 2) perform busulfan pharmacokinetic analysis for their own patients, or on behalf of other clinical centers. Transplant centers routinely collect 6 or more busulfan plasma samples for analysis – 3 samples are considered the minimum for calculation of AUC. The PK laboratories (or the clinical sites) use validated computer programs, such as WinNonlin, to calculate and report busulfan AUC, C_{ss} and other PK

parameters. The AUC or C_{ss} results are then used by clinicians to determine if dose adjustment is required. The usual formula for dose adjustment is

[redacted] dose (mg) = Actual Dose (mg) x Target AUC (μMol•min)/Actual AUC(μMol•min)

A sample laboratory report from [redacted] is provided in Attachment 3. [redacted] is considered a reference laboratory for busulfan pharmacokinetic analysis and acted as the central PK laboratory for the Busulfex development program.

In consideration of the above, OMI is not aware of any clinical center or accredited pharmacokinetic laboratory in the USA who use single sample plasma-concentration data to dose-adjust busulfan-based treatment. Further we are not aware of any published literature where a single busulfan plasma-concentration sample is applied for dose-adjustment.

Dose Adjustment using one plasma sample:

We acknowledge that limited sampling strategies (LSS) are widely accepted and clinically appropriate. All published and current clinical applications of LSS utilize at least 3 data points which is considered clinically appropriate for the following reasons:

1) At least 3 data points are needed to determine AUC, which (as described above) is the key PK parameter for estimation, analysis and comparison of individualized systemic exposure.
2) Validated computer models allow identification of potentially erroneous samples, and allow AUC estimation even if 1 sample was erroneously collected

In addition, clinical application of the proposal to use a single 2hr sample may not be ideal for the following reasons:

1) Due to institutional variations in nursing & IV administration practice or technical difficulties C2hr may not always represent the end of the Busulfex infusion
2) Even in patients whose infusions run exactly 2 hrs, the scheduled sample may not always be taken at the correct (2hr) time-point due to competing medical priorities
3) There is no mechanism to correct the dose-adjustment calculation for late samples (samples not taken at 2 hrs)
4) This single time point may not be applicable in patients whose clearance and volume term are not highly correlated (in contrast to the BUS-5 patients, n=24).

Summary and Alternative Proposal:

In conclusion OMI feels that the FDA proposal is not consistent with current clinical & pharmacokinetic practice, would require significant educational efforts and may be associated with a greater margin for error (compared to AUC based methods). In view of the above OMI proposes to revert to our proposed labeling of 13th June 2002 in relation to dose adjustment:

[ ]

dose (mg) = Actual Dose (mg) x Target AUC (μMol•min)/Actual AUC (μMol•min)"

OMI feels that this approach is reflective of current clinical practice and allows increased safety in interpretation and clinical application of these time-critical clinical data..In addition provision of a formula for calculation of AUC is not required given that most labs automatically calculate this parameter for the clinician.

FDA: You will need to provide the following information to support your approach for therapeutic drug monitoring:

1. *Justify the choice of the 2, 4 and 6 hr times for sampling Busulfex concentrations.*

2. *Demonstrate that the use of these three samples can be used to accurately determine AUC. Please provide a comparison of the Busulfex AUC derived from the complete data for each patient in the OMC-BUS-5, with the AUC derived using the three samples at the proposed time points for each patient.*

3. *Provide Busulfex labeling instructions that explain how to take the samples and how to calculate the AUC.*

The important thing is to provide recommendations based on results of the study. Your recommendations for the therapeutic drug monitoring need to be justified based on the results of the data collected in study OMC-BUS-5.

ORPHAN MEDICAL: It will take us some time to prepare and submit this information to the Agency and this will not be possible until after the review clock has expired. Given the current time restraint, what does the Agency suggest as the best course of action?

FDA: We can issue an APPROVABLE letter for this supplement that will specify the additional information that you need to submit to the Agency in order for us evaluate your proposal for the content of the label to complete the review of your supplement. We will also fax this information to you this afternoon.

The teleconference concluded at 2:20 PM, EST. There were no unresolved issues or discussion points.

Minutes prepared by: /S/
Sean Bradley, R.Ph., Project Manager

Concurrence Chair: /S/
Ramzi Dagher, M.D., Medical Reviewer

Bradley, Sean

From: Carol Curme [CCURME@orphan.com]
Sent: Wednesday, June 26, 2002 12:15 PM
: 'bradleys@cder.fda.gov'
ubject: RE: Busulfex S-004: Response to Fax 062502


Schuler US--2001.pdf


Hassan M--1996a.pdf

Please find the attached journal articles that are referenced in this response. We will follow up this e-mail response with an official submission.

-----Original Message-----
From: Carol Curme
Sent: Wednesday, June 26, 2002 11:04 AM
To: 'bradleys@cder.fda.gov'
Subject: Busulfex S-004: Response to Fax 062502

Dear Sean,

Here is our response to the FDA's fax dated June 25, 2002. An e-mail with the referenced journal articles and a list of meeting attendees will follow.

Sincerely,
Carol

British Journal of Haematology, 2001, 114, 944–950

Intravenous busulphan for conditioning before autologous or allogeneic human blood stem cell transplantation

ULRICH S. SCHULER,[1] ULF D. RENNER,[1] FRANK KROSCHINSKY,[1] CHRISTINE JOHNE,[2] ANDREAS JENKE,[1] RALPH NAUMANN,[1] MARTIN BORNHÄUSER,[1] H. JOACHIM DEEG[3] AND GERHARD EHNINGER[1] [1]*Department of Internal Medicine I,* [2]*University Hospital Pharmacy, Technical University, Dresden, Germany, and* [3]*Clinical Research Division, Fred Hutchinson Cancer Research Center, University of Washington School of Medicine, Seattle, WA, USA*

Received 3 January 2001; accepted for publication 30 May 2001

Summary. This study was undertaken to evaluate the toxicity and pharmacokinetics of a dimethyl sulphoxide (DMSO)-based intravenous formulation of busulphan in the conditioning of 45 patients undergoing allogeneic or autologous stem cell transplantation (SCT). Busulphan was given as a single daily dose. In 15 patients a single dose of intravenous busulphan, given over 3 h in 1 d, was combined with additional oral (single daily) doses. Thirty patients received all four daily doses intravenously. Busulphan plasma levels were analysed using high performance liquid chromatography. There was no major acute toxicity with daily intravenous doses of 2·8–3·1 mg/kg infused over 3 h. No veno-occlusive disease (VOD) was seen in 30 patients receiving busulphan as an intravenous formulation over 4 d. In the group of 15 patients receiving three oral doses and one intravenous single daily dose, one patient experienced mild VOD. Pharmacokinetic samples were taken over at least 2 d of treatment in 44 patients. The area under the concentration time curve (AUC) values normalized for a dose of 1 mg/kg were 7000 ng/ml × h on d 1 and 5890 ng/ml × h on d 4, thus showing a moderate decrease over time. This was accompanied by a moderate increase of the clearance from 2·6 to 3·0 ml/min/kg. Administration of busulphan as a DMSO-based intravenous formulation was well tolerated. The total dose of busulphan can be given in four (rather than the typical 16) doses. With such a regimen, the intravenous administration becomes feasible on an outpatient basis.

Keywords: intravenous busulphan, HSCT, pharmacokinetics.

Oral busulphan (BU) was introduced into conditioning regimes for bone marrow transplantation in the early 1980s. Several aspects of BU pharmacokinetics have been correlated with outcome. The area under the concentration time curve (AUC) has been correlated with the frequency of hepatic complications, especially veno-occlusive disease (VOD), by some investigators (Grochow *et al*, 1989), although not all subsequent reports confirmed these associations (Schuler *et al*, 1994). Other groups found exposure to high BU levels to be associated with permanent hair loss or overall poor outcome (Ljungman *et al*, 1995; Ringden *et al*, 1999). Conversely, low levels were found to be associated with an increased incidence of graft rejection or relapse (Slattery *et al*, 1995, 1997), but again not in all studies (Baker *et al*, 2000). These observations suggest that individualization of therapy may be necessary, a goal which in our experience may be difficult to achieve with oral dosing because of the poor predictive value of one oral AUC for AUC after later doses. In order to reduce both intra- and interindividual variability of BU pharmacokinetics, an intravenous BU formulation using a dimethyl sulphoxide (DMSO) base was developed and initially evaluated in a canine model (Ehninger *et al*, 1995; Deeg *et al*, 1999). In human patients, using a BU/DMSO preparation as intravenous standard, we determined the bioavailability of oral BU to be 70% (Schuler *et al*, 1998). Here we present data on 45 patients receiving intravenous BU as single daily infusions (rather than four daily doses), either only for 1 d out of four treatment days (with 3 d of oral administration, $n = 15$) or as a complete 4-d course ($n = 30$).

Correspondence: Dr Ulrich Schuler, Medizinische Klink und Poliklinik I, Fetscherstr. 74, D-01307 Dresden, Germany. E-mail: schuler@mk1.med.tu-dresden.de

PATIENTS AND METHODS

Patients

Patients were eligible for this protocol if their conditioning would have normally included oral busulphan for 4 d in a total dose of 16 mg/kg. Forty-five patients were included in

Table I. Patient characteristics.

	Number of patients	
Male/female	27/18	
Age (years)		18–63, median 40
Weight (kg)		51–100, median 72
Diagnoses		
CML	19	3 blast crisis, 3 accelerated phase, 13 chronic phase
AML	13	2 secondary AML after HD, 6 in CR1, 5 > CR1
ALL	7	5 > CR1, 2 bcr/abl positive CR1
Lymphoma	3	1 HD, 2 NHL
Myelodysplastic syndrome	3	
Source of stem cells		
Family matched	11	
Family mismatched	3	
Unrelated	19	
Autologous	12	

CML, chronic myelogenous leukaemia; AML, acute myelogenous leukaemia; ALL, acute lymphoblastic leukaemia; HD, Hodgkin's disease; NHL, non-Hodgkin's lymphoma; CR1, first complete remission.

the study. The protocol was approved by the ethics committee of the University of Dresden (reference number EK220396). All patients had given informed consent. Patient characteristics are summarized in Table I.

Medication

Busulphan/dimethyl sulphoxide (DMSO) infusions were prepared as follows: 200 mg of BU (Sigma Chemical, St. Louis, USA) were dissolved in 10 ml of DMSO (Cryoserv, WAK Chemie, Bad Homburg, Germany). After sterile filtration (Minisart SRP 25, Sartorius AG, Göttingen, Germany), the resulting stock solution was further diluted with isotonic saline to a constant final concentration of 0·2 mg/ml BU. Based on the observation of an average bioavailability of 70% observed in our previous study (Schuler et al. 1998), patients initially received 2·8 mg/kg body weight BU/DMSO (study patient number (SPN) 1–14) as a single daily infusion over 3 h and, in addition, received single oral doses of 4 mg/kg on the other 3 d. Single daily dosing has been shown to be feasible (Shaw et al. 1994) and we observed equivalent AUCs compared with the more usual four daily doses in a small series (data not shown). We preferred this dosing interval because there are principal limitations in carrying out pharmacokinetic analyses of a drug with a terminal half-life of 2–3 h in a dosing interval of less than three half-lives (for discussion see Schuler et al. 1994). The remaining 31 patients were scheduled to receive the entire BU of the conditioning as four intravenous infusions on four consecutive days. Beginning with SPN 21, the dose was increased from 2·8 to 3·1 mg/kg body weight because the AUCs at the 2·8 mg/kg dose were slightly lower than expected. In order to eliminate possible chronopharmacological effects, all infusions were started at 9:00 a.m. The infusion solution was prepared by the hospital pharmacy immediately before infusion. The DMSO dose administered amounted to 140 μl/kg body weight (bw) for the first 20 patients and 155 μl/kg bw for patients 21–45.

Patients received benzodiazepines for seizure prophylaxis, usually 10 mg diazepam/d. Phenytoin was not given because of the known induction of BU metabolism by this drug (Hassan *et al.* 1993). For anti-emetic prophylaxis, serotonin antagonists (± dexamethasone) were used. Conditioning in addition to busulphan included cyclophosphamide (CP) 60 mg/kg/d i.v. on two consecutive days in patients transplanted from a human leucocyte antigen (HLA)-identical sibling. In patients to be transplanted from an unrelated or HLA-mismatched donor, the dose of CP was 50 mg/kg/d for 4 d and additional antithymocyte globulin (ATG) was added to the conditioning regimen. In patients with advanced leukaemia given a graft from a HLA-identical sibling, etoposide was administered at a dose of 30–45 mg/kg in addition to 120 mg/kg of CP and BU. Patients with allogeneic transplants received cyclosporin A (CSA) either alone or in combination with methotrexate (short course, eight patients with unrelated donors) or mycophenolate mofetil (13 patients with related donors) for graft-versus-host disease (GVHD) prophylaxis.

Pharmacokinetic studies

Sample collection. Blood samples [7–8 ml of heparinized (ammonium heparinate) monovettes, Sarstedt, Nürmbrecht, Germany] were collected before and 1, 2, 3, 4, 5, 6, 9, 12, 15 and 24 h after administration of BU. Blood samples were drawn at least on 2 d of the four treatment days. The monovettes were immediately chilled. After centrifugation at 4°C for 10 min, the plasma was removed and portioned into sterile Cryo vials. The plasma samples were stored at −20°C and assayed within 3 weeks of freezing.

Sample pretreatment. BU in plasma was quantified using a high performance liquid chromatography (HPLC) assay, as

described in detail before (Blanz *et al*, 1990), except that the extraction procedure was modified as follows. For the 'clean-up' of plasma samples at 2, 3 and 4 h after administration of BU, 3 ml preconditioned extraction columns were used (in order to prevent overloading of the solid phase extraction column with the higher concentrations). The external standards in final concentrations from 3000 ng/ml to 5000 ng/ml were also extracted using 3-ml cartridges. For the 3-ml columns, 1·5 ml of methanol was used to elute drug into a glass reaction vial.

Reagents and materials. Methanol and acetonitrile (HPLC grade) were obtained from J. T. Baker (Mallinckrodt Baker, Griesheim, Germany). Busulphan (98% pure), 1·4-diiodobutane (99% pure), n-heptane (HPLC grade) and 2-methoxyethanol (99·9+ %, HPLC grade) were purchased from Sigma-Aldrich (Steinheim, Germany) and sodium iodide (99·5%) and acetone (LiChrosolv, HPLC grade) from Merck (Darmstadt, Germany). Water was deionized and filtered with a Milli-Q™ Plus PF water purification system (Millipore, Eschborn, Germany).

HPLC. The chromatographic system consisted of an analytical HPLC pump 626 (Waters, Eschborn, Germany), a gradient controller 600S (Waters) and an autosampler 717plus (Waters). A Spectra System UV1000 spectrophotometer (Spectra-Physics, Darmstadt, Germany) was used for drug detection. Acquired data were processed using MAXIMA software (Waters). Solvent was degassed on-line using a vacuum membrane degasser (Spectra Physics). Separations were performed on a Grom Sil100 Cyano-2 PR (5 μm) HPLC cartridge (250 × 4·6 mm; Grom, Herrenberg, Germany) protected with a LiChrospher 100 CN (5 μm) guard column (10 × 4·6 mm; Grom). The equipment for post-column derivatization by photochemical reaction was placed between the column and detector.

Chromatographic conditions. The isocratic solvent system consisted of water–acetonitrile (80:25; v/v) at a flow rate of 1 ml/min. The solvents were used without further purification. The detection wavelength was 226 nm.

Data analysis. Data were analysed using TOPFIT (Heinzel *et al*, 1993). $t_{1/2}$, clearance and volume of distribution (Vd) of BU were calculated using a one-compartment model for the intravenous formulations. Intravenous AUCs presented here are also model-derived and extrapolated to infinity. Non-compartmental estimates (not shown) using the trapezoidal rule and limited to 24 h were almost identical, because the degree of accumulation was negligible. For oral doses, non-compartmental estimates of AUC were used in some cases, when there was evidence of multisegment absorption. For comparisons of pharmacokinetic parameters over time, the Wilcoxon signed rank test was used for paired variables. As this was a *post hoc* decision for testing, only *P*-values $< 0{\cdot}01$ were considered significant.

RESULTS

Toxicity

No major acute toxicity was associated with the intravenous BU preparation. One female patient experienced facial flushing on the first day and was subsequently switched to oral BU (SPN 25, no pharmacokinetic data available). No seizures were observed. With serotonin–antagonist prophylaxis, nausea during the days of BU administration was usually mild and only a few patients had minor episodes of vomiting. As expected, the typical DMSO odour was perceived for about 24 h after the infusion.

Mucositis was seen in all patients and was mild in most cases, nevertheless the majority received opiates for some days in order to allow as much enteral feeding as possible in these circumstances. There was no apparent correlation between opiate requirements (as a measure of severity of mucositis) and BU AUC, which probably reflects the non-homogeneous nature of the patient population and cytostatic drugs used in the conditioning other than BU. No patient needed intubation. No VOD was seen in 30 patients who received intravenous BU only. Non-fatal VOD was observed in one patient receiving a single dose of intravenous BU preparation and three oral doses.

Pharmacokinetics

Data were available on 44 patients. One patient was switched to oral dosing after first dose, and was not sampled. Overall, 74 d of i.v. dosing and 22 d of oral dosing were monitored. For 30 patients data from 2 d with i.v. dosing were available, which allowed the use of test statistics for paired samples in the analysis of temporal trends. For eight patients 2 d with oral and 1 d with i.v. dosing, and for six patients 1 d with oral and 1 d with i.v. dosing, were analysed. Of the data sets after i.v. BU, 29 were from d 1, 17 from d 2, 18 from d 3 and 10 from d 4 of the 4-d course.

Average peak BU levels (2·8 and 3·1 mg/kg dose levels combined) were 3250 ± 910 ng/ml after intravenous single daily doses and 4275 ± 1040 ng/ml after single oral doses of 4 mg/kg. In all patients, BU concentrations after 24 h were close to or below the detection limit. About half the

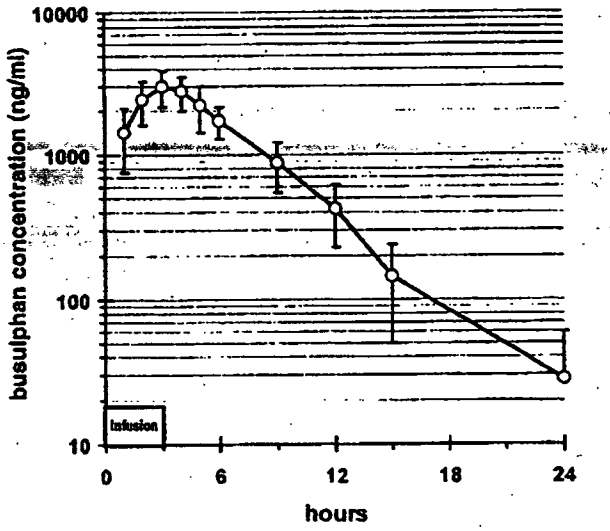

Fig 1. Semilogarithmic plot of average concentrations (± STD) of 20 patients receiving the dose of 2·8 mg/kg busulphan as a 3-h infusion. Patients with sampling on d 1 and d 2 are combined.

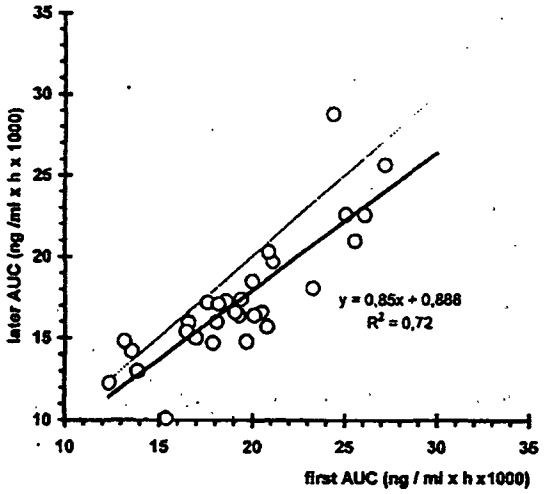

Fig 2. Correlation between the two AUCs measured in individual patients after intravenous doses of 2·8–3·1 mg/kg. Solid line, linear regression; thin line, expected line of identity.

values at 24 h were below 20 ng/ml (62% after oral doses, 52% of i.v. doses), the remaining levels were 64 ng/ml on average. Thus, no relevant accumulation occurred and the proportion of the AUC extrapolated to infinity was negligible.

A typical BU–plasma concentration curve is displayed in Fig 1. Intravenous AUCs, as measured in the first 20 patients, were lower than expected from studies using the conventional 4 × 1 mg/d oral BU dosing. In the subgroup of 14 patients with both i.v. (2·8 mg/kg) and oral doses (4 mg/kg), the mean AUCs for i.v. doses were 22300 ± 5640 and for oral doses were 26440 ± 6620; therefore, the i.v. dose was increased to 3·1 mg/kg.

There was a good correlation between AUCs of earlier and later i.v. doses ($y = 0{\cdot}85x + 0{\cdot}888$, $R^2 = 0{\cdot}72$, Fig 2). However, there was a deviation from the line of identity, with the later AUCs being lower than earlier measurements (Wilcoxon signed ranks test, $P = 0{\cdot}0004$). This was accompanied by a minor increase in clearance from 2·6 to 3·0 ml/min, and a corresponding reduction of $t_{1/2}$ from 2·89 to 2·68 (for patients with two i.v. data sets the *P*-values were $P = 0{\cdot}006$ and $P = 0{\cdot}066$, respectively, by Wilcoxon signed rank test). Table II shows mean AUCs as measured for the two dose levels on d 1–4. Table III gives comparisons for Vd, Cl and $t_{1/2}$.

Given the observed decline of AUC over time, a measure of individual exposure could not be derived directly from the measured AUC, as the days of sampling differed between patients. Therefore i.v. AUCs had to be standardized for dose and day of treatment. The proportions of a standardized AUC (AUC divided by dose) with the means of the respective days were used to estimate the AUCs on those days that were not measured in individual patients. This enabled the comparison of estimates of exposure for patients, e.g. with sampling on d 1 and 2 (assuming lower exposure on d 3 and 4), and patients with measurements on d 3 and 4 (assuming higher exposure on d 1 and 2). Table IV gives the means of AUC/dose (mg/kg) for the respective days. For patients with i.v.-only conditioning, this led to an estimate of an average overall AUC of 18060 ± 3310 ng × h/ml (range 12130–25640) per d.

Outcome

Diagnoses were heterogeneous and the numbers of patients did not enable the assessment of the antileukaemic potency of the intravenous BU formulation. D 100 mortality in this series was 28% (13/45 patients). Causes of death in relation to BU-AUCs are outlined in Table V.

Four patients had primary or secondary graft failure (three unrelated donors, one haploidentical family donor, all grafts done with CD34 selection). Two of these four patients were in the lower range of exposure with estimated AUCs of 12950 and 14100 ng × h/ml (SPN 17 and 22). The patient with the haploidentical transplant (SPN 37) recovered after transfusion of the autologous back-up marrow, but died from progressive chronic myeloid leukaemia (CML). The fourth patient (SPN 33) was a 20-year-old man with a predominantly pancreatic form of cystic fibrosis, a disease known to be associated with altered metabolism of some drugs. His blood levels were close to the observed means of the entire group (AUC d 1 19300, d 4 16400 ng/ml × h). He engrafted with stable chimaerism after a second stem cell infusion but died at 5 months from chronic GVHD complicated by aspergillosis.

DISCUSSION

Here we describe a series of 30 patients receiving all BU doses of pretransplant conditioning protocols in the form of single daily infusions on four consecutive days. An additional 15 patients received at least 1 d of i.v. BU in addition to oral doses. The acute tolerability of the infusions was excellent. In accordance with oral single daily dosing (Shaw *et al*, 1994), high peak levels were not associated with an increased risk of seizures using standard prophylaxis with benzodiazepines.

The administration of single daily infusions of BU of 3·1 mg/kg rendered handling of this regime easier than the strict adherence to the usual 6 h intervals (i.e. four times a

Table II. Measured AUCs (± SD) for two BU dose levels on the respective days.

Dose	d 1	d 2	d 3	d 4
2·8 mg/kg	23200 ± 5070 ($n = 9$)	17900 ± 3370 ($n = 11$)	15940 ± 2670 ($n = 5$)	22900 ± 14990 ($n = 2$)
3·1 mg/kg	20600 ± 3800 ($n = 19$)	19290 ± 3650 ($n = 7$)	17400 ± 2770 ($n = 12$)	16890 ± 4960 ($n = 9$)

Table III. Changes in volume of distribution, clearance and $t_{1/2}$ (± SD) in individual patients.

	Initial dose	Subsequent dose	
Volume of distribution (l/kg)	0·62 ± 0·18	0·62 ± 0·17	n.s.
Clearance (ml/min/kg)	2·64 ± 0·56	3·02 ± 0·72	Mann–Whitney $P = 0·0095$ (Wilcoxon $P = 0·0004$)
$t_{1/2}$ (h)	2·87 ± 0·64	2·64 ± 0·59	Mann–Whitney $P = 0·096$ (Wilcoxon $P = 0·0150$)

'Initial dose' refers to that dose in an individual patient for which we have the first data set of concentrations, irrespective of whether it was dose 1, 2 or 3. 'Subsequent dose' refers to the second available data set in the same patient (dose 2, 3 or 4).

day dosing) of BU administration. AUCs were close to the expected range on average. We have, however, evidence that AUCs and peak levels decreased along with increasing clearance and possibly shortened half-life over the 4 d of treatment. Self-induction of BU metabolism has been described before, but seems to be of minor importance, at least in patients in whom phenytoin is not used for seizure prophylaxis (Hassan *et al*, 1989, 1993). Decreasing AUCs could be as a result of the co-administration of DMSO affecting the pharmacokinetics of BU. An increasing Vd of BU caused by DMSO would be an explanation, but there was no significant change of Vd over time. However, given the comparison of AUCs after single daily oral doses with i.v. doses, an increase of Vd (independent of time) with the intravenous formulation compared with that observed at lower doses seems possible. In the small group receiving both oral and intravenous BU, the observed AUCs after 1 × 4 mg/kg orally were higher than expected compared with our previous experience with 4 × 1 mg/kg/d (Schuler *et al*, 1994), which renders intraindividual comparisons difficult. For mathematical reasons it was impossible to estimate volume of distribution after oral dosing independently from the bioavailability. As a consequence, a possible influence of DMSO on BU-Vd cannot be ruled out completely; this would require the administration of i.v. DMSO together with oral BU in a separate study. Our data suggest an increase of the BU clearance over time as a cause of decreasing AUCs.

Overall, the observed AUCs after intravenous BU administration were slightly lower than anticipated. We considered that our results were affected by the stability of the BU preparation; however, the minor changes over the period of administration (data not shown) were insufficient to explain the observed effects. A more probable explanation is that the actual bioavailability of oral busulphan is higher than our original estimate of 70% (Schuler *et al*, 1998). Hassan *et al* (1994) found a bioavailability of 80% in seven adults (range 47–103%) and 68% (range 22–120%) in nine children. Nevertheless, the observation of two patients (SPN 17, SPN 22) with non-engraftment and estimated AUC in the lower range of observed values is of concern.

No VOD was observed in patients who received BU intravenously only. This may be for several reasons. First, AUCs were lower than expected. Second, interpatient variability may be somewhat lower in patients who are treated intravenously, reducing the likelihood of extreme values. However, if variability is expressed as SD/mean the available data show only a minor reduction of interpatient variability compared with our previous series. A third explanation would be that by the intravenous route a possible contribution of 'first pass-toxicity' of gastrointestinal absorption of BU (by high concentrations in the portal blood) was avoided. Despite this lack of hepatic toxicity, the d 100 mortality of this series was high, but the majority of fatal outcomes occurred in situations with other obvious risk factors.

Further evaluation of the current i.v. BU preparation should include a further moderate dose increase, in order to obtain AUCs closer to or even higher than the target range based on levels obtained with oral dosing. This should be possible, as the current formulation was not associated with hepatic side-effects. Cumulative DMSO doses were within the range usually administered with previously cryopreserved stem cell grafts. It has recently been shown that no major accumulation of DMSO is to be expected, but that accumulation of the metabolites of DMSO, dimethylsulphone and dimethysulphide may occur (Egorin *et al*, 1998). A study aimed at elucidating the impact of DMSO on drug distribution and elimination in this setting is under way.

Other intravenous formulations of BU have been studied in animal models (Bhagwatwar *et al*, 1996; Hassan *et al*, 1998) and, more recently, in patients (Andersson *et al*, 2000; Olavarria *et al*, 2000). In the latter study an almost identical dose recommendation (0·8 mg/kg body weight in

Table IV. Means (± SD) of estimated AUCs, standardized for a dose of 1 mg/kg body weight i.v. BU.

	d 1	d 2	d 3	d 4
AUC/dose (ng × h/ml)	7000 ± 1510	6150 ± 1080	5460 ± 1010	5890 ± 1590

Table V. Causes of death in relation to BU AUC.

	SPN	Age (years)	Underlying disease	Graft	Day of death (day post transplant)	ANC (> 0·5 × 10^9/l)	Platelets (< 20 × 10^9/l)	Estimated average AUC	Causes of death
One single daily intravenous + three oral doses	2	42	NHL	UD	10			35680	Gram-positive septicaemia + suspected aspergillosis
	11	36	Secondary AML after HD	MM-FD	94		13	24510	CMV pneumonitis
	12	19	CML in BC	UD	34		30	21920	Progression of leukaemia
	14	21	NHL	Auto	62	10	6	20190	Progression of lymphoma
Four single daily intravenous infusions	16	63	AML 2.CR	SD	89	14	9	18080	Progression of leukaemia
	17	32	MDS, RAEB-T	UD	88			14100	Non-engraftment, CMV pneumonitis + MOF after second graft
	22	41	CML	UD	65	37		12950	Non-engraftment → second graft*Aspergillus niger* infection
	24	31	CML	SD	87	10	7	17650	aGVHD, CMV infection
	28	37	ALL, 1. REL	UD	44	19		23430	Interstitial pneumonia
	37	45	CML, 2nd CP	Haplo-id FD	79	18		17340	Progression of leukaemia after non-engraftment + aspergillosis → autologous backup graft given
	38	49	AML	MM-FD	90	15		19170	GVHD
	42	43	AML, 1. REL	UD	41	18		16930	MOF (suspected aspergillosis)
	43	42	ALL, 1. REL	UD	97	9	11	18050	Relapse, intracerebral bleeding

AML, acute myelogenous leukaemia; CML, chronic myelogenous leukaemia; BC, blast crisis; CP, chronic phase; HD, Hodgkin's disease; ALL, acute lymphoblastic leukaemia; NHL, non-Hodgkin's lymphoma; CR, complete remission; REL, relapse; MDS-RAEB–T, myelodysplastic syndrome, refractory anaemia with excess of blasts in transformation; MM-FD, mismatched family donor; SD, sibling donor; UD, unrelated donor; auto, autologous; haplo-id, haploidentical; CMV, cytomegalovirus; MOF, multiorgan failure; aGVHD, acute graft-versus-host disease.

6-h intervals, corresponding to 3·2 mg/kg/d) was reached. The formulation presented here has the obvious advantage of simple preparation and low cost. Furthermore, administration of BU as a single daily dose (rather than four doses) simplifies treatment substantially. Even with the i.v. route, such a regimen can be administered on an outpatient basis.

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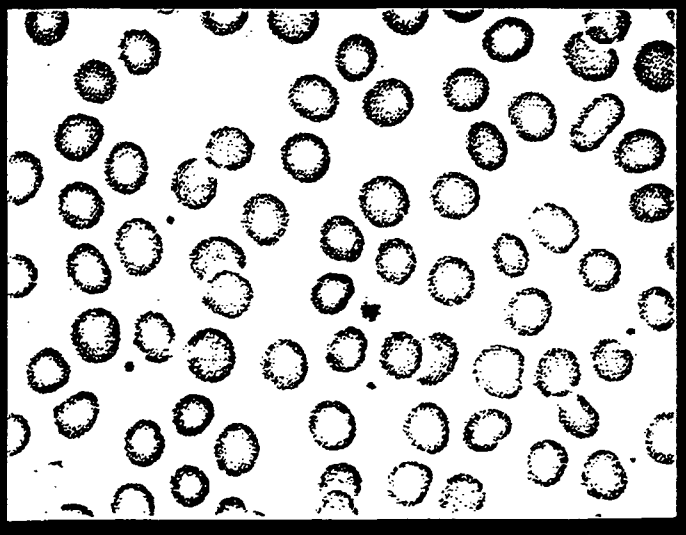

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Aspects Concerning Busulfan Pharmacokinetics and Bioavailability

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Busulfan as a high-dose therapy is an important component of many of the myeloablative regimens for both allogeneic and autologous bone marrow transplantation (BMT) in adults and children. During the last decade, several studies have shown a wide inter- and intra-patient variability of busulfan disposition in adults and children. Some of the factors affecting the interpatient-variability were identified as circadian rhythmicity, age, disease, drug interaction, alteration in hepatic function and recently busulfan bioavailability. In adults, pharmacodynamic studies have shown a positive correlation between high-systemic exposure of the drug and venocclusive disease (VOD). However, pharmacodynamic studies in children did not establish any correlation between the systemic exposure and VOD. Drug-monitoring and dose adjustment in adults were used successfully to decrease the occurrence of VOD and mortality. It was observed that about 20% of the busulfan dose crosses the blood brain barrier. The high amount of the drug which enters the brain can probably be involved in the CNS toxicities reported.

In children, a low rate of toxicity combined with a high rate of engraftment failure were observed. Several investigators have expressed their concern about the dosage in children and many suggested higher doses based on the body surface area for young children. However, recently it was shown that busulfan bioavailability varied by 2-fold in adults (0.5–1.03) while in children a 6-fold variation was observed (0.22–1.20). The access to an intravenous form of busulfan and a deeper understanding of pharmacodynamics of the drug might be essential to optimize the treatment, reach a successful engraftment and lower the therapy related toxicities.

KEY WORDS: busulfan bioavailability pharmacokinetics

INTRODUCTION

Busulfan is a difunctional alkylating agent introduced in the early 1950's by Haddow and Timmis.[1] For more than three decades, busulfan was used for the treatment of chronic myelocytic or granulocytic leukemia (CML),[2-9] polycythemia rubra vera,[10-12] essential thrombocythemia,[13,14] myasthenia gravis[15] and chronic granulomatous disease in children.[16] Excessive bone marrow depression is the main toxic effect.[17-20] However, some very important but less common side effects occurring during low dose treatment are pulmonary or alveolar lung fibrosis i.e. "busulfan lung",[21-26] cataract,[27,28] Addison-like disease, hyperpigmentation[29,30] and endocardial fibrosis.[31,32]

The myelosuppressive properties of busulfan were used when Santos *et al.*[33] introduced the drug in combination with cyclophosphamide (BuCy), as an alternative for total body irradiation (TBI), in the myeloablative regimen for bone marrow transplantation (BMT). Busulfan in high doses (4mg/kg) for four days is currently used in BMT[34-37] for hematological malignancies and nonmalignant disorders such as immunodeficiencies,[38-40] thalassemia[41,42] and osteopetrosis.[43] This combination has the advantage that TBI is avoided which is important especially in pediatric patients. The anti-leukemic effects and the therapeutic efficacy of the BuCy regimens are considered to be equivalent to cyclophosphamide and TBI.[44,45] However, toxicity is still the dose limiting factor.[46-49] In high dose therapy, hepatic veno-occlusive disease (VOD) was reported and correlated to high levels of busulfan in plasma.[50] Interstitial pneumonia (IP) occurred in about

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10% of the bone marrow transplantation recipients and was fatal in many cases.[48] Furthermore, hemorrhagic cystitis, cataracts, convulsions and mucositis were reported as side effects during or after the high dose therapy.[51–56]

Busulfan is administered only by the oral route and is not available as parenteral drug. It is extensively metabolised in the liver.[57,58] In recent studies it was shown that busulfan crosses blood brain barrier in the humans and it accumulates in the liver and lungs of the monkey.[59] During the last decade the disposition of the drug has been investigated in both adults[50,60] and pediatric patients.[61–66] The kinetic behaviour of busulfan has also been studied in hematological malignancies and nonmalignant disorders.[67] Recent studies have shown alterations in busulfan disposition in children compared to adults: including lower plasma concentrations, minimal toxicity and higher rates of failure to achieve marrow engraftment.[38,68,69] A wide inter-patient variability in busulfan kinetics was observed by several investigators. Age, drug-drug interaction, variability in absorption, hepatic function, circadian rhythmicity and the disease were pointed out as factors affecting the variability of busulfan disposition. Because of the lack of a parenteral administration form of busulfan, most of the pharmacokinetic studies assumed the complete bioavailability of the drug. In a recent study, we have shown that busulfan's bioavailability varied by about 2-fold in adults and about 6-fold in children. Considering these factors with a fixed dose of busulfan of 16 mg/kg, systemic exposure and thus toxicity and therapeutic efficacy would be variable from one patient to another. To achieve a better outcome with a lower toxicity in BMT pharmacologically-guided dose adjustment was discussed by many authors. However, because of the lack of information about the pharmacodynamics of the drug and its mechanism of action, the dose-adjustment is still neither the optimized method to improve BMT outcome nor to lower the drug toxicity. To improve the drug efficacy, minimize inter-individual variation, improve the outcome from BMT especially in young children and to lower the drug related toxicities i.e. VOD, seizures and interstitial pneumonia there is an urgent need of a parenteral form of busulfan in combination with pharmacodynamic studies. Our aim is to illustrate some of the problems and difficulties using the oral form of busulfan in bone marrow transplantation.

BUSULFAN ASSAY

Busulfan is thermolabile and with a very low UV absorption and no fluorescence properties. This has made the detection of the intact compound very difficult. In early pharmacological and metabolic investigations radiolabelled busulfan was used. These methods could not distinguish between the parent compound and its metabolites. During the last decade many analytical methods have been developed,[70–80] which probably was the first step to understand the pharmacokinetics, pharmacodynamics and transplant-related toxicities of busulfan.

In the early 1980's new chromatographic techniques for the determination of busulfan were developed. Most of these methods converted busulfan to either thermally stable derivatives to be analyzed by gas chromatography or to a derivative with UV absorption properties that could be analyzed by liquid chromatography.[75–78] Only one method was reported to analyse busulfan as an intact drug using a liquid chromatography system and mass spectrometry.[80] The first method[70] reported by our laboratory which consisted of an initial extraction with dichloromethane, derivitization with sodium iodide to convert busulfan to 1,4-diiodobutane and its internal standard (1,5-bis-(methanesulfonoxy) pentane) to 1,5-diiodopentane. Gas chromatography in combination with mass spectrometry (GC-MS) was used for the analysis. The detection limit is about 1 ng/ml. Vassal *et al.*[71] also utilized GC-MS after conversion of busulfan and deuterated busulfan (internal standard) to the corresponding iodo-derivatives.

Gas chromatography with electron capture detection is a very sensitive but less expensive technique which has also been used in the determination of busulfan.[72–74] The conversion of busulfan and its internal standard to 1,4-diiodobutane and 1,5-diiodopentane, respectively was modified so that the extraction and the conversion to the iodo-derivatives were performed in one step. The reaction was performed in plasma for 40 min at 70°C in the presence of n-heptane and sodium iodide. The organic phase was subsequently used for the GC analysis. The GC-ECD method is currently used in our laboratory because of its high sensitivity, selectivity and the minimum assay time required to achieve results which is probably of a great importance when dose-adjustment is considered. Other reagents such as 2,3,5,6-tetrafluorothiophenol are used to convert busulfan and its internal standards to tetrafluorothiophenol derivatives after either an extraction from plasma or from ultrafiltrate. The derivatives were analyzed either by GC-ECD or LC/UV. The reaction with iodide was utilized by Blanz *et al.*[78] in a precolumn derivatization yielding 1,4-diiodobutane, which was analyzed and detected after on-line photolysis.

BUSULFAN KINETICS AND BIOAVAILABILITY

The early studies about the pharmacokinetics parameters of busulfan have been carried out in mice, rats, rabbits and humans by the administration of busulfan labelled

with ^{35}S, ^{14}C or ^{3}H.[80–86] However, these studies did not distinguish between the parent compound and its metabolites.

In the early 1980's new analytical techniques were developed which were able to distinguish between the parent compound and its metabolites. The first pharmacokinetic study[87] after oral doses of 2, 4 and 6 mg in patients with CML showed that busulfan was eliminated with a half-life of 2.6 hr. Busulfan was excreted as unchanged drug by about 1% in urine over 24 hours. The kinetics obeyed a zero-order absorption process: a constant fraction of the dose reaches the central compartment per time unit, in an one compartment model. It was also shown that busulfan demonstrated linear kinetics within the range 2–6 mg. Considerable interest has been focused on busulfan since its introduction as a part in the myeloablative regimen by Santos *et al.*.[33] Several studies dealing with busulfan pharmacokinetics in both adults and children have been reported. However, all these investigations assumed the complete bioavailability of the drug because of the lack of a parenteral form.

Bioavailability, especially for those drugs involved in the conditioning regimen prior to BMT, is an important parameter that defines the efficacy of ablation of BMT and the regimens related toxicities. Bioavailability refers to the rate and extent of absorption of the administered active compound, where absorption encompasses the processes between administration and site of measurement which usually is the systemic circulation. There are many factors which can alter the bioavailability of a drug including the dissolution and absorption characteristics of the administered form, the dosage form, the route of administration, the stability of the active compound in the gastrointestinal tract and the extent of the hepatic drug metabolism. In a serie of 16 patients[88] we have shown that the oral availability after a 2 mg dose varied from 47–103% in adults and from 22 to 120% in children. One child with Hurler's disease showed a bioavailability of 22%, while the bioavailability for the other children was similar to that for adults. It was reported by Vassal *et al.*[67] that children with lysosomal storage diseases like Hurler's and San Filippo's disease have a significantly higher clearance than other children (8.7 versus 6.3 mL/min/kg). Clinical studies have also showed that patients with Hurler's or San Flippo's disease have a higher rate of engraftment failure when conditioned with a standard dose of busulfan. Altered drug absorption and/or increased first-pass effect could probably explain the low bioavailability of the drug observed for children with lysosomal storage diseases.

According to the venous equilibrium or sinusoidal model, the hepatic clearance of a drug may be increased due to: higher metabolic activity as a function of the hepatic mass; decreased plasma protein binding and/or increased hepatic blood flow. On the other hand the distribution volume may be influenced by body composition, protein binding and tissue binding of the drug. After an i.v. administration of busulfan, both the clearance normalized to body weight and the distribution volume adjusted to body weight appear to be age dependent (Fig. 1 and Fig. 2). Clearance and distribution volumes were significantly higher in children than in adult patients (45% and 34%, respectively). Busulfan protein binding does not explain these differences since it was shown that the binding is very low at both high and low doses.[60,89] No difference in the elimination rate constant was observed. Busulfan administered by the i.v. route confirmed the results reported by several investigators after oral administration of the drug[61–65] regarding busulfan disposition in relation to age (table).

The values for distribution volumes obtained after i.v. administration corresponded to total body water and the difference between adults and children was equal to that reported for body water.[90,91] This might indicate that in spite of the lipophilicity of busulfan,[92] it is still distributed to body water. In this respect, busulfan might be like caffeine, which is also a rather lipophilic compound and has a distribution volume equal to body water.[93,94] The differences in clearance could be explained, if the liver blood flow or hepatic mass is different in children compared to adults when normalized to body weight, but not to body surface area. It was shown that the children have a liver volume (examined by ultrasound scans) averaged 30–35 mL/kg while in adults the liver volume is 19.5–22 mL/kg.[95–97] Because of the very limited information available concerning busulfan bioavailability we will limit our discussion to factors affecting availability to absorption, drug-drug interaction and the metabolic pathway for busulfan since it is known that the first pass effect can alter drug bioavailability.

ABSORPTION

Busulfan is only available as 2 and 25 mg tablets. This particularly affects the administration in very young children. The methods used in different bone marrow transplantation centers have been reported to be: crushed tablets in a water suspension given through a gastric tube,[61] mixed with food or applesauce[50] and/or enclosed in gelatine capsules.[62] After oral high-doses of busulfan in young children as well as in the low dose therapy, the absorption kinetics were described by a zero-order kinetic model.[62] However, the kinetics of absorption varied and was also described by a first-order absorption model.[65] The absorption half-life for